EFFECT OF SOIL TEXTURE AND MATRIC POTENTIAL ON THE MOVEMENT OF *POLYMYXA GRAMINIS* ZOOSPORES

by

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Introduction

Polymyxa graminis Ledingham, a plasmodiaphoraceous fungus, is an intracellular obligate parasite that infects roots of plants in several genera of the family Gramineae. It does not produce any external symptoms in the host, but it is a vector that has been implicated in the transmission of a number of destructive viruses of cereals. The fungus is distributed world-wide and has been found on almost all the continents of the world (4, 7, 11, 13, 22, 23, 25).

The viruses P. graminis vectors include wheat soil-borne mosaic virus (WSBMV) (4), wheat spindle streak mosaic virus (WSSMV) (22), oat mosaic virus (7), barley yellow mosaic virus (11) and rice necrosis mosaic virus (10). Among the diseases caused by these viruses, wheat soil-borne mosaic (WSBM) has long been recognized in Kansas (17). This disease, synergistically associated with wheat spindle streak mosaic (WSSM), has become a potential threatening problem to the wheat crop in Kansas (14). They are particularly serious in eastern and central Kansas and have caused an estimated average annual loss of 2.7% statewide since 1976 (W. G. Willis, personal communication). Prolonged cool spring weather increases disease severity.

The fungus P. graminis has been known since 1939 (13). However it has not been well studied. This situation may be due to methodological problems. P. graminis is not a pathogen itself, and

therefore its growth in plant tissues does not lead to any noticeable change in the appearance of the host plant. Host bioassay methods by examination of the fungal structures in the host plants cannot be applied without tedious work. Additionally P. graminis structures are very small; viz. a Polymyxa graminis zoospore (20) is only one third the size of a Phytophthora zoospore (5). Therefore great difficulties exist in the observation of fungus development and activity.

Most previous work on P. graminis has concerned the biology and morphology of the fungus in relation to the transmission of WSBMV. The work of Ledingham shows that the fungus enters root hairs and epidermal cells of wheat plants via motile, biflagellate zoospores (13). The zoospores have two unequal flagella. While swimming, the spores have various shapes and average 4.2 μ in diameter (20). In the penetrated host cells of wheat roots P. graminis expands into plasmodial bodies. These plasmodia begin to differentiate into sporangia within a few days and eventually segment into zoospores or develop into smooth, thick-walled resting spores 5 to 7 μ in diameter. After release from zoosporangia, the zoospores may reinfect other wheat roots and develop into zoosporangia or resting spores. Under low-power magnification the clustered resting spores (cystosori), in cortical and epidermal root cells, resemble bunches of grapes and can be easily recognized.

In plant disease epidemiology, dissemination of inoculum is a critical stage in the initiation of disease cycles. In diseases

caused by zoosporic fungi, the ability of zoospores to move as dispersal units through soil is considered to be important for epidemic development (18) and may be partially characterized by length of motile period, tactic response to host plants, and swimming distance in the environment. Motile zoospores of P. graminis are considered the primary and secondary infective propagules in the field. Ledingham reported (13) that P. graminis zoospores swim for a few minutes at 28 C, whereas at 18 C, they remain motile much longer. But there is no definite time recorded in the literature. In the field, it is frequently observed that more wheat plants are infected with WSBMV in low, poorly-drained spots than in other areas (25). It has been speculated that high moisture conditions in these areas create a more favorable environment for zoospore movement through soil. However, little research has been done concerning zoospore motility under conditions typical of the natural soil environment.

P. betae, another species in the genus Polymyxa, has been found to be morphologically identical to P. graminis (1). P. betae is also an obligate parasite. It infects sugar beets and, interestingly, is also associated with a virus disease, beet necrotic yellow vein virus. However, compared with P. graminis much less research has been done on the biology of P. betae in relation to virus transmission.

Although Polymyxa zoospores are the only infective dispersal units in the field for the virus vectors, their motility has been little studied. In contrast, there is considerable information on the

motility of other zoosporic fungi, specifically the Phytophthora species. This is probably due to the wide distribution and economic importance of the diseases caused by Phytophthora, and the relative ease with which the large zoospores typical of this genus can be observed. Several studies have indicated that Phytophthora zoospores remain motile longer at cool temperatures than at higher temperatures. For example, P. palmivora zoospores swim for up to 24 hours at 17 C, and some even remain motile for as long as 84 hours; at 30 C, motility does occur but it is much reduced (3). In another species, P. megasperma var. sojae, the period of motility is 48 hours at 15 C, 24 hours at 25 C, and markedly reduced at either 5 or 36 C (9). Clearly temperature has a great influence on zoospore motility.

In addition to the effect of temperature, Benjamin and Newhook (2) have shown that abundant obstacles, (viz. glass beads and, possibly, soil particles) may lead to more rapid encystment of zoospores, presumably through collision with the solid surfaces of these obstacles. In the field, the percentage and size distribution of water-filled pores are determined by soil structure and water status (24). These factors are perhaps the most influential in zoospore movement, since zoospores swimming in soil water are likely to encounter numerous obstructions (soil particles). Duniway (6) has shown that Phytophthora cryptogea zoospores readily swim 25-35 mm through a coarse-textured (UC-type) soil mixture (16) at $\Psi_m >= -1$ mb. At $\Psi_m = -10$ mb zoospores moved lesser distances, and $\Psi_m = -50$ mb is probably the lower limit for measurable zoospore motility in the soil

mixture. In comparison to that in the UC-type soil mix, movement of P. cryptogea zoospores is very much reduced in loam soils, which contain fewer large pores than does the UC-type soil mix. At Ψ_m =< -10 mb, active zoospore movement is not observed. Duniway suggested that the limitation of active zoospore movement to high Ψ_m values is due to the large size of soil pores that must be filled with water to accommodate swimming zoospores, and probably the larger and less tortuous the water-filled pores the more suitable they will be for active zoospore movement.

Another study has further demonstrated the effects of various sizes of pore space on Phytophthora zoospore motility. Young et al (26) studied the effects of "ideal soils", composed of glass microbeads of various sizes, on zoospore movement by Phytophthora cinnamomi. Different sized beads create passages (pore necks) of various sizes connecting the voids between the beads. P. cinnamomi zoospores can be drawn passively through columns of beads with pore necks of 25-35 μ or larger and still retain their motility. But active movement among beads of this fine grade is severely restricted. Conversely, with pore necks 150-200 μ both passive and active movement of P. cinnamomi zoospores occurs.

Since zoospores are the only infective propagules produced by Polymyxa (25), studies on zoospore movement in relationship to soil physical factors will improve our understanding of diseases caused by the viruses it vectors. In this regard, studies on zoospore movement

of Phytophthora species may be instructive. However, information about zoospore movement by Phytophthora can not necessarily be extrapolated to Polymyxa, due to the differences in size of zoospores and lack of information on tactic reaction to host stimuli. Thus, the objective of this study was to investigate the influence of soil water status, soil texture and pore size on active zoospore movement of Polymyxa graminis to plant roots. As a complementary study, the influence of temperature on the motile period of P. graminis zoospores was investigated.

Materials and Methods

1. Fungal Culture Maintainance

The isolate of Polymyxa graminis used in this research, originally isolated from wheat plants in the state of Michigan, was supplied by W. G. Langenberg. This isolate was presumed to carry WSSMV although this has not been verified. The fungus was maintained on wheat plants (var. Ionia) according to Larsen's culture methods (12) as modified by W. G. Langenberg (personal communication). Wheat seedlings, pre-germinated for 2 to 3 days, were placed in 0.1-strength Hoagland's solution in a petri dish together with 1- to 2-month old P. graminis-infected wheat plants. The dish was painted black and had a small opening in the lid for the shoot of the source plant to stick out. Wrapped in a plastic bag, the dish was placed in an incubator at 20 C. After 3 to 4 days of incubation, source plants were removed, and inoculated seedlings were transferred to several similarly modified dishes. Two seedlings were placed in each dish, and allowed to grow in Hoagland's solution for several days. When the leaves were approximately 15-20 cm long, each seedling was transferred to a brown jar (Qorpak amber wide mouth bottles, 473 ml, Fisher Scientific) with an opening in the cap for the plant's leaves to escape. Hoagland solution was maintained at a depth of 1.5-2.0 cm in each jar and replaced regularly. After 1-2 months of growth these inoculated seedlings were used as P. graminis-infected source plants in further experiments (later in the paper, simply referred as source

plants). These culture transfers were made at 1- to 1.5-month intervals. All source plants were cold-treated by placing the jars at 3 C for 2-4 days before use in experiments, unless otherwise specified. This treatment is thought to stimulate zoospore release (W. G. Langenberg, personal communication).

2. Soil Materials

Three soil materials of different textures were used: a coarse silica sand (sand blasting grade), a soil-sand mixture, and a silt loam (Reading fine silt loam). The soil was collected from the upper 10-15 cm of a WSBM-infested field at the Ashland experimental farm of Kansas State University. Soil was air-dried and passed through a No.20 sieve (0.84 mm openings) before use. The soil-sand mixture was made by blending the sieved soil and a coarse sand at a 1:1 ratio. All three soil materials were autoclaved 1 hour at 121 C several days before use. Soil moisture release characteristics of all three types of soil materials were investigated by using 8-cm Buchner funnel tension plates (24).

3. Influence of Matric Potential on Zoospore Movement

The influence of matric potential on active zoospore movement of P. graminis was examined by arranging bait plants at various distances from a source of zoospores in soil materials at different matric potentials. The percentages of bait plants that had become infected at these distances from the source of zoospores presumably represented the amount of infective zoospores having reached the bait

plants after having moved various distances. A baiting method for detecting P. graminis zoospores was developed in this study (Fig 1).

Zoosporangia that formed on the roots of source plants were the source of zoospores. In this experiment, 1.5- to 2-month old source plants used in all treatments were collected from the brown jars, which had originally been inoculated with the same source plant. Four matric potentials and three types of soil materials in all combinations were used, totaling 12 treatments. The 0 mb (i.e. at saturation) treatments were done in glass petri dishes. Matric potentials ranging from -10 mb (water level 10 cm under roots of source seedlings) to -50 mb were controlled by using 8-cm-diameter Buchner funnel tension plates. A 15-mm layer of soil material was placed in each funnel following the method described by Duniway (6). After soil was in place, tension plates were adjusted to 0 mb and saturated from below. After 12 hours of saturation 12 small holes (to accommodate bait seedlings) were made with a dissecting needle in the soil layer in each funnel. The holes were made in two rows parallel to the diameter of the circular funnel, and traversed 2/3 of the length of this diameter (Fig 2). The distance between the two rows was 2.5 cm. Wheat seedlings (2-3 days old) were used as bait plants, and all the roots except one from each seedling were removed. Twelve bait seedlings were used per funnel, and the single root of each bait seedling was placed into one of the holes. The funnels were then adjusted to the desired matric potentials (-10 mb, -20 mb, and -50 mb) and allowed to equilibrate. After 12 hours, a trough (5 mm in

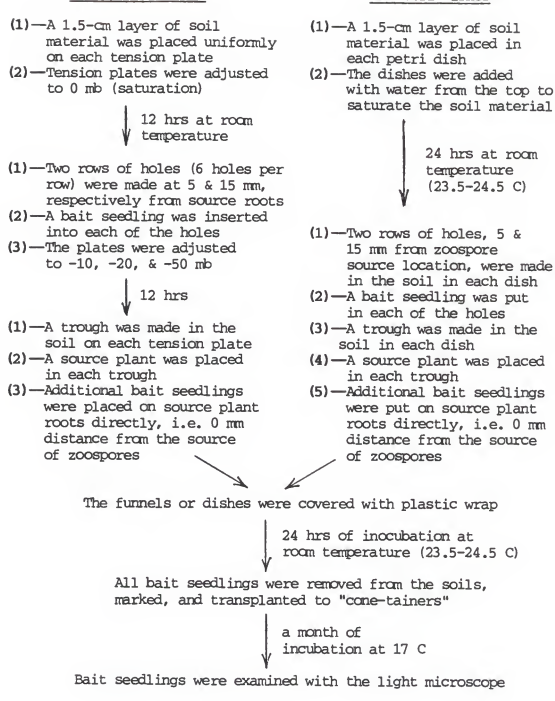

Figure 1. Flow chart of inoculation procedures used in the soil experiments

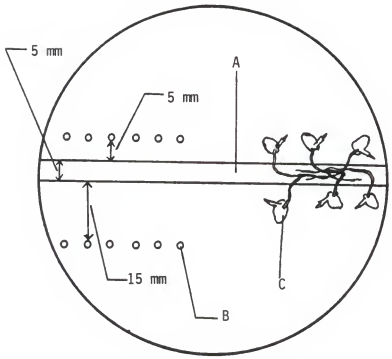

Figure 2. Spatial arrangement of bait seedlings and source of Polymyxa graminis zoospores in soil on tension plates or petri dishes. A, trough for placement of infected source roots. B, holes for placement of bait seedlings. C, bait seedlings at 0 distance from zoospore source.

width, 10-12 mm in depth, and 80 mm in length) was cut along the diameter of the soil layer, and roots of an intact source plant (1.5-2 months old) that were folded into 60-80 mm lengths were buried in the trough. On that portion of the source roots with no neighboring holes, roots of 6 bait seedlings were placed directly on the roots of each source plant (designated as the 0 mm distance from zoospore source), and covered with soil. Five to ten ml of water was added to consolidate the soil around the source roots. The 0 mb treatments were done in the same way except water was added from the top to saturate the soil in the dishes, and the soil was maintained at saturation (0 mb) continuously during the inoculation. In addition, bait seedlings in this treatment were put in at the same time as the source plants, 12 hours later than in the treatments done in the funnels. Funnels and dishes were covered with plastic wrap to reduce evaporation, and maintained at room temperature (23.5 to 24.5 C). After 24 hours of incubation, bait seedlings were taken out, and the tips (approximately 1-2 mm long) of their inoculated single roots were removed to differentiate the inoculated roots from roots which would form subsequently. The seedlings were then transplanted to sand in tubular seedling containers ("Cone-tainer", Ray leach, Canby, Oregon; top dia. 2.5 cm, length 12 cm). Each tube contained only one seedling. After a minimum of one month of growth in a growth chamber at 17 C, inoculated seedlings were taken out and rinsed with tap water. The marked roots, easily recognized because they were usually much shorter than the rest of the root system due to pruning, were examined with low magnification light microscopy. Seedlings with

cystosori of P. graminis in the roots were considered infected, and the number of infected seedlings was recorded. This experiment was repeated once, thus there were 2 replicate dishes or funnels for each treatment.

The raw data from these experiments were expressed as percent of bait seedlings infected and analyzed as a factorial experimental design with split plots. The main effects were soil medium and matric potential and the split plots were the distances.

4. Influence of Pore Size on Zoospore Movement

Effect of various sizes of pore space on zoospore motility of P. graminis was investigated by using glass microbeads (Fig 3). Two grades of glass microbeads, 0.10-0.11 mm and 1.00-1.05 mm in diameter (Braun Instruments, Burlingame, California), were used: the pore necks between the particles of each of the two grades of glass beads were 25-35 μ and 200-300 μ in diameter, respectively (26). Prior to use, beads were soaked in concentrated HCl (11.6 N) for 12 hours and then rinsed with distilled water several times till pH came close to neutral. Forty ml of beads were used in each petri dish and saturated with 0.1-strength Hoagland's solution. Two types of inoculum, representing different treatments, were used. In one treatment, roots of each 1.5-month old source plant were severed into pieces 4-7 cm in length, and all pieces from each source plant were buried in the beads in a straight line in the middle of a dish. In the other treatment intact source plants of the same age were used and placed

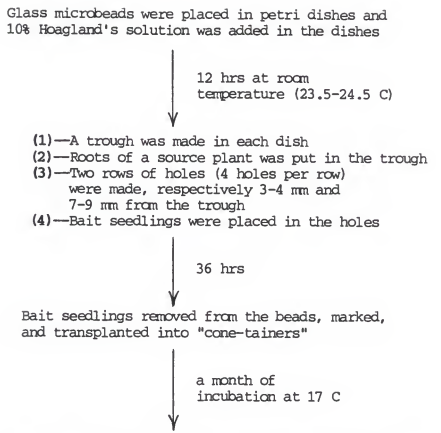

Figure 3. Flow chart of inoculation procedures used in the glass microbead experiments

in dishes in the same way as the severed inoculum. Combining the two grades of microbeads and two types of inocula, there were a total of four treatments. Bait seedlings with single roots were prepared as described in the preceeding section. These seedlings were placed in the glass beads with their single roots at specified distances from the inoculum. Methods similar to those described in the above experiment were used to bury roots of the source plants and the bait seedlings. There were two replicates (two petri dishes) in each treatment, and in each of the replicates 4 bait seedlings were used at each distance. All the inoculations were done at room temperature (23.5 to 24.5 C). After a 36-hour incubation, seedlings were removed and marked as previously described. They were then transplanted in sand and allowed to grow for a minimum of one month in a growth chamber at 17 C. Seedlings were then removed from the tubes, rinsed free of sand with tap water, and the marked roots of bait seedlings examined with the light microscope. Seedlings observed to have P. graminis cystosori in their roots were considered infected, and the number of infected seedlings was recorded. The experiment was repeated once.

The data collected in the experiments were expressed as percent infected bait seedlings and analyzed as a factorial experimental design with split plots. The main effects were the size of glass microbeads and the type of inocula, and distances were the split plots. The two experiments were analyzed together as two blocks.

5. Length of Zoospore Motile Period

This experiment was conducted to determine the effect of temperature on zoospore motility period of P. graminis; the procedures developed in this experiment are illustrated in Figure 4. The source of zoospores for the experiment was a 6-week old source plant that had been chilled at 3 C for 3 days. The source plant was placed in a brown jar with 40 ml of fresh, autoclaved 10% Hoagland's solution and placed at room temperature (23.5-24.5 C). After 24 hours, the source plant was removed and the suspension, assumed to contain released zoospores, was initially transferred to a beaker and then divided into four portions in smaller beakers. These beakers were put in incubators at two different temperatures, with two beakers at each temperature. Separate beakers at individual temperatures were considered replicates. Starting at the time that zoospore suspension was placed into the beakers, samples were taken every 4 hours, and assayed by the following procedure. Using a micropipeter (Gilson Pipetman), samples of the suspension were immediately transferred from these four beakers into microcapillary pipets (1.68 mm dia., Fisher Scientific) which were used as the inoculation apparatus. Each capillary was filled with 120 µl of the zoospore suspension which was pipeted from the middle and upper parts of the beakers. Each capillary was inserted with a single root of a 2- to 3-day old bait seedling, and 4 capillaries were prepared from each beaker. The baited capillaries were placed in moist chambers and incubated at 17 C. After 36 hours, the inoculated seedlings were marked by removal of root tips as described earlier and transplanted

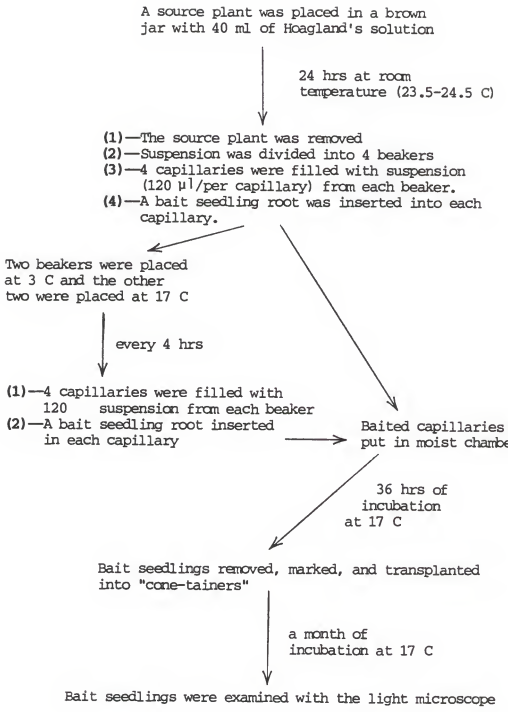

Figure 4. Flow chart of baiting procedures in Polymyxa graminis zoospore motility period experiment

to sand in "cone-tainers". After a minimum of one month of growth, seedlings were removed, rinsed with tap water, and the marked roots examined with the light microscope. Data were collected by the methods described in the previous experiments and expressed as percent of bait seedlings infected.

Results

1. Soil Moisture Release Characteristics

Figure 5 illustrates soil moisture release characteristics for the three soil materials used in this study. Water loss was expressed as percentage of soil volume for each soil sample (volume of water drained from soil sample / volume of the soil sample) at each matric water potential, at which pores over a certain range of sizes were expected to be drained of water. Table 1 gives the volume of these soil pores in numerical form, as shown by the cumulative percentage of drained water in each soil material. Clearly there were more large pores in the sand than in either the soil or the soil-sand mix, and there was no appreciable difference in percentage and size distribution of pore spaces between the soil and the soil-sand mix (Figure 5). Table 1 shows that there were 19.8% soil pores >= 147 μ in diameter in the sand, but only 4.9% and 4.1% in the soil and the soil-sand mix respectively.

2. Influence of Matric Potential on Zoospore Movement

Results from the experiment on effect of matric water potential on zoospore movement by P. graminis, which was duplicated, were expressed as percent of seedlings infected, presumably indicative of the amount of zoospores present. Similar results were found in both experiments although the absolute number of infected bait seedlings was not identical for every treatment. Thus the two experiments were analyzed together as replications. Table 2 expresses the effect of

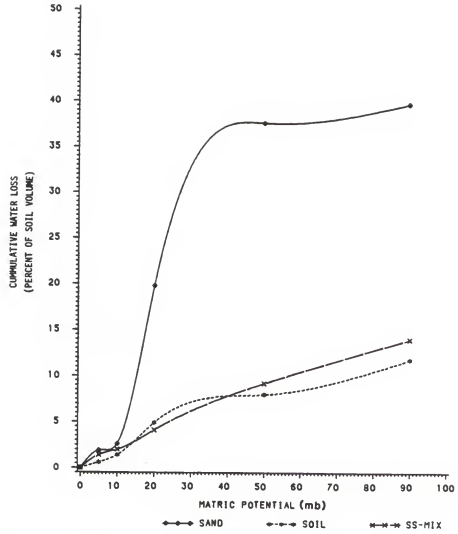

Figure 5. Cumulative water loss from Reading fine silt loam (SOIL), the soil-sand mix (SS-MIX), and the coarse sand (SAND) plotted as a function of decreasing matric potential. Cumulative water loss is expressed as percentage of total soil volume.

Table 1. Percentage and Size Distribution of Water-filled Pores in Three Soil Materials[a]

Matric Potential (mb)	Pore Size (μ)[b]	Cumulative Water Loss (% Total Soil Volume)[c]		
		Soil	S-S[d]	Sand
0		0	0	0
-10	> 294	1.4	2.0	2.6
-20	> 147	4.9	4.1	19.8
-50	> 59	8.0	9.2	37.6

[a] Matric potential was controlled by using 8-cm tension plates in Buchner funnels which were placed at room temperature (23.5-24.5 C). Soils were steam sterilized at 121 C for 1 hour before use.

[b] Size of idealized capillary pores which are expected to drain water at corresponding matric potentials

[c] Cumulative water loss as a percentage of the soil sample volume (cm^3 H_2O/cm^3 soil)

[d] Soil-Sand mix

Table 2. Effect of Matric Water Potential on Zoospore Movement by Polymyxa graminis[a]

Medium	Distance (mm)[b]	Percent of Plants Infected			
		Matric Potential (mb)			
		0	-10	-20	-50
Soil	0	8[c]	9	0	0
	5	17	25	0	0
	15	0	0	0	0
Soil-Sand Mix	0	8	25	0	0
	5	8	8	0	0
	15	0	0	0	0
Sand	0	58	92	0	0
	5	58	67	0	0
	15	91	58	0	0

[a] Treatments at 0 mb were done in petri dishes. Matric potentials ranging from -10 to -50 mb were controlled by using tension plates in Buchner funnels. Infected wheat source plants (1.5-2 months old) were used as the source of Polymyxa graminis zoospores.

[b] Distances from roots of bait seedlings to source of zoospores.

[c] Each value is the average of two replications, with 5 or 6 seedlings used in each replication.

soil matric potential and soil type on the movement of P. graminis zoospores. The analysis of variance and the overall influence of soil matric potential and soil texture on infection of bait seedlings are shown in Tables 3 and 4. In sand, P. graminis zoospores readily moved at least 15 mm at Ψ_m = 0 and -10 mb as indicated by high infection rates (Table 2). At Ψ_m = -20 and -50 mb no zoospore movement could be detected in the sand, i.e. no bait seedlings were infected at any of the three distances (0, 5, and 15 mm) from the zoospore source. In the loam soil, zoospores moved only 5 mm at Ψ_m = 0 and -10 mb. The number of infected bait seedlings at each distance (0 and 5 mm) from the zoospore source was obviously lower in the soil than in the sand. Zoospore movement in the soil was significantly ($p = 0.05$) less than that in the sand at matric potentials of 0 and -10 mb (Tables 3 & 4). Similar to what was found in the sand, no infected seedlings were found from the treatments at Ψ_m = -20 and -50 mb in the soil (Table 2). In the soil-sand mixture, a result similar to that in the soil treatments was obtained (Tables 2, 3 & 4). In these experiments, -10 mb was the lowest matric potential at which zoospore movement by P. graminis was detected in all three types of soil materials (Table 2).

3. Influence of Pore Size on Zoospore Movement

The results obtained from this test were also expressed as percent of bait seedlings infected, indicative of the number of P. graminis zoospores present. Table 5 shows results from two experiments which were analyzed together. Analysis of the effect of pore size and inoculum type, is shown in Table 6. The data (Table 5)

Table 3. Analysis of Variance for Effect of Matric Water Potential on Zoospore Movement by *Polymyxa graminis*[a]

Source	df	F-value	PR > F
Medium	2	89.40	0.001
Matric Potential	1	0.80	0.406
Distance	2	0.94	0.419

[a] Experiments were analyzed as a factorial design with split plots and only the data from the 0- and -10-mb treatments were used in the analysis. The main effects are medium and matric potential. Distances are split plots.

Table 4. Percentage of Plants Infected by Polymyxa graminis Zoospores as Influenced by Medium Type and Matric Water Potential

Medium	Percent of Plants Infected[x] Matric Potential (mb) 0	-10
Soil	8 a[z]	12 a
Soil-Sand Mix	6 a	11 a
Sand	69 b	72 b

[x] Each value is the average over the outcomes at three distances (0, 5, 15 mm) for each combination of medium type and matric potential.

[z] Percentages within each column followed by different letters are statistically different by a LSD test at $p = 0.05$.

Table 5. Effect of Bead Size on the Zoospore Movement by *Polymyxa graminis*[a]

Bead Diameter (mm)	Pore neck size (μ)	Distance (mm)[b]	Percent of Plants Infected			
			Source of Inoculum			
			Intact		Severed[d]	
			1[c]	2	1	2
1.00-1.05	230-300	3.5 ± 0.5	100[e]	100	38	43
		8 ± 1	100	75	100	75
0.10-0.11	25-35	3.5 ± 0.5	63	57	25	43
		8 ± 1	38	50	25	57

[a] Glass microbeads were placed in petri dishes. In the dishes zoospore sources were 1.5-month old *Polymyxa graminis*-infected plants. The beads were saturated with Hoagland's solution.

[b] Distance between roots of bait seedlings and the source of zoospores. In the first experiment, the two distances 3 and 7 mm were used, whereas in the second, 4 and 9 mm were used for each treatment.

[c] Numbers 1 and 2 refer to Experiment 1 and 2, respectively.

[d] Roots of source plants were cut into segments 5-7 mm long and placed in each petri dish.

[e] Each value is the average of two replications with 4 bait seedlings per replication.

Table 6. Main Effects of Bead Size and Inoculum Source on the Movement of Polymyxa graminis Zoospores[x]

Treatment Factor	Percent of Plants Infected	F-value	P > F
Bead Size			
1.00-1.05 mm	79[y]	11.95	0.009
0.10-0.11 mm	43		
Inoculum Source			
Intact	71	3.52	0.097
Severed	51		
Distance			
3.5 ± 0.5 mm	59	0.17	0.690
8 ± 1 mm	63		

[x] The data in Table 5 were analyzed as a factorial design with split plots. The two experiments were used as two blocks. Size of beads and type of inoculum were main effects. Distances were split plots.

[y] Each value is the average of two experiments for each factor or split plot (Table 5).

shows that in 1.00-1.05-mm beads with pore necks of 230-300 μ, almost all bait seedlings were infected when placed at either 3-4 mm or 7-9 mm from the zoospore source when intact source plants were used, indicating that zoospores moved for 9 mm through pore spaces with necks of 200-300 μ. In the smaller beads (0.10-0.11 mm) having pore necks of 25-35 μ, zoospores also moved for 9 mm. However, the movement was significantly reduced in the smaller beads as shown by lower infection rate of bait seedlings (Table 6). The data also indicated that cutting of the source roots affected the infection rate of bait seedlings. Cutting could have affected the physiological condition of the source roots, which may have resulted in a decrease in the number of zoospores released since Polymyxa is an obligate parasite (13).

4. Length of Zoospore Motile Period

The period of P. graminis zoospore motility was measured by the percentage of bait seedlings infected at each time period (Table 7). The number of infected seedlings in each treatment was used as a relative measure of the quantity of motile zoospores at each time period. However, because of the great variability in the data among treatments, no statistical analysis was conducted to compare the length of motility period at the two temperatures. Nonetheless, an indication of overall motility period and the influence of temperature is presented in Table 7. P. graminis zoospores swam as long as 12 hours at 17 C as shown by 25% of bait seedlings infected. At 3 C, the zoospores remained motile and infective for approximately

Table 7. Length of Motile Period of Polymyxa graminis Zoospores at Two Temperatures[a]

Temperature (C)	Percent of Plants Infected			
	Time Period (hr)			
	0	4	8	12
3	8[b]	18	18	0
17	33	50	50	25

[a] Zoospores were released in Hoagland's solution from a 6-week old Polymyxa graminis-infected source plant which had been treated at 3 C for 3 days. The process of discharge was allowed to proceed 24 hr at room temperature (23.5 -24.5) before collecting the suspension and starting the experiment. A single root of each bait seedling was inserted into a capillary tube (1.68 mm in diameter) which had been filled with the zoospore suspension.

[b] Each value is the average of two replications with 4 bait seedlings per replication.

8 hours.

DISCUSSION

This study showed that P. graminis zoospores could move through sieved or reconstituted soil (Table 2), however the movement was greatly influenced by soil texture (Tables 3 & 4). Zoospores could move greater distances, and more bait seedlings were infected by P. graminis zoospores at every distance from the inoculum, in the coarse sand than in either the fine loam or the fine-textured soil mix. Thus zoospore movement was restricted in soils having fewer large pores (Tables 1 & 4). These findings are similar to Duniway's results on the study of Phytophthora cryptogea zoospores (6), which indicated that soil texture is a determining factor in the active zoospore movement, and that only the large pores in soil are suitable for zoospore motility.

As has been reported for Phytophthora (6), zoospore movement by Polymyxa graminis increased when Ψ_m was raised from -20 to 0 mb (Table 2). The relatively high levels of infection observed in the present study at matric potentials from 0 to -10 mb is consistent with the observed association of WSBM with wet areas in the field (25). Although research on some Phytophthora spp. (6) has demonstrated that flooded or saturated soils (i.e. 0 mb) are most suitable for active zoospore movement, this study did not indicate any differences between 0 and -10 mb in terms of active zoospore movement of P. graminis in all the three soil materials used in this study (Table 3). This could be due to the limitation of the bioassay

method used in the experiments: the number of bait seedlings for each treatment may have been too small, and the distance at which the bait seedlings were placed from source of zoospores too short to detect the difference. However, the lack of difference in zoospore movement between 0 and -10 mb is also possibly due to the small difference in volume of large pores between these two matric potentials (0 and -10 mb) in all the soil materials (Table 1). Even in the sand material, which had the largest volume of large pores (>294 μ dia) among all the three soils, these large pores constituted only 2.6% of the total pore volume. This amount of large soil pores present in any soil sample is unlikely to make significant difference in zoospore movement.

The confinement of active zoospore movement to high Ψ_m values (Table 2) is most likely attributable to the large size of soil pores that must be filled with water to accommodate swimming zoospores. Duniway (6) found that Ψ_m = -50 mb is the lower limit for measurable zoospore motility of P. cryptogea in UC mix soil. At this matric potential, soil pores on the order of 60 μ in diameter are expected to be drained of water. A Phytophthora zoospore, even with its flagella extended, is considerably less than 60 μ in diameter and water requirements for its movement in soil are considered to be related more to swimming habit than to size, and more to the continuity and tortuosity of water-filled channels that can accommodate zoospores than to the absolute number of individual large pores. This was confirmed for Polymyxa in this study. Glass bead test

indicated that P. graminis zoospores could move for at least 9 mm through pores having pore necks 35 μ (Tables 5), but in the soil or the soil-sand mix, active zoospore movement was limited to 5 mm at Ψ_m = 0 and -10 mb (Table 2). Thus movement was restricted in soils, even when water-filled pores larger than 300 μ in diameter should be present and even though even at Ψ_m = -10 mb there was still a good portion of large pores at least three times larger in diameter than those in the beads (Table 1). This discrepancy may be explained by considering that the pores in the glass beads are undoubtedly more uniform in size and the water-filled channels more continuous in comparison to those in soils. Therefore not only large pores, but also continuous and less tortuous water-filled channels appear to be required to accommodate active zoospore movement by P. graminis.

In the soils drier than Ψ_m = -20 mb, active zoospore movement of P. graminis was not detected (Table 2). This value is higher than the matric potential (-50) mb at which Phytophthora cryptogea zoospores can move 5 mm in a coarse textured UC-type soil mix (6), indicating that Polymyxa graminis zoospores, like other zoosporic fungi (6), apparently require high Ψ_m values for active movement in soil. It is possible that a lack of large water-filled pores was responsible for the absence of zoospore movement at Ψ_m < -20 mb because the remaining pores in the soils might be too small to accommodate zoospore movement. But since none of the bait seedlings, even at 0 distance from the source of zoospores, were infected at Ψ_m < -20 mb (Table 2), it is more likely that the low soil moisture

conditions limited the production of P. graminis zoospores, or even possibly the infection process of the bait seedlings. Previous study (15) on Phytophthora spp. has demonstrated that zoospore discharge is very sensitive to soil matric water potential. Whereas release proceeds normally at 0 or -1 mb Ψ_m , it is greatly impaired at -5 mb Ψ_m ; a matric potential of -25 mb is limiting to all stages of indirect germination of Phytophthora sporangia. Therefore, it is plausible that discharge of Polymyxa graminis zoospores may be inhibited at a low soil matric potential, such as -20 mb.

The unexpectedly low infection rate of bait seedlings in soil and soil-sand mix at 0 distance from the zoospore source at 0 and -10 mb Ψ_m (Table 2) might be due to one of several factors. The most likely explanation is that a lack of oxygen in the soil and the soil-sand mix may have affected production (release) or motility of zoospores. In either the soil or the soil-sand mix, bait seedlings often had poorly-developed root systems, suggesting conditions of insufficient oxygen in the soils. Alternatively it is possible that, in burying the bait seedlings, some soil did inadvertently get placed between source and bait roots (Fig 2). The soil particles in between might have limited some zoospores from reaching the bait roots.

The results obtained from the glass bead test demonstrated that P. graminis zoospores could move through pores with pore necks of 25-35 μ in diameter, just larger than the greatest dimension (23.5 μ) of a zoospore with its flagella extended (20). In comparison,

Phytophthora cinnamomi zoospores cannot move through this grade of glass beads (26) probably because of the excessive size (30 μ) of the zoospore. In addition, it has been demonstrated that Phytophthora zoospores take helical paths during swimming, and the space required for one cycle of locomotion is a cylinder 180 μ long x 50 μ in diameter, much bigger than the pores in the beads. Thus it would be very difficult for Phytophthora zoospores to pass through such a limited space. However, nothing is known about the swimming pattern of Polymyxa graminis zoospores except that they move in a gentle, rotatory motion when unrestricted in their movement (13).

Although P. graminis zoospores could move through small pore space (35 μ dia), the movement was significantly less compared with that in the larger pores (300 μ dia) (Table 6), as indicated by lower infection rate of bait seedlings in the smaller than in the larger beads (Table 6). This result suggests that pore size had a major influence on the zoospore movement of P. graminis. However, it is not clear whether this was due to loss of motility as a result of more frequent collisions of the spores with solid surfaces in the small glass beads or constraints of the small pore space on the zoospore movement. Imagining the piled glass beads as stacked screens, the smaller the pores on the screens, the slower and more difficult for the zoospores to pass through them, possibly leading to a decrease in the number of zoospores to reach bait seedlings. In contrast with the results of Ho & Hickman (9) and Bimpong & Clerk (3) who found that zoospores encyst rapidly when placed in contact with glass beads, the

present study did not show that P. graminis zoospores markedly lost motility, since a high infection rate was observed in either large or small beads in my experiments (Tables 5). In the Phythophthora studies (3, 9), the premature encystment was considered to be due to "contact stimulus" and to result from zoospore repeated contacting or colliding with numerous solid surfaces. The results from the glass bead test suggested that Polymyxa graminis zoospores may not be very sensitive to contact stimulus.

The period of P. graminis zoospore motility in this study was assessed by infectivity of the spores. Samples of zoospore suspension taken from upper parts of the suspension in beakers were assumed to contain only motile zoospores and that the encysted (non-motile) zoospores of P. graminis had settled to the bottom or adhered to other solid surfaces. Although this feature is not known in P. graminis, it has been found in Phythophthora (21). Zoospores become adhesive early in encystment, and if they come into contact with a solid surface at this time, they become firmly attached. However, if they fail to contact a solid surface during the adhesive phase, zoospores produce cysts that remain unattached.

Only two temperatures were used in the motility test, and 17 C was found to be more favorable for P. graminis zoospore motility. This temperature is the same as the optimal temperature for maintaining the fungus, as reported previously (20). Similar optimal temperature for the motility of Phythophthora zoospores has been

reported (3,9). However since only two temperatures were used in the experiment, further study would be needed to fully understand the influence of temperature on motility of Polymyxa graminis zoospores.

The wide-spread distribution of WSBMV in the field is, clearly, not attributable to zoospore movement through the soil, given the limited swimming distance of P. graminis zoospores found in this study (Table 2). However, zoospore movement through the soil may be a very important factor in the initiation of disease infection and further development because, unlike most other zoosporic fungi such as Phytophthora, P. graminis does not produce any mycelial structures in its life cycle. Therefore, P. graminis zoospores would be the only infective structures capable of not only carrying viruses, but also responding to host stimuli by moving toward potential infection sites. The final stage of locating infection courts on susceptible host plants probably depends, to a great extent, on the active zoospore movement in the soil. Several centimeters of swimming distance may be enough for the zoospores to accomplish this.

Two soil factors - soil texture, and soil matric water potential - are critical for P. graminis zoospore movement as found in this study. These two factors are interrelated functionally (24) and determine the number of water-filled large pores, which is the key component in active zoospore movement. Present study shows that P. graminis required saturated or wet (Ψ_m = -10 mb) soils for zoospore movement, and that the coarser the soil texture, the more

suitable for zoospore movement the soil was under this high moisture condition. Whereas these two optimum conditions may be seen in coarse-textured soils as in the sand and glass microbeads, they may not be found in fine-textured natural soil in the field. However, this does not exclude the possibility that P. graminis zoospores can move in fine-textured soils. In fact, present study showed that P. graminis zoospores could move in fine soil materials used in this study and through small pore spaces in glass microbeads. Probably this is what happens in natural soil conditions since in nature WSBM does occur in clay or loam soil. The original soil material used in this study, a fine loam soil, was collected from a WSBM-infested field. This type of soil would certainly have fewer large soil pores than sandy soil and glass beads do, but fine clay or loam soils may favor zoospore movement in that they retain water for a longer period after rain or irrigation. Since coarse-textured soils may drain water more quickly than fine-textured soils do (8), matric water potentials in fine-textured soils will be maintained at a higher level for a longer time than in coarse soils. Thus under high moisture conditions, there will be more water-filled large pores in fine-textured soils than in coarse soils. However, this does not necessarily imply that active zoospore movement of P. graminis does not occur in sandy soil under natural conditions. Certain conditions may permit high moisture levels in coarse-textured soils. For example, the percentage of water-filled soil pores in a given soil type may be affected by relative position of a field and soil profiles. High moisture conditions can occur in low areas or layered

soils in the field after rain or irrigation. In layered soils, the drainage of water is slower than in uniform soil profiles (8), and water may accumulate in the upper soil layer due to low infiltration to the sublayer and retarded water movement at interface between the two layers. The retarded drainage will lead to an increase in soil matric potential in the upper soil layer. In this kind of conditions, a much significantly higher zoospore movement may be expected in sandy soil than in clay or loam soils.

In any situation of poor drainage, whether in layered or fine-textured soils, the length of time that soil remains wet will be critical to zoospore movement. Since zoospores may be capable of remaining motile for 12 hours (Table 9), prolonged soil saturation would give the zoospores increased opportunity to locate host roots and allow the infection process to be completed, as in the inoculation in the laboratory (Table 2).

In addition to the influence of soil particle size, soil pore structure may also be affected by a number of other factors which are not usual criteria for soil texture, i.e. aggregated structure, degree of compaction, and prevalence of channels left by dead roots and burrowing animals, etc (6). Thus, a simple relationship between soil texture and zoospore motility is unlikely. The information developed in this study does, however, clarify certain key factors that must be considered in relating active zoospore movement by P. graminis to soil physical parameters.

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EFFECT OF SOIL TEXTURE AND MATRIC POTENTIAL ON THE MOVEMENT OF *POLYMYXA GRAMINIS* ZOOSPORES

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Abstract

The influence of soil matric water potential, soil texture and pore size on active zoospore movement of Polymyxa graminis to plant roots was investigated by using a baiting technique; the length of P. graminis zoospore motility period was also investigated. Active zoospore movement was examined by arranging wheat seedlings at various distances from a source of zoospores (P. graminis-infected wheat plants) for a 24-hr inoculation period. Infection of bait seedlings by P. graminis was assessed by observation of cystosori in the roots of inoculated bait seedlings after a minimum of one month's growth post inoculation.

Three types of soil materials were used to study the effect of matric water potential and soil texture on active zoospore movement of P. graminis. Soil matric water potential was controlled by using Buchner funnel tension plates. At Ψ_m = 0 and -10 mb, P. graminis zoospores readily moved 15 mm in a coarse sand, whereas in a fine silt loam or a fine-textured soil-sand mix, zoospore movement was detected only over a distance of 5 mm. Zoospore movement was significantly reduced in the loam soil or the soil-sand mix compared with the sand, indicated by a low frequency of infection of bait plants. At Ψ_m = -20 and -50 mb, no P. graminis-infected bait plants were detected in any of the three types of soil materials used in the study.

Influence of pore size on P. graminis zoospore movement was investigated by using two different sizes of glass microbeads saturated with 0.1-strength Hoagland's solution. Either severed roots or intact P. graminis-infected wheat plants were used as sources of zoospores. P. graminis zoospores moved 9 mm through beads providing pore necks with diameters 25-35 or 230-300 μ, however zoospore movement was restricted (infection frequency of bait plants reduced) in the beads with pore necks of 23-35 μ. Lower infection rate of bait plants was also obtained in treatments with excised inoculum.

Length of P. graminis zoospore motility period was examined by using a host bioassay method. Zoospore suspensions at two temperatures were sampled regularly, at an interval of 4 hours. Microcapillary tubes containing samples of zoospore suspension were baited with wheat seedlings. By this host bioassay, motile zoospores of P. graminis were detected in suspension that had been placed at 17 C for up to 12 hours, or at 3 C for approximately 8 hours.