

Competitive Colonization of Organic Matter in Soil by *Phytophthora megasperma* f. sp. *medicaginis*

J. P. Stack and R. L. Millar

Former graduate research assistant and professor, Department of Plant Pathology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca 14853.

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## ABSTRACT

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The ability of *Phytophthora megasperma* f. sp. *medicaginis* to colonize root segments (RS) of various plants in unsterile silt loam soil was investigated. A metalaxyl-insensitive isolate (Pm20) of *P. m. f. sp. medicaginis* was added to soil at different temperatures and moistures together with RS of various plants. Over time, the RS were retrieved and plated on a selective medium containing metalaxyl. Pm20 grew 2.0 cm through unsterile soil and colonized alfalfa RS at low soil temperatures (4 and 15 C) and high soil moistures (0 and -10 mb matric potential). At 24 C and -100 mb matric potential, Pm20 grew only 0.5 cm to colonize RS. Root

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tissues of black medic, birdsfoot trefoil, and corn also were colonized in soil. Colonization occurred in sieved, reconstituted soil, as well as in soil with an intact field structure. RS colonized by Pm20 in soil served as sources of inoculum for alfalfa plants; RS colonized at 4 C were more effective than those colonized at 15 C. In soil columns, zoospores of Pm20 could not move through the silt loam soil. Therefore, it was concluded that hyphae of Pm20 grew through the soil to effect colonization. Based upon these studies, it appears that *P. m. f. sp. medicaginis* may have an active nonparasitic phase in its life history.

*Phytophthora* root rot of alfalfa, which is caused by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan and Erwin, can severely impair stand establishment and contribute to stand decline in subsequent years. In New York State, alfalfa is grown in rotation with corn and a small grain, usually oats. As many as four or more years may occur between alfalfa crops. Despite this period, the pathogen survives sufficiently well to cause significant damage when alfalfa is again planted. The length of time that propagules of *P. m. f. sp. medicaginis* survive in a dormant state or the pathogen remains active in soil has been investigated (3,13,25,28,29). However, little is known about the mechanisms by which it survives. For example, does it have an active nonparasitic phase in its life history? This activity could greatly contribute to persistence for long periods of time in the absence of a host.

The term commonly used to describe nonparasitic activities of pathogens in soil is "competitive saprophytic ability." This term usually connotes a capacity to compete effectively in the colonization of dead organic matter. In this report, the term "organic matter" is used in a broad sense to include a range of substrates from those still-living plant materials recently turned under soil, to substrates of uncertain origin that have been in the soil for a long period of time. During the sequence for a field in an alfalfa-corn-oats rotation, plants of the particular crop as well as volunteer plants of another crop or weeds will be present in the field and either living or in various stages of decomposition. This diversity of organic matter incorporated into soil offers a myriad of substrates to soilborne organisms. An ability to utilize any part of this range of substrates may be significant to the ecology and survival of a soilborne pathogen. Since both living and dead tissues may be involved, the phenomenon will be considered competitive colonization of organic matter rather than competitive saprophytic ability, which usually implies that only dead organic matter is involved.

This paper originally appeared in the September issue of *Phytopathology* (pp. 1020-1025), but an error occurred during the electronic processing such that the pathogen trinomial was incorrectly printed as *P. m. f. sp. megasperma* instead of *P. m. f. sp. medicaginis* in two manuscripts that were companions to this paper. The paper is printed here in entirety, along with the corrected versions of the companion papers, to preserve the sequence of the three related papers.

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It is generally accepted that most species of *Phytophthora* have little if any competitive colonizing ability (17,31,37), little ability to grow through an unsterile field soil (33,37-39), and that long-term survival is accomplished by durable propagules such as chlamydospores and oospores (10,30,33). There is, however, some evidence to indicate colonization activity by *P. cinnamomi* (42), *P. palmivora* (38), *P. parasitica* (39), *P. cactorum* (as reported in Weste [40]), *P. cryptogea* (5), and *P. nicotianae* (Jensen, 1917, as reported in DeBruyn [8]). Other members of the oomycete family Pythiaceae have strong competitive colonizing ability (1,22).

The objectives of this research were to determine whether *P. m. f. sp. medicaginis* could grow through an unsterile field soil to competitively colonize organic matter and to determine the environmental conditions under which colonization might occur. As the study progressed, it was determined that colonization did occur under conditions of high moisture and low temperature. Because silt loam soils have been reported to greatly impede zoospore activity even at high water potentials (11,23,41), an additional objective was to determine whether zoospores could move through this silt loam soil to affect colonization. The data obtained support the conclusion that hyphal growth rather than zoospore motility is involved in movement of the pathogen through soil. A preliminary report of part of this work has been published (35).

## MATERIALS AND METHODS

**Media, isolate, and soil.** In all experiments, a metalaxyl-insensitive isolate (Pm20) of *P. m. f. sp. medicaginis* was used (36). The origin and characterization of this isolate has been described (36). The media used for culture maintenance, zoospore production, and selective isolation of Pm20 were also described (36). The selective medium was a modification of the medium of Masago et al (26). Potato-dextrose agar (Difco laboratories, Detroit, MI) and prune agar were used. The ethanol-streptomycin agar medium consisted of agar, 15 g/L, amended with ethanol at 5.5 ml/L and streptomycin at 100 µg/ml (Sigma Chemical Corp., Little Rock, AR). All colonization experiments were conducted in the Mt. Pleasant silt loam field soil (36).

**Soil moisture.** A soil moisture characteristic curve was developed by using 600-ml sintered glass tension plate Büchner funnels (11). Sieved (2.3-mm openings) soil at 25% moisture was gently packed into tension plate funnels to a depth of 1.0 cm, saturated from the bottom with distilled, demineralized water, and covered loosely with plastic. Continuous water columns were established between

the tops of the tension plates and water reservoirs through thick-walled tubing. The water column heights were adjusted to 100, 75, 50, 25, 10, and 0 cm above the reservoir. There were two funnels per water column height. After equilibration for 18 hr at room temperature, three soil samples from each funnel were dried at 110 C for 24 hr. After determining the dry weights, the mean percentage moisture was calculated and plotted versus the corresponding matric potential (Fig. 1). In experiments, soil moisture was adjusted on a gravimetric basis using values obtained from the funnel system.

**Substrates for colonization.** In most experiments, root segments from 8-wk-old alfalfa (*Medicago sativa* L.) plants were used as substrates for colonization. Alfalfa plants were grown in an unsterile potting mix (36) for at least 8 wk in the greenhouse. The roots were harvested, washed thoroughly in water, and cut into 0.5-cm segments. They were stored in distilled, demineralized water at 4 C until used. Root segments of black medic (*Medicago lupulina* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and corn (*Zea mays* L.) were prepared in the same manner.

**Organisms associated with alfalfa root segments.** Because *P. m. f. sp. medicaginis* would have to tolerate and possibly compete with organisms associated with the root segments to utilize them as substrates, organisms associated with the root segments were identified. Root segments (5 mm) from 8-wk-old alfalfa plants prepared as described above, were plated (10 per medium) on seven different media: (PDA, V-8A, prune agar, ethanol-streptomycin agar, Masago's medium, Masago's minus hymexazol, and Masago's plus metalaxyl). When necessary, organisms were transferred to other media or microscope slides for further observations and attempted identifications.

**Colonization of substrates in soil.** Most colonization experiments were performed in soil contained in 600-ml Pyrex storage dishes (Fisher Scientific Co.). Sieved Mt. Pleasant field soil (100 ml) equilibrated to a specific temperature (4, 15, or 24 C) was added to a storage dish. The surface was left uneven to avoid creating any artificial interface. Five root segments not colonized by Pm20 were placed on the soil surface at a specific distance (0.5, 1.0, or 2.0 cm) from the center of the dish. These served as bait segments. A Pm20-colonized source root segment, obtained by placing root segments on an agar culture of Pm20 for 3–5 days, was placed on the soil at the center of the dish. An additional 100-ml of soil was added to cover the root segments carefully so as not to disturb their position. The soil then was leveled and gently firmed. For each soil temperature, there were three dishes with five root segments per dish for each distance. The percentage moisture was adjusted to correspond to a specific matric potential. The dishes were covered with glass lids, placed in loose-fitting plastic bags to retard moisture loss, and held at constant temperatures (4, 15, or 24 C) for up to 3 wk. Over time, all segments were retrieved, washed with distilled, demineralized water, blotted dry, and plated on the selective medium that contained metalaxyl. Growth of Pm20 from a bait segment onto the selective medium was taken to indicate prior movement of Pm20 through soil from the source segment to the bait segment and colonization of the bait segment. Some colonies were transferred to V-8 medium for positive identification.

**Effect of soil structure on colonization.** To determine if Pm20 could grow through an undisturbed field soil to colonize a new substrate, intact soil cores (10.2-cm diameter, 6.0 cm depth) were taken by means of a golf cup cutter from an alfalfa field on the Mt. Pleasant research farm. Cores were placed in waxed cardboard cups (10-cm diameter, 8.0-cm depth) in incubators at 4 or 15 C for several days to allow equilibration of temperature. A 0.9-cm-diameter cork borer was used to make wells about 2–3 cm deep at the center and at 0.5 cm or 1.0 cm distant from the center of the core. The surface of each well was gently scraped with a scalpel to break any compaction caused by the borer. A Pm20-colonized source segment was placed in the center well. Root segments not colonized by Pm20 were placed in the peripheral wells. Soil was added to each well to bury the root segments approximately 2 cm deep. The soil cores were saturated with distilled, demineralized water equilibrated to 4 or 15 C, covered with a plastic bag to retard moisture loss, and held at 4 or 15 C. After 3 wk, all root segments

were retrieved, washed thoroughly with distilled, demineralized water, blotted dry, and plated on the selective medium with metalaxyl. The plates were observed in 2–4 days for the presence of Pm20 colonies. Isolations were made to confirm identifications.

**Colonized bait segments as a source of inoculum.** The suitability of Pm20-colonized bait segments to serve as a source of inoculum for infection of alfalfa plants was determined in Styrofoam planting trays. Each tray had 196 wells (2.5-cm diameter, 13-cm deep) for individual plants. Nonsterile greenhouse potting mix was added to each well to a depth of 10 cm. The potting mix was used instead of field soil to insure that symptom development could be attributed to infection by Pm20. In a field soil, symptoms might derive from either added Pm20 or indigenous *P. m. f. sp. medicaginis*.

A bait segment that had been colonized by Pm20 in field soil was added to each well and covered with 4.0 cm of potting mix. Noncolonized root segments served as controls. Surface-sterilized (15 min in 0.5% NaOCl) alfalfa seeds (one to three seeds per well) were placed on the top, covered with 1.0 cm of potting mix, and the mix was firmed. The trays were placed in the greenhouse and watered daily. After 4 wk, the planters were flooded for 3 days and allowed to drain for 3 days, and the plants were harvested. The roots were washed thoroughly in distilled, demineralized water, blotted dry, and plated on the selective medium with metalaxyl.

**Zoospore movement through soil.** Whether or not movement through soil is accomplished by motile zoospores was investigated. Field soil was added to plastic columns (1.0-cm diameter) covered at one end with four layers of cheesecloth. The soil was firmed into the columns to a height of 2.0 cm. In some columns a root segment was placed at the bottom. The soil in each column was saturated with distilled, demineralized water. The columns were placed in a saturated atmosphere at 15 C. After equilibration of temperature, a 0.5-ml suspension of motile zoospores (about 50,000 per milliliter) was applied to the top of the soil column and allowed to infiltrate. The columns then were placed horizontally in a saturated atmosphere at 15 C and sampled over time.

At 0, 0.5, 1.0, 2.5, and 5.0 hr after infiltration, the soil was pushed from some of the columns and sectioned (bottom to top) into 3-mm disks. Each 3-mm soil disk was suspended in 0.5 ml of sterile distilled, demineralized water and the suspension was spread evenly over the surface of selective medium with metalaxyl in petri plates. After 3–4 days, these plates were observed for the presence of Pm20 colonies. The root segments present in some columns were also plated.

At the same sampling times, soil was pushed from other columns. The second 3-mm soil disk from the top of the column was suspended in 1.0 ml of sterile distilled, demineralized water. The large soil particles were allowed to settle out for 1.0 min. The supernatant liquid was transferred to a clean petri dish and observed microscopically for the presence of motile zoospores. The soil from this disk and the supernatant liquid were plated on the selective medium with metalaxyl and observed for the growth of Pm20.

**Statistical treatment of data.** Unless otherwise stated, experiments were performed three times. For analysis of the effects of temperature, moisture, and distance on colonization, the arcsin square root transformation was calculated for the mean proportion of each treatment. Prior to their transformation, proportions of zero and one were converted to 0.05 and 0.95, respectively, by the formulas  $\frac{1}{4}n$  and  $(n-\frac{1}{4})/n$ , respectively. An analysis of variance was performed on the transformed data to determine the significance of treatments and possible interactions (32).

## RESULTS

**Organisms associated with root segments.** Fungi, bacteria, nematodes, protozoans, and algae were recovered from the root segments at the start of the experiments; the fungi included *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Geotrichum candidum*, *Gliocladium roseum*, *Mortierella* sp., *Mucor* sp., *Penicillium* sp., *Pythium* sp., and several unidentified species. Additional organisms were isolated at the end of the experiments.

Some of these organisms were extremely antagonistic to Pm20 on the selective recovery medium as determined by inhibition of linear growth and the gross distortion of Pm20 hyphae. *Gliocladium roseum* Bain., which was frequently isolated from the root segments, greatly inhibited Pm20 growth on the selective medium but had no effect on Pm20 growth on V-8 agar.

**Effects of temperature, moisture, and source-to-bait distance on colonization.** Pm20 grew through field soil and colonized bait segments up to 2.0 cm distant from the source segments. Under conducive conditions (high soil moisture and low temperature), colonization was not significantly affected by the source-to-bait distance ( $\alpha = 0.05$ ); the proportions of baits colonized at 0.5, 1.0, and 2.0 cm from the source were not significantly different (Table 1). Under restrictive conditions, colonization was affected by distance. At low moisture and/or high temperature, colonization was limited to the shortest distance (0.5 cm). Colonization occurred most frequently at high moisture levels (61 and 79% moisture content = -10 and 0 mb, respectively) and low temperatures (4 and 15 C) (Table 1). At 35% moisture content (-100 mb) regardless of temperature, and at 24 C regardless of moisture level, little colonization occurred.

To more accurately control matric potential, two experiments were conducted in tension plate funnels. The bait segments were buried 1.0 cm from the source. The soil was saturated and the water column heights were adjusted to 0 or 10 cm above the reservoir. Colonization occurred within funnels at 15 and 19 C. Proportions of baits colonized within funnels at 0- and 10-cm water column heights, were comparable to those occurring in storage dishes.

Between -10 and -25 mb, there was a sharp decrease in the proportion of baits colonized. This was true at 4 and 15 C. There was no significant difference ( $\alpha = 0.05$ ) from -25 to -100 mb in the proportion of baits colonized.

In one additional experiment, washed-mycelium disks were used as a source of Pm20 rather than colonized root segments to determine if a food base was necessary for growth through soil. There was no difference in the proportion of baits colonized (0.30) at 15 C whether root segments or mycelium disks were used as the source of Pm20.

**Nature of bait.** Substrates other than alfalfa root tissue also were colonized by Pm20 under the same experimental conditions. Root segments of black medic and birdsfoot trefoil became colonized in proportions comparable to alfalfa root segments, 0.58 and 0.45, respectively. This also was true for birdsfoot trefoil tissue that had been air-dried for several weeks; the proportion of baits colonized was 0.40. Root and stalk tissue of corn became colonized by Pm20 in soil, but much less frequently than other tissues, with a proportion of 0.15 for both.

There was no difference in the proportions of baits colonized between the two alfalfa cultivars Iroquois and Oneida, 0.58 and

0.45, respectively. Oneida was developed from Iroquois as a *Phytophthora*-resistant cultivar and yields well under field conditions in soils infested with *Phytophthora*.

On two occasions, native soil organic matter of uncertain identity and origin was collected from experimental soil and Pm20 was isolated from it.

**Colonized baits as sources of inoculum.** We wanted to know whether substrates colonized as above could be important in the life cycle of the pathogen or contribute to development of *Phytophthora* root rot. In one experiment, bait root segments that had been colonized by Pm20 in soil were reburied in unsterile soil mix to determine whether they could serve as a source of inoculum for infection of alfalfa. After 4 wk, many of the plants grown in these soils showed typical *Phytophthora* root rot symptoms; some plants were killed. Pm20 was recovered from nearly 50% of all the plants, indicating that some of the colonized segments had served as a source of inoculum. More diseased plants resulted from bait segments colonized at 4 C (94%) than from those colonized at 15 C (30%). This was significant at  $\alpha = 0.05$ .

**Effect of an intact soil structure.** Sieving and reconstituting soil markedly alters its structure, which may profoundly influence results obtained in it. To determine if colonization could occur in a soil with an undisturbed aggregate structure, experiments were conducted in soil cores removed from the field in a way to maintain the field structure. Pm20 grew 1.0 cm through three of 12 intact soil cores and colonized 57% of the root segments in these cores after 16 days at 15 C. No additional colonization occurred after 25 days. In a second experiment, Pm20 grew 1.0 cm through two of 17 intact soil cores to colonize 22% of the bait segments in these cores after 14 days at 4 C. In both experiments, the soil was at saturation. The proportion of baits colonized in intact soil cores where colonization occurred, was comparable to the proportion colonized in sieved soil. The proportion of total baits colonized in all cores (0.06) was lower than the proportion observed in sieved soil.

**Zoospore movement through soil.** Zoospores of Pm20 were unable to move through the silty loam soil except in a moving front of water. At time 0 the zoospores had moved almost 1.0 cm into the column as a result of infiltration (Table 2). At 2.5 hr after infiltration, few zoospores remained motile. For the duration of zoospore motility (2.5-5.0 hr), zoospores did not move through the soil column beyond the point of infiltration. This was true whether or not a bait root segment had been placed at the end of the column 1.0 cm away from the point of infiltration. By continually adding more zoospore suspension to the top of the column, zoospores eventually passed all the way through the column. From these columns, Pm20 was recovered from all sections and the bait root segment, indicating there were sufficient pores of sufficient size to allow zoospore movement, at least in a moving front of water.

TABLE 1. Colonization of root-tissue baits by metalaxyl-insensitive isolate Pm20 of *Phytophthora megasperma* f. sp. *medicaginis* at different temperatures, source-to-bait distances, and water potentials in a silt loam soil with an analysis of variance of the data

Temperature	Source-to-bait distance (cm)	Soil water potential (mb) and proportion of baits colonized <sup>a</sup>			Analysis of variance <sup>b</sup>			
		0 mb	-10 mb	-100 mb	Due to:	d.f.	Mean square	F statistic
4	0.5	0.34 a	0.57	0.04	Factor	3	0.93482	12.614**
	1.0	0.68	0.38	0.0	Temperature	1	0.81516	10.999**
	2.0	0.42	0.36	0.0	Moisture	1	1.65764	22.367**
15	0.5	0.24	0.72	0.09	Distance	1	0.33166	4.475*
	1.0	0.32	0.58	0.0	Error	77	0.0741	
	2.0	0.26	0.15	0.0	Total	80		
24	0.5	0.15	0.32	0.03				
	1.0	0.00	0.05	0.0				
	2.0	0.02	0.00	0.0				

<sup>a</sup>Bait root segments (0.5 cm) were buried in a silt-loam soil at specific distances from the center of the 600-ml storage dish. A Pm20-colonized source root segment was buried in the center. The soils were equilibrated to specific temperatures and moistures. Over time, the segments were retrieved, washed, and plated on the Pm20-selective medium containing metalaxyl to determine colonization. Each proportion is the mean of three experiments.

<sup>b</sup>An analysis of variance of the arcsin square-root transformation of the proportions of baits colonized was done. Two asterisks (\*\*) indicate significance at  $\alpha = 0.01$ ; a single asterisk (\*) indicates significance at  $\alpha = 0.05$ . There were no significant interactions.

Pm20-colonized root segments were retrieved after a few days in soil, gently washed, and observed microscopically. Frequently soil aggregates remained attached to the root segments via hyphal strands. Some hyphal strands emerging from an opposite side of a soil aggregate appeared to have grown through the soil aggregate. Occasionally a Pm20-like sporangium extended from the soil aggregate that was still attached to the root segment. Some hyphal strands extended at least 7 mm into the soil from the root segment.

## DISCUSSION

*P. m. f. sp. medicaginis* grew through unsterile soil and colonized new organic matter substrates in the absence of intact host plants. It colonized a variety of substrates including alfalfa, black medic, birdsfoot trefoil, and corn. These are among the many plant species present in alfalfa fields during a rotation sequence. Since black medic occurs commonly and birdsfoot trefoil less frequently in alfalfa fields, these tissues may be available as substrates for *P. m. f. sp. medicaginis*. Corn tissues were colonized less frequently, but this still may be very significant since corn is often grown for 2 yr in rotations with alfalfa, and tremendous amounts of corn tissue are incorporated into the soil. Any colonization of these tissues may be significant to the survival of *P. m. f. sp. medicaginis*. In a field soil, the organic matter is likely to be very diverse with respect to nature, origin, and state of decomposition. Even obligate saprophytes are not omnivorous (15,18). Their ability to utilize a substrate depends upon its molecular and macromolecular constituents as well as their ability to compete. Similarly, pathogens with an active nonparasitic phase will be able to utilize tissues from certain plant species but not from all (2). Failure to colonize organic matter substrates in soil may be more an indication of the inability to utilize the tissue than an indication of a pathogen's impotence as a saprophyte.

Cutting the roots into segments and storing them before use, even at 4 C, may have allowed changes in the microflora, qualitative and/or quantitative. This could have affected the level of antagonism associated with the root segments. Colonization occurred with freshly cut plant tissues, tissues that had been stored for up to 3 wk, tissues that had been air dried for several weeks, and with native soil organic matter. Therefore, if microfloral changes did occur, *P. m. f. sp. medicaginis* was not noticeably affected by them. During a rotation sequence, a vast amount of organic matter, living and dead, is incorporated into the soil. If the term "competitive saprophytic ability" refers only to the utilization of dead substrates, it does not adequately describe the activity observed in this study. If one accepts a broader description of organic matter that includes the spectrum from living tissues to

decomposed tissues of uncertain identity and origin, then the term "competitive colonization of organic matter" better describes all the possibilities of substrate utilization in soil.

Many organisms are associated with these organic matter substrates, including fungi, bacteria, nematodes, protozoans, and algae. Some of them demonstrated a high degree of antagonism to Pm20 on an agar medium. Clearly, *P. m. f. sp. medicaginis* encounters intense competition in its attempts to reach and colonize new substrates. That it is an effective competitor is indicated by the frequency with which it was successful. That it has this capability is especially significant since the colonized pieces of organic matter served as sources of inoculum for infection of alfalfa plants.

Colonization of organic matter in soil may enhance the survival capacity of *P. m. f. sp. medicaginis*. At one location in New York, severe Phytophthora root rot occurred in a 1-yr-old stand in a field that had been out of alfalfa production for 13 yr. Based on the data reported here, it is likely that *P. m. f. sp. medicaginis* was an active competitor for substrates in the interim and not just surviving as dormant propagules. Long crop rotations have been suggested for controlling Phytophthora root rot of alfalfa (3). In New York, this is not likely to be effective unless substantial modification in the crops grown and weed control practices are effected.

Results obtained for a sieved, reconstituted soil may or may not be the same as those obtained for a soil with an intact structure. *P. m. f. sp. medicaginis* was capable of growing through and colonizing organic matter in both types of soil. The proportion of total baits colonized was much lower in the soil with an intact structure. This may in part be due to the physical barriers present in many New York soils. In several of the cores, large stones comprised a large percentage of the total mass of the core. This prevented the setting of wells in some cores. Often, when the root segments were retrieved, a large stone formed a barrier between the source segment and the bait segment. In cores where colonization was successful, the proportion colonized was comparable to that in sieved soil. Therefore, *P. m. f. sp. medicaginis* seems well-equipped to deal with the many possibilities of soil structure and substrate diversity it could encounter.

Some consider *P. megasperma* to be a species that is not yet clearly defined (4,14,19). Brasier has suggested that this species is

TABLE 2. Motility and movement of zoospores of isolate Pm20 of *Phytophthora megasperma* f. sp. *medicaginis* in horizontal columns of a reconstituted silt loam soil

Sample time (hr)	Zoospores motile <sup>c</sup>	Column sections <sup>a,b</sup>							
		Top	1	2	3	4	5	6	Bottom
0.0	+	+	+	+	+	-	-	-	-
0.5	+	+	+	+	+	-	-	-	-
1.0	+	+	+	+	+	-	-	-	-
2.5	+	+	+	+	+	-	-	-	-
5.0	-	+	+	+	+	-	-	-	-

<sup>a</sup> Sieved (23-mm openings) field soil was firmed into 1.0-cm diameter columns to a depth of 2.0 cm and saturated with distilled, demineralized water. After equilibration to 15 C, a 0.5-ml suspension of motile zoospores was added to the top of the vertical column and allowed to infiltrate. After infiltration, the columns were placed horizontally in a saturated atmosphere. Over time, the soil was pushed from the column, and 3-mm sections were plated as a slurry on the selective medium.

<sup>b</sup> + = recovery of Pm20 on the selective medium from a section of the soil column; - = no recovery.

<sup>c</sup> Microscopic observations were made over time to determine the duration of zoospore motility. + = motile zoospores observed; - = no motile zoospores observed.

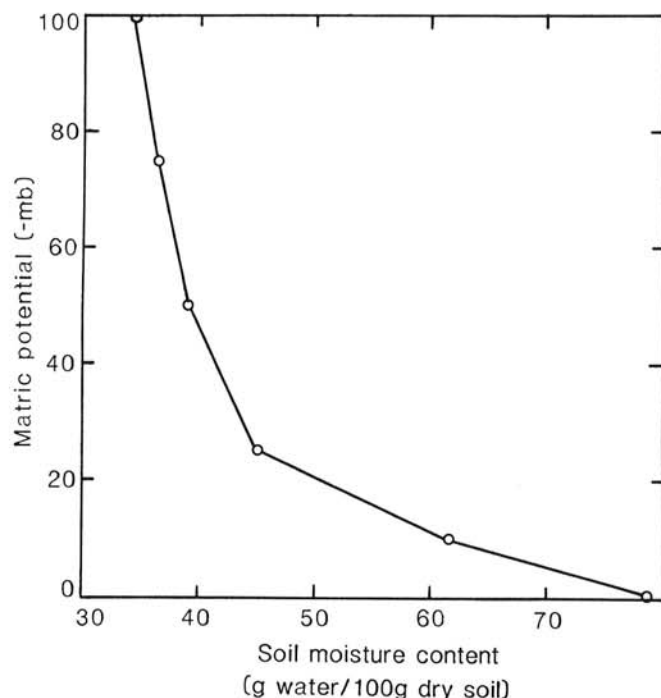


Fig. 1. Relation of soil moisture content to water potential as determined by using a tension plate funnel-water column system for a silt loam soil. Each point is the mean of six soil samples.

still evolving and that the soil phase of its existence is very important to species development (4). He proposed that where a population of *P. megasperma* is surviving in soil cropping that soil (especially with monoculture) tends to select subpopulations that can adapt to parasitization of a given crop. That *Phytophthora* species can exist in soil apart from cultivated hosts is supported by reports that they have caused diseases of crops newly planted in virgin soils (7,12). It seems likely that the inoculum was already present in these soils at the time the soil was first cultivated. The pathogen may have existed saprophytically or in association with other plant species, or both. As stated earlier, many *Phytophthora* species have some saprophytic capability. Association with alternate hosts has been reported for *P. megasperma* and *P. drechsleri* (7,12,20). Based on Weste's (40) view of the genus *Phytophthora*, *P. megasperma* should be included with *P. cactorum* and *P. cinnamomi* as having a significant nonparasitic phase in its life history.

The full significance of hyphae of *Phytophthora* in soil has not yet been determined. Most studies have shown that hyphae of *Phytophthora* rapidly lyse in unsterile soil (30,33,37). Because of their ephemeral nature in soil, their significance in movement and survival has generally been discounted. Gregory (17) feels that hyphae are of little if any importance outside of host tissues. Weste (40), on the other hand, reports that *P. cinnamomi* can grow uphill through soil and increase the area of infected plants. Various *Phytophthora* species have shown varying abilities to grow through unsterile soil: *P. parasitica* (39) and *P. cactorum* (16) are capable of no growth, *P. palmivora* (38) and *P. erythroseptica* (39) are capable of limited growth from a food base, and *P. cinnamomi* (42) is capable of growth over short distances. The successful colonization of organic matter in soil by *P. cinnamomi* (30,42) has been attributed to hyphal growth through soil.

In this study, an attempt was made to determine whether zoospores or hyphae were responsible for movement through soil and colonization of organic matter. Previous studies have shown that zoospores are not released from sporangia and do not remain motile or move in a soil of this texture (silt loam) at -100 mb or even at high moisture levels (11,23,24). Maximum movement of zoospores under favorable conditions apparently occurs when they are motile and in a moving front of water (41). In the present study, such zoospores moved at least 2.0 cm through this silt loam soil. However, without moving water, motile zoospores failed to move through soil beyond the point of infiltration even though the soil was at saturation. This leads to the conclusion that hyphae of *P. m. f. sp. medicaginis* grew through soil at least 2.0 cm. As Carlile (6) points out, the nature and function of hyphae of *Phytophthora* suggest that they may function as a motile propagules. Although hyphae of *P. m. f. sp. medicaginis* do rapidly lyse in soil (34) they still may be especially significant to the ecology and survival of *P. m. f. sp. medicaginis* by accomplishing movement through soil, colonization of organic matter, and perhaps initiating infections of whole plants (21) prior to lysing.

The optimal temperature for growth of *P. m. f. sp. medicaginis* in vitro is 24 C, which also was conducive to the activity of soil organisms, especially bacteria. This may account for the very low degree of colonization by *P. m. f. sp. medicaginis* at 24 C; in vitro, it grows slower at 15 C than at 24 C and very slowly at 4 C. However, colonization in soil readily occurred at these temperatures. Other organisms, especially bacteria, were noticeably less active at the lower temperatures. This relationship of high competitive colonizing ability at temperatures suboptimal for growth (in vitro) has been observed with other pathogenic fungi (1,9,27). Being active at low soil temperatures may give *P. m. f. sp. medicaginis* a competitive advantage in colonizing substrates in soil and perhaps in initiating infections.

In New York, combinations of low soil temperatures and high soil moistures occur in mid- to late-autumn and early- to late-spring. For some soils, the temperature remains between 4 and 15 C for up to 5 mo each year. These periods also coincide with the times organic matter is most likely to be incorporated into soil. In a separate investigation, oospores of *P. m. f. sp. medicaginis* were observed to germinate in soil at 4 and 15 C, and the rate of hyphal

lysis in soil was greatly reduced at 4 C (34). Apparently *P. m. f. sp. medicaginis* can initiate activity and remain active longer at temperatures suboptimal for growth in vitro, which may provide it the opportunity to colonize new substrates.

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