Relative Survival Potential of Propagules of *Phytophthora megasperma* f. sp. *medicaginis*

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ABSTRACT

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Pouches of nylon screen containing zoospores, hyphae, sporangia, or oospores of *Phytophthora megasperma* f. sp. *medicaginis* were added to an unsterile soil maintained at different levels of temperature (4, 15, and 24 C) and moisture (0, −10, −100 mb ψ_m) to determine the relative survivability of the propagules. The rates of propagule lysis correlated positively with soil temperature. There was no statistically significant effect ($P = 0.05$) of soil moisture upon the rate of lysis. Complete hyphal lysis occurred in 9 days at 24 C and in 15 days at 15 C, but took longer than 35 days at 4 C. Encysted zoospores, hyphae developed from germinated cysts, and sporangia responded similarly to hyphae. Oospores survived at least 140 days in soil. Oospores were capable of germination in unsterile soil after 6 days at 24 C, 9 days at 4 C, or 16 days at 15 C. Hyphal lysis and formation of new hyphae occurred concurrently in soil at all three temperatures; some of these new hyphae gave rise to sporangia and/or oospores, depending upon temperature and moisture conditions. Relative persistence in unsterile field soil of zoospores, hyphae, oospores, or root segments colonized by a metalaxyl-insensitive isolate Pm20 of *P. m.* f. sp. *medicaginis* was determined by means of two alfalfa seedling bait bioassays. Only oospores free in soil or in colonized root tissue appear capable of long-term survival.

Phytophthora megasperma Drechsler f. sp. *medicaginis* Kuan & Erwin causes root rot of alfalfa in many soils throughout the world (1,7,33,37). The pathogen is widespread in New York State and has been detected in fields either planted to alfalfa, seeded to other crops, or left fallow (37). In New York, alfalfa is grown in rotation with corn and a small grain crop, commonly with 4 yr or more between alfalfa crops. Apparently *P. m.* f. sp. *megasperma* is well-adapted to persist in many soil environments and for long periods in the absence of its host.

In the life history of a pathogen, survival in the absence of its host can be considered long-term survival. Activity of *P. m.* f. sp. *megasperma* as determined by the alfalfa seedling bait bioassay of Marks and Mitchell (20), declines markedly with time to negligible or zero levels in soils either left fallow or replanted to crops such as corn and oats (24,25,33). Replanting these soils to alfalfa results in activity of *P. m.* f. sp. *megasperma* that may increase rapidly to the original level. The same observation has been made for New York soils (R. L. Millar, *unpublished*). However, which propagule(s) of the pathogen is most important to long-term survival and whether different types of propagules are important for its survival in different soil environments has not been clearly established.

Propagules of *Phytophthora* differ markedly in ability to persist in soil (19). For *P. m.* f. sp. *megasperma*, hyphae, sporangia, zoospores, chlamydospores, and oospores have been described as propagules. For certain soils, oospores of *P. m.* f. sp. *megasperma* have been implicated as the survival propagule (8); for other soils, chlamydospores have been indicated as the long-term survival structure (4).

The purpose of the work reported here was to determine, for New York alfalfa field soils conducive to the development of

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Phytophthora root rot, the relative survival potential of the various propagules and which of the propagules is most important to long-term survival of the pathogen. During this study the New York isolate of *P. m.* f. sp. *megasperma* did not produce chlamydospores.

MATERIALS AND METHODS

Isolates, media, and soil. Most experiments were conducted with metalaxyl-insensitive isolate Pm20 of *P. m.* f. sp. *medicaginis* (31). This was necessary to make possible selective isolation from a field soil harboring an indigenous population of *P. m.* f. sp. *megasperma* as well as interfering, fast-growing *Pythium* spp. Isolate Pm20 was obtained by mass screening of zoospores on cornmeal agar amended with metalaxyl at 10 mg/ml. This was a natural selection procedure; no artificial methods were used to induce mutagenesis. It was equal to the wild type in total growth, growth rate, sporulation, propagule germination, and pathogenicity. In some experiments, isolate AFI, the wild type and progenitor of Pm20, also was used.

The isolates were cultured and maintained on V-8 juice agar (200 ml of Campbells's V-8 juice, 3 g of $CaCO_3$, 15 g of agar, and 800 ml of distilled demineralized water). Production of zoospores, hyphae, sporangia, and oospores also was on V-8 agar. The selective medium used was a modification of the medium of Masago et al (21).

The soil used was from the Mt. Pleasant Research Farm in Tompkins County, NY; it had a silt loam texture, pH 7.4, and an organic matter content of 6.0% (31). A soil-moisture release curve for this soil was determined (32). Soil moisture in these experiments was adjusted gravimetrically to a specific percentage moisture corresponding to a given matric potential.

Qualitative assessment of propagule survival in soil. *Zoospores.* Zoospore suspensions produced by flooding V-8 agar cultures of Pm20 at 15 C were agitated (Vortex-Genie, Scientific Industries Inc., Springfield, MA) to induce zoospore encystment. Concentrations were determined by hemacytometer counts and adjusted by dilution to approximately 2,000 zoospores per milliliter. Encysted zoospores (about 1,000) were added to nylon screens (5-mm^2; 10-μm pore size) resting on a moist sintered-glass filter. Vacuum was applied to draw the cysts tightly onto the screen. The nylon screens were cut from a sheet of Nitex fabric (Tetko Inc., Elmsford, NY), autoclaved for 20 min at 121 C, and kept sterile until used.

Field soil (25 ml) equilibrated to specific temperatures (4, 15, or 24 C), was added to glass petri plates and the soil moisture was adjusted to 34.4, 61, and 79% (grams moisture/dry weight of soil) corresponding to −100, −10, and 0 mb, respectively. Nylon screens with zoospores were placed on the soil. An additional 25 ml of soil at the appropriate temperature and moisture was added to bury the screens and the soil was gently firmed. The plates were placed in plastic bags to retard moisture loss. Plates at each of the three moisture levels were placed at each of the three temperatures.

Hyphae and sporangia. Sterile nylon screens (5-mm^2, 100 μm pore size) were placed on the surface of V-8 agar plates. A suspension of encysted Pm20 zoospores, prepared as described above, was pipetted onto the agar surface adjacent to the screens. The plates were kept at 24 C to promote hyphal development; in 3 days the screens were uniformly covered with hyphae. Screens with hyphae were removed from the agar surface; washed with sterile distilled, demineralized water; and placed in soil under the same conditions and in the same manner as the screens with zoospores described above. Early trials were similarly conducted with hyphae of isolate AFI.

At high soil moisture content (61 and 79%), sporangia formed abundantly on screens that had only hyphae at the time of placement in soil. At 35%, many fewer sporangia formed in soil. These sporangia also were observed for survival potential.

If screens were allowed to remain on the agar surface for longer periods (7–10 days), sporangia and oospores also developed on the screens. When these screens were added to soil an assessment of the survival of all three propagule types could be made on the same screen. This was done with both isolates, AFI and Pm20.

Oospores. In addition to oospores produced on screens, a second method of oospore production was used. Hyphal mats were peeled from the surface of 14-day-old agar cultures of Pm20; care was taken to exclude agar from the mats. These mats contained high concentrations of oospores. The mats were blended three to five times for 45 sec at high speed (Waring blender) and the resulting suspension was frozen overnight (−20 C). The preparation was thawed, filtered through nylon mesh (100-μm pore size), and centrifuged (15 min at 3,000 rpm, clinical centrifuge) to concentrate the oospores. These oospores were then pipetted onto screens (5-mm^2, 10 μm pore) which were subjected to light vacuum (0.69 bar [10 psi]), to provide approximately 100 oospores per screen. These screens were placed in soil under the same conditions and in the same manner as described above for zoospores.

Observations. At the stated times, the screens were removed from the soil; gently washed with distilled, demineralized water to remove adhering soil particles; and either mounted in a brightener solution at 300 mg/l (Calcofluor; American Cyanimid Co., Bound Brook, NJ) on glass microscope slides or blotted dry and plated on the selective medium containing metalaxyl. Screens on slides were observed microscopically with white light and UV fluorescence. The general appearance and integrity of propagules was assessed subjectively and viability was determined by growth on the selective medium.

Quantitative assessment of propagule survival in soil. From the observations providing a qualitative assessment of propagule survival in soil, it was apparent that the behavior of hyphae, zoospores, and sporangia was quite similar. With time, these propagules lyse and disappear from the screens under all conditions of temperature and moisture tested. An attempt was made to quantify this lysis and hyphae were selected as the representative propagule. Initially, a determination of hyphal viability was attempted by using as criteria: induction of plasmolysis with high concentrations of salts or sugars coupled with uptake of neutral red, observation of protoplasmic streaming, and the use of vital stains and fluorescent dyes (e.g., Trypan blue and fluorescein diacetate). These methods failed to give any clear determination of viability.

The disappearance of hyphal strands from the screen has been used by others (26,34) and was selected as the best means for quantification. Viability is lost and lysis initiated prior to disappearance of a hyphal strand, but since the time frame for disappearance was short, 3–5 days, the difference in time between loss of viability and complete lysis was judged insufficient to affect the conclusions.

Screens (1.0-cm^2, 100-μm pore size) colonized by hyphae of Pm20 were prepared as described above (Fig. 1A). Results of preliminary experiments showed negligible variability among replicate screens for a given treatment. For each treatment in the quantification experiment, there were three screens buried in each soil plate with three plates for each sampling time. At 0, 3, 6, 9, 12, and 15 days, the nine screens for each treatment were retrieved from soil, washed gently with distilled, demineralized water, and mounted on glass microscope slides in 300 ppm brightener solution. For each screen, five observations were made at random locations across the entire screen. Each observation consisted of counting the number of intact hyphal strands per one pore (100-μm) of the nylon screen. This was done microscopically with both white light and UV fluorescence.

Determination of the propagules important to long-term survival. Two forms of a seedling bioassay were used to distinguish propagules capable of long-term survival. The alfalfa seedling bait bioassay previously described (20) and designated here as the abbreviated bioassay was used with the following modification: 3- to 5-day-old alfalfa seedlings were used as bait, but they were not pinched with forceps to restrict further development. In this assay, 30 ml of Mt. Pleasant field soil was added to a glass petri plate. The soil was flooded by adding 40 ml of distilled, demineralized water and six seedlings were floated in the water above the soil for 3 days. The seedlings were removed from the soil/water plates, observed microscopically for sporangia of *P. megasperma*, and plated on the selective medium containing metalaxyl.

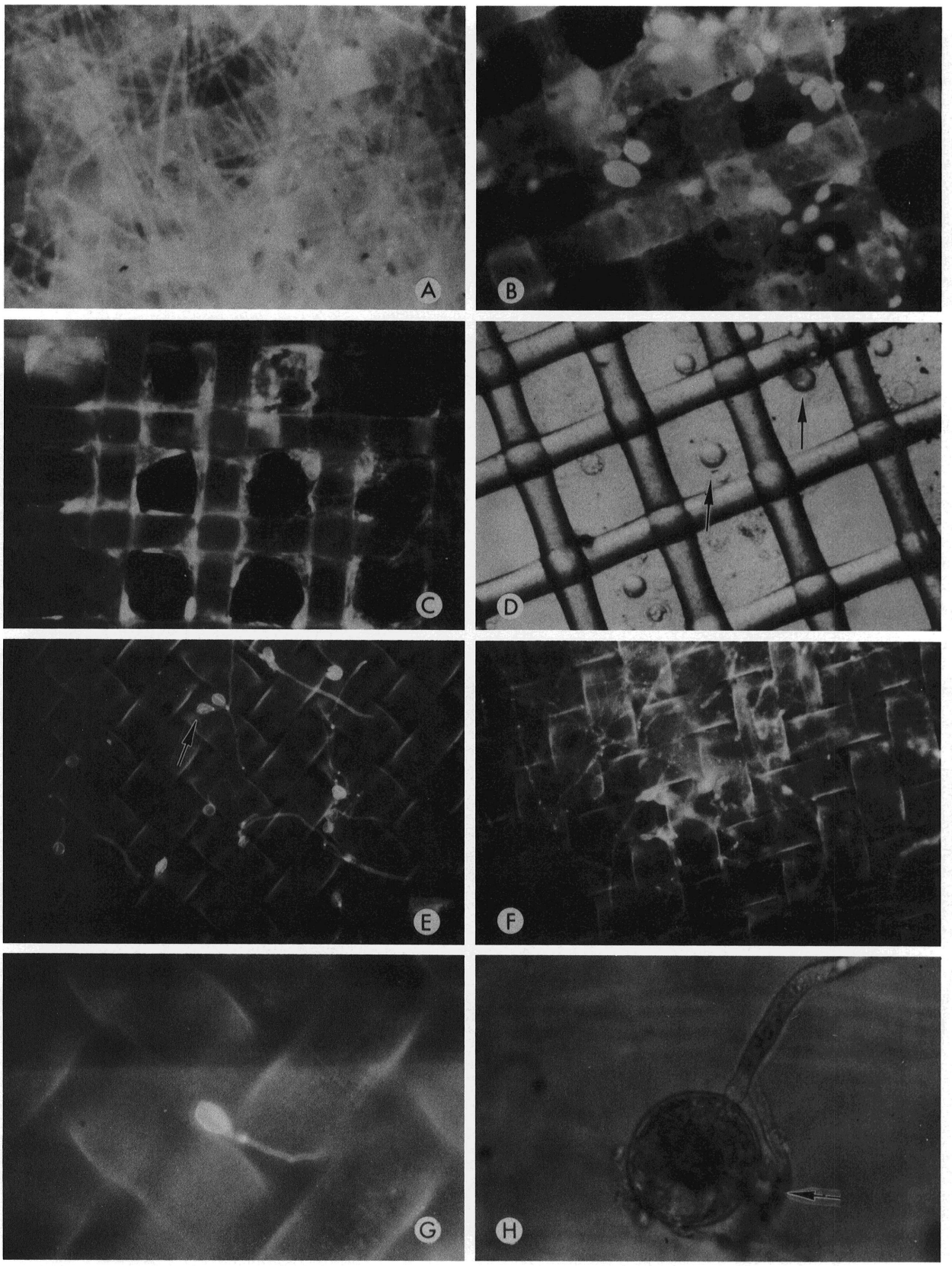

Fig. 1. Microscopic observations of propagules of *Phytophthora megasperma* f. sp. *medicaginis* in soil. **A,** Nylon screen prior to placement in soil. Hyphae are abundant. **B,** Nylon screen after 3–5 days in field soil at 24 C. Sporangia remain after hyphae have lysed. **C,** Nylon screen after 10 days in field soil at 24 C. All sporangia and hyphae have lysed. **D,** Nylon screen after 10 days in field soil at 24 C. Only oospores remain (arrows). **E,** Nylon screen from soil showing germination of zoospore cysts and hyphal development. After germination and hyphal elongation, the germ tube walls off and and the cyst collapses (arrows). **F,** Nylon screen after 3–5 days in field soil at 24 C. Cysts and the hyphae that developed from the germinated cysts are in a stage of lysis. **G,** Nylon screen from soil showing germination of zoospore cyst. Septation of the germ tube and cyst collapse can be seen. **H,** Oospore germination in field soil at 4 C. **A, B, C, E, F, and G** are UV-fluorescent photomicrographs. Calcofluor brightener solution was used at 300 mg/ L. **D and H** are white light photomicrographs. This was necessary since oospores did not consistently fluoresce.

The abbreviated bioassay also was modified and this modified procedure is referred to here as the extended bioassay. In the extended bioassay, field soil (30 ml) was added to a glass petri plate and allowed to dry for 7–10 days until the soil reached 3–5% moisture. The soil then was moistened with 10–15 ml of distilled, demineralized water. After 3 days, the soil was flooded by adding 40 ml of distilled, demineralized water and six 3- to 5-day-old alfalfa seedlings were floated above the soil. The seedlings were removed after 3 days, observed microscopically for sporangia of *P. megasperma* and plated on the selective medium containing metalaxyl.

To test the ability of propagules of Pm20 to survive and also to detect their activity with the two bioassays, propagules were placed on nylon screens in soil, and that soil was subjected to the two bioassays. Zoospore cysts and oospores were vacuum infiltrated (0.69 bar [10 psi]) onto separate screens. Nylon screens with hyphae were obtained from agar cultures as described. For each propagule type, there were three screens per soil plate and five soil plates for each of the two bioassays. For each propagule type, the mean number of infected seedlings for the five soil plates was determined. Screens were retrieved, observed microscopically, and plated on the selective medium to determine propagule viability.

Statistical treatment of data. All experiments were performed at least three times. For comparing treatments in the quantification experiments, the means of the five observations per screen were determined for each of the three screens per sample time per treatment. A linear regression analysis was performed with time, temperature, and moisture as the independent variables. Coefficients were tested for significance at $P = 0.01$ (28). For the long-term survival experiments that involved the two bioassays, treatment means were compared by a Duncan's multiple range test subsequent to a determination of significance by an F-test (28).

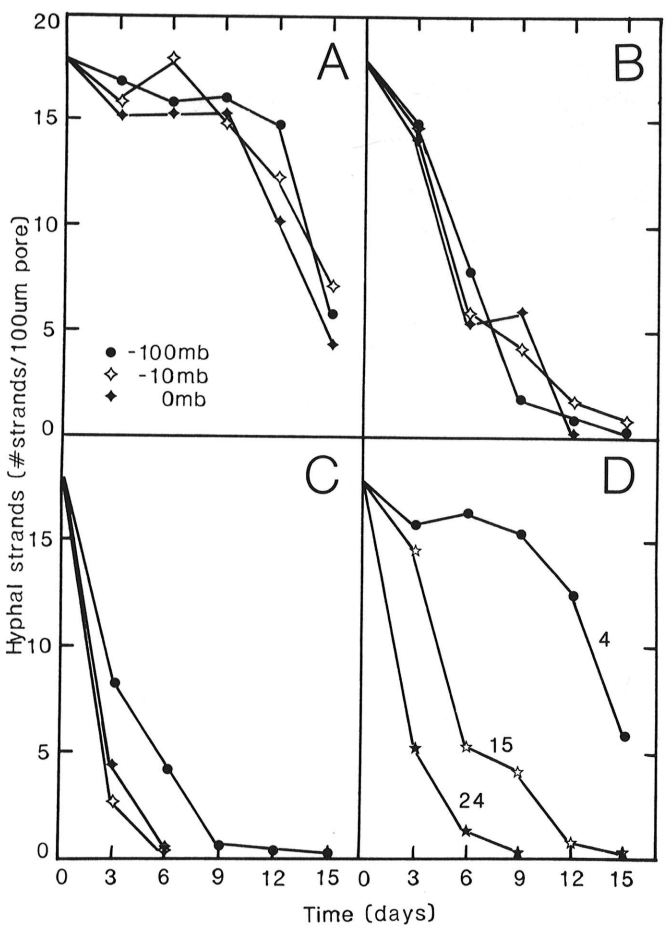

Fig. 2. Quantification of hyphal lysis of *Phytophthora megasperma* f. sp. *medicaginis*. **A, B, and C** indicate the number of hyphal strands per 100-μm pore of nylon screen over time at 4, 15, and 24 C, respectively. At each temperature, the three curves represent −100 mb, −10 mb, and 0 mb matric potential. **D,** Each curve represents the means of the three moisture curves at the indicated temperature for the curves shown in **A, B, and C**.

RESULTS

Qualitative assessment of propagule survival in soil. *Zoospores.* Encysted zoospores on nylon screens readily germinated in soil under all conditions of temperature and moisture tested (Fig. 1). The rate and extent of hyphal development from germinated cysts was positively correlated with soil temperature and soil moisture. The rate of lysis of the resultant hyphae also was positively correlated with soil temperature. The cyst collapsed after germination and growth of the germ tube (Fig. 1E and G). Cysts and the resultant hyphae rarely survived longer than 7 days in the field soil at high temperatures (15 and 24 C) and high moistures (0 and −10 mb) (Fig. 1F). Occasionally an oospore was formed on a screen from the hyphae that developed from a cyst.

Hyphae, sporangia, and oospores. There was no detectable difference in behavior between isolates AFI or Pm20. Subsequent to their placement in soil, hyphae on nylon screens were capable of further growth and development as indicated by hyphal elongation at 4, 15, or 24 C and the formation of sporangia and oospores in soil at 15 or 24 C and 0 or −10 mb. At −100 mb, oospores readily formed but sporangia rarely did. Lysis of hyphae occurred under all conditions tested (Fig. 1B, C) but the rate of lysis increased with increasing temperatures. At 24 C, lysis was rapid; most hyphae were affected within 3–5 days and all hyphae had lysed within 7–10 days. Hyphae in soil at 4 C showed very little change at 7–10 days. If hyphae at 4 C were transferred to 24 C at any time, lysis then proceeded at a rapid rate. The rate of lysis of hyphae in soil at 15 C was intermediate between that at 4 and 24 C but closer to that at 24 C.

Sporangia in soil behaved similarly to hyphae with respect to the relationship of rate of lysis to soil temperature and moisture. Sporangia persisted a few days longer than hyphae, but ultimately lysis occurred under all conditions tested (Fig. 1B and C). This was the case also for the sporangia produced on screens in soil.

When screens bearing hyphae, sporangia, and oospores were placed in soil, the relative abilities of the propagules to persist was readily observed. Hyphae rapidly lysed leaving sporangia and oospores on the screen. Soon the sporangia also lysed leaving only oospores (Fig. 1D). Oospores were capable of persisting for long periods in soil, at least 140 days. These oospores were still viable as indicated by their germination when transferred to the selective medium. The length of oospore persistence did not differ for the two methods of oospore production.

Though oospores as a population persisted much longer than other propagules, individual oospores were parasitized by what appeared to be a chytrid and a filamentous organism resembling an

TABLE 1. Analysis of variance table and the linear regression model for the quantification of hyphal lysis of *Phytophthora megasperma* f. sp. *medicaginis*[a]

Due to	d.f.	Sums of squares	Mean square	F statistic
Regression	3	6,580.50	2,193.98	172.3 **[b]
Times (X_1)	1	3,919.98	3,919.98	307.9 **
Temperature (X_2)	1	2,632.31	2,632.31	206.8 **
Moisture	1	28.21	28.21	2.2
Residual	158	2,011.47	12.73	
Total	161	8,591.96		

Linear regression equation[c]
$y = 20.9 - 0.891\ X_1 - 4.94\ X_2$[d]

[a] Nylon screens with Pm20 hyphae were buried in soil at different temperatures and moistures. Over time, the number of hyphal strands per unit area of screen was determined. See text for details.
[b] Asterisks (**) indicate statistical significance at $\alpha = 0.01$.
[c] The linear regression equation for the significant variables of time and temperature.
[d] The significant coefficient of X_1 indicates that the number of hyphal strands per unit area decreased with time. The significant coefficient of X_2 indicates that the number of hyphal strands decreased with increasing temperature.

actinomycete. The filamentous parasite completely colonized the oospore. The chytrid sporulated both internal and external to the oospore wall.

Quantification of hyphal lysis in soil. To quantify the rate of propagule lysis in soil, screens with hyphae as the representative propagule were buried in soil at different temperatures and moistures, and the number of hyphal strands per unit area of screen was determined over time. For all treatments, lysis increased with time. The rate of lysis was not significantly different at 0, −10, or −100 mb at 4, 15, or 24 C (Fig. 2A,B,C; Table 1). However, the rate of lysis increased with increasing temperatures. The rate of hyphal lysis at 15 and 24 C for all moisture levels was significantly greater than the rate at 4 C (Fig. 2D). It took 9 and 15 days to reach 100% lysis at 24 and 15 C, respectively. At 4 C, it took more than 35 days to reach 100% lysis.

Although hyphae, as a population, underwent rapid lysis in soil, new growth in the form of hyphal elongation occurred at all three temperatures. Significantly, some of this new growth resulted in the production of sporangia and/or oospores. Germinated oospores (Fig. 1H) were observed after 6 days at 24 C and 0 mb, after 9 days at 4 C and −10 or −100 mb, after 13 days at 4 C and 0 mb, and after 16 days at 15 C and −10 or −100 mb. Sporangia were produced abundantly at 15 C after 6 days. At 4 C, no sporangial production was observed at any moisture level throughout the 16 days. At 24 C, many sporangia were observed at 3 and 6 days, but not after 6 days. The sporangia produced in soil lysed as readily as did culture-grown sporangia that were placed in soil.

Determination of the propagules important to long-term survival. Mt. Pleasant field soil artificially infested with propagules of Pm20 was subjected to the abbreviated and extended alfalfa seedling bioassays to determine if the propagules could survive the two procedures and if the activity resulting from the propagules would be detected by either or both of the bioassays. Propagules which survived the extended bioassay and which resulted in activity that was detected by it were designated long-term survival propagules.

When the alfalfa field soil that had been left fallow for several months was subjected to the two bioassays, no seedlings became infected in the abbreviated bioassay but a high proportion of seedlings became infected in the extended bioassay (Table 2). This is a typical response of alfalfa field soil left fallow or cropped to oats or corn for several weeks. With the extended bioassay we have repeatedly detected and isolated *P. m.* f. sp. *megasperma* in soils for which the abbreviated bioassay has failed to do so.

The fallow soil was artificially infested with propagules of Pm20 and subjected to the two bioassays. The seedling baits were plated on the selective medium with metalaxyl to distinguish the activity of the added Pm20 from the indigenous *P. m.* f. sp. *megasperma*. Zoospores survived the abbreviated bioassay; a high proportion (0.97) of seedlings became infected (Table 2), and a high proportion (0.94) of the screens had viable Pm20. Activity in zoospore-infested soil was dramatically reduced to almost zero in the extended bioassay.

Mycelium yielded low proportions (0.05 for abbreviated; 0.10 for extended) of infected seedlings in both assays (Table 2). For mycelium, the proportion of screens with viable Pm20 was moderate (0.67) as determined by the abbreviated bioassay and low (0.20) by the extended bioassay.

Oospores survived both bioassays well as indicated by moderate (0.43) and high (0.78) proportions of seedlings infected in the abbreviated and extended bioassays, respectively (Table 2). The proportion of screens with viable Pm20 was moderate (0.47 for the abbreviated; 0.70 for the extended) in both assays.

At intervals during the extended bioassay, screens with zoospores or mycelium were removed from the soil and observed microscopically to assess viablity. In the period that the soil was drying, the hyphae and zoospores became granular and vacuolate, and then empty and collapsed. After the 7-day drying period, no hyphae or zoospores appeared to be viable. Even so, a small proportion of screens from these treatments gave rise to colonies of Pm20 on the selective medium when removed from the soil at the end of the extended bioassay procedure.

DISCUSSION

Fungal propagules in any environment have a finite lifetime. The ability to persist is a function of the properties inherent in the propagule as well as the environment in which it exists. *P. m.* f. sp. *medicaginis* produces at least four propagule types. In this investigation, the relative survival potential of these propagules was determined under an array of environmental conditions. Under all conditions tested, the greater ability to persist was in the order (least to greatest): hyphae, zoospores, sporangia, and oospores. This is in good agreement with that observed for California soils under less controlled conditions (8). At different soil temperatures the length of persistence varied, but the relative order of propagule survival potential remained the same. Unlike observations for Canadian isolates in Canadian soils (4), chlamydospores were not observed with the New York isolate in New York soil.

Hyphae, zoospores, and sporangia all behaved similarly in soil; they lysed in a short period of time. Though increasing moisture seemed to increase the rate of hyphal lysis, the effect was not statistically significant. Temperature dramatically affected the rate of lysis; as temperature increased, the rate of lysis increased. This temperature effect on the rate of propagule lysis has been reported for *P. cactorum* (29) and *P. cinnamomi* (22). At 24 C, complete lysis of hyphae of *P. m.* f. sp. *megasperma* occurred in 9 days. At 4 C, complete lysis took longer than 35 days. In many New York alfalfa field soils, the temperature at the 15-cm depth remains between 4 and 15 C for up to 5 mo each year. This may provide an environment conducive to activity of *P. m.* f. sp. *megasperma*.

Like *P. cinnamomi* (26), *P. parasitica* (34), *P. megasperma* f. sp. *glycinea* (11), and *P. cactorum* (29), propagules of *P. m.* f. sp. *megasperma* were capable of further development subsequent to their placement in soil. Sporangia formed from hyphae in soil primarily at high moisture levels and the higher temperatures (15 and 24 C). Direct and indirect germination were observed. Oospores formed at 4, 15, and 24 C in soil from hyphae and germinated zoospore cysts. Because of this ability to form oospores in soil, it is possible that the oospores that germinated on the selective medium after 140 days in soil were the result of development subsequent to placement of screens in soil. Some oospores were capable of germination in soil within 4–9 days after their production. The majority did not germinate during the course of the experiments. When oospores were plated on the selective medium, it often took 3–5 days for them to germinate and initiate growth. This slow development from oospores on agar may be why direct plating of soil for enumeration of *Phytophthora* has been

TABLE 2. Propagule activity of *Phytophtora megasperma* f. sp. *medicaginis* as determined by the abbreviated and extended alfalfa seedling bioassays

	Propagules of *P. m.* f. sp. *medicaginis* added to fallow soil[a]				
Seedling bioassay	None	Oospores	Colonized tissue[b]	Zoospores	Hyphae
Abbreviated[c]	0.0 c	2.6 b	5.6 a	5.8 a	0.3 c
Extended[d]	4.3 a	4.7 a	6.0 a	0.6 b	0.6 b

[a] Mt. Pleasant field soil left fallow for several months was bioassayed. Pm20 propagules (zoospores, hyphae, or oospores) were added to the fallow soil and the soil subsequently subjected to the two bioassays. These values are the means of three experiments. They represent the mean number of seedlings infected for the five soil plates per propagule type per experiment. Means within a row not followed by the same letter are significantly different, $P = 0.05$, according to Duncan's multiple range test.

[b] Alfalfa root segments colonized in vitro by Pm20 were incorporated into soil and subjected to the two bioassays. This treatment was tested once.

[c] The abbreviated bioassay involved adding 30 ml of soil to a petri plate, flooding the soil by adding 40 ml of distilled, demineralized water, and floating six alfalfa seedlings in the water above the soil for 3 days. The seedlings then were plated on the selective medium with metalaxyl. There was no metalaxyl in the medium for the plating of seedlings from fallow soil not supplemented with propagules.

[d] The extended bioassay involved first drying the soil for 7 days followed by a 3-day moistening period, then proceeding as for the abbreviated bioassay.

successful only with chlamydospore-producing species (3). Germination of oospores and subsequent hyphal development was observed at 4 C. At this temperature, the rate of lysis was very slow. In field studies, activity of *P. m.* f. sp. *megasperma* was not detected at soil temperatures less than 12–15 C (38). This apparent lack of activity may be due to a lack of sporangium formation at low temperatures (4 C) as was observed in this study. Colonization of organic matter by *P. m.* f. sp. *megasperma* readily occurred in soils at 4 C, probably a result of hyphal activity (32). Low temperatures may favor survival and activity of *P. m.* f. sp. *megasperma* by being unfavorable to antagonists as indicated by the slower rates of propagule lysis at low temperatures. Low temperatures have also been reported to be conducive to survival and activity of other *Phytophthora* species (5,23,29,35).

Oospores germinated by production of sporangia or hyphae which often branched close to the oospore. Germination could be initiated from anywhere on the oospore with respect to the antheridium. Forster et al (9) reported similar observations in solution cultures of *P. m.* f. sp. *megasperma*. Kuan and Erwin (15) reported the sensitivity of oospore germination of *P. m.* f. sp. *megasperma* to soil matric potential. In their study, sporangial production from oospores (criterion for germination) at −100 mb ψm was not observed. Similar observations were made in this study, however oospores did germinate at −100 mb ψm by production of hyphae.

An attempt was made to determine which propagules survive in soils not cropped to alfalfa. Some workers have concluded that *P. m.* f. sp. *megasperma* persists poorly in the absence of alfalfa based upon an inability to detect its activity with the seedling bait bioassay (24,25,33). An alternative conclusion could be that *P. m.* f. sp. *megasperma* survives very well in the absence of alfalfa but that the active or motile propagules, zoospores, and hyphae, are less active or inactive in the absence of alfalfa. An assay was developed (extended bioassay) which, unlike the routinely used seedling bioassay (abbreviated bioassay), could detect *P. m.* f. sp. *megasperma* in soils not cropped to alfalfa. Zoospores and hyphae of Pm20 added to field soil were detected by the abbreviated bioassay but not by the extended bioassay. When a naturally infested soil left fallow or cropped to oats or corn is subjected to the two assays, activity is readily detected by the extended assay but rarely by the abbreviated bioassay. If zoospores and hyphae were present and active in the oat or fallow soil they should have been detected by the abbreviated bioassay.

When oospores of Pm20 were added to field soil they were detected by both abbreviated and extended bioassays. Since, in a population of oospores produced either in culture or in soil, a small proportion germinate without any requirement for a maturation period, these might account for the difference in responses of a naturally infested soil versus the soil infested with oospores of Pm20 subjected to the two assays. In the artificially-infested soil, the oospores capable of early germination are present and can give rise to activity. In the naturally infested soil, the early germinating oospores would have been spent. Root tissue colonized by Pm20 also resulted in activity in both assays. It was not determined whether hyphae could survive the extended bioassay within the colonized tissue, therefore both hyphae and oospores could have been responsible for the resultant activity.

The extended bioassay includes a drying period of 7–10 days in which the soil reaches about 5% moisture content. It is generally believed that *Phytophthora* species quickly lose viability upon exposure to drying (35). Zoospores, hyphae, and sporangia are very sensitive to drying and do not persist in dry soil (17,18,29,36). There are, however, reports which indicate that *Phytophthora* species persist well in dry soil but are merely inactive until the soil is wetted (4,6,10,12,13,23,27). Two reports actually indicate increased activity as a result of drying and moistening soil (Jensen 1917, as reported in references 6 and 27). The activity of *P. m.* f. sp. *megasperma* was observed in this study to increase after a drying and rewetting of the soil. This drying and rewetting made possible the detection of *P. m.* f. sp. *megasperma* in soils in which it could not be detected with the abbreviated assay. Based on data derived from observations of oospores on the nylon screens, the drying and rewetting stimulated oospore germination. Further experimentation is needed to verify this interpretation. Air-drying of an unsterile soil extract was reported to stimulate oospore germination of *Pythium ultimum* (2), whereas viability of its other propagules was lost upon drying (16).

The activity of pythiaceous fungi in response to drying and rewetting may be ecologically significant at two points in their life cycle, at least in New York. A typical growing season commonly involves alternating periods of dry and wet soil. By adapting to be activated by drying and rewetting, *P. m.* f. sp. *megasperma* may have gained a competitive edge by being active when microbial activity in general has been decreased by the drying. Perhaps more significant to its survival is overwintering. With respect to a soil's physical properties, freezing and thawing are equivalent to drying and wetting (14). When a soil freezes, water is drawn out of the soil aggregates and replaced with air, in effect drying the soil. During the thawing process, water gradually reenters the soil aggregates and upon complete thawing the soil may be flooded. The thawing of soil in spring after being frozen during the winter, may enhance activity by stimulating germination of oospores.

In this investigation, the survival of propagules of *P. megasperma* f. sp. *medicaginis* was considered from two perspectives. The first evaluated the capability of the pathogen's propagules to persist when placed in a soil under specific conditions of temperature and moisture. The second attempted to determine which propagules existed in a soil known to be harboring the long-term survival propagules. Both approaches led to the same conclusion, that is, only oospores can persist for long periods of time in soil. However, other propagules may play a role in long-term survival by their ability to produce oospores in soil, by effecting colonization of organic matter, and by associating with alternate hosts (30,32).

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