Factors Affecting Oospore Germination of *Phytophthora megasperma* f. sp. *medicaginis*

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ABSTRACT

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Oospores of *Phytophthora megasperma* f. sp. *medicaginis* were produced on carrot broth and purified of mycelium by digestion in Glusulase® (β-glucuronidase and aryl sulfatase). Incubation of oospores in alfalfa root extract, root exudate, or soil extract at 24 C under blue light (450–475 nm) increased germination markedly compared to incubation in distilled water. Oospore germination increased with increasing age of the culture from 11 to 50 days. Glucose inhibited germination, but the inhibition could be partially or completely overcome by the addition of acetate or asparagine. Ca^{2+} at 1 and 10 μg/ml had no effect but at 50 μg/ml inhibited oospore germination. Metabolic inhibitors of RNA and protein synthesis and respiration reduced germination.

Additional key words: aborted oospores, metabolic inhibitors, root extract, root exudate, soil extract.

Oospores are one of the important survival structures of *Phytophthora* species. Consistent success in obtaining germination of oospores has not been realized. The germination rate usually has been low and variable, often not exceeding 10%. Oospores of homothallic species usually germinate more rapidly and in greater abundance than those of heterothallic species (28). Nevertheless, the frequencies of oospore germination of the homothallic species *P. megasperma* f. sp. *medicaginis* are usually low. Erwin (8) observed about 10% germination on water agar; Baumer (3) also reported 5–10% germination on water agar; however, the high incidence of disease obtained with low levels of oospores in the soil indicated that there was a much higher amount of germination in the soil. Kuan and Erwin (18) reported up to about 20% germination in soil. However, up to 93% of the oospores ingested by the land snail, *Helix aspersa*, germinated (22). Factors that affect germination of oospores of various species of *Phytophthora* have been summarized by Ribeiro (19).

In this paper we shall report the effects of root and soil extracts and certain metabolic inhibitors on oospore germination of *P. megasperma* f. sp. *medicaginis*. Some of these results were briefly reported (13).

MATERIALS AND METHODS

Oospores. Isolates P1057 and P844 of *P. megasperma* f. sp. *medicaginis* (PMM) were obtained from alfalfa plants in California and isolate P410 from F. Frosheiser (University of Minnesota, St. Paul). The isolates were grown on sterile carrot broth, which consisted of 200 g of scraped fresh carrots, blended in 1 L of double distilled water (DDW), and filtered through four layers of cheesecloth (15 ml per 90-mm-diameter petri dish), in the dark at 24 C for 4 wk. Mycelium-free oospores were isolated after various times by the following method. Mycelial mats containing oospores were comminuted in distilled water for 1 min with a Sorvall mixer (Ivan Sorvall Inc., Newton, CT 06470) at medium speed. The suspension was sonicated (Braun-Sonic 1510, S. Braun Instruments, South San Francisco, CA 94080) for 30 sec at 50W, washed twice with DDW and incubated in Glusulase® (5% v/v), a mixture of β-glucuronidase and aryl sulfatase (Calbiochem-Behring, La Jolla, CA 92037), at 27 C on a reciprocal shaker adjusted to make 200 excursions per minute. After 12 hr, the suspension containing the partly digested mycelium was sonicated for 10 sec at 30W to remove mycelial fragments still attached to the oospores; in most cases, the antheridium was also removed. Incubation of oospores in Glusulase for longer than 24 hr greatly reduced the percentage of germination (*unpublished*). Mycelial fragments and aborted and empty oospores were removed by repeated sucrose density gradient centrifugation (gradients: layers of 0, 10, 20% sucrose with the most dilute on the top). The oospores were collected in the pellet at the highest sucrose concentration. The oospores were washed by centrifugation on a low-speed clinical centrifuge (International Equipment Co., Needham Heights, MA 02194) at least 10 times to remove the sucrose. Oospores were either used immediately or were stored in water at 3 C for future experiments. A droplet of the oospore suspension was mixed with 3.5 ml of sterile DDW in plastic petri dishes (60 mm in diameter), incubated under blue light at 800 $\mu W\ cm^{-2}$ (Sylvania F1 5T8-B bulb) (21) at 24 C for 4–6 days and oospore germination was microscopically examined directly in the petri dishes. Oospores usually adhered to the bottom of the dish. Oospores were either used immediately or stored in water at 3 C for up to 5 mo until required for experiments.

Alfalfa root extracts. Alfalfa plants, either susceptible (cultivar Moapa 69) or resistant (germ plasm line A77-10B) (10) to Phytophthora root rot, were grown in steamed soil (Hanford coarse sandy loam) in the greenhouse for various periods varying from 7 to 87 days. Plants were fertilized with a slow-release fertilizer (Osmocote® [NPK = 18-6-12]). The soil was washed off the roots and free water was removed by blotting with filter paper. Root extracts were prepared by blending roots (10 g fresh weight) for 1 min in 100 ml of DDW in a Sorvall omnimixer, incubating the homogenate overnight at 3 C, centrifuging 5 min in a clinical centrifuge, diluting the supernatant 1:9 with DDW, and passing the extract through a Nalgene filter (0.2 μm, Nalgene Sybron Corp., Rochester, NY 14602). DDW passed through a Nalgene filter was used as a control. In some experiments the extract was autoclaved.

Alfalfa root exudates. Alfalfa seeds (cultivar Moapa 69 and germ plasm line A77-10B) were surface sterilized for 30 min in 50% bleach (5.25% sodium hypochlorite), 20% ethanol, and 30% water, washed in DDW, and germinated on water agar. After incubation for 24 hr in the darkness at 24 C under sterile conditions, 120

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uniform seedlings were placed in a sterilized aluminum foil container (85 mm in diameter with holes in the bottom) floating on sterile DDW (40 ml) in petri dishes (90 mm in diameter). Five petri dishes of each alfalfa cultivar were prepared. The control consisted of DDW from petri dishes containing aluminum foil without seedlings. The petri dishes were incubated under a bank of fluorescent lamps (two 40W Vitalite Powertwist bulbs, Durotest Corp., North Bergen, NJ 07047), with spectral peak at 550 nm, at 1,200 $\mu W\ cm^{-2}$, operating on a 12 hr light/12 hr dark cycle. After 2 days seed coats were removed to avoid effects of seed coat leachates, and the DDW was replaced. After 6 days the volume of the root exudate was reduced by half with a Rotavapor RE 120 (Brinkman, Westbury, NY 11590) at 40 C. The solution was passed through a Nalgene filter (0.2 μm, Nalgene Sybron Corp., Rochester, NY 14602). The absence of contaminating bacteria was ascertained by incubating an aliquot in Difco Eugon nutrient broth and by streaking on potato-dextrose agar.

Soil extracts. Raw soil (Hanford coarse sandy loam) (300 g) from an alfalfa field was incubated for 8 hr in 180 ml of DDW with frequent stirring. After centrifugation (5 min in a clinical centrifuge) the supernatant was passed through a Nalgene filter (0.2 μm). Dilutions were made with DDW. DDW, passed through a Nalgene filter, was used as a control.

Filtering the crude root and soil extracts through filter paper was avoided since filter paper extracts had a stimulating effect on germination. Extracts from Nalgene filters had a much smaller effect. Stimulation of germination by filter paper extracts was also reported for rust uredospores (1).

Chemicals. Antimycin A (Sigma Chemical Co., St. Louis, MO 63178), ascorbic acid (Mallinckrodt Inc., St. Louis, MO 63134), asparagine (Sigma), 8-azaguanine (Calbiochem-Behring, La Jolla, CA 92037), 6-azauracil (Calbiochem-Behring), biotin (Calbiochem-Behring), $CaCl_2 \cdot H_2O$, ethylene glycol-*cis*-(β-aminoethylether)-*N*, *N'*-tetraacetic acid (EGTA) (Sigma), 5-fluorouracil (Calbiochem-Behring), KCN (Mallinckrodt), nystatin (Sigma), pyridoxine HCl (Schwarz-Mann Inc., Spring Valley, NY 10977), and thiamine (Calbiochem-Behring) were dissolved or suspended in DDW. Concentrated stock solutions of chloramphenicol (Sigma) and cycloheximide (Sigma) were made in 95% ethanol; oligomycin (Charles Pfizer & Co., Inc., Groton, CT 06340) and β-sitosterol (United States Biochemical Corp., Cleveland, OH 44128) were dissolved in dichloromethane. Dilutions were made with DDW. Stock solutions of oleic, linoleic, and palmitic acids (United States Biochemical) in ether were diluted with DDW. The ether was allowed to evaporate in the petri dishes before the oospores were added. Controls included water alone and water containing the same amount of solvent used to dissolve the chemicals.

Germination tests. Oospore germination tests were done in

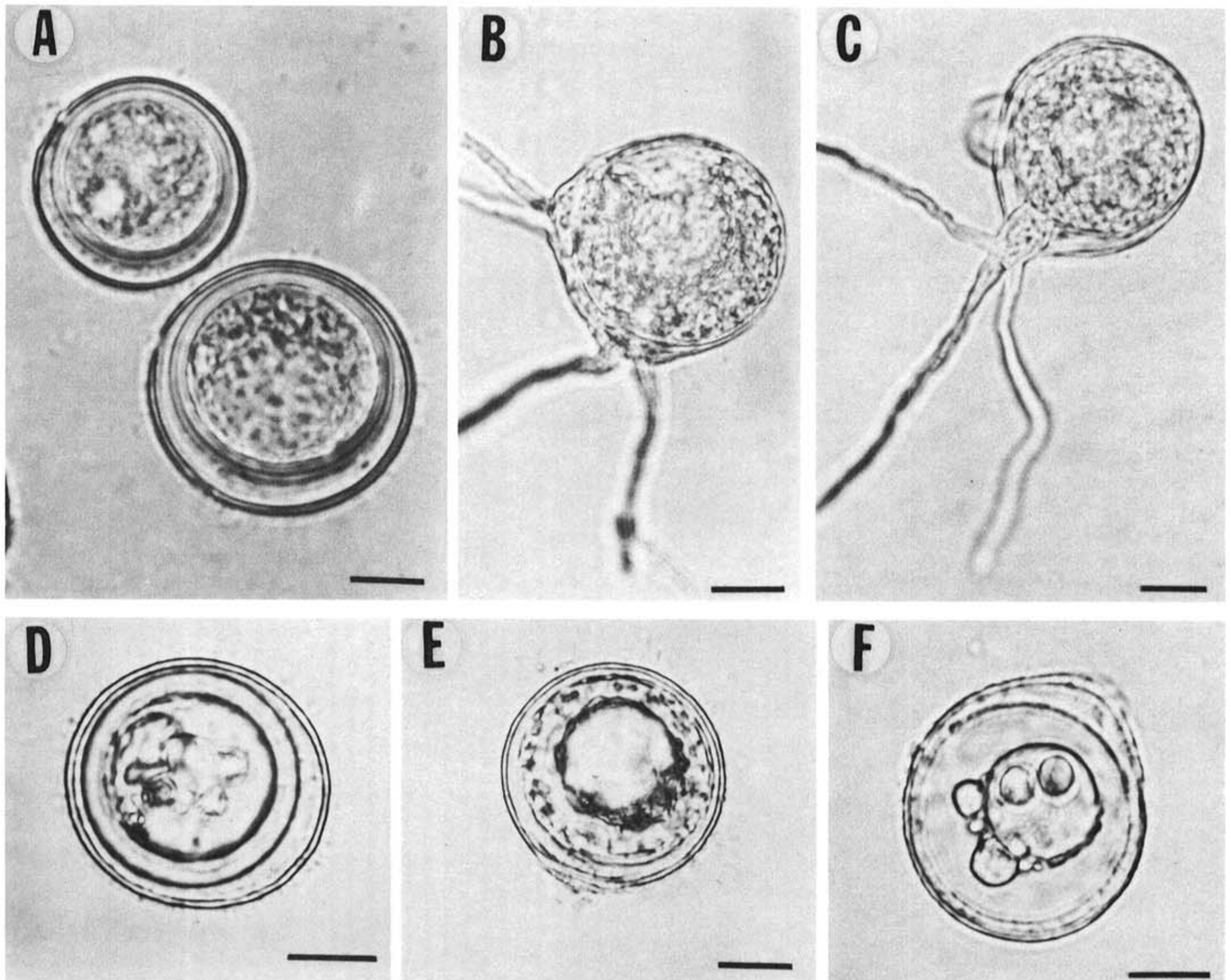

Fig. 1. Oospore germination of *Phytophthora megasperma* f. sp. *medicaginis* P1057 (bar = 10 μm). **A,** Ungerminated oospores after Glusulase® enzyme treatment and sonication. **B,** Germinated oospore showing multiple germ tubes. **C,** Germinated oospore showing branched germ tube and antheridium. **D, E,** and **F,** Various stages of oospore abortion.

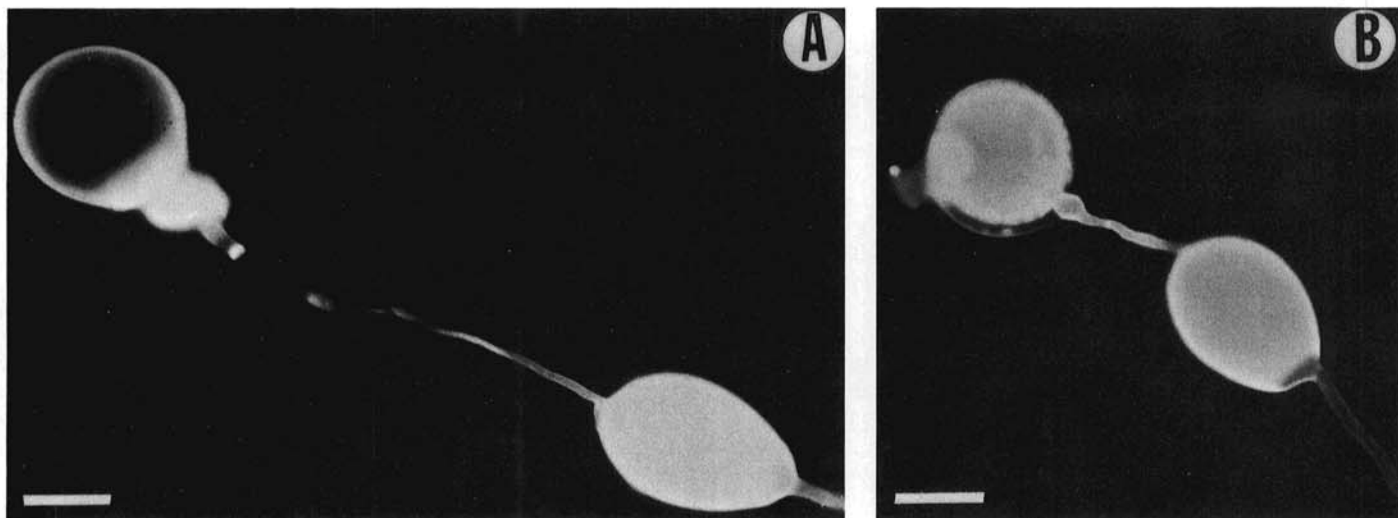

Fig. 2. Germination of *Phytophthora megasperma* f. sp. *medicaginis* P1057 (bar = 15 μm) oospores, which were stained with diethanol and observed by fluorescence microscopy. **A,** Germ tube emergence through the antheridium. **B,** Germ tube emergence through the oogonial wall.

TABLE 1. Germination and abortion of oospores of three isolates of *Phytophthora megasperma* f. sp. *medicaginis* in distilled water and alfalfa root exudate

			Incubation medium			
			Double distilled water		Root extract	
Isolate	Culture age (days)	Incubation (days)	Germinated[a] (%)	Aborted[a] (%)	Germinated[a] (%)	Aborted[a] (%)
P1057	62	6	15	43	87	6
P844	28	8	20	61	87	10
P410	28	8	1	50	73	7

[a] Data for isolate P1057 were combined from four experiments and for P844 and P410 from two experiments. Differences between the double distilled water control and the root extract incubation medium were highly significant ($P = 0.01$).

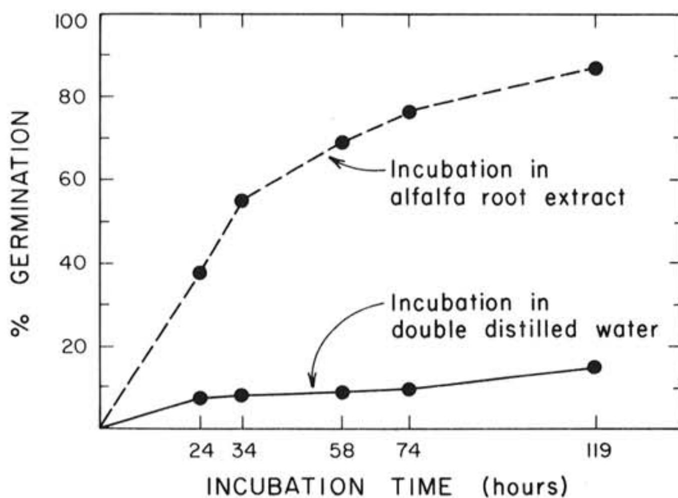

Fig. 3. Increase in rate and percentage of oospore germination of *Phytophthora megasperma* f. sp. *medicaginis* (P1057) in alfalfa root extract compared to that in distilled water.

plastic petri dishes (60 mm in diameter) containing 3.5 ml of solution, which was mixed with one drop of the oospore suspension. The petri dishes were incubated under continuous blue light at 800 μW cm^{-2} (Westinghouse 15-W blue lamp) and were examined microscopically for germination. Data were based on at least 100 oospores per replicate. Each treatment was replicated twice and experiments were repeated two or three times. Since oospores adhered to the bottom of the petri dishes, it was possible to recheck the same oospores by marking the bottom of the dish in timed experiments. Oospores were classified as unchanged, aborted (with inner structures disorganized), or germinated, ie, with one or several germ tubes from which sporangia sometimes developed.

RESULTS

The germination process. The germination of oospores of PMM proceeded as described by other authors (4,5,12). The protoplasm became granular in appearance while the thick inner oospore wall eroded, the two pellucid bodies and the ooplast disappeared, and the oospore swelled, filling the entire oogonium. This was followed by the emergence of one or several germ tubes on which sporangia sometimes formed (Figs. 1B and C, 2A and B). The germ tubes protruded from any site, not necessarily only where the antheridium had been attached (Fig. 2A and B). During the early germination phases many oospores disintegrated internally, collapsed, and failed to germinate. Some oospores burst. The inner oospore wall of these aborted oospores was still thick in some cases, and all degrees of degradation were observed (Fig. 1D–F).

Effect of alfalfa root extracts and exudates and a soil extract on germination. Oospore germination in DDW varied between 3 and 50% but generally varied from 10–20%. Incubation of oospores of three isolates in root extracts resulted consistently in higher and more rapid germination than in water (Table 1). The germination rate also significantly increased (Fig. 3). When root extracts from 7-, 25-, and 87-day-old plants from the greenhouse were compared, the percent germination increased from 13% in DDW to 50% on root extract from 7-day-old, 64% from 25-day-old, and 62% from 87-day-old plants. Root extract from the resistant germ plasm line A77-10B induced about the same percentage germination as Moapa 69. Natural root exudates from seedlings of both Moapa 69 and A77-10B increased germination from 20% in water to 58%. Soil

extract increased germination from 17% in water to 56%. Differences between the water control and the root extracts, root exudates, or soil extract were highly significant ($P=0.01$). Since the natural materials were not chemically quantified, no attempt was made to compare them statistically.

Almost all germinated oospores formed sporangia when incubated in root and soil extracts and in root exudates, but none were formed in DDW. Oospore abortion during the early germination stages was greatly reduced by incubation in root extracts (Table 1).

Effect of culture age. Oospore germination increased with increasing age (11–50 days) of the cultures from which the oospores were harvested (Fig. 4). In DDW, germination did not exceed 28%, but in root extract, germination increased to 86%. In root extracts, oospores from 11-day-old cultures germinated well, but the germination rate was slower.

Effect of metabolic inhibitors. Germination was completely or partially prevented by inhibitors of RNA and protein synthesis and respiration (Table 2) with the exception of oligomycin, which was inactive. Although data are not given here, tests of the effects of

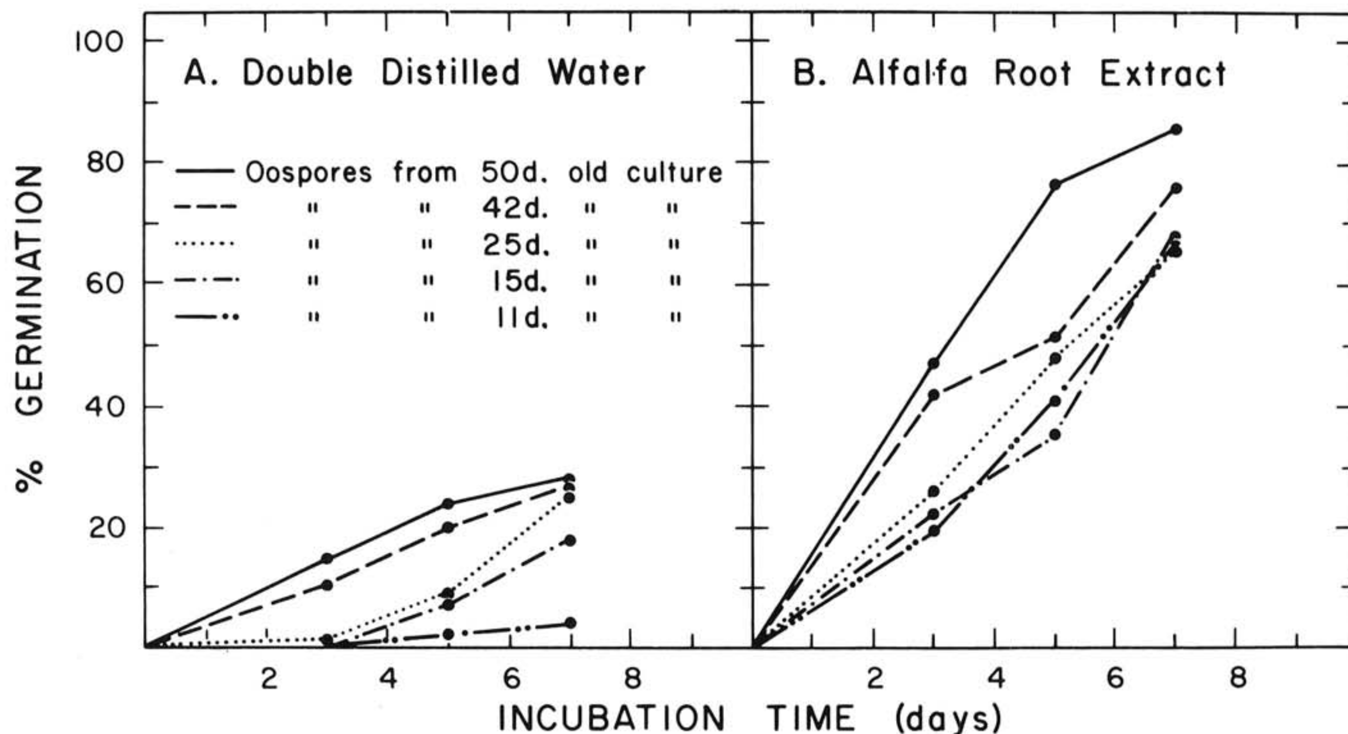

Fig. 4. Increase in rate and percentage of oospore germination of *Phytophthora megasperma* f. sp. *medicaginis* (P1057) of different culture ages in alfalfa root extract compared to that in distilled water.

TABLE 2. Germination of oospores of *Phytophthora megasperma* f. sp. *medicaginis* in aqueous solutions of several metabolic inhibitors

Experimental treatments	Concentration (μg/ml)	Oospore germination[a] (%)	Percentage of control	Statistical significance[c]
Exp. A. Respiration inhibitors:				
Control[b]	0	22	100	r
KCN	600	0	0	u
	200	19	86	s
Oligomycin	50	22	100	r
Antimycin A	50	0	0	u
	10	14	63	t
Exp. B. Protein synthesis inhibitors:				
Control[b]	0	37	100	v
Cycloheximide	10	0	0	w
	1	29	78	vw
Chloramphenicol	50	28	78	vw
	10	38	102	v
Exp. C. RNA synthesis inhibitors:				
Control[b]	0	28	100	x
5-fluorouracil	10	0	0	z
	1	0	0	z
6-azauracil	100	0	0	z
	10	1	4	yz
	1	23	82	yz
8-azaguanine	100	0	0	z
	10	16	57	xy
	1	50	178	x

[a] Based on a count of not less than 400 oospores for each treatment.
[b] Control was double distilled water.
[c] Means (average of three experiments) followed by different letters differ significantly ($P=0.01$) according to Duncan's multiple range test.

TABLE 3. Germination of oospores of *Phytophthora megasperma* f. sp. *medicaginis* in double distilled water and in aqueous solutions of several chemicals

Treatment	Concentration (μg/ml)	Oospore germination (%)[b]				Comments
		Control[a]	Chemical treatment	Percentage of control	Statistical significance[c]	
Glucose	10,000	18	0	0	+	Most oospores aborted
	45	32	4	1	+	
Sucrose	10,000	18	0	0	+	Most oospores aborted
Oleic acid	100	40	0	0	+	Most oospores aborted
	10	32	23	72	+	
Linoleic acid	100	40	0	0	+	Most oospores aborted
	10	32	40	125	0	
Palmitic acid	100	36	33	109	0	
	10	36	36	100	0	
Asparagine	10,000	18	10	56	+	
	1	32	44	138	+	
β-sitosterol	100	40	35	88	0	
	10	40	50	125	+	
Thiamine	1	40	28	70	+	
	0.1	40	34	85	+	
Ascorbic acid	1	40	20	50	+	
	0.1	40	33	82	+	
Pyridoxine	1	18	8	44	+	
	1	40	15	37	+	
Biotin	0.1	40	33	82	0	

[a]Control was double distilled water.
[b]Based on a count of not less than 400 oospores for each treatment.
[c]Differences between the control and chemical treatment significant level = +; no difference = 0. Since data were compiled from different experiments, treatments were not compared statistically.

TABLE 4. Effect of glucose, asparagine, and acetate on oospore germination of *Phytophthora megasperma* f. sp. *medicaginis* (P1057)

Treatment	Oospore germination (%)	Percentage of control[a]	Statistical significance[b]
Control, double-distilled water	31	100	vx
Glucose (2.5×10^{-4}M)	3	13	z
Asparagine (7.5×10^{-6}M)	35	113	v
Acetate (10^{-6}M)	28	110	x
Glucose (2.5×10^{-4}M) plus asparagine (7.5×10^{-6}M)	22	70	y
Glucose (2.5×10^{-4}M) plus acetate (10^{-6}M)	31	107	vx

[a]Based on a count of not less than 400 oospores for each treatment.
[b]Means (average of two experiments) followed by different letters differed statistically ($P = 0.01$) according to Duncan's multiple range test.

inhibitors were the same in root extract as in DDW.

Effect of various chemicals. The source of distilled water used to incubate oospores significantly affected oospore germination; however, the reason was not determined. Glucose completely inhibited oospore germination, but this inhibition was completely or partially overcome by the addition of acetate or asparagine (Tables 3 and 4). Diluted media (1:100) such as cleared V-8 juice broth, carrot broth, and synthetic media (11,20) increased germination approximately four times over that obtained in the DDW control.

Since calcium salts seem to play an important role in the developmental stage of PMM (9,11), the effect of calcium on oospore germination was investigated (Table 5). $CaCl \cdot 2H_2O$ at 1 or 10 μg/ml had no effect on germination, but was inhibitory at 50 μg/ml. EGTA, a calcium chelator (14,15), inhibited germination in distilled water at 50 and 100 μg/ml, but in root exudate had no effect except at 100 μg/ml. $CaCl_2 \cdot 2H_2O$ did not negate the EGTA effect, nor did $MgSO_4$ or KNO_3 in other experiments.

DISCUSSION

The primary events responsible for initiation of germination in oospores are still unknown. Blackwell (6) stated that germination of oospores of *P. cactorum* could not take place until the spore had matured and the wall was rendered permeable to oxygen and water. One of the first visible signs in the germination process of oospores, as observed by light microscopy, was the digestion of the thick inner oospore wall. Beakes (5), working with oospore germination of *Saprolegnia ferax*, believed that one of the keys to the breaking of dormancy may be the mobilization of wall glucans; environmental conditions such as light and temperature might be the triggers for induction of glucanase production. However, recent reports (17,25) indicate that light is not necessary for germination of *P. megasperma* f. sp. *glycinea* in soil or root extracts.

Oospore germination of PMM was strongly affected by certain external nutritional factors in soil and root extracts. Germination in DDW was variable and usually low, but it was markedly increased by root extracts and root exudate from susceptible and resistant alfalfa plants and by soil extract (Figs. 3 and 4, Table 1). Jimenez and Lockwood (17) and Sneh et al (25) also reported that oospores of *P. megasperma* f. sp. *glycinea* germinated better in natural or sterilized soil than in deionized water, and that soybean seedlings and other plant tissues stimulated rapid and high germination of oospores incubated in soil extract either in light or darkness. In our work, autoclaved root extracts (data not presented here) had the same stimulatory effect as filter-sterilized extracts indicating that the stimulating factor(s) may be heat stable. Storage of oospores in a nonsterile soil extract for 3 mo also increased germination significantly (H. Förster, *unpublished*). Similar results were reported with *P. cactorum* oospores by Banihashemi and Mitchell (2).

Sporangia formed on germinated oospores only when oospores were incubated in root and soil extracts, diluted cleared V-8 juice broth, or carrot broth, but not in DDW or in diluted synthetic media. Sporangia either differentiated into zoospores or germinated by forming new sporangia by internal proliferation. Apparently, oospores contain enough endogenous reserves to form one or several germ tubes, but exogenous nutrients and/or stimulants are necessary for sporangia formation. Sporangia also developed when oospores germinated on water agar. Since impurities in the agar may stimulate sporangia formation, this may have been the cause for formation of sporangia from oospores that germinated on water agar in a previous study (8). Thus, for use in physiological studies, oospores should be completely cleansed of mycelial fragments, as we have done, since nutrients that leak from mycelial fragments could stimulate sporangium formation.

TABLE 5. Oospore germination of *Phytophthora megasperma* f. sp. *medicaginis* (P1057) as affected by calcium ($CaCl_2 \cdot 2H_2O$) and EGTA, a chelator of calcium

Experimental treatment	Concentration (μg/ml)	Oospore germination (%)[a]	Percentage of control	Statistical significance[b]
Experiment A				
Control[c]	0	36	100	y
$CaCl_2 \cdot 2H_2O$	1	41	113	y
	1 plus EGTA[d] (10 μg/ml)	0	0	z
	10	32	88	y
	10 plus EGTA (10 μg/ml)	0	0	z
	50	16	44	z
	50 plus EGTA (10 μg/ml)	0	0	z
EGTA	1	35	97	y
	50	0	0	z
	100	0	0	z
Experiment B				
Control	0	42	100	w
Root extract	0	45	107	w
	0 plus EGTA (1 μg/ml)	48	114	w
	0 plus EGTA (10 μg/ml)	42	100	w
	0 plus EGTA (100 μg/ml)	15	35	x

[a] Based on a count of not less than 400 oospores for each treatment.
[b] Means followed by different letters differed significantly ($P = 0.01$) according to Duncan's multiple range test.
[c] Control was double distilled water.
[d] EGTA = ethylenglycol-*bis*-(β-aminoethylether)-*N,N'*-tetraacetic acid.

Externally supplied factors in root extracts reduced oospore abortion (Table 1) as well as enhanced the germination and stimulation of sporangia formation. Abortion of *P. fragariae* oospores at high temperatures was also observed by Duncan (7). He attributed this phenomenon to physical or chemical damage during the processing cycles or to localized impurities in the agar. The effect of exogenous factors on the occurrence of oospore abortion in other *Phytophthora* spp. has not previously been reported.

The process of oospore germination of PMM could occur in at least two steps. The first step is partly independent of external nutrients and therefore germination can take place in DDW although the incidence of germination is stimulated by exogenous factors. The initiation of germination probably begins with the mobilization of the glucans in the thick inner oospore wall. During the enzymatic degradation of the inner oospore wall, a physiological process for some oospores might depend on external nutrients. In the second stage of germination the degradation of the inner oospore wall appears to have been completed; synthesis and mobilization of various compounds take place, and finally a germ tube emerges.

The absence of nutritional factors could cause many oospores to abort. Aborted oospores with all stages of wall degradation were often observed in our experiments (Table 1). However, abortion of oospores does not seem solely to be due to lack of external factors because several nontoxic compounds like sugars (glucose, sucrose, 0.5%), oleic and linoleic acid (100 μg/ml), chlortetracycline at 10 μg/ml (13), and polymyxin B (10 μg/ml) had the same effect. The compounds cycloheximide, 5-fluorouracil, and 6-azauracil (Table 2), and a N_2-atmosphere (H. Förster, *unpublished*) inhibited oospore germination, but the oospores remained unchanged and did not abort. Results with these metabolic inhibitors indicate that RNA, protein synthesis, and respiration are necessary for initiation of germination.

Protein synthesis seems to be essential for germ tube formation in all fungal spores (26). However, chloramphenicol, which inhibits protein synthesis in mitochondria, did not inhibit germination. Some fungi have an absolute requirement for RNA synthesis to form germ tubes while others do not (16,26). PMM oospores appear to belong to the former group and therefore either lack m-RNA, contain ribosomes deficient in r-RNA, or lack certain t-RNAs. Actinomycin D did not inhibit oospore germination. This might be due to the sensitivity of this compound to the light used for germination of oospores.

According to Smith et al (24) the physical act of germ tube emergence in fungal spores is not yet fully understood. These authors suggest that the germ tube passes through the spore wall by way of enzymatic digestion rather than by mechanical rupture. However, observation of germinating oospores of PMM suggest that germ tube emergence is, at least partially, mechanical since the outer oospore wall and the oogonium wall were sometimes greatly extended by the germ tube before it broke through. Electron micrographs of PMM by Hemmes (14) showed ruptured walls after the germ tube had emerged. Beakes (5) suggests a combination of physical force and enzymatic action in germ tube emergence of *Saprolegnia ferax*.

The inhibition of germination of oospores by glucose, sucrose, oleic, and linoleic acid (Table 3) is not understood. The inhibition of sporulation and germination processes by glucose and sucrose was reviewed by Ribeiro (19). D-Glucose or sucrose (0.5%) inhibited germination of oospores of *P. cactorum* (2) and *P. heveae* (20). Jimenez and Lockwood (17) and Sneh et al (25) reported that glucose inhibited oospore germination of *P. megasperma* f. sp. *glycinea*. Oospores of *Phytophthora* spp. behave differently from spores of most other fungi because usually glucose strongly enhances germination. The inhibitory effect of glucose might be based on a general catabolic repression because the effect was reversed by the addition of asparagine or acetate (Table 4). Yoshikawa and Masago (27) reported that formation of sporangia by *P. capsici* was inhibited by glucose but could be overcome by cyclic AMP. Fatty acids can probably also be regarded as general catabolic repressors, although specific processes such as inhibition of succinoxidase and uncoupling of oxidation from phosphorylation in respiration by oleate (23) are affected by these compounds.

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