Enzymatically Induced Germination of Oospores of Phytophthora megasperma

M. A. Salvatore, F. A. Gray, and R. B. Hine

Research Assistants and Professor, respectively, Department of Plant Pathology, University of Arizona, Tucson 85721.

The authors gratefully acknowledge the assistance of Albert R. Mead, Department of Biological Sciences, University of Arizona, Tucson 85721.

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ABSTRACT

Germination of oospores of *Phytophthora megasperma* consistently increased from 5% to 93% after ingestion by the land snail, *Helix aspersa*. Similar results were obtained using the snail enzyme complex, beta-glucuronidase/aryl sulfatase, and the snail enzyme, beta-glucuronidase. Maximum germination occurred when cultures 35 days old or older were used.

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Previous studies have shown aquatic and terrestrial snails to be useful tools for obtaining mycelium-free oospores, and increasing oospore germination of some fungi. Gregg (2) reported germination of oospores of *Phytophthora erythroseptica* and *P. cactorum* in the excreta of the land snail, *Helix aspersa*. Shaw (3) used the water snail, *Planorbarius corneus*, to induce germination of oospores of *P. cactorum*. Bhalla & Mitchell (1) used another aquatic snail (*Helisoma* sp.) to obtain mycelium-free oospores of *Aphanomyces euteiches*. Most recently, Stanghellini & Russell (4) used aquatic snails (*Planorbis* sp.) and the commercially available enzymes of the land snail, *H. pomatia*, to increase the germination of oospores of *Pythium aphanidermatum*.

This note describes techniques using the land snail, *Helix aspersa* Müller, which consistently induce high percentage germination of the oospores of *Phytophthora megasperma* (Drechs.), and reports the effect of a snail enzyme, beta-glucuronidase, on stimulation of oospore germination.

Initial studies were designed to determine the effect of ingestion by land snails and oospore age on germination. Tests were made with four pathogenic isolates of *P. megasperma* recovered from rotted taproots of alfalfa. *P. megasperma* was grown at 24 C, under two 50W incandescent bulbs, on V-8 agar (100 ml V-8 juice, 2 g $CaCO_3$, 17 g agar/liter). Snails which had been previously starved for 48 hr were allowed to graze on cultures varying in age from 7 to 56 days. Feces collected 6 hr after the cultures were fed to the snails contained large numbers of oospores and lysed mycelial fragments. The feces were placed in plastic petri dishes (60 × 15 mm) containing 10 ml of sterile distilled water (SDW). After 12 hr of incubation at 24 C, the oospores which were embedded in the mucilaginous agar-like feces germinated. Germination of 93% of the observed oospores occurred in the snail feces after 72 hr in cultures 35 days of age or older; younger oospores germinated at a lower percentage (Table 1). Approximately 50% of the germinating oospores produced a short germ tube with a terminal sporangium. Similar results were obtained using the land snail, *Rumina decollata* (Linn.), and the water snail, *Helisoma tenue* Say. Oospores which were mechanically separated from either V-8 agar or infected alfalfa seedlings germinated less than 5% when suspended in SDW and in a number of nutritional substrates, including glucose (100 ppm), asparagine (100 ppm), yeast extract (100 ppm), Hoagland's solution (10%), and various water extracts from germinating alfalfa seedlings.

Further studies were made using the enzyme complex, beta-glucuronidase/aryl sulfatase, obtained from *H. pomatia* (Calbiochem, La Jolla, Calif.). Forty-day-old cultures of *P. megasperma* grown on V-8 agar were blended in 500 ml SDW, passed through a 45-μ mesh sieve and washed four times by centrifugation in SDW to remove most hyphal fragments.

Washed oospores were suspended in SDW and varying concns of the enzyme complex for 24 hr, then washed four times by centrifugation in SDW. Oospores were incubated at 24 C in plastic centrifuge tubes containing 1 ml SDW. Observations of germination were made at 24 hr intervals for 192 hr. Maximum germination of 60%

TABLE 1. Germination of *Phytophthora megasperma* oospores of various ages in excreta of the land snail *Helix aspersa*

Oospore age (days)	Germination[a] (%)
7	2.6
14	8.4
21	48.6
28	75.6
35	92.0
42	93.2
49	93.2
56	93.4

[a] Three hundred oospores were scored in each of three microscopic observations of snail excreta; the mean percentage is shown for counts made 72 hr after ingestion by snails.

TABLE 2. Germination of *Phytophthora megasperma* oospores as affected by a 24-hr exposure to the snail enzyme, beta-glucuronidase

	Germination (%)[b]				
	Enzyme concn (units/ml)				
Time (hr)[a]	5000	2000	1000	500	0 (ck)
24	<1	<1	<1	<1	<1
48	3.3	10.2	4.0	3.0	2.3
72	20.3	29.2	5.0	4.2	3.5
96	33.6	41.9	5.3	4.6	3.8
120	34.3	48.6	6.1	4.6	4.2
144	40.3	52.3	6.3	4.7	4.6

[a] Number of hours after initial exposure to the enzyme; oospores were washed four times in sterile distilled water after a 24-hr exposure to the varying concentrations.

[b] Three hundred oospores were scored in each of three microscopic observations; the mean percentage is shown.

occurred after 144 hr at a concn of 1% (v/v) and a pH of 6.2. Enzyme concns higher than 2% inhibited oospore germination at the same pH. Mycelial fragments remaining in the preparation after the initial washing were lysed after a 24 hr exposure to enzyme concns higher than 0.5%.

Similar results were obtained using the snail enzyme, beta-glucuronidase (Calbiochem). Maximum germination of 52.3% occurred after 144 hr at a concn of 2000 units/ml and pH of 6.0 (Table 2). In both cases, oospores mechanically separated from V-8 agar and suspended in SDW, but not exposed to either the enzyme complex or the specific enzyme, germinated less than 5%.

This technique can be a useful tool in studies of oospores of *Phytophthora* spp. where low germination has hampered observations. Studies of the interaction of enzymes and nutrition on oospore germination are in progress.

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