A Method of Obtaining Viable, Mycelium-Free Ooospores of Aphanomyces euteiches Using Live Water Snails

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ABSTRACT

The use of water snails to prepare oospore suspensions free of mycelia from cultures of *A. euteiches* is described. The oospores are infectious and appeared to initiate infections over a period of several days. A difference among strains of the fungus in oospore production is noted. Phytopathology 60:1010-1012.

Oospores appear to be a major factor in the survival of *Aphanomyces euteiches* Drechs. between pea crops (7). Germination of oospores occurs under varying conditions. Jones & Drechsler (2) observed that a considerable proportion of oospores produced in cornmeal agar germinated without passing through a period of dormancy. Scharen (5) and Olofsson (4) reported a maximum germination of 40% of the oospores of *A. euteiches* in pea root debris from soil. Knowledge of the factors affecting the survival and inoculum potential of this pathogen has been limited in part by lack of a method of obtaining inoculum consisting entirely of oospores free of mycelium. Yang (8) isolated oospores of *A. euteiches* from mycelial mats by coupling sonification and isopycnic centrifugation. Gregg (1) obtained oospores of *Phytophthora erythroseptica* and *P. cactorum* in the excreta of garden snail (*Helix aspera*) fed on the young cultures of these fungi grown on oat agar. Shaw (6) used the water snail (*Planorbarius corneus*) to obtain oospores of *P. cactorum*. This note describes a method now being used in this laboratory to obtain viable oospores of *A. euteiches*.

Mycelial mats of five different isolates of *A. euteiches* (A100, PR-4, Q, GMX, and GMX-2) were grown in 50 ml of corn-extract broth (45 g sweetcorn seed/liter of distilled water) in 4-oz screw-capped bottles. Discs (5 mm) were transferred to the bottles from the periphery of 2-day-old cultures of *A. euteiches* isolates on potato-dextrose agar. These bottles were incubated for 4 weeks at room temp in a slanting position to allow maximum spread of mycelial mats in the broth and development of oospores. Mycelial mats were then placed into petri dishes and washed three times with 10-ml aliquots of distilled water. At the end of the final wash the mycelia were suspended in the dishes in approximately 10 ml of water. Snails (*Helisoma* sp.) that had been washed and then starved overnight were placed in the dishes containing mycelial mats. The mycelia were nearly completely consumed in 2 to 3 days, during which period snails produced the feces consisting almost entirely of oospores. The snail feces were collected with tweezers and the oospores dispersed in distilled water with the aid of a glass tissue grinder or a sonifier.

The average yield of feces per mat of the isolate GMX was 82% on a dry wt basis after 3 days' snail feeding. The oospores in the feces appeared to be embedded in a mucilaginous matrix. When the feces were homogenized in a glass tissue grinder partial dispersion occurred with many clumps remaining. When exposed to sonification for less than 1 min nearly complete dispersion occurred. There was no visual evidence that any mycelium survived the digestive process in the snails and no evidence of growing mycelium when the oospores were tested for germination in dilute agar.

The oospore preparations of the different isolates varied in appearance. Relatively few mature oospores were present in the initial culture of isolate A100, and this was even more evident in the snail feces where many emptied oogonia were found (Fig. 1-A). It appears that the oogonia are resistant to digestive action of the snail whether the oospore itself is intact and mature or absent. The oospores from isolates PR-4 and GMX-2 (Fig. 1, C, D, E, F) were similar in appearance; most appeared to be mature and there were relatively few empty oogonia present.

Viability of oospores was determined by the germination test and by infectivity assay. Oospores were placed on water agar discs in contact with the root of 5-day-old pea seedlings, and germination occurred by means of a germ-sporangium or by germ tubes in 5 days. The number of germinating oospores was low in all cases. Infectivity of oospores of isolate A100 in snail excreta

TABLE 1. Infectivity of oospores of *Aphanomyces euteiches* (isolate A100) present in snail excreta after varying periods of incubation

Inoculation period	Postinoculation incubation period	% Pea seedlings infected
days	*days*	%
1	12	0
3	10	30
4	9	30
6	7	30
7	6	40
8	5	60
9	4	60
10	3	50
12	1	70

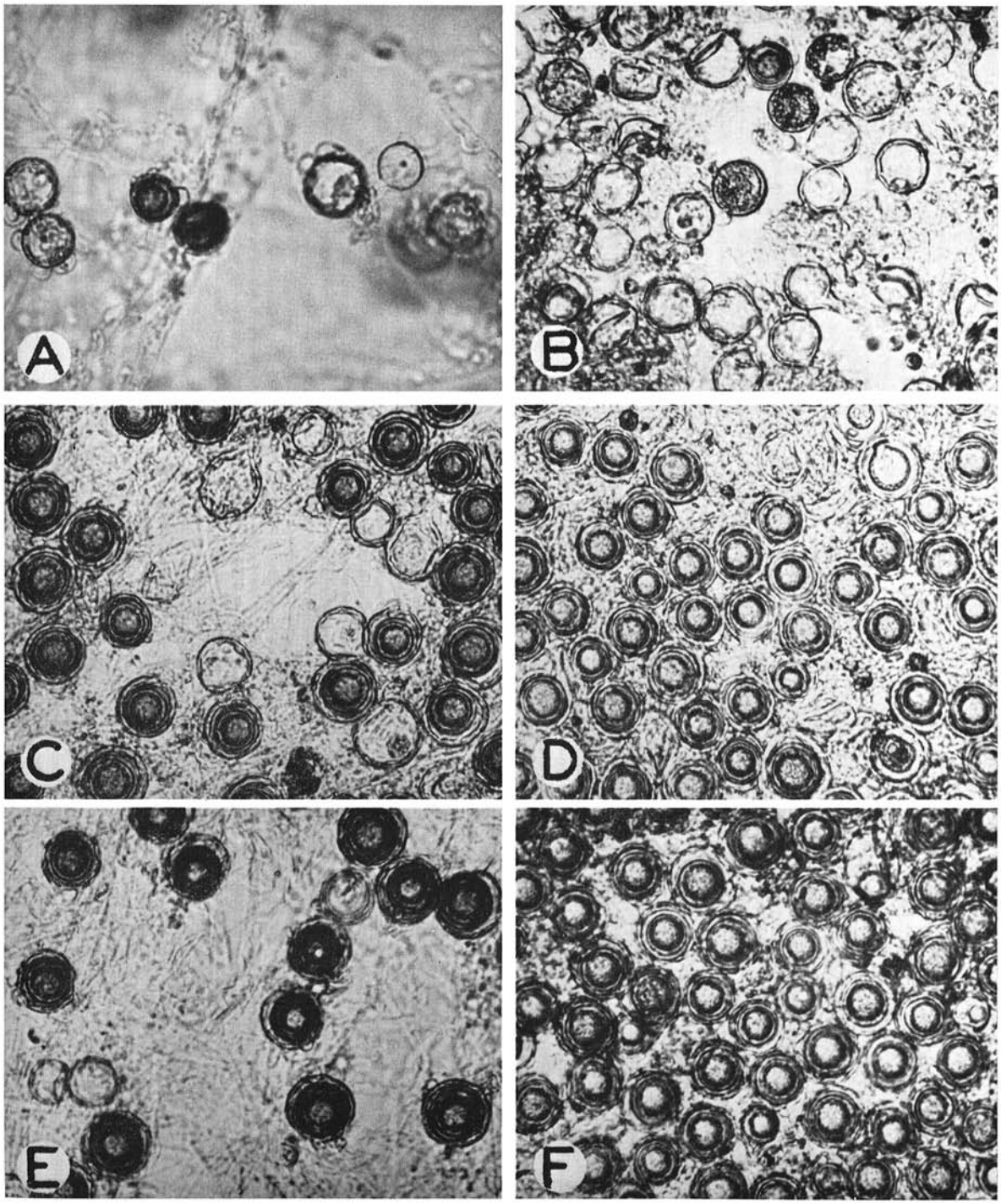

Fig. 1. Oospores from cultures of isolates A100, PR-4, and GMX-2 of *Aphanomyces euteiches* before (A, C, E) and after (B, D, F) ingestion by snails (*Helisoma* sp.).

was tested on 5-day-old pea seedlings grown in vermiculite by the paper towel technique of Mitchell et al. (3). Five pea seedlings on each of 18 paper towels were inoculated with pieces of snail excreta. At intervals during the subsequent 12 days, the roots of seedlings on two towels were washed to remove the inoculum and placed in fresh towels. After 12 days, the number of infected seedlings was determined and the presence of *A. euteiches* in roots was confirmed by the presence of its characteristic oospores. It is apparent from the data (Table 1) that the inoculum was viable and infective, and that the number of infected seedlings increased

with increased period of exposure of pea roots to oospores. The results indicate that the time required for germination of oospores ranged upward from 3 days, and that it was still occurring at the end of the experiment. Studies of factors affecting oospore germination and inoculum potential of *A. euteiches* in soil are in progress.

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