

Pythium aphanidermatum Oospore Germination as Affected by Time, Temperature, and pH

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ABSTRACT

Germination of *Pythium aphanidermatum* oospores, obtained from a synthetic liquid medium, was studied in autoclaved soil. Oospores began to germinate within 2 hr. After a 10- to 12-hr incubation period, hyphal growth obscured the oospores, making observation of germination difficult. The optimum temperature and pH for germination was 30 C and 7.5, respectively. *Phytopathology* 61:1149-1150.

Oospores of most soil-borne phycomycetes are considered to be one of the resistant structures which allow the organism to survive adverse conditions in soil. This is particularly true for many soil-borne phytopathogenic *Pythium* spp. Despite the apparent importance of these structures, little is known about the factors affecting their germination in soil. This paper reports the effect of temperature, pH, and age of the culture on the germination of oospores of *P. aphanidermatum* (Edson) Fitzp. in soil.

The isolate of *P. aphanidermatum* was obtained from a greenhouse soil by R. D. Lumsden, and was

highly virulent to snapbeans. Oospores were obtained from cultures grown for about 21 days in 40 ml of a synthetic liquid medium at 30 C. The medium, developed by A. F. Schmitthenner (*personal communication*), was composed of the following: sucrose, 2.5 g; asparagine, 270 mg; KH_2PO_4 , 150 mg; K_2HPO_4 , 150 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 100 mg; CaCl_2 , 55 mg; thiamine hydrochloride, 2 mg; cholesterol, 10 mg; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 4.4 mg; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.07 mg; and distilled water to make a final volume of 1,000 ml. Aqueous suspensions of oospores were prepared by mincing mycelial mats in a blender for 30 sec. The resulting suspension was filtered through four layers of cheesecloth and twice through lens paper to produce a suspension of oospores reasonably free of hyphal fragments.

The buried filter method (1) was used to study germination in soil. Approximately 20,000 oospores were placed on each of two filters. Three counts of 100 spores each were made on each filter, and the average per cent germination of the six counts was determined.

The soil used in all experiments was a sandy loam with a water-holding capacity (WHC) of 25% and a pH of 5.8. Hydrated lime was used to adjust the pH of the soil. The moisture level of the soil in all experiments was ca. 50% (WHC). Prior to each experiment, the soil was autoclaved for 30 min at 121 C.

After many preliminary experiments in which the percentage of oospore germination was very low (5-20%), it became evident that the following two conditions must be met in order to obtain substantial germination. First, the final count of germinated oospores must be taken at the end of a 10- to 12-hr incubation period. When the incubation periods were longer than

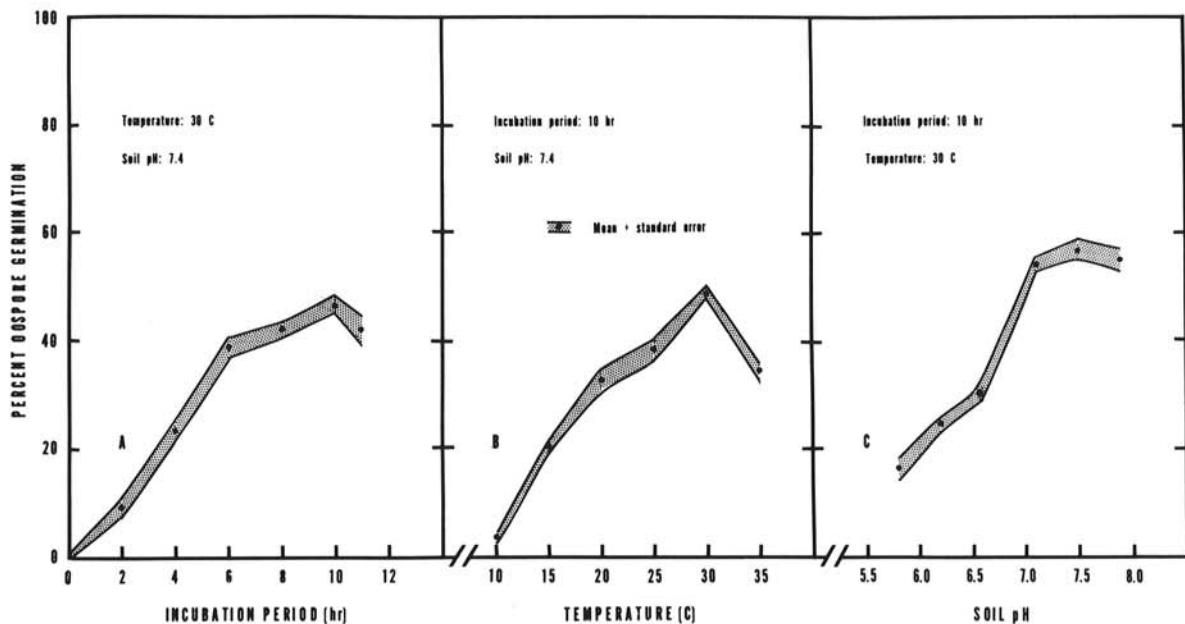


Fig. 1. Germination of oospores of *Pythium aphanidermatum* in autoclaved soil as affected by A) time; B) temperature; and C) soil pH.

10-12 hr, hyphal growth obscured the oospores. Second, substantial germination in soil was obtained only when the pH of the soil was at or above 7.0. When these two conditions were met, substantial germination of oospores in autoclaved soil was obtained.

An experiment was performed to determine the effect of the age of the culture from which the oospores were obtained on subsequent germination. Oospores from 14-, 20-, and 27-day-old cultures resulted, respectively, in 24, 49, and 45% germination on 2% water agar (pH 7.2); 31, 58, and 53% in autoclaved soil (pH 7.8); and 55, 71, and 56% on cornmeal agar (pH 6.0). As a result of this experiment, it was decided to use 3-week-old cultures in subsequent experiments.

The data in Fig. 1 indicate that the optimum conditions for oospore germination in autoclaved soil are a temperature of 30 C and a soil pH of 7.5 when the oospores are incubated for 10 hr. The percentage of germination of oospores declined with incubation periods greater than 10 hr (Fig. 1-A). This apparent decline in the percentage of germination was observed to be due to lysis of the germ tubes. Fragmented germ tubes were considered lysed.

Pythium aphanidermatum is known as a "high temperature *Pythium*" (3), and thus, an optimum temperature of 30 C (Fig. 1-B) for oospore germination was expected. Likewise, the rapid rate of oospore germina-

tion (Fig. 1-A) was not surprising, in view of the rapid growth rate (3). However, the optimum pH for oospore germination (Fig. 1-C) seemed unusually high, considering the extensive damage which this pathogen causes on numerous crops in soils with a broad range of pH values. Chang-Ho (2) reported that encysted zoospores of *P. aphanidermatum* germinated equally well and to a high degree (65-90%) in buffered solutions at pH levels ranging from 3.5 to 7.5. In contrast, my data (Fig. 1-C) indicate that the pH range for optimum oospore germination in autoclaved soil was ca. 7.0 to 8.0.

The effect of soil pH on oospore germination will have to be confirmed in studies in which natural soil is used. However, before such a study can be made, the nutrients required to stimulate oospore germination in natural fungistatic soil will have to be determined.

LITERATURE CITED

1. ADAMS, P. B. 1967. A buried membrane filter method for studying behavior of soil fungi. *Phytopathology* 57:602-603.
2. CHANG-HO, Y. 1970. The effect of pea root exudate on the germination of *Pythium aphanidermatum* zoospores cysts. *Can. J. Bot.* 48:1501-1504.
3. MIDDLETON, J. T. 1943. The taxonomy, host range, and geographic distribution of the genus *Pythium*. *Mem. Torrey Bot. Club* 20:1-171.