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Inhibition of Germination of Oospores of Peronospora Manshurica

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Inhibition of Germination of Oospores of Peronospora Manshurica[1]

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Abstract. Oospores of *Peronospora manshurica* have not previously been observed to germinate. In the present tests, however, oospores on seed coats of soybeans washed in running tap water for 1 week germinated. Each germinating oospore produced a germ tube, which developed into typical branched mycelium. Oospores were placed in the center of water-agar plates, some of which were immediately sprayed with a dilute suspension of conidia of *P. manshurica*. Other plates were sprayed later. Germination of conidia was inhibited in an area around the oospores. The size of the area of inhibition was proportional to the elapsed time between placing oospores on the agar and spraying the agar with conidia. A germination inhibitor has been described for the conidia of *P. manshurica* and the same inhibitor may be active in preventing oospore germination.

Oospores of members of the Peronosporales germinate with difficulty and some, such as *Peronospora manshurica* (Naom.) Syd. ex Gaum. which causes downy mildew of soybeans, have not previously been observed to germinate (Grabe, 1957). Several workers have reported oospore germination in other Peronospora species. Person and Lucas (1953) observed that the oospores of *P. tabacinia* Adams germinated by forming one to three sessile sporangia from which more than 100 zoospores emerged. Wolf and Lehman (1924) reported that oospores of the same fungus germinated by forming a germ tube. Other genera of the Peronosporales in which oospores have been observed to germinate are Plasmopara (Gregory, 1912; Arens, 1929) and Sclerospora (Hiura, 1935), both of which germinate by formation of a germ tube.

Studies of the factors affecting germination have ranged from alternating temperatures (Lucas and Person, 1954) to age (Tasugi, 1933) and aeration (Gregory, 1912; Evans and Harrar, 1930; Tasugi, 1933). The present study was initiated to determine the type of germination of oospores of *P. manshurica* and, if possible, to find the stimulus for germination.

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Materials and Methods

The soybean variety Blackhawk was used throughout the study. Physiologic race 8 of *P. manshurica* was used as a source of oospores. Oospores were obtained from seed coats of mature seed from systematically infected soybean plants. They were washed by placing either the spores alone or the spores and seed coats in a collodion tube, sealing the ends and placing the tube in a beaker under slowly running tap water.

Medium used for all germination and inhibition studies was 1.5 percent water agar. Germination of conidia in the inhibition studies was determined along circular paths described by radii of 5 and 30 mm from the center of the agar plate. Percentage germination of conidia was taken as the mean of germination counts of 100 spores at each of four locations on each of four plates.

Results

Volunteer soybeans in fields that had contained downy mildew-infected plants the previous year were observed to have as high as 40 percent systemic mildew infection. Seedlings inoculated with oospores usually showed only one to five percent infection in the greenhouse. Age or aeration of oospores appeared to be less important than temperature and excessive moisture.

Oospore-encrusted soybeans were placed at –20° C and removed to room temperature on alternate days for 4 weeks. The oospores were then scraped from the seed, distributed on the surface of agar plates and examined for germination each day for 2 weeks. No germination was observed.

Oospores scraped from seed were placed in collodion sacks and washed under running tap water. Because of the difficulty in collecting the oospores in the flooded sacks for examination, oospores were left on the seed oats in later germination tests. Oospores were examined daily, and the first signs of germination

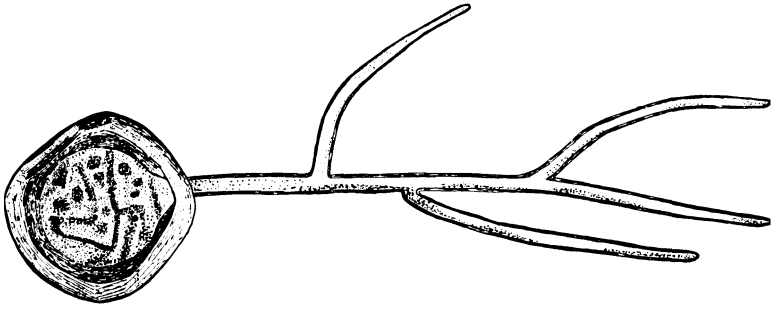

Figure 1. Drawing of a germinating oospore of *Peronospora manshurica*

occurred after 1 week. Germinating oospores produced a simple germ tube which developed into typical branched mycelium (Fig. 1). Germination progressed very slowly over the 4-week period that oospores were observed. Only a few additional oospores were observed to germinate each day and no germination was recorded on five of the 21 days when observations were made after the first germination was recorded.

Pederson (1961) reported a germination inhibitor in the conidia of *P. manshurica.* Results of the washing experiment suggested that the same inhibitor might be present in oospores and was removed or diluted by washing.

Oospores were placed in the center of 24 agar plates and four plates were sprayed immediately with a dilute suspension of *P. manshurica.* Additional sets of four plates were sprayed at intervals of 30 minutes up to 6 hours after the oospores had been put in place. Spores were incubated at 10° C for 18 hours and percentage germination of conidia was determined 5 and 30 mm from the oospores in each plate (Fig. 2). Conidia were

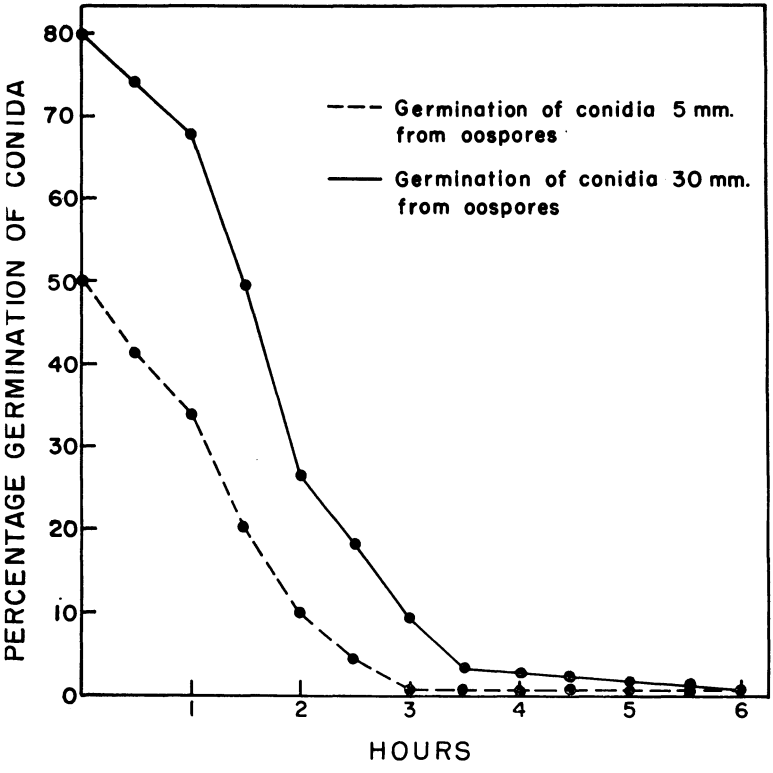

Figure 2. Germination of conidia of *Peronospora manshurica* sprayed on agar plates at 30-minute intervals after oospores of the same fungus had been placed in the centers of the plates

inhibited from germinating in an area around the oospores. The size of the area of inhibited conidia was proportional to the time elapsed between placing oospores in the center of the plates and spraying in the plates with conidia. The greatest difference in percentage germination of conidia 5 and 30 mm from the oospores occurred in the plates sprayed immediately after the oospores had been placed on the plates. This difference became progressively smaller with time. After 6 hours there was no difference in germination of conidia at the two locations.

Discussion

It is apparent that a substance diffused from the oospores into the surrounding agar during the 6-hour period that conidia were sprayed on the plates. Conidia inhibited from germinating had the same granular appearance as that described by Pederson (1961) for conidia in the presence of conidium-produced inhibitor. Additional tests will be required before it can be established that the conidium-produced inhibitor is the same as the oospore-produced inhibitor, but present evidence indicates that they are the same.

Results of the study indicate that planting soybeans in a field that contained downy mildew-infected plants the previous season is much more likely to result in a mildew outbreak than planting oospore-encrusted seed in a noncontaminated field. Drainage of rain and melted snow through soil containing oospores would reduce the concentration of the inhibitor during the fall and spring, and thus give a higher number of germinating oospores than might be expected on oospore-encrusted seed sown in the spring.

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