

On the Influence of Temperature to
the Germination of Zoosporangia of
Phytophthora capsici LEONIAN*

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1. Introduction

Germination of the zoosporangia of the genus *Phytophthora* occurs by the development of germ-tubes or zoospores which are differentiated within the zoosporangium. Both methods of germination will not occur at the same time within the same zoosporangium. Experiments on the influence of temperatures to the sporangial germination were first carried out by MELHUS (5,6) on *Phytophthora infestans* (MONT.) deBARY. His results showed that germination by zoospores occurs comparatively at a low temperature and that with germ-tubes at a higher temperature. CROSIER (1,2) obtained nearly the same results as those of MELHUS. Other similar experiments were followed by other investigators using related fungi. Summarizing these experiments, the zoosporangium seems to have a tendency to germinate by the development of germ-tubes at high temperatures, and by zoospores at low temperatures. But as a matter of fact, these are the only references that are available to this day, and it was the desire of the writers to determine the meaning of such differentiation into the two types of zoosporangial germinations.

In this paper, the writers carried out experiments on how the temperature have effects upon the zoosporangial germination of the fungus, *phytophthora capsici* LEONIAN, which causes the rot of pumpkin fruit reported by the author KATSURA (3) recently. Our laboratory having been poorly equipped for securing a series of temperatures in the incubators, the experiments were forced to be performed in the winter. As the genus *Phytophthora* seems to be especially sensitive to environmental factors, there were some doubts as to the use of zoosporangia produced artificially on the pumpkin fruit for determining the influence of temperatures upon the germination of zoosporangia. But

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the results obtained, nevertheless, were of some interest so it was decided to be reported herein as added information upon this fungus.

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2. Methods of experiments

The fungus *Phytophthora capsici* was obtained in its pure state from a pumpkin fruit, having been isolated by the writer on August 10, 1948. Stock 1 of our laboratory was used for the entire experiments. All test zoosporangia used were formed on the fruits of pumpkin which were artificially inoculated with this fungus. The experiments extended during the period between early December of 1948 and the end of February 1949.

In preparing for inoculating, the surface of the pumpkin fruits were sterilized by soaking in 80% alcohol for 30 seconds and rinsing thoroughly with sterile water. The fruits were then inoculated artificially with the fungus previously cultured on the potato extract agar medium in Petri dishes, and placed in a damp desiccator kept at 25°C for 24 hour incubation. After confirming the positive infection, the cover of the desiccator was removed and transferred to an incubator. The incubator was kept dry at temperatures between 12°-18°C, or an average of 15°C. Under these conditions, it was very favorable for the formation of the zoosporangia on the lesion of the fruit by controlling the growth of aerial mycelium. Also, there was no germination of the sporangium on the lesion as confirmed by the microscopic observations. Of course, no secondary zoosporangia were formed. The sporangia began to appear on the lesion around the inoculated point as frosty white colony in about 8 days.

The germination experiments were carried out by using a hanging drop of zoosporangial suspension on a slide glass. The incubators were kept at respective temperatures of room temperature (8°-12°C), 16°, 20°, 22°, 24°, 26°, 28°, 30°, 32°, 34°, 36°, and 40°C. Petri dishes were placed beforehand in each incubator held at above temperatures and quickly enclosed a hanging drop slide glass in these Petri dishes. The rate of growth of germ-tubes or zoospores was observed after 2 hours of incubation. The experiment was repeated five times using three petri dishes in each test.

3. Results of the experiments

In these experiments germ-tubes issued chiefly from the papilla of zoosporangium, and often from the other parts of it. Zoospores issued without exception from the opening pore of the papilla leaving behind the empty zoosporangium. The experiments were repeated five times but due to the faulty adjustment of the incubator for the treatment at 36°C, only two readings were recorded for this group.

The gathered results are shown in table I and figure 1.

Table I. Effect of temperature upon the zoosporangial germination of *Phytophthora capsici* LEON.

Temperature in °C.	R.T.*	16	20	22	24	26	28	30	32	34	36	40
Sporangia used	3578	4173	4407	4321	4832	5194	4708	5258	5338	4150	1649	4317
Sporangia germinated	9	186	351	554	506	702	526	225	182	83	6	0
Germ-tubes												
Number	9	158	274	359	421	693	526	225	182	83	6	0
Percent	0.25	3.78	6.22	8.31	8.71	13.34	11.17	4.27	3.40	2.00	0.36	0
Zoospores												
Number	0	28	77	195	85	9	0	0	0	0	0	0
Percent	0	0.67	1.74	4.51	1.76	0.17	0	0	0	0	0	0

* Room temperature (11°-12°C).

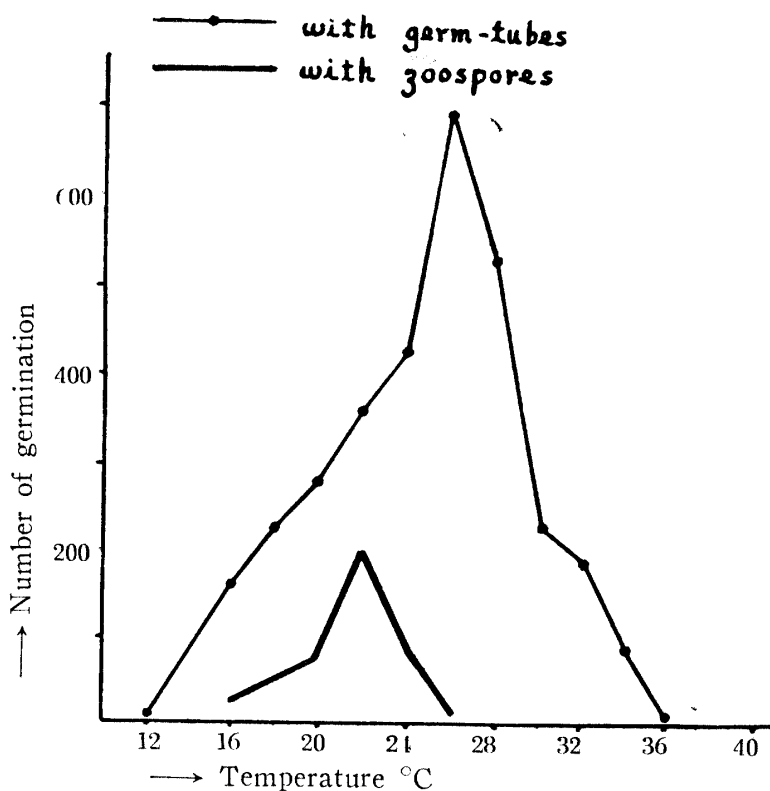


Fig. 1. Graph showing the effect of temperature upon the zoosporangial germination of *Phytophthora capsici* LEON.

According to table 1 and fig. 1, the zoosporangia of this fungus germinated considerably after two hours by either germ-tubes or zoospores, but the percentage of germination with the germ-tubes was greater than that with the zoospores. At or about the optimum temperature, the germ-tubes measured some 8μ , but at the minimum and maximum, they appeared only as a slight projection on the zoosporangium more or less difficult to observe. The respective temperatures of the room at each test were 8° , 10° , 12° , 10° , and 11°C . In these experiments zoosporangia germinated with germ-tubes was only 1 at 11° and 8 at 12°C , but none at 8° nor 10°C . From the above, the minimum temperature for germinating with germ-tubes is about 11°C , the maximum at about 36°C or a little higher, and the optimum of 26°C or a little higher. The germination with zoospores occurred between 16° and 26°C as shown in table 1 and fig. 1 but the minimum temperature should be considered a little lower than 16°C of between 16° and 12°C , and the optimum temperature of about 22°C .

4. Discussion

In these experiments, sporangia germinated more by germ-tubes than by zoospores. But LEONIAN (4) described *Phytophthora capsici* generally germinating with zoospores, only in rare cases with germ-tubes. TOMPKINS and TUCKER (7) reported that the germination of the same fungus normally occurs with zoospores or germ-tubes but they did not state the proportion of the two methods of germination.

The results of the writer indicated germination with zoospores at temperatures between 16° and 26°C , which approached those given by earlier investigators. The germinations with germ-tubes, on the other hand occurred at temperatures between 11° and 36°C , which is contrary to the general accepted idea that it takes place at a comparatively high temperature in genus *Phytophthora*. The optimum temperature was, however, higher for the zoospores as shown in table 1 and fig. 1. The maximum and minimum temperatures for the germination with germ-tubes was same as that for the growth of the fungus in culture media as KATSURA (3) reported. The optimum temperature for germ-tubes was 26°C , in comparison to 30°C for the mycelial growth in culture media. It is of interest that the sporangial germination with zoospores, unlike with germ-tubes and the growth of the mycelium in culture media, was quite peculiar at low temperatures. Of course, throughout the experiments, the sporangial germination was either with zoospores or with germ-tubes, and there was no case where both methods took place within the same zoosporangium at the same time.

Summary

1. This paper deals with the experimental results on the influence of temperature on the germination of zoosporangia, formed on the fruits of pumpkin artificially inoculated with the fungus *Phytophthora capsici* LEONIAN.

2. The experiments were carried out by using a hanging drop of zoosporangial suspension on a slide glass enclosed in Petri dishes respectively kept at room temperature (11°-12°C), 16°, 20°, 22°, 24°, 26°, 28°, 30°, 32°, 34°, 36° and 40°C.

3. From the experiments the zoosporangia germinated with either zoospores or germ-tubes. The germination with zoospores took place between 16° and 26°C, with an optimum of 22°C, and that of germ-tubes between 11° and 36°C, with an optimum of 26°C.

4. Maximum and minimum temperatures of the zoosporangial germination with germ-tubes were similar to that of the mycelium growing on agar media but those for the germination with zoospores were somewhat different.

5. In these experiments the germination with the germ-tubes was found to be more vigorous than that with the zoospores.

Literature cited

- (1) CROSIER, W.: Culture of *Phytophthora infestans*. *Phytopath.* **23**, p. 713-720, 1933.
- (2) —————: Studies in the biology of *Phytophthora infestans* (MONT.) DE BARY. Cornell Agr. Exp. Sta. Mem., **155**, P. 1-40, 1934.
- (3) KATSURA, K.: Studies on Phytophthora disease of economic plants, I. Phytophthora disease of pumpkin and squash. *Sci. Rep. Saikyo Univ. Agr. Ser.* **1**, p. 51-76, 1951.
- (4) LEONIAN, L. H.: Stem and fruit blight of pepper caused by *Phytophthora capsici* sp. nov. *Phytopath.* **12**, p. 401-408, 1922.
- (5) MELLIUS, I. E.: The factors governing germination and infection with *Phytophthora infestans*. *Phytopath.* **2**, p. 46, 1912.
- (6) —————: Germination and infection with the fungus of the late blight of potato. *Wiscon. Agr. Exp. Sta. Res. Bull.* **37**, p. 64, 1915.
- (7) TOMPKINS, C. M. and TUCKER, C. M.: Phytophthora rot of honeydew melon. *Jour. Agr. Res.* **54**, p. 933-944, 1937.