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ZOOSPORE IN *PHYTOPHTHORA PARASITICA* VAR. *SABDARIFFAE*

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The formation of zoospores in the species under consideration has been studied in detail. Voluminous literature has accumulated with regard to the role of temperature factor in zoospore formation of the various genera of Oomycetes. A. de Bary (1860) has reported first that zoospore formation in *Cystopus* takes place in any temperature between 5°–25°C. In *Saprolegnia mixata*, Klebs (1899) has shown that at 1°–2°C. zoospores are liberated after 24 hours, while the optimum temperature lies between 24°–28°C. At the latter temperature zoospores are said to have formed within 5–6 hours while the maximum temperature for the process lies between 32°–33°C. Melhus (1911) has shown that zoospores may be formed by the sporangia of *Cystopus candidus* at temperature ranging from 0°–25°C. and that the optimum temperature is 10°C. A number of investigators have mentioned the temperature relation for zoospore formation in the genus *Phytophthora*. Dastur (1913) has shown that in *P. parasitica* zoospores came out from sporangia in 5 minutes at 25°C. and any temperature higher than that did not favour the formation of zoospores. Melhus (1915) has shown that the optimum temperature for zoospore formation in *P. infestans* lies between 12°–13°C. with a minimum range between 2°–3°C. and the maximum between 24°–25°C. Rosenbaum (1917) working with various species of *Phytophthora* has stated in the discussion on formation of zoospores by conidia that "the suspension of spore was held at a temperature of about 15°C. and further that at the end from two to five hours a larger number of conidia had germinated". Gadd (1923) in *P. faberi* has found 20°–25°C. as the optimum temperature for zoospore formation and at temperature below or above this, formation of zoospores is retarted. Ashby (1929) obtained zoospores of *P. palmivora* by keeping a fresh culture (5–7 days) in cold water. Cotner (1930) working with an undescribed species of *Phytophthora* has found that the temperature range of zoospore formation extends from 4°–6°C. to 34°–36°C., the optimum being 25°–27°C. At the optimum temperature zoospores are liberated in well aerated water within five minutes. He has also found that *P. cactorum* and *P. terrestris* took ten minutes to form zoospores under optimum condition (25°–27°C). The optimum temperature for zoospore formation in *P. palmivora* lies between 23.5°–25.5°C. when zoospores are liberated in nine to ten minutes. In *Saprolegnia anisospora*, Coker (1923) reports that zoospores are formed in spring water at a temperature of 13°C. He also reports that zoospores are formed in *Isoachlya toruloides* at 26°C., *I. unispora* at 30°C., *A. klebsiana* at 18°–24°C. and *A. orion* at 21.5°C. Reviewing the literature on zoospore formation in the genus *Plasmopara*, Nishimura (1926) has shown that there exists great divergence in the data obtained by different investigators who have studied temperature relation swith zoospore formation.

Aerial mycelium of the fungus under consideration from several weeks' old culture on *potato–dextrose–agar* was taken on a clean slide in few drops of water, teased and examined under microscope for locating sporangia. Several hanging drop cultures were also prepared simultaneously in van Teighem cells. Time was recorded as soon as mycelium was placed in water on the slide. Under the high power of the microscope a cluster of sporangia, preferably 4–5 were kept under observation at room temperature.

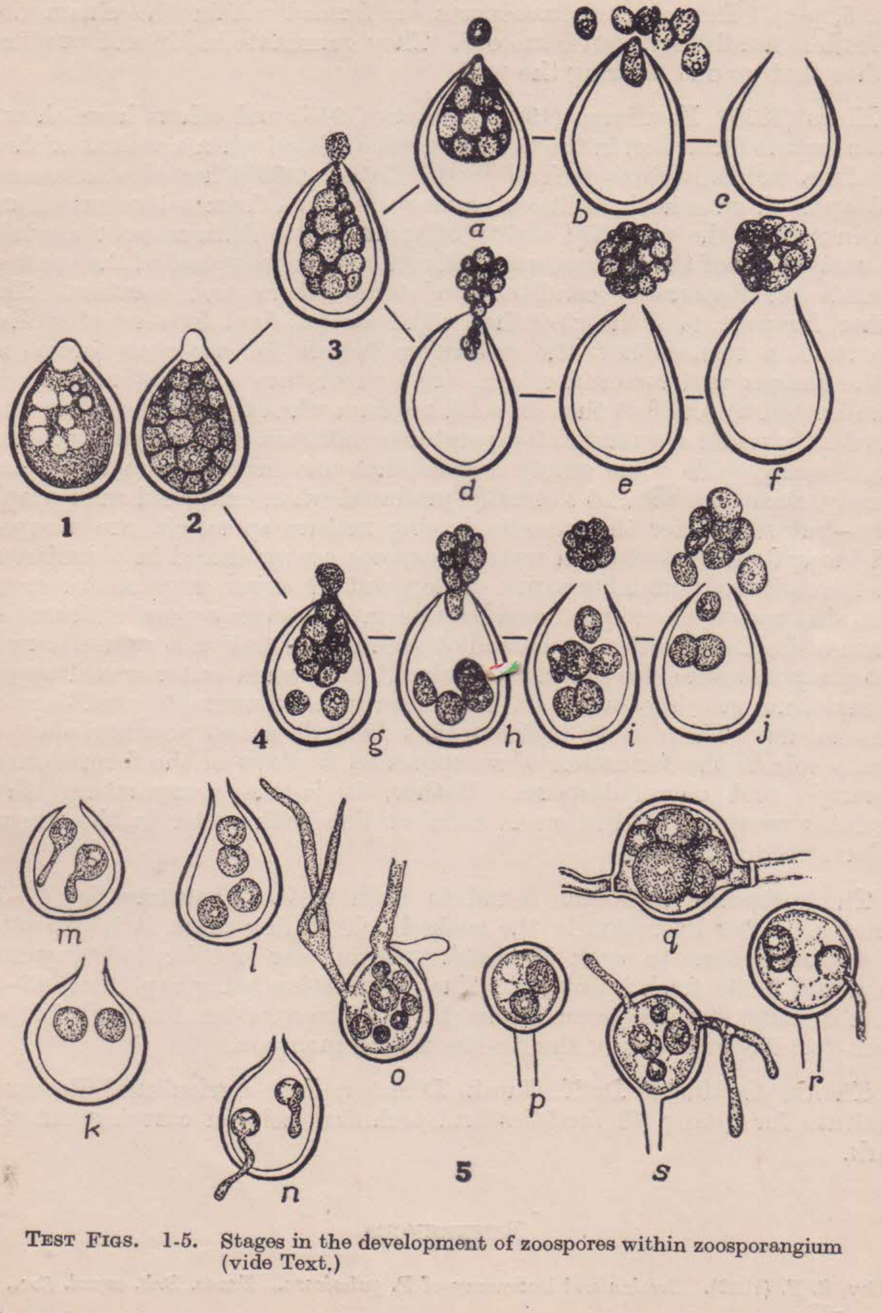
A change in the protoplasm of the sporangia is found to develop soon. It contracts from the wall, becomes denser, undergoes cleavage and develops a tendency to elongate along the main axis of the sporangium (TEXT-FIGS. 1–2). After about 8–10 minutes the papilla bursts forming a narrow passage. The protoplasm at this end becomes very narrow and develops a high tension, as if something is pulling it out or an inside pressure thursting it from below. Zoospores are usually formed in the following three ways :—

a) The protoplasm passes slowly through the narrow passage and swells as soon as it comes out of the sporangium. The entire protoplasm comes out wherefrom zoospores single out themselves or the whole protaplasm may form a very big single, round or irregularly shaped zoospore which may show a sluggish movement or may be immobile (TEXT-FIG. 3, d–f).

b) Zoospores are cut off one by one from the outcoming protoplasm (TEXT-FIG. 3, a–c).

c) Zoospores are cleaved out from some portion of protoplasm while still inside the sporangium and the remaining mass of protoplasm produces zoospores by either of the above processes (TEXT-FIG. 4, g–j).

It has been observed that zoospores are formed from mature sporangia when the temperature ranges between 23°–27°C. but the optimum temperature may be said to vary between 24°–27°C. This temperature range has been worked out during the winter months in the laboratory. At 22.5°C. or a little below, zoospores are not truly formed though the protoplasmic contents of the sporangia show tendencies to form zoospores by rounding of protoplasm, its cleavage and final bursting of the papillar region. The number of zoospores produced inside a sporangium is not constant and depends mainly upon the size of the sporangium and amount of protoplasm it contains. Usually, from 4 to 16 or more zoospores are produced but the number is always found to be a multiple of 2. They are more or less spherical or somewhat pyriform. Flagella cannot be seen without staining but as soon as zoospores are liberated they become active and begin to swim vigorously. The diameter of the zoospore varies between 5.16–14.76μ. It is interesting to note that zoospores having a size from and over 8.94 $\times$ 8.94μ generally cannot come out of the sporangium when formed inside. Besides, it is further noticed that zoospores with smaller dimensions sometimes fail to come out, though motile. In all probability they are produced from the left over mass of protoplasm that already has given a crop of zoospores and becomes depleted in the interior pressure that may be responsible for thrusting the zoospores out normally (TEXT-FIG. 5, k–o).



TEST FIGS. 1-5. Stages in the development of zoospores within zoosporangium (vide Text.)

They ultimately come to rest and germinate inside the sporangium. Usually, the zoospores which cannot come out of the sporangium germinate within giving rise to new hyphae which come out through the papillar end or by piercing the wall of the sporangium (TEXT-FIG. 5, m–n). Chlamydospores, in many cases are also found to produce zoospores, (TEXT FIG. 5, p–s). In such cases, zoospores are formed within the chlamydospores but hardly they can come out. They germinate inside and produce hyphae that go out piercing the wall.

Klebs (1899), Kauffman (1908), Pieters (1915) and others have shown that zoospore formation in the Oomycetes is initiated when a well nourished mycelium suddenly faces lack of food. Cotner (1930) has also expressed that apart from other conditions, removal of food from active mycelium sets in motion the potential ability of sporangia to form zoospores, while the completion of the process, especially in a manner typical of the species, depends on favourable conditions of temperature and aeration. The author, however, is of the view that withdrawal of food does not play that important a role, rather, the governing factors for zoospore formation in the fungus under consideration, are temperature and water. This is corroborated by the fact that on solid medium when the fungus is growing luxuriantly under favourable temperature conditions for zoospore production, leaving aside some empty vesicle that one may come across occasionally, zoospores are not normally produced when examined microscopically—but no sooner the mycelia having mature sporangia are scooped and brought in contact with water zoospores are produced in abundance, other conditions remaining same. The point, however, remains for argument that zoospores are produced because mycelia were separated from the source of food. In order to contradict such interpretation it was observed that zoospores were also produced in abundance, when water was dropped on mycelium growing on the solid medium under favourable conditions of temperature. These facts indicate that food does not possibly play as vital a role in the formation of zoospores as it plays in the formation of sporangia and chlamydospores. Rather, it is the temperature that regulates zoospore production in collaboration with water in the fungus under consideration.

The zoospores have been found to swim in the laboratory for 40–50 minutes. After liberation in the soaked field during rains, it is expected to get ample time to swim to healthy plants near and far, lodge against them and incite fresh infections. The germination being rapid i.e., 45–60 minutes under favourable conditions, they can be considered as the principal agent for dissemination of the disease during monsoon.

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