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EFFECT OF CULTURE ENVIRONMENT UPON SPORANGIUM AND ZOOSPORE PRODUCTION OF THREE SPECIES OF PHYTOPHTHORA¹

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Species of *Phytophthora* are cosmopolitan in nature and are responsible for economically important diseases of potato, soybeans, tobacco, and tomato. The *Phytophthora* spp. are Phycomycetes and reproduce sexually by the formation of oospores, and asexually by the production of sporangia and zoospores.

Since species of *Phytophthora* are common and of economic importance, conditions favoring their growth and reproduction have been studied rather extensively. These fungi have also been used for teaching purposes as examples to demonstrate asexual reproduction by zoospores.

Various techniques have been used to promote oospore and sporangium production within the genus *Phytophthora*. For sporangium and zoospore production Gooding and Lucas (2) grew *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan) Tucker on solid oatmeal agar and stripped aerial mycelium from cultures after 6-20 days growth. The mycelium was then transferred to plates and moistened or floated on water at 24-26°C for 6-10 days. Fresh distilled water was then added to the cultures, they were chilled for 25 min at 8°C, and then returned to room temperature. Zoospores were released after about 15 min. This procedure requires from 16-32 days. Sporangia and zoospores can also be obtained from oatmeal agar cultures by taking small squares of agar with the organism and floating them on water in Petri dishes for 10-12 days, during which time additional mycelium and sporangia develop. The cultures are then chilled for about 30 min and the mycelium and sporangia are then transferred to water at room temperature and zoospores are released. This latter method is also time consuming, and sporangium development and zoospore release are often erratic.

Reproduction on semi-solid media has been studied and is used less extensively (3, 4). The authors have successfully used such media for a number of years for both research and teaching purposes. For sporangium production by *Phytophthora parasitica* Dast., the causal organism of a tomato disease known as buckeye rot, very

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good results have been obtained by growing the organism on 1/10 strength lima bean agar (LBA). After 7-10 days, when sporangia have been produced, a small volume of mycelium and residual media are put on a microscope slide and an equal or slightly larger volume of distilled water is added to the slide. The mycelium is teased apart with needles to expose the sporangia to the distilled water, and the preparation is covered with a cover slip. Zoospores normally begin to be released within a few minutes after the slide is prepared. If the film of water is maintained under the cover slip, the zoospores will usually stop swimming, encyst, and germinate by the formation of a germ tube within an hour.

The authors have used *P. parasitica* for instructional purposes because it is readily available from diseased plants or professional sources and it is easy to culture. The optimum conditions for reproduction of this organism on semi-solid media have not been fully determined. The present study was undertaken to further delineate conditions favoring sporangium and zoospore production.

MATERIALS AND METHODS

The cultures used in the study were: an isolate of *P. parasitica* obtained locally from tomato, two isolates of *P. parasitica* var. *nicotianae* from tobacco (NC 1156 and NC 1030),¹ and an isolate of *P. cactorum* (Leb. & Cohn) Schroet.² Isolates were maintained on plates of solid LBA (Difco). A 4 mm disc of each organism on agar was aseptically transferred to a 125 ml Erlenmeyer flask containing 20 ml of sterile 1/10-strength LBA. Appropriate numbers of such inoculated flasks were immediately incubated in the dark at temperatures of 20, 24, 28, and 32°C, and a series of flasks were also incubated at room temperature under normal light conditions. At 3-day intervals, for a period of 18 days, individual flasks of each organism at each temperature were checked for sporangium formation and zoospore release. Forty ml of sterile distilled water was added to each flask, the flasks were gently shaken on a shaker for 20 min., and a 1 ml sample was scanned under low magnification to check for presence of zoospores. The zoospores in the flasks were then inactivated by the addition of 1 ml of 1-1000 HgCl₂ per flask. The numbers of zoospores in the diluted medium were determined by the use of a hemacytometer, and the results were transferred into number of zoospores per ml of original growth medium.

¹Cultures supplied by Dr. J. L. Apple, North Carolina State University.

²Culture supplied by Dr. Donald C. Erwin, University of California, Riverside.

RESULTS AND DISCUSSION

The results of tests are shown in Figures 1 and 2. Since few zoospores were produced at 32°C, this temperature was omitted from the graphs. The results show that considerable variation occurred among the isolates in number of zoospores released and that sporangium production and zoospore release was affected by time, temperature, and light conditions. The isolate from tomato, *P. parasitica*, produced the largest number of zoospores, with 187,000/ml being released from flasks incubated 15 days at room temperature in the light (Fig. 1-A). Isolate NC 1030 of *P. parasitica* var. *nicotianae* produced the second largest number of zoospores, with 176,000/ml released from cultures incubated in the dark at 20°C for 18 days (Fig. 2-A). Isolate NC 1156 from tobacco produced consistently low numbers of zoospores at all temperatures (Fig. 2-B). Except for *P. cactorum* the cultures were somewhat cyclic in zoospore release, with periods of high zoospore release followed by periods of low zoospore release. This undoubtedly resulted from the fact that zoospores were released in the flasks during incubation even though no distilled water was added, and a time interval was necessary for production of additional sporangia. Interestingly, *P. cactorum* released a maximum number of zoospores after 9 days incubation at all but room temperature (Fig. 1-B). Except for *P. parasitica*, the largest numbers of zoospores were generally produced by the cultures incubated in darkness, regardless of temperature.

The results in the present study differ somewhat from those obtained by other workers. Dukes and Apple (1), using the technique of Gooding and Lucas (2), reported that tobacco isolate NC 1156 was more pathogenic and produced more sporangia than isolate NC 1030. This contrasts with the low number of zoospores produced by NC 1156 and the high number produced by NC 1030 in the present work. Harnish and Barnett (3) reported that *P. cactorum* grown on dilute LBA produced only a few sporangia when grown in darkness. Although grown on the same medium in the present work, a different isolate of the organism produced more sporangia and zoospores in darkness than under conditions of fluctuating darkness and light.

Results obtained in the present work thus indicate that cultural conditions have a pronounced effect on sporangium and zoospore production, and that the reaction of isolates is dependent upon the technique used. The results also indicate that individual isolates may vary even when grown under similar cultural conditions.

For instructional purposes to demonstrate zoospore release and activity, the utilization of 1/10 strength LBA as a growth medium is more simple, rapid, and reliable than use of other methods. With *P. parasitica*, the cultures can be grown under normal conditions of light and room temperature, and abundant zoospores for obser-

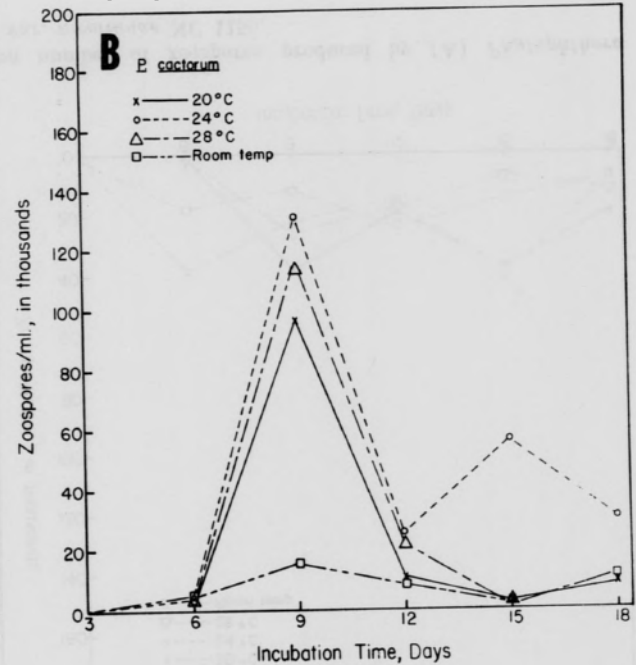
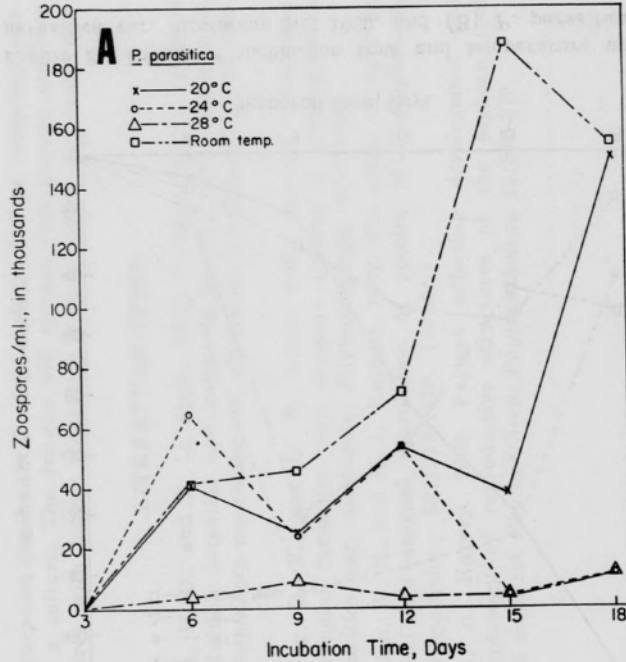


Figure 1. Effect of incubation time and temperature upon number of zoospores produced by (A) *Phytophthora parasitica*, and (B) *P. cactorum*.

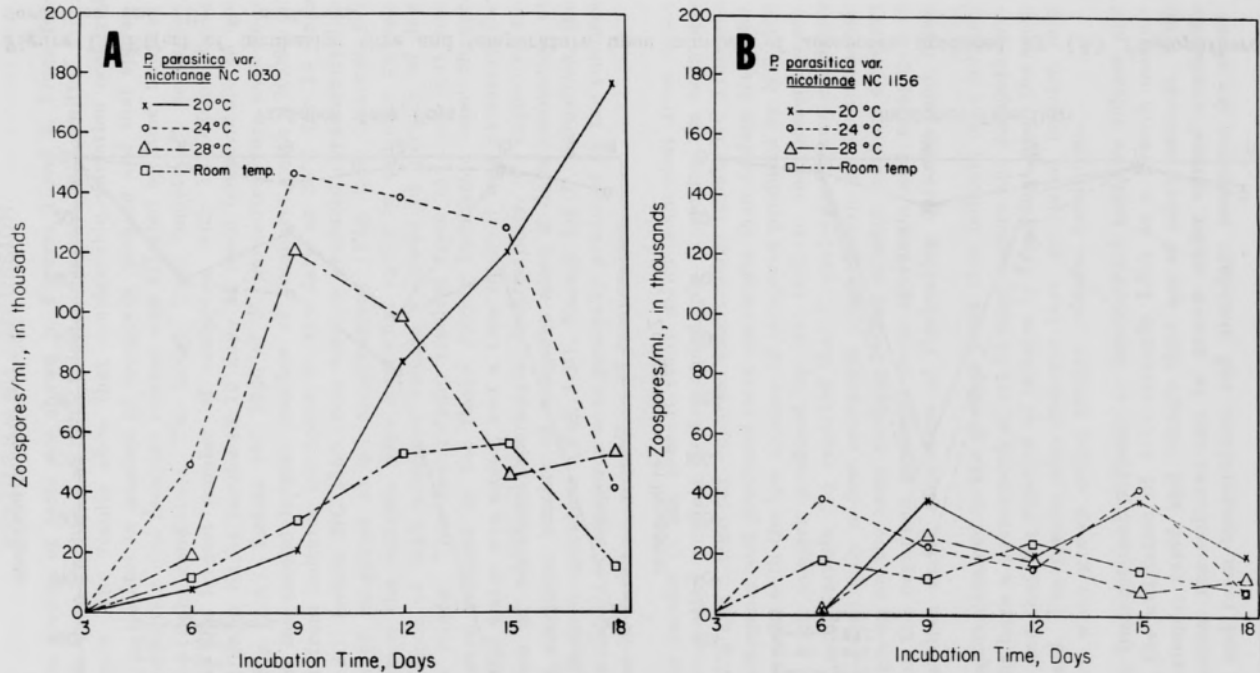


Figure 2. Effect of incubation time and temperature upon number of zoospores produced by (A) *Phytophthora parasitica* var. *nicotianae* NC 1030, and (B) *P. parasitica* var. *nicotianae* NC 1156.

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vational purposes can be obtained as early as 6 days following incubation of a culture. The fungus will release even greater numbers of zoospores with longer incubation periods under the same conditions.

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