# Overwinter Survival of *Phytophthora cactorum* in Infected Strawberry Fruit

G. G. GROVE, Graduate Research Associate, M. A. ELLIS, Associate Professor, L. V. MADDEN, Assistant Professor, and A. F. SCHMITTHENNER, Professor, Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691

**ABSTRACT**

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Mummified strawberry fruits (cultivar Midway) infected with *Phytophthora cactorum* were exposed to winter conditions on the ground and 1 cm beneath the soil surface. Germinating oospores were observed in water suspensions prepared from mummified fruit retrieved the following spring; strawberry seedlings originating from seed in overwintered, infected mummies were also infected with *P. cactorum*.

Epidemics of strawberry leather rot, caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., have resulted in significant yield and quality losses in Ohio (2). In 1981, an estimated 20–40% of Ohio's strawberry crop was lost to leather rot (2). Very little information is published on this disease, and the overwintering nature of *P. cactorum* in strawberry fields has not been determined (6).

Survival of several *Phytophthora* spp. in infected host tissue has been reported (1,8,10). *P. palmivora* has been reported to survive in root tissues of infected rubber trees (10). *P. cinnamomi* has been recovered from *Banksia grandis* roots up to 2 yr after plant death (8). Gerlach et al (3) reported survival of *P. citrophthora* in diseased *Pieris japonica* tissue. Kuske and Benson (5) reported the survival of *P. parasitica* in infected rhododendron tissue.

Our observations in Ohio indicate that strawberry fruits infected with *P. cactorum* eventually dry to form mummies. We have observed oospores in fruit mummies obtained from laboratory inoculations and naturally infected fruit in the field (2). The purpose of this study was to determine if infected, mummified fruit could serve as a source of overwintering inoculum for leather rot.

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## MATERIALS AND METHODS

Laboratory inoculations were performed with cultures of *P. cactorum* freshly isolated from infected strawberry fruit (cultivar Midway) on pentachloronitrobenzene-benomyl-neomycin sulfate-chloramphenicol medium (PBNC) (7). Mycelia from the edges of 3-day-old cultures were transferred to lima bean agar. Cultures were incubated 7 days. All incubations were conducted at 22 C in continuous light at 2.7 W/m$^2$. Sporangia were produced after 4 days. Indirect sporangial germination was then induced by flooding each culture with 20 ml of sterile distilled water (SDW) followed by refrigeration for 30 min at 5 C. Plates were removed from refrigeration and brought to 22 C, and zoospores were produced within 30 min. Zoospore concentration was adjusted to 10,000/ml in SDW using a hemacytometer.

Fruits were obtained from Midway strawberry plants grown to reproductive maturity in a 1:1:1 (v/v) peat:sand:steam-disinfested silt-loam mixture in the greenhouse. Immature (green) fruit were harvested and washed with detergent in SDW, rinsed in SDW, surface-sterilized in 100 ml of a 0.5% NaOCl solution for 30 sec, and rinsed again in SDW.

Twenty milliliters of inoculum was transferred to 9-cm-diameter plastic petri plates, and five fruits were placed in each for 4 hr. Inoculated fruit were placed in plastic containers (50 × 50 cm) sealed with clear plastic wrap and incubated 72 hr. Containers were then uncovered, and inoculated fruit were allowed to incubate an additional 11 days. Relative humidity in the room was 30–40%. Tissue sections were taken daily from infected fruit and observed microscopically at ×40 for oospore production. Oospores were first observed 4 days after inoculation.

Five mummified fruit were then placed between pieces (2–6 cm) of 130-μm Nitex nylon cloth (Tetko Inc., 420 Saw Mill River Rd., Elmsford, NY) and sewn together with monofilament fishing line. Sixteen fruit pouches were prepared in this manner and buried 1 cm below the soil surface in a Midway strawberry field at the end of the fruiting season. Locations of buried fruit pouches were marked with wooden stakes. Pouches were buried on 1 July 1982 and retrieved on 15 April 1983. The experiment was repeated in 1983–1984.

After retrieval, three mummies from each pouch were placed in 50 ml of SDW and ground for 1 min at high speed in a Sorval Omni Mixer. Each suspension was diluted by adding 250 ml of SDW. Ten milliliters of suspension were then transferred to petri plates and incubated 14 days, and daily microscopic examinations were made.

The remaining two fruits from each pouch were incubated 72 hr in moist chambers. Strawberry seedlings were observed emerging from seed in the mummified fruit tissue. Each seedling was transferred to a petri plate containing 10 ml of SDW. Covered plates were incubated 24 hr, then seedlings were transferred to PBNC medium and incubated an additional 72 hr.

Twenty-five mummified Midway strawberry fruits that were formed naturally during the previous season were collected from the soil surface of a strawberry field at Wooster, OH, in April of 1983 and 1984. The field had a history of leather rot. Twenty mummies were chosen at random, and suspensions from each were prepared and incubated as described previously. From the five remaining mummies, seedlings were produced and treated as described previously.

## RESULTS AND DISCUSSION

*P. cactorum* was recovered from infected fruit mummies after a 9-mo burial beneath or exposure at the soil surface. Germinating oospores were observed in all overwintered mummified fruit suspensions 4–14 days after placement in SDW. Numerous papillate sporangia, characteristic of *P. cactorum* (11), were observed microscopically at the distal portions of germ tubes emerging from oospores contained in the suspensions (Fig. 1). Indirect germination of several sporangia connected via a germ tube to oospores was observed.

Sporangia of *P. cactorum* were observed in or on the root tip and hypocotyl regions of strawberry seedlings

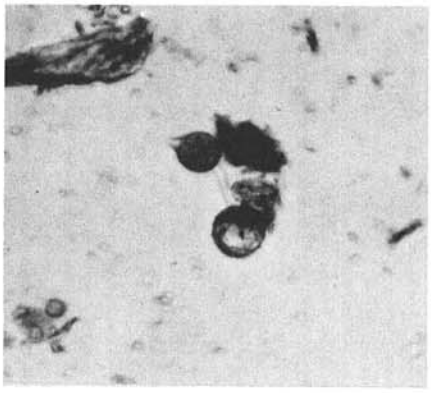

**Fig. 1.** Germinating oospores (×2,500) from an overwintered strawberry fruit mummy infected by *Phytophthora cactorum*. Note the sporangium at the distal end of germ tube.

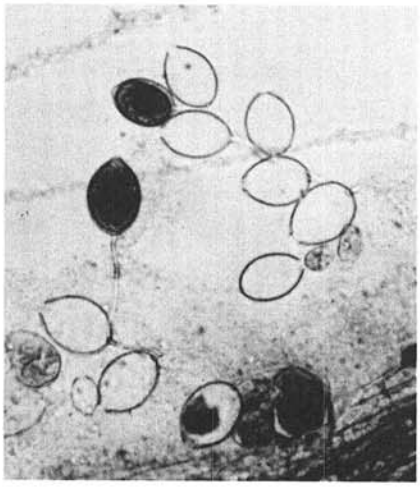

**Fig. 2.** Sporangia of *Phytophthora cactorum* (×2,500) on the root surface of strawberry seedling. Seedling originated from a seed in an overwintered fruit mummy.

obtained from seed in overwintered mummies (Fig. 2). Strawberry seedlings emerging from naturally occurring mummies in the field were also colonized by *P. cactorum*. *P. cactorum* was isolated from all strawberry seedlings that developed from mummified fruit.

These results suggest that *P. cactorum* can overwinter as oospores in mummified, infected strawberry fruit. Sporangia, mycelia, encysted zoospores, and oospores of *P. cactorum* have all been reported to have survival value in soil (9). Survival in soil for as long as 30 and 35 days has been reported for mycelia and sporangia, respectively, at 4 C (4,9). Sporangial and mycelial viability in soil has been shown to depend on soil conditions. Sneh and McIntosh (9) reported that mycelia were unable to survive freezing and that the ability of sporangia to survive low temperatures was affected by soil moisture. They concluded that oospores, because of their structure and nature, were undoubtedly able to survive in soil for longer periods than sporangia but that sporangia should be regarded as the most important short-term inoculum unit. Mycelium and hyphal fragments were not important in persistence of the pathogen in soil.

The mean soil temperatures for December through February in 1982 and 1983, respectively, were 1.6, 1.1, and 5.0 C; and 0, −2.7, and −1.1 C. It should be noted that the winter temperatures during 1983 were abnormally low and the soil was frozen for at least 3 mo. These long-term, adverse conditions make it highly unlikely that *P. cactorum* survived as sporangia or mycelia in mummified fruit tissue. In addition, only oospores were observed in tissues from overwintered mummies. On the basis of these observations, we conclude that oospores are the most probable survival propagules of *P. cactorum* in strawberry fields under Ohio conditions.

The implications of mummified fruit as a potential source of primary inoculum for leather rot are obvious. In areas where leather rot is a serious problem, sanitation (removal of infected fruit) may be an important disease control measure.

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