Sporulation and Germination of Phytophthora lateralis

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Cooperative Investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the Oregon Agricultural Experiment Station, Technical Paper No. 3743.

This investigation was supported in part by the Oregon Forest Severence Tax Funds provided through the Oregon Forest Protection and Conservation Committee.

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Accepted for publication 8 July 1974.

ABSTRACT

In culture on seven different agar media, the growth rate of *Phytophthora lateralis* increased from 3 to 20 C, then decreased at 25 C. Moisture was essential for the induction of sporangia, which developed only when vegetative growth ceased or was reduced to a minimum. Mature sporangia remained firmly attached to sporangiophores and liberated 25-40 fully developed zoospores. In contrast to sporangia, abundant production of chlamydospores depended on rich media. On a medium containing a protein hydrolysate, chlamydospores averaged 51 μm in diameter and germinated readily by producing several germ tubes. Oospores were produced on media containing cedar foliage and averaged 40 μm in diameter. A single paragynous antheridium was formed per oogonium. Colonies arising from single zoospores produced oospores, demonstrating the homothallic nature of this fungus.

Phytopathology 64:1531-1533

Additional key words: sporangia, chlamydospores, oospores, *Chamaecyparis*, white cedar.

Phytophthora lateralis Tucker & Milbrath causes a serious root disease of Port Orford cedar, *Chamaecyparis lawsoniana* (A. Murr.) Parl. (3, 5). This disease is threatening this valuable timber species throughout its native range in southwestern Oregon (3). This beautiful white cedar tree, known also as Lawson cypress, is used extensively in ornamental plantings in North America, New Zealand, and Europe, and *P. lateralis* poses a threat to those ornamental trees. Thus, plant pathologists outside of Oregon are occasionally concerned with the identification of this pathogen. Tucker and Milbrath's description of *P. lateralis* (5) was accurate, but limited, and additional cytological and physiological data on this fungus would be of value. Furthermore, the validity of *P. lateralis* remains questionable, inasmuch as only the asexual stage has been described (5). The sporulation and germination characteristics, as well as the first description of the oospores of this fungus, are reported here.

METHODS AND RESULTS.—*Mycelial growth.* The physiological characteristics of *Phytophthora lateralis* were investigated to gain information on the capacity of this pathogenic fungus to survive and spread under different climatic conditions. In culture on seven different media containing plant materials (peas, potatoes, wheat germ, lentils, V-8 juice, or cedar foliage), the growth rate increased from 3 to 20 C, then decreased at 25 C, which was near the maximum temperature allowing growth of the mycelia.

The young, vigorously growing hyphae are coenocytic, 5 μm in diameter, and smooth, but often become septate and contorted with age. The morphology of the vegetative colony depends on the substrate. The nitrogen level of the medium seems to exert the greatest influence. Thin, spreading colonies develop on media with low nitrogen levels, whereas dense, compact colonies develop on high-nitrogen media (5 g mixed amino acids per liter of medium). All 120 isolates of *P. lateralis* examined were morphologically indistinguishable on potato-dextrose agar, as were 30 single-zoospore isolates.

Sporangia. Sporangia typical of this species are shown in Fig. 1. After sporangia had formed the sporangiophores were observed to continue development in either of two ways: (i) by branching at the base of the sporangium, or (ii) by growing through an empty sporangium. Unlike many other species of *Phytophthora,* the mature sporangia of *P. lateralis* usually remain firmly attached to the sporangiophores. The sporangia germinate directly by germ tube formation and indirectly by zoospores. Zoospores are always fully developed within the sporangium, and each sporangium produces 25 to 40 zoospores.

Tucker and Milbrath (5) did not observe sporangia on agar substrates, but in my investigation sporangia were frequently seen on many agar substrates of low nutritional value. Aerial sporangia did not occur, even though aerial hyphae were common, on media favorable for sporangial production.

The capacities of many liquid solutions and agar media to support sporangial formation were tested. The most notable feature associated with the formation of sporangia in cultures of *P. lateralis* was the low nutritional level of the medium. Good vegetative growth and sporangial formation were never associated. Usually sporangia developed only when vegetative growth ceased or was reduced to a minimum. Moisture was essential for the production of sporangia, and excess water increased the abundance of sporangia.

Sporangia, numerous enough for identification, were easily obtained on many agar media low in nutrients; for example, one liter of water agar containing 10 ml commercial V-8 juice, or 25 g of fresh cedar foliage, or 25 g of corn meal, or 20 ml whole milk. When, in the preparation of these minimal media, Hoagland's mineral solution number 1 (1) was used in place of distilled water,

the production of sporangia was greatly increased.

Initial culture in pea broth (broth of 15 g dried peas autoclaved in 1.0 liter water) proved to be the greatest stimulus to sporangial development. Sporangia were not formed in pea broth, but when mycelial colonies were cultivated in pea broth at room temperature for 4 days, then washed and covered with distilled water or Hoagland's mineral solution number 1, abundant sporangia developed within 24 hours. The fruiting response was prompt on small mycelial colonies. On large mycelial colonies, well supplied with reserve nutrients, sporangial production was rare and was limited to the periphery of the colony.

Sporangia were produced from 5 to 25 C in culture, and from 5 to 20 C on infected foliar twigs. The formation of sporangia, both in culture and on host tissue, was slow and sparse below 10 C. The sporangia were capable of germinating indirectly to produce motile zoospores from 5 to 25 C. Zoospores as well as sporangia perished very quickly as the water surrounding them evaporated.

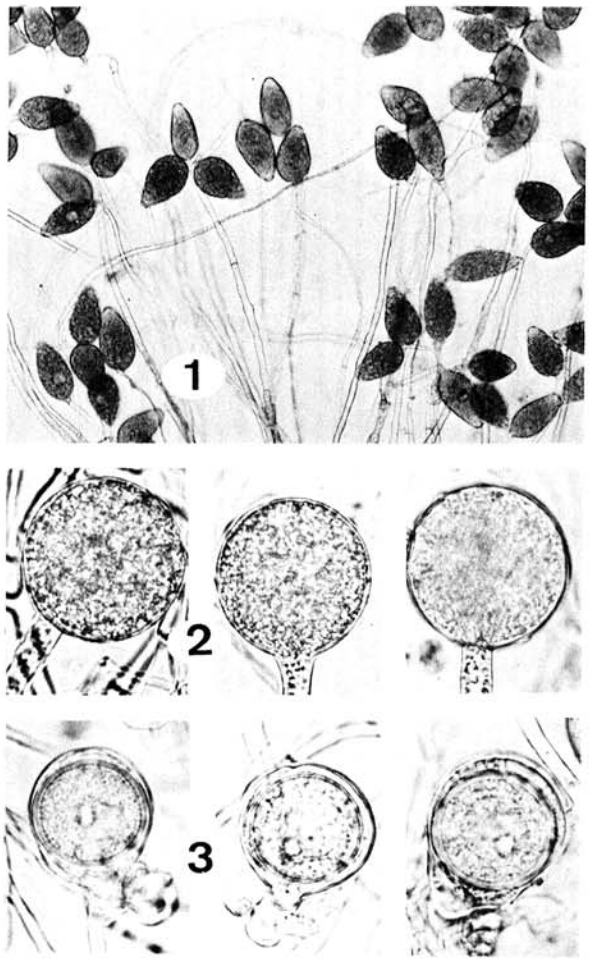

Fig. 1-3. Cultural characteristics of *Phytophthora lateralis*. 1) Sporangia of *P. lateralis* that grew into a mineral solution from infected cedar foliage (× 236). 2) Terminal and intercalary chlamydospores of *P. lateralis* that developed on the chemically-defined medium, overlain with cedar foliage (× 422). 3) Oospores of *P. lateralis* that developed on the chemically defined medium, overlain with cedar foliage (× 422).

Chlamydospores. In contrast to sporangia, the production of chlamydospores was closely associated with vegetative growth of *P. lateralis*. Abundant chlamydospore production depended on high food reserves in the medium and incubation temperatures of 15 to 25 C. Chlamydospores were produced on practically all types of media that supported good vegetative growth. Abundant chlamydospores were produced in a V-8 broth (100 ml V-8 juice, 15 g glucose, 900 ml water), and also in a chemically-defined medium (4) containing 25 μg/ml β-sitosterol or stigmasterol. Chlamydospores that formed in cedar foliage agar (25 gm cedar foliage per liter) were an intense cinnamon-brown color due to pigments that accumulated in the cell walls of the spores and to a lesser extent in their cytoplasm. Pigmentation was greatest at 15 C.

Chlamydospores (Fig. 2) produced on the chemically-defined medium ranged in diameter from 37.8 to 63.1 μm and averaged 51.4 μm. These chlamydospores germinated readily at 20 C on a medium containing 2 g free amino acids (0.2% Edamin, an enzymatically digested lactalbumin, Sheffield Chem. Co., Norwich, N.Y.), 20 g glucose, 5 mg thiamin, and a mixture of inorganic salts (4). Usually several germ tubes emerged from each chlamydospore. The capacity of different isolates of *P. lateralis* to produce spores was very uniform for sporangia and oospores, but the abundance of chlamydospores varied several fold among the 10 isolates tested.

Oospores. Oospores were observed most frequently on agar containing *C. lawsoniana* foliage or decoctions of the foliage. The decoction agar was prepared by steaming 200 g of fresh cedar foliage in 500 ml tap water at 95 C for one hour, decanting the liquid, adding 15 g of glucose, 15 g of agar, and enough water to bring the volume to one liter. A single oospore filled the oogonial cavity of each oogone (Fig. 3). The oogonia were smooth, spherical, and terminal. A single paragynous antheridium formed per oogonium. The granular protoplasm of the oospore contained one or more reserve globules. The oospores were spherical, with a smooth wall 2-μm thick. The diameter of 100 oospores formed on cedar foliage agar varied between 34.5 μm and 45.3 μm with an average of 40 μm. Oospore color depended on the medium. In alfalfa agar (50 g dried alfalfa, 15 g agar per liter of medium) oospores were amber, whereas in cedar foliage agar they were cinnamon brown. Brown intramatrical oospores were observed in naturally infected cedar foliage and in the cortex of young twigs. Colonies resulting from single zoospore isolates of *P. lateralis* produced oospores, thus demonstrating the homothallic nature of this fungus. Isolates of *P. lateralis* from ten different locations in Oregon were paired in all possible combinations on cedar foliage agar. Oospores were produced by all isolates, but their numbers were not increased as a result of the pairing. Although excellent vegetative growth and chlamydospore production were obtained on the chemically-defined medium (4), containing sterols (25 μg/ml stigmasterol or β-sitosterol), oospores were not produced on those media.

Oospores were rarely seen to germinate. The oospores that did germinate after 2 months' incubation at 5 C

produced a single germ tube, which gave rise to a single sporangium. A recent report indicates that hydrolytic enzymes may stimulate the germination of *Phytophthora megasperma* oospores (2).

CONCLUSIONS.—This research on the physiological characteristics of this pathogen indicated that 15 C to 20 C is optimum and 25 C maximum, for most infection processes: sporulation, germination, reinfection and growth within the host. In nature, mycelial growth probably occurs only in the cool and mild seasons of the year and probably is restricted by warm temperatures in summer. These cool-temperature requirements agree well with the rapid development of the disease that occurs in the spring season. Oospores and chlamydospores in infected cedar tissue presumably function as an important oversummering stage of this pathogen (3).

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