

# The Maturation and Germination of *Phytophthora ramorum* Chlamydospores<sup>1</sup>

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## Abstract

Chlamydospores are a distinctive feature of *Phytophthora ramorum*. They are formed quickly in agar, and within colonized leaves. We followed their development and maturation *in vitro* and *in vivo*, and studied conditions affecting their germination. Cell walls of mature *P. ramorum* chlamydospores are thicker than reported for other *Phytophthora* species, although thin-walled chlamydospores are also formed. Chlamydospores formed within rhododendron leaves are smaller with thicker walls than spores formed *in vitro*. Chlamydospore development begins on hyphae less than two days old, and chlamydospores reach maximum size in about 10 days. Chlamydospores are formed continuously as *P. ramorum* develops, so spores of all ages are mixed in a colony. Chlamydospores of *P. ramorum* germinate *in vitro* at a low but highly variable frequency. In our experiments, the maximum germination obtained was about 13 percent, and the overall average was closer to 3 percent. Germination was higher on V8 agar than on cornmeal agar, and lowest on water agar. Smaller chlamydospores germinated more frequently than larger spores.

Key words: *Phytophthora ramorum*, chlamydospores, germination.

## Introduction

Chlamydospores are a distinctive feature of *Phytophthora ramorum*. They are formed quickly in agar and in V8 juice broth (V8JB). In other *Phytophthora* species, chlamydospores have allowed the organism to survive periods of natural environmental extremes such as: temperature, microbial antagonism, and the absence of host tissue. When conditions conducive to vegetative growth are encountered, the chlamydospore can germinate and create new colonies vegetatively or through the production of sporangia and subsequent release of zoospores. For many species of *Phytophthora*, the chlamydospore is an important survival mechanism that is not well understood. We followed chlamydospore development and maturation *in vitro* and studied conditions affecting their germination.

## Materials and Methods

**Wall thickness and diameter development**—Chlamydospores were grown in V8 and V8JB cultures for 2 to 120 days. Chlamydospore wall thickness and diameter were measured at 1000x under bright field illumination.

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**Chlamydospore germination**—Chlamydospores were grown for 10 days in 25 ml V8JB. V8JB cultures rinsed of medium were rinsed three times for 30 seconds with ionized, distilled water on a 100 µm sieve, the rinsate was diluted to 400 ml. Then 100 ml of chlamydospore suspension was filtered through a 5 µm polycarbonate filter. Filters were placed face-down on the surface of V8, cornmeal with antibiotics (CAR), and water agar plates and peeled off. Petri plates were sealed and incubated for 24 hours at 20°C in the dark. The number of germinated and intact chlamydospores was tallied in multiple 100x fields of view. Percent chlamydospore germination was calculated by the formula: percent germination = (number germinated chlamydospores) / (number germinated chlamydospores + number intact non-germinated chlamydospores).

## Results and Discussion

**Chlamydospore maturation in V8JB**—Mean chlamydospore wall thickness increased from 1.8 µm in 10-day-old age class chlamydospores to an average of 2.3 µm in chlamydospores in the 20 to 120 day old age classes. Mean maximum chlamydospore wall thickness continued to increase from 2.7 µm in the 10-day-old age class to 4.0 µm in the 90- and 120-day-old age classes. Mean diameter of chlamydospores formed in V8JB averaged 53.2 µm in 10- to 120- day age classes. This pattern was repeated in mean maximum chlamydospore diameter values that averaged 71.6 µm in chlamydospores 10 to 120 days old.

### **Relationship between chlamydospore diameter and wall thickness**—

Chlamydospore diameter increased with wall thickness in chlamydospores grown on V8 agar for two to 31 days and in chlamydospores grown in V8JB for 10 to 120 days (fig. 1). The r-values for V8 agar and V8JB-grown chlamydospores were 0.69 and 0.60 respectively.

**Chlamydospore germination**—Chlamydospore germination began within 24 hours of plating on agar. Chlamydospores germinated to form mycelial colonies, or directly to sporangiophores and sporangia (fig. 2). The effect of nutrients on chlamydospore germination was compared in three experiments (fig. 3). In each experiment, germination was highest on V8 agar, and lowest on water agar.

**Germinated and V8JB chlamydospores**—Germinated mean chlamydospore wall thickness was thinner at 1.6 µm than parent V8JB culture mean chlamydospore wall thickness at 1.8 µm. Germinated mean chlamydospore diameter was smaller at 42.7 +/- 1.0 µm than parent V8JB culture mean chlamydospore diameter at 54.6 +/- 1.0 µm (Data not shown).

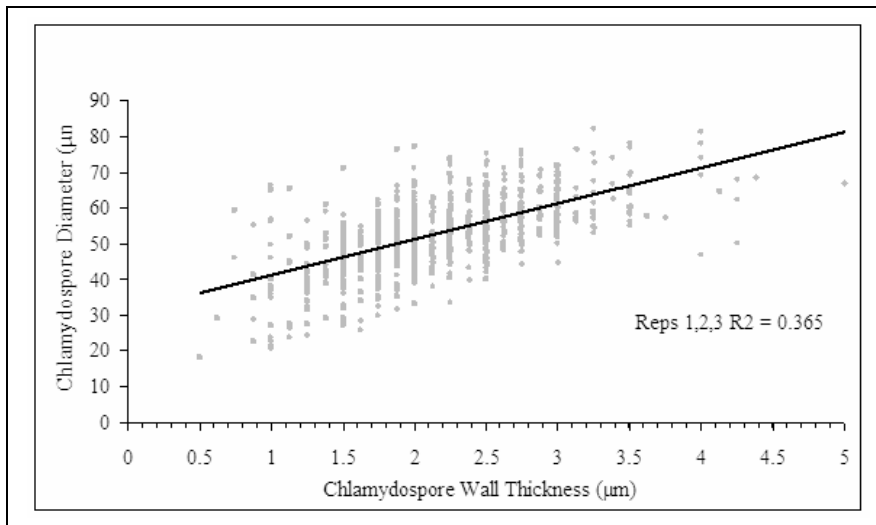


Figure 1—Diameter vs. wall thickness relationship in V8JB-grown chlamydospores aged 10 to 120 Days.

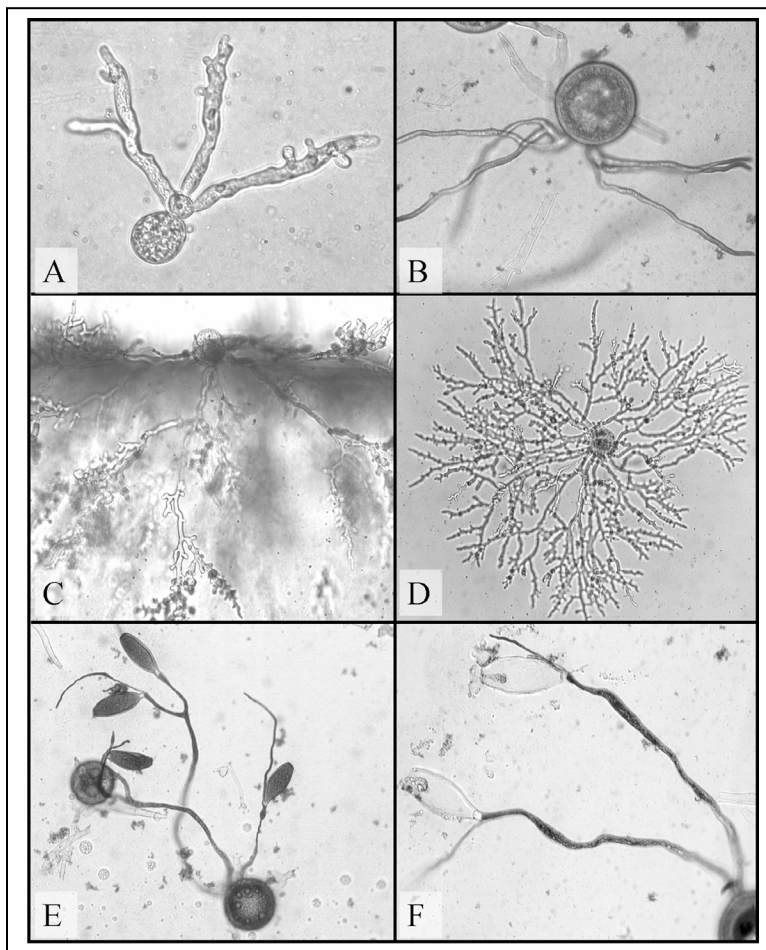


Figure 2—Chlamydospore germination after 24 hours.

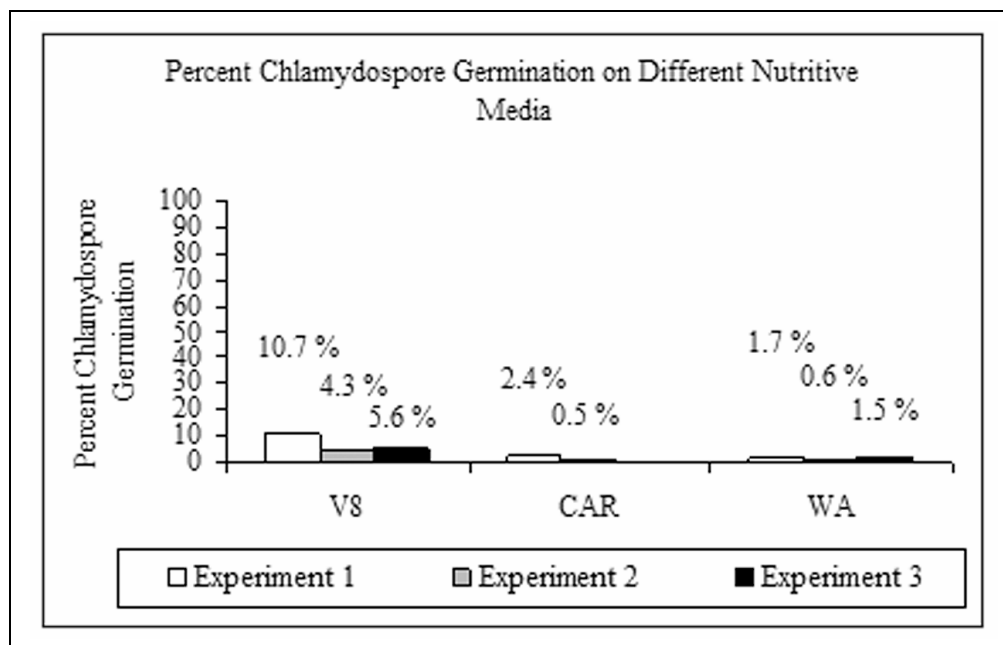


Figure 3—Chlamydospore germination on V8 agar, cornmeal agar with antibiotics, and water agar.

## Conclusions

- The chlamydospore wall of *P. ramorum* is thicker than most species of *Phytophthora*.
- Most chlamydospores of *P. ramorum* were fully developed within 10 days, though subsequent wall thickening occurred up to 120 days in V8JB.
- Chlamydospore wall thickness increased with spore diameter.
- Chlamydospores with thinner walls and smaller diameters were more likely to germinate.
- The rate of germination of *P. ramorum* chlamydospores is low and variable compared with published chlamydospore germination rates of other *Phytophthora* species.
- *P. ramorum* chlamydospore germination is controlled, at least in part, by the presence of exogenous nutrients.

These results represent an important first step in understanding the basic biology of the chlamydospore in *P. ramorum*, but further research is necessary to better understand their true biological significance to the organism.