Germination of *Phytophthora ramorum* Chlamydospores: A Comparison of Separation Method and Chlamydospore Age[1]

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Abstract

Phytophthora ramorum characteristically produces large amounts of chlamydospores *in vitro*, but the role of these propagules in the disease cycle remains unclear. Germination is difficult to observe and quantify if chlamydospores are not free of mycelium, and the low frequency of germination commonly reported suggests that requirements for germination may not have been entirely met. Here, we conducted germination experiments factoring for chlamydospore age, chlamydospore isolation method, and nutrient medium. Chlamydospore germination frequency in each of the treatments ranged from 0 to 70 percent. Results suggest that chlamydospore age, isolation method, and nutrient media significantly affect chlamydospore germination.

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

Introduction

Chlamydospores are thick-walled, resistant structures produced by many *Phytophthora* species. These structures can be a key component in the ecology and epidemiology of *Phytophthora* species by ensuring pathogen survival during adverse conditions. While *P. ramorum* characteristically produces large amounts of chlamydospores *in vitro*, the role of these propagules in the disease cycle in both natural and managed systems remains unclear. Germination is difficult to observe and quantify if chlamydospores are not free of mycelium. Moreover, the low frequency of germination commonly reported suggests that endogenous and exogenous requirements for germination may not have been entirely met. Finally, findings of high-germination frequency by some researchers have not been universally reproducible. Reports of germination frequency vary from 5 to 10 percent on V8 (Smith and Hansen 2008) to 40 to 47 percent on V8 agar + PARPH (Tooley et al. 2008). These differences may be due to variation in inoculum type, media, or chlamydospore maturity. Given the potential importance of chlamydospores to the life cycle of *P. ramorum*, there is a need for comparison and refinement of methods regarding chlamydospore germination.

Methods

Phytophthora ramorum isolate 4581 (NA1, A2 mating type) was grown on 10 percent V8 agar to obtain 1-month- and 8-month-old cultures. Plates were sealed with parafilm to reduce formation of sporangia. Each plate was marked at 3 and 6 days to demarcate growth of similar, defined colony age. For both culture ages, two types of inoculum were prepared from within the marked areas: chlamydospores in agar and chlamydospores in suspension.

Aqueous media consisted of corn meal broth + PAR (PAR), reverse osmosis (R/O) water (H_2O), and 20 µm-filtered creek water. Each treatment was replicated five times. Treatment plates were randomized and incubated at room temperature (19 to 21 °C). Germination was quantified at 24 hours

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by counting the number of germinated chlamydospores out of 100 using a stereomicroscope. The experiment was repeated once.

A second experiment was conducted using an identical design, but comparing only agar plug chlamydospore inoculum of young (2-week-old) and old (6-month-old) cultures, between R/O water and the same creek water. Each treatment was replicated 16 times. Treatment plates were randomized and incubated at room temperature (19 to 21 °C). Germination was quantified at 96 hours as above. The experiment was repeated once.

Results

In the first experiment (8 months vs. 4 weeks), chlamydospore germination ranged from 7 to 78 percent (figs. 1, 2). There was no significant effect of inoculum type on chlamydospore germination; however, age, aqueous media type, and the interaction between age and aqueous media significantly affected germination ($F_{1,45} = 3.79$, $p = 0.058$; $F_{2,45} = 21.44$, $p < 0.001$; $F_{2,45} = 10.06$, $p < 0.001$, respectively). In creek water and R/O water, germination frequencies for old and young chlamydospores were similar (62 to 78 percent), but in PAR germination, frequencies of young chlamydospores were reduced relative to old ones (fig. 2). In the second experiment (6 months vs. 2 weeks), chlamydospore germination ranged from 34 to 68 percent (fig. 3). Age and aqueous media type significantly affected germination ($F_{1,55} = 7.73$, $p = 0.007$; $F_{1,55} = 16.17$, $p < 0.001$).

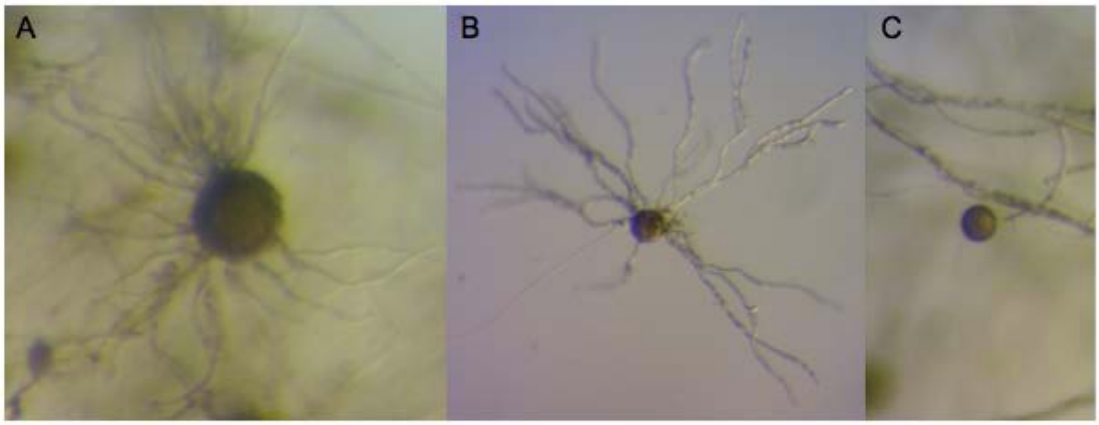

Figure 1—Chlamydospore germination at 24 hours. (A) Old (8-month) chlamydospore germinating in an agar plug submerged in creek water. (B) Old (8-month) chlamydospore from a suspension germinating in PAR broth. (C) Young (1-month) chlamydospore in an agar plug not germinated.

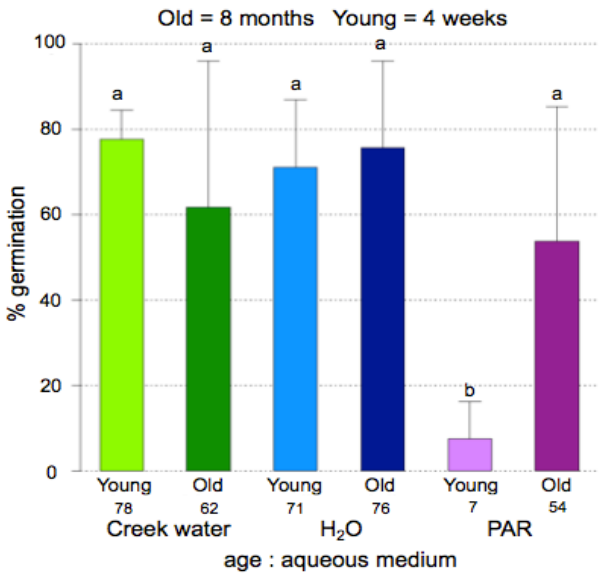

Figure 2—Chlamydospore germination (percent) among treatments after 24 hours. Data for agar plug and suspension treatments were pooled among age and liquid media combinations. Letters represent significant differences among means displayed below bars (Tukey's HSD, $p < 0.0001$).

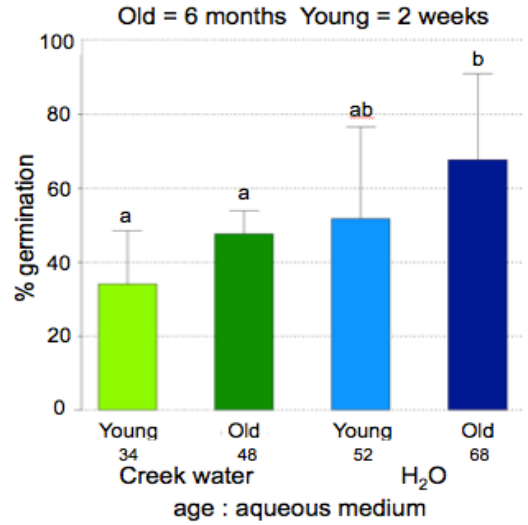

Figure 3—Chlamydospore germination (percent) among treatments at 96 hours. Letters above bars represent significant differences among means displayed below bars (Tukey's HSD, $p < 0.05$).

Discussion

Chlamydospore germination in R/O water and creek water was greater than has been reported previously for *P. ramorum* (Smith and Hansen 2008, Tooley et al. 2008). Germination in corn meal agar + PAR was similar to levels reported by Tooley et al. (2008) working with 11- to 12-week-old chlamydospores, in which 40 to 47 percent of chlamydospores germinated on V8 agar + PARPH. Germination was somewhat lower for comparable treatments in the second experiment relative to the first (figs. 2, 3), perhaps due to the younger chlamydospore ages. Chlamydospore age has been shown to influence germination frequency of other *Phytophthora* species (Tsao 1971), and chlamydospore wall thickness, which is a function of age, has been related to germination of *P. ramorum* (Smith and Hansen 2008).

Interestingly, the germination frequency of young chlamydospores incubated in creek water and R/O water was significantly greater than that of young chlamydospores incubated in corn meal broth + PAR. These results demonstrate that exogenous nutrients are not required for chlamydospore germination, but suggest that some component of corn meal broth + PAR (antibiotics and/or nutrients) reduces the germination of young (4-week-old) chlamydospores. Germination of older chlamydospores incubated in corn meal broth + PAR was not affected, implying that germination ability may be related to chlamydospore maturity.

Literature Cited

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