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Temperature effects on growth of fungal symbionts of the western pine beetle, *Dendroctonus brevicomis*

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ARTICLE INFO

Article history:
Received 29 September 2014
Revision received 4 April 2015
Accepted 10 May 2015
Available online 1 July 2015
Corresponding editor:
Ann E Hajek

Keywords:
Bark beetle
Ceratocystiopsis brevicomi
Entomocorticium
Mutualism
Symbiosis
Temperature tolerance

ABSTRACT

Differences in temperature ranges and optima among poikilothermic partners in symbioses can have profound effects on their interactions and stability. In this study, we investigated how the two mutualist mycangial fungi (*Ceratocystiopsis brevicomi* and *Entomocorticium* sp. B) associated with the western pine beetle, *Dendroctonus brevicomis*, respond to temperature *in vitro*. Little variability in growth rate at the various temperatures tested occurred among isolates of *C. brevicomi* either within or among sites. In contrast, *E.* sp. B exhibited highly variable responses to mid-range temperatures among sites and within some sites, and, unlike *C. brevicomi*, grew not at all or only very poorly at the highest and lowest temperatures tested. This variability affected both optimal temperature and maximum growth rate. The high variability in response to some temperatures among isolates of *E.* sp. B in some populations indicates that the ability to capture spatial and nutritional resources can vary greatly within this species which may have considerable impact on the outcome of both inter- and intra-specific competition among the fungi within trees and the short- and long-term dynamics of the fungi with the host beetle.

Introduction

Several species of bark beetles (Coleoptera: Curculionidae, Scolytinae) in the genus *Dendroctonus* are associated with mutualistic fungi that provide nutrient supplementation to their host insects (Six and Paine, 1998; Ayers et al., 2000; Bleiker and Six, 2007; Cook et al., 2010). These fungi are transported from tree to tree by adult beetles in specialized structures (mycangia) maintaining the continuity of the association among generations (Whitney and Farris, 1970; Six, 2003). *Dendroctonus*-mycangial fungus symbioses typically involve two, or less commonly, three fungal partners specific to each beetle host (Lee et al., 2005; Rice and Langor, 2009; Six, 2012). The various fungal partners exert differential effects on the host insect's fitness with one typically superior to the other(s) (Goldhammer et al., 1990; Coppedge et al., 1995; Bleiker and Six, 2007). The relative prevalence of each fungus is, therefore, predicted to affect the host beetle's population dynamics (Six and Bentz, 2007).

The factors that affect the relative prevalence of mycangial fungi within and among populations of a host beetle remain poorly studied. However, recent work indicates that temperature plays a major role (Hofstetter et al., 2006; Six and Bentz, 2007; Rice et al., 2008; Addison et al., 2013). This should not be surprising as fungi are poikilotherms, with their growth rates and sporulation highly dependent upon temperature. The effect of temperature on *Dendroctonus*-mycangial fungus symbioses has only been studied in detail in one system. The

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http://dx.doi.org/10.1016/j.funeco.2015.05.010

relative prevalence of the two fungi associated with developing and dispersing *Dendroctonus ponderosae* (mountain pine beetle) (a univoltine beetle) is highly dependent upon temperature (Six and Bentz, 2007; Addison et al., 2013). One partner, *Grosmannia clavigera*, grows best and dominates under cooler conditions, while the opposite is true for the other partner, *Ophiostoma montium*. These differences lead to dissimilar rates of growth and resource capture by the fungi in the host tree over the larval development period as well as differential rates of dissemination when brood adults disperse (Six and Bentz, 2007). It also results in some beetle populations being dominated by one fungus, although both are typically present (Six and Bentz, 2007). The temperature dependent nature of this symbiosis is predicted to provide a mechanism for the maintenance of its multipartite structure (Addison et al., 2013); temperature fluctuations within and among years constraining any one fungus from completely dominating over time. It also implies that global warming has the potential to decouple the symbiosis as one or both fungi are marginalized or lost as conditions become too warm to support their growth and/or reproduction (Addison et al., 2013).

Temperature also likely plays an important role in the dynamics of other *Dendroctonus*-fungus symbioses. For example, the two mycangial fungi associated with *D. frontalis* (southern pine beetle) (a multi-voltine species with as many as seven generations a year) fluctuate in prevalence among generations over the course of a year in response to temperature (Hofstetter et al., 2006). An understanding of the effects of temperature on the symbionts is critical to understand how *Dendroctonus*-fungus symbioses have remained stable over evolutionary time, how environmental conditions influence short- and long-term fluctuations in symbiont prevalence, and hence beetle fitness and population dynamics, and how climate change may affect the continued persistence of these symbioses.

In this study, we investigated how the two mycangial fungi associated with *D. brevicomis* (western pine beetle) respond to temperature *in vitro*. This system is similar to the *D. ponderosae*-fungus system in that two mycangial fungi are associated with the beetle across its geographic range (Bracewell and Six, 2014). However, it differs in several important ways. Instead of feeding entirely within the phloem layer as larvae as does *D. ponderosae*, *D. brevicomis* feeds in phloem only briefly before moving into the outer bark. In addition, the mycangia of *D. ponderosae* are located on the mouthparts and are functional in both sexes (Whitney and Farris, 1970), while the mycangia of *D. brevicomis* are located on the anterior pronotum and are functional only in females (Whitney and Cobb, 1972; Paine and Birch, 1983). This indicates an independent origin of the structures and symbiosis with fungi. The fungi associated with the two beetles are also quite different. The fungi associated with *D. ponderosae* (*G. clavigera* and *O. montium*) are bionecrotrophic blue-staining ascomycetes in the Ophiostomatales. In contrast, *D. brevicomis* is associated with a non-staining saprotrophic ascomycete in the Ophistomatales, *Ceratocystiopsis brevicomi*, and a saprotrophic basidiomycete, *Entomocorticium* sp. B, in the Corticiales (Hsiau and Harrington, 1997, 2003).

Only one paper to date has looked at growth rates of the fungi associated with the western pine beetle. Davis et al. (2010) compared growth of isolates of the two mycangial fungi from a population of the beetle in California with isolates from a population in Arizona. Growth rates were determined from radial growth measurements, which may be problematic with *E.* sp. B given the erratic rather than uniform growth margins it produces in culture. The high end of temperature tolerances for the fungi was also not tested; however, it was at higher temperatures that differences in the growth rates of the two fungi began to become apparent.

Our main objective was to investigate whether the two mycangial fungi associated with *D. brevicomis* differ in response to temperature which may influence their prevalence and thus their interaction with the beetle in nature. Furthermore, we were interested in determining if there was any evidence of geographic variation in response between isolates from different parts of the western pine beetle's range. Fungi from different populations likely encounter different biotic and abiotic conditions which may shape genetic variation and fungal response (Mueller et al., 2011). For our study, we build on Davis et al. (2010) and use a larger number of isolates from a broader geographic range of sites. We measured area rather than radial growth to more accurately capture growth rates and explicitly test if responses to temperature by the two symbiotic fungi vary among populations.

Materials and methods

Fungal isolates

Ceratocystiopsis brevicomi and *E.* sp. B isolates used in our study originated from isolations from the mycangia of western pine beetle in Bracewell and Six (2014). Four isolates of *C. brevicomi* and *E.* sp. B from each of four geographically distinct sites (SB, SierraII, LA, SU) extending along a north-south latitudinal gradient from British Columbia to southern California were selected for use in growth studies (Fig 1). Methods used to initially culture fungi from beetles and information about the beetle collection method and location are described in Bracewell and Six (2014). Cultures of *C. brevicomi* were purified using single spore isolations by removing a small mass of spores from each culture and placing them into 1.5 ml microcentrifuge tubes containing 150 μl of sterile water. The tubes were agitated for 1 min to separate the spores and the suspension was then poured onto the surface of 2 % malt extract agar (MEA) in individual Petri dishes. Once spores germinated, single spores were lifted from the culture medium surface and placed individually onto new medium for growth. *Entomocorticium* sp. B does not readily produce spores in culture; therefore, single hyphae were removed from the growing edge of a culture and transferred to new medium.

Temperature effects on growth of E. sp. B *and* C. brevicomi

In growth rate studies it is important that cultures are in an actively growing state. When a fungus is inoculated onto new medium, there is initially a lag period when growth is absent or occurs only slowly (Lilly and Barnett, 1951). The length of this lag phase is affected by the age and vigor of the existing culture and the conditions under which it has been stored.