Effects of water potential and solute on the growth and interactions of two fungal symbionts of the mountain pine beetle

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ABSTRACT

We investigated the effect of water potential (WP) on the growth of, and interaction between, two ophiostomatoid fungi, *Grosmannia clavigera* and *Ophiostoma montium*, associated with the mountain pine beetle (*Dendroctonus ponderosae*). The WP of malt extract agar was amended by adding potassium chloride (KCl) or sucrose. Growth of both fungi decreased with WP on KCl-amended media. Growth of *G. clavigera* also decreased with WP on sucrose-amended media, although growth was stimulated on these media compared to unamended treatments. Growth of *O. montium* remained relatively constant on sucrose-amended media, confounding the effect of WP on this species. Both fungi were able to colonize media occupied by the other species, but at a slower rate than on unoccupied media, indicating competition. In most treatments, *G. clavigera* grew faster than *O. montium* and colonized a greater area when the two fungi were inoculated concurrently but distant to one another on a Petri dish. However, when each fungus was inoculated adjacent to a 10-d-old well-established colony of the other species, *O. montium* colonized occupied media more effectively than *G. clavigera* considering the growth rate of each species alone. Thus, *G. clavigera* dominated primary (uncolonized) resources on most media, whereas *O. montium* was more effective in colonizing secondary (occupied) resources. The differential response of the two fungi to sucrose indicates that they may use different carbon sources, or use different carbon sources at different rates, in the tree. Fine-scale resource partitioning, differences in primary and secondary resource capture abilities, and the non-equilibrium dynamics in an attacked tree over time, could all act to promote the co-existence of two unit-restricted dispersers on a discontinuous resource.

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Introduction

The importance of competition in shaping ecological communities is well known. Competition occurs when one species negatively affects another by consuming a common limited resource (exploitation) or controlling access to a limited resource (interference) (Wicklow 1981). These types of competition are difficult to dissociate for many fungi because nutrient acquisition is often dependent upon competition for space for fungi (e.g. Boddy 2000). As a result, competition between fungi has been categorized as either primary resource capture (colonization of unoccupied habitat) or secondary resource capture

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(colonization of habitat that is already occupied) (Rayner & Webber 1984). Regardless of the terms applied, most research on competitive interactions has focused on plants growing at high densities and on animals competing for the same resource; comparatively less is known about the effect of competition on fungal communities (Shearer 1995).

Many fungi in the *Ophiostomataceae* (*Ascomycotina*) have symbiotic associations with phloeophagous bark beetles (*Coleoptera*: *Curculionidae*, *Scolytinae*). Often more than one species of fungus is associated with a single bark beetle species (reviewed in Six 2003a). The fungi are dependent on the beetle for transport to new trees that are either recently dead or moribund after being attacked *en masse* by beetles. Fungal spores are carried by adults on the exoskeleton or in mycangia (structures of the integument for transporting microorganisms) (Beaver 1989) and inoculated into tree tissues during construction of egg galleries in the phloem and inner bark. The fungi rapidly colonize the phloem and sapwood of trees successfully attacked by beetles (e.g. Reid *et al.* 1967; Solheim 1995; Bleiker & Six 2008). Teneral (young, sexually immature) adults acquire fungal spores just prior to dispersing from the natal host when pre-emergence they feed on thick layers of spores that commonly line the pupal chambers (Whitney 1971; Six & Paine 1998; Bleiker & Six 2007). Thus, sporulation in pupal chambers must coincide with the beetle's pre-emergence feeding in order for the fungi to be transported to the next host tree. Conditions change rapidly in trees following attack, which may affect sporulation (Mathiesen-Käärik 1960). Resources (e.g. nutrients and moisture) may also be limited by the time beetles are pre-emergence feeding (Klepzig *et al.* 2004; Kim *et al.* 2005; Bleiker & Six 2008) potentially affecting the ability of the fungi to sporulate in the pupal chambers. Limited resources in the tree and variation in resource use or requirements of different species of fungi (Mathiesen-Kaarik 1960) may affect the ability of fungi to compete for resources and sporulate in the pupal chambers.

In beetle-attacked trees, the ophiostomatoid fungal associates apparently share the same limiting resources, typically nutrients that are easy to assimilate (Seifert 1993). Thus, the fungi may be expected to compete with one another when growing together. Competition may result in species persisting on a resource at some equilibrium level, or in competitive exclusion where the victor is the species that can survive on the lowest level of a resource (e.g. Gause 1934). These classic outcomes of competition may be most applicable to systems where resources are, at least periodically (e.g. seasonally), replenished. However, a beetle-attacked tree is a discontinuous resource (or unit) for the fungi: resources within the tree decline over time, which results in non-equilibrium conditions (Schmit 1999). Once resources within a tree are consumed, the individual fungi that inhabit the tree will die because they inhabit a discrete resource (Rayner & Webber 1984). Ophiostomatoid fungi do not disperse vegetatively (e.g. using rhizomorphs or mycelium to locate new habitat patches); instead, they are unit-restricted dispersers (Rayner 1994) that rely on bark beetles for transport to the next suitable habitat. Thus, the challenge for the fungi is twofold: (1) capture sufficient phloem resources to maximize contact with insects in pupal chambers; and (2) coordinate sporulation in the pupal chambers with the beetle's pre-emergence feeding period, a time when resources (e.g. moisture) are most likely to be limited.

In this study, we examine the interactions between two ophiostomatoid fungi, *Grosmannia clavigera* (previously *Ophiostoma clavigerum*) and *Ophiostoma montium*, which are commonly associated with the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (e.g. Whitney & Farris 1970; Whitney 1971; Six 2003b; Bleiker & Six 2007). The mountain pine beetle is an eruptive, tree-killing bark beetle that causes significant economic losses in mature pine forests in western North America (Safranyik & Carroll 2006). The fungi may aid the beetle in overwhelming tree defences and conditioning phloem (e.g. detoxifying tree defence chemicals, altering water relations) for brood development (Reid *et al.* 1967; Ballard *et al.* 1982, 1984; Raffa & Berryman 1983; Owen *et al.* 1987; Solheim 1995). *G. clavigera* is described as moderately pathogenic and can tolerate lower oxygen conditions compared with *O. montium*, and may be the primary invader of tree tissues (Solheim 1995; Solheim & Krokene 1998). *G. clavigera* also grows faster than *O. montium* at temperatures between 3 and 22 °C; however, *O. montium* grows faster than *G. clavigera* at temperatures above 27 °C (Six & Paine 1997; Solheim & Krokene 1998; Rice *et al.* 2008). Both species appear to benefit the beetle through nutritional supplementation; however, *G. clavigera* confers greater benefits than *O. montium*. A larger brood was produced by insects developing with *G. clavigera* compared with *O. montium* (Six & Paine 1998), and *G. clavigera*-carrying beetles emerging from attacked trees were larger than beetles carrying *O. montium* (Bleiker & Six 2007). Given the difference in the magnitude of the benefits provided by the two fungi to the beetle, competitive interactions between the fungi could indirectly affect the beetle's fitness by altering the degree of contact the beetles have with each species.

The objectives of this study were two-fold: (1) determine the nature of the interaction between *G. clavigera* and *O. montium*; and (2) determine the effect of the water potential (WP) on the growth of each fungus and on interspecific fungal interactions. We tested the nature of the interaction between *G. clavigera* and *O. montium* by growing each fungus alone and with each other under controlled conditions in the laboratory. Because one of the most prominent changes in beetle-attacked trees over time is a decrease in moisture content, we tested for an effect of WP on the ability of each fungus to colonize unoccupied resources (primary resource capture) and resources already occupied by the other species (secondary resource capture) (Klepzig & Wilkens 1997; Rayner & Webber 1984).

Materials and methods

Fungal cultures

Cultures of *Grosmannia clavigera* and *Ophiostoma montium* used in the following experiments were isolated during a previous study (Bleiker & Six 2007) from the maxillae (includes the mycangia) of mountain pine beetles emerging from beetle-attacked lodgepole pine (*Pinus contorta* var. *latifolia*) trees. A representative culture, in terms of morphology and growth rate on 2 % malt extract agar (MEA), of each species was selected. Inocula used in the following experiments were 4 mm diam plugs taken from the actively growing margins of cultures maintained on MEA. All cultures and experiments