EFFECTS OF TREE PHYTOCHEMISTRY ON THE INTERACTIONS AMONG ENDOPHLOEDIC FUNGI ASSOCIATED WITH THE SOUTHERN PINE BEETLE

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Abstract—We examined the interaction between host trees and fungi associated with a tree-killing bark beetle, Dendroctonus frontalis. We evaluated (1) the response of four Pinus species to fungal invasion and (2) the effects of plant secondary metabolites on primary growth of and secondary colonization of three consistent fungal associates. Two of these fungi, Entomocorticium sp. A and Ophiostoma ranaculosum, are obligate mutualists with D. frontalis, and the third associate is a blue-staining fungus, O. minus, that is commonly introduced by beetles and phoretic mites. O. minus negatively affects beetle larvae and in high abundance can impact D. frontalis population dynamics. Size of lesions formed and quantity of secondary metabolites produced in response to fungal inoculations varied significantly among Pinus species. However, monoterpene composition within infected tissue did not significantly vary across treatments. While all eight tested metabolites negatively affected the growth rate of O. minus, only 4-allylanisole, p-cymene, and terpinene reduced the growth of the mycangial fungi. Surprisingly, growth rates of mycangial fungi increased in the presence of several secondary metabolite volatiles. O. minus out-competed both mycangial fungi, but the presence of secondary metabolites altered the outcome slightly. O. ranaculosum out-performed E. sp. A in the presence of dominant conifer monoterpenes, such as α- and β-pinene. Volatiles from the mycangial fungi, particularly E. sp. A, had a negative effect on O. minus growth. In general, phloem phytochemistry of particular Pinus species appeared to alter the relative growth and competitiveness of mutualistic and non-mutualistic fungi associated with D. frontalis. The outcome of interactions

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among these fungi likely has important consequences for the population dynamics of *D. frontalis*.

**Key Words**— *Dendroctonus*, *Ophiostoma*, *Entomocorticium*, monoterpenes, plant defenses, competition, mycelial interactions, antagonism, symbiosis, resin.

**INTRODUCTION**

The oleoresin system of pines is a primary mechanism of tree resistance against subcortical invasion by insects and pathogens (Paine et al., 1997; Trapp and Croteau, 2001). The physical properties of resin, particularly the quantity of resin flow, are important in deterring and excluding attacking insects such as tree-killing bark beetles (Smith, 1966; Raffa and Berryman, 1982; Christiansen and Horntvedt, 1983; Paine and Stephen, 1987; Franceschi et al., 2000). Alternatively, chemical properties of oleoresin are likely more important in disrupting the activity of insects within trees by altering the nutritional quality of subcortical tissues (Smith, 1963; Coyne and Lott, 1976; Hodges et al., 1985). Differences in the chemical composition and physical properties of oleoresin among pine species are thought to be related to differences in resistance to insects and pathogens (Hodges et al., 1979; Tisdale et al., 2003).

More specifically, differences in the oleoresin chemistry among tree species may indirectly affect tree-killing bark beetles by altering the colonization success (Christiansen, 1985; Raffa and Smalley, 1988), growth rate (Bridges, 1987; Ross et al., 1992), and competitiveness of fungi associated with beetles (Raffa, 2001). Mutualistic fungi are thought to enhance the access of beetles to phloem tissues either by helping kill the tree (pathogenic; Solheim, 1992; Paine et al., 1997), neutralizing secondary metabolites within phloem (Paine, 1984), or providing phloem nutrients directly or indirectly to progeny (Barras, 1973; Beaver, 1989). Additionally, mutualistic fungi that are antagonistic to, or resist displacement by, non-mutualistic fungi could contribute to the successful development of beetle larvae (Goldhammer et al., 1990; Klepzig and Wilkens, 1997).

Variation in primary phytochemistry (e.g., N, P, carbohydrates) and secondary phytochemistry (e.g., terpenes, phenolics) likely leads to differences in the relative success and abundance of mutualistic and non-mutualistic fungi (Raffa, 2001), which would influence beetle fitness and subsequently the initiation and speed of beetle population growth (Showalter and Filip, 1993; Trapp and Croteau, 2001). The growth of and interactions between beetle-fungal associates on substrates varying in primary and secondary phytochemistry, however, have not been thoroughly studied.

Here, we focus on three questions dealing with the effects of tree metabolites on the growth and competitiveness of fungi associated with bark beetles: 1. Do trees vary in their response to various fungi associated with bark beetles? 2. Are