PHENYLOXIDASE PRODUCTION BY *AMYLOSTEREUM AREOLATUM* AND ITS ROLE IN PATHOGENESIS IN LOBLOLLY PINE

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ABSTRACT

The woodwasp *Sirex noctilio* and its symbiotic fungus *Amylostereum areolatum* constitute an exotic pathosystem recently introduced into North America. Capable of killing living, healthy trees, *S. noctilio* has ravaged pine plantations of the Southern Hemisphere and poses an immediate threat to the conifer forests of the United States, notably the commercially important loblolly pine (*Pinus taeda* L.) forests of the Southeast.

Our investigations have shown that the fungus involved in the symbiosis, *A. areolatum*, produces enzymes capable of oxidizing the phenoloxidase substrates, ABTS, guaiacol, and syringaldazine, in solid media and ABTS in liquid media. The responsible enzymes are likely laccases, as well as one or more peroxidases. We hypothesize that:

1. *Amylostereum areolatum*, like many other white-rot fungi, produces laccase.

2. This laccase is involved in the pathogenicity of the fungus to loblolly pine.

We have developed and optimized a defined medium for culturing *A. areolatum* in the laboratory and are currently establishing growth curves for the fungus. Conditions have been established to increase dry weight of mycelium, as well as increase phenoloxidase enzyme production. We have also established a bioassay system with *P. taeda* seedlings to demonstrate susceptibility to *A. areolatum* infection.

To more completely investigate the role of specific phenoloxidases in the disease process, we are working to isolate *A. areolatum* mutants that do not express these enzymes. Subsequent work will focus on characterizing the enzymes involved, as well as elucidating the specific role played by these enzymes in the disease process. Having a better understanding of the specific interactions between individual players in this pathosystem will enable us to pursue multiple approaches to subverting key interactions, which should provide a more powerful approach to tree protection.