PHENOLIC COMPONENT OF LOBLOLLY PINE DEFENSE RESPONSE TO BLUE-STAIN FUNGI ASSOCIATED WITH ROOT-FEEDING BEETLES

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Southern pine species are integral components to southeastern forests, comprising a significant percentage of the forested lands. Among these species, loblolly pine (*Pinus taeda* L.) is the dominant timber species grown commercially, thus being extremely important for both the economy and the ecology of southeastern regions. Extensive studies on pathogens and insect pests affecting this species have been conducted, but for obvious reasons, most of them have focused on major tree-killing species, such as the southern pine beetle (SPB) [*Dendroctonus frontalis* Zimmerman (SPB)] and its associated fungal complex. However, many other beetles and associated fungi colonize the species, and are still worth being investigated, as they too are integral components of the loblolly pine planation system. The root-feeding beetle system and associated fungi, for instance, have largely been overlooked in the past, and only recently become of interest (Coyle et al. 2015, Eckhardt et al. 2007). These beetles are generally secondary invaders, colonizing tree hosts only after their defenses have already been weakened (Matusick et al. 2013). Additionally, their associated ophiostomatoid fungi, which are carried externally on the exoskeleton, are usually considered weak pathogens (Jacobs and Wingfield 2001). However, there is still relatively too little known about the system, and in particular, nothing is known about how loblolly pine responds to colonization by the fungal species.

In this study, we focused on the phenolic metabolisms of loblolly pine, which, together with the terpenoid component, is one of the main defense mechanisms of the species. In fact, while terpenoids of loblolly pine have been widely explored, surprisingly, phenolics are currently totally unexplored. Objectives of the study were: (1) to characterize the constitutive phenolic profile of loblolly pine phloem, and (2) to investigate the qualitative and quantitative induced responses of the phenolic metabolism to inoculation with blue-stain fungi commonly associated with root feeding beetles in the region.

Using a nested randomized complete blocked design, 45 mature loblolly pines were selected from a planted stand in Whitehall Forest (Athens, GA). Trees were assigned to one of five different treatments: (1) inoculation with *Grosmannia alacris*, (2) *G. huntii*, (3) or *Leptographium profanum*, (4) sterile wounding and (5) non-wounded control. For inoculations, four plugs of phloem were removed from each tree with a cork borer, and then substituted with another bark plug that had been previously sterilized and then colonized by the fungi (fig. 1). All fungal isolates used in this study had been isolated from the surface of root-feeding beetles collected in the area. For sterile wounding, the plug was not replaced after removal, while control trees were left unwounded. Plugs collected at the time of treatment (September 2017) were used for the characterization of the constitutive phenolic profile. Four weeks after treatment, the outer bark surrounding inoculation sites was removed and vertical length of each induced lesion (fig. 2) was measured. Phloem tissues surrounding the inoculation sites and sterile wounding sites were collected and immediately stored in liquid nitrogen. Also at this time, phloem samples from non-wounded control trees were collected. All samples were then transported to the lab and stored at -80˚C until further processing.

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Figure 1—Loblolly pine bark plugs previously sterilized and then colonized by one of the fungal species used in this experiment. Inoculations consisted in removing bark plugs from the experimental trees and substituting them with colonized ones. (courtesy photo by Caterina Villari)

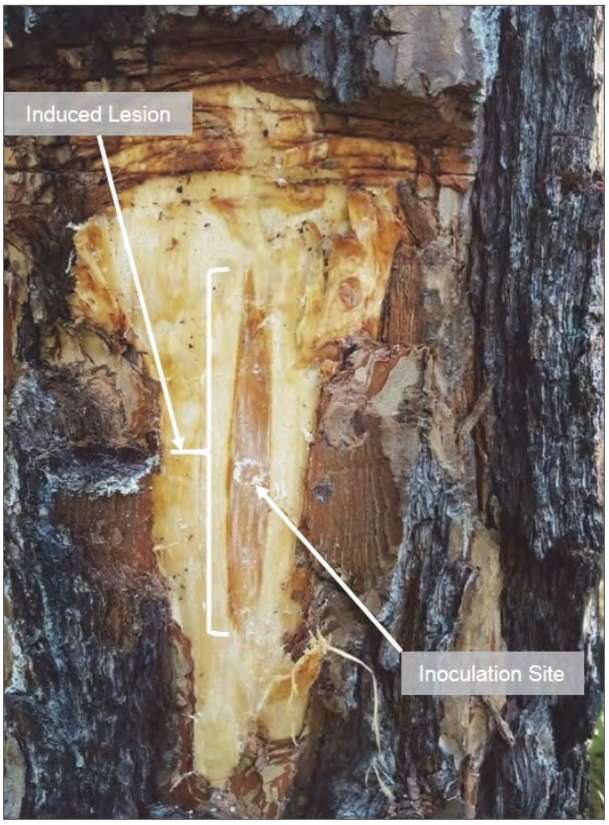

Figure 2—Fungal induced lesion in loblolly pine phloem. Lesions were measured in centimeters and used to estimate virulence of pathogens. (courtesy photo by Zack Parker)

Collected tissues were ground to powder in liquid nitrogen, and phenolics were extracted in methanol. Extracts are currently being analyzed using a combination of ultra-high pressure liquid chromatography—diode array detector (UHPLC-DAD) and high-pressure liquid chromatography—mass spectrometry (HPLC-MS) approaches.

Preliminary results show that all fungal inoculations produced significantly longer lesions than sterile wounding alone, and that inoculations with *G. huntii* produced the longest lesions. Additionally, preliminary observations of the phenolic profile indicate distinct quantitative changes in specific compounds after inoculation. Results from this study will provide valuable insights into the phenolic response of loblolly pine to fungal infections. Findings will also allow for further investigations regarding the effects of those phenolic compounds most responsive to the fungal induction on the survival and fitness of the same inoculated fungal species.

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