A Comparison of Host Response in the Roots and the Stem to Various Root Pathogenic Fungi

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Abstract

Premature mortality has been observed in two southern pine species, loblolly pine (*Pinus taeda*) and longleaf pine (*Pinus palustris*), two of the most important commercial forest species in the southeastern United States. The ecosystems of both loblolly and longleaf pine are also very diverse, supporting many endangered and sensitive organisms. The observed large-scale mortality negatively impacts not only timber production but the ecosystem as well. One factor contributing to this mortality is the group of root-inhabiting pathogenic fungi, particularly those of the genus *Leptographium*. The relationship between these fungi and southern pines is relatively unknown. The purpose of this study is to better understand the relationship between these pathogenic fungi and their hosts, and to compare two inoculation methods. This purpose will be addressed by studying the ability of these fungi to produce an infectious disease in southern pines (its pathogenicity) by observing the host's reaction to the pathogens, and by comparing the response of the host in the stem and roots. The main objective of this study is to observe and characterize host response differences between root and stem wound inoculations.

Introduction

Pine decline is an occurrence that is believed to result from a combination of abiotic factors, such as stand age, topography and soil condition, as well as biotic ones, such as insects or fungi. Due to the widespread planting of loblolly pine throughout the southeastern U.S, primarily for timber production, loblolly pine decline is the most prevalent. However, decline has also been observed in longleaf pine[1]. *Leptographium* spp. are a genus of fungi that have been found in association with the roots of trees experiencing decline[2]. When testing the pathogenicity of these fungi, stem inoculations are the most common method[2,3,4]. However, due to these fungi commonly being vectored directly into the roots by bark beetles[5], it is hypothesized that a more accurate measure of the virulence would be to test by directly inoculating into the root tissue.

Fig 2a - Root Harvest

Fig 2b - Stem Harvest

Fig 1 - Inoculated stems after 8 weeks (Left: *L. terebrantis;* Right: *L. serpens*)

Methods

Inoculum of the 5 fungal species (*L. procerum, L. terebrantis, L. serpens, L. huntii and Heterobasidion annosum*) was prepared and plated on pine twig agar. 50 loblolly pine and 50 longleaf pine trees were selected for the experiment, with the height and diameter at breast height of each tree recorded. Two fungal treatments, along with one wound control treatment, were inoculated on to each tree. Also, three roots were selected on each tree, with one fungal or wound control treatment per root. Each tree was inoculated with the same two fungi in the bole as well as the roots. For the stem inoculations, the bark was removed using a bark scraper, after which a small section of the cambium was removed. A fungal plug was inserted using a sterile spatula, after which the cambium was replaced, and the area was sealed with duct tape. Each root also had a small section removed in which a fungal plug was inserted, re-covered, and sealed with duct tape. The roots were then re-covered with soil.

After 8 weeks, the roots were uncovered, harvested and brought to the lab, where the root diameter, lesion depth and occluded area will be measured, as well as tissue samples taken for attempted re-isolation of the fungi. For the stems, the trees were felled and a section approximately one foot above and below the inoculated area was removed. The bolts of wood were then transported back to the lab for the lesion depth, tissue collection and occluded area measurements. These methods were performed once each in consecutive years (2008 and 2009)

Fig 3 - Inoculated roots after harvest with occluded tissue (from left: *L. huntii, L. serpens, L. procerum*)

Fig 4 – Depth of lesion (clockwise from top right: stem with *L. terebrantis*, root with *H. annosum,* root with *L. serpens*)

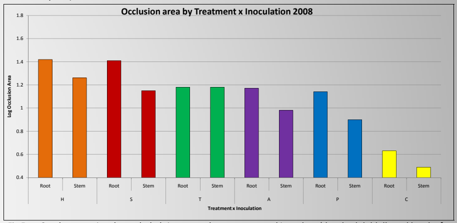

Fig 5a – Graph comparing the occluded tissue area by treatment and inoculum (data both loblolly and longleaf; 2008)

Results

Figure 5a shows the 2008 data of occluded area by both treatment and fungi species. The host response appears to be greater in the root tissue than in the bole for all species, with the exception of *L. terebrantis*. Figures 5b and 5c show the occluded area by both treatment and fungi species for 2009, respectively. The graph displaying the inoculations on loblolly pine shows the same general trend as the 2008 graph. However, the graph for longleaf pine displays no discernable trends.

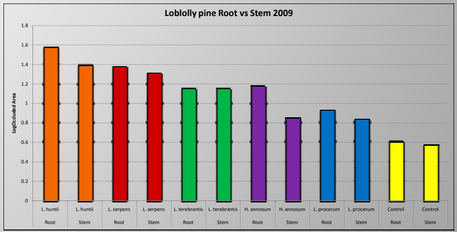

Fig 5b – Graph comparing the occluded tissue area by treatment and inoculum, for loblolly pine (2009)

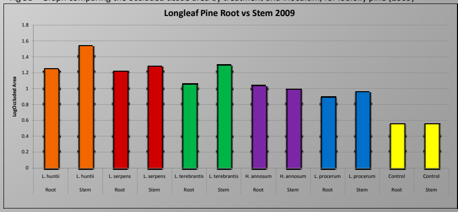

Fig 5c – Graph comparing the occluded tissue area by treatment and inoculum, for longleaf pine (2009)

References

1. Otrosina, William J., Bannwart, Diana, and Roncadori, Ronald W.. "Root-infecting fungi associated with a decline of longleaf pine in the southeastern United States." Plant and Soil 217(1999): 145-150. Print.
2. Nevill, R. J, Kelley, W.D, Hess, N. J, and Perry, T. J. "Pathogenicity to Loblolly Pines of Fungi Recovered from Trees Attacked by Southern Pine Beetles." Southern Journal of Applied Forestry. 19(1995): 78-83. Print.
3. Eckhardt, L. G., Jones, J. P., and Klepzig, K. D. 2004. Pathogenicity of *Leptographium*species associated with loblolly pine decline. Plant Dis. 88:1174-1178.
4. Matusick, G., Eckhardt, L. G., and Enebak, S. A. 2008. Virulence of *Leptographium serpens*on longleaf pine seedlings under varying soil moisture regimes. Plant Dis. 92:1574-1576.
5. Zhous, X. D., De Beer, W., Wingfield, B. D. and Wingfield, M. J. (2002). "Infection sequence and pathogenicity of *Ophiostoma ips, Leptographium serpens* and *L. lundbergii* to pines in South Africa." In: *Fungal Succession* (eds. K. D. Hyde ad E. B. G. Jones). Fungal Diversity 10: 229-240

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