The Effects of Nicaraguan Ophiostomatoid Fungi on Southern Pines.

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Abstract

Decline is a progressive loss of vitality and is a natural part of the life cycle of all higher organisms (Sinclair and Lyon 2005). In the district of Nueva Segovia in Nicaragua pine species are being affected by decline diseases. Two previously unknown ophiostomatoid species were recovered from declining trees (Eckhardt et al 2008). The bark beetle *Dendroctonus approximates* Dietz was shown to be in association with this *Leptographium* spp. Fruiting structures of these ophiostomatoid species contain sticky spores, which increses the possiblity of being vectored by Scolytides (Jacobs et al 2006). However, information is lacking concerning either *D. approximates* or the Nicaraguan ophiostomatoids. In March of 2009, isolates of the two unknown Nicaraguan ophiostomatoid species, *Leptographium procerum* (Kendrick) Wingfield, and *Leptographium terebrantis* Barras and Perry were cultured on 2% malt extract agar. Inoculations began in the spring of 2009, in order to obtain the optimum temperature for fungal growth. Fifty slash (*Pinus elliottii* Engelm), loblolly (*Pinus taeda* L.), and longleaf pine (*Pinus palustris* Mill) seedlings were inoculated with one of the selected fungal isolates.

Figure 1: Loblolly and Longleaf pine growing in a greenhouse without being inoculated.

Introduction

In 2002, over 30,000 ha of pine forest was lost in the district of Nueva Segocia in Nicaragua due to *Dendroctonus frontalis* Zimmermon. Shortly after, wildfires erupted in the region eliminating much of the natural pine regeneration. Since then, survival of pine species has continued to decrease. Trade with Nicaragua and the United States increases the threat that the pathogen could be transported into the forests in the United States.

The objectives of this research are to assess the impact of the ophiostomatoid spp. from Nicaragua on southern pine species and to identify factors that lead to poor survival of pine species in Nicaragua.

Two hypotheses are proposed: 1) Ophiostomatoid spp. isolated from Nicaragua are more pathogenic to native southern pines than that of either *Leptographium procerum* (Kendrick) Wingfield or *Leptographium terebrantis* Barras and Perry and, 2) The ophiostomatoid spp. isolated from Nicaragua are pathogenic to slash (*Pinus elliottii* Engelm), loblolly (*Pinus taeda* L.), and longleaf pine (*Pinus palustris* Mill) seedlings.

Figure 2: Stump of an infected pine tree from Nicaragua as can be seen by the dark staining.

Figure 3: Perithecia collected from *Pinus oocarpa* decline in Nicaragua.

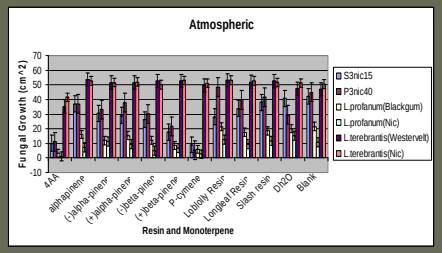

Figure 4: Results of the atmospheric test showing fungal growth area in centimeters squared after seven days of growth.

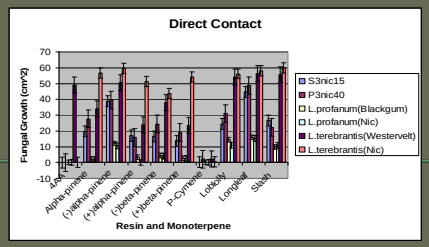

Figure 5: Results of the direct contact test showing fungal growth area in centimeters squared after seven days of growth.

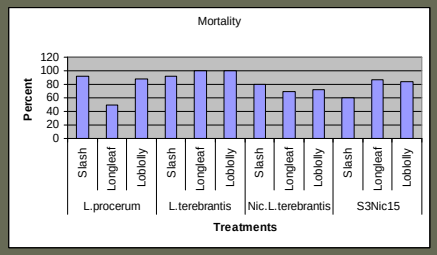

Figure 6: Percent mortality of pine seedlings inoculated with fungi after ten weeks of growth.

Methods

Experiment 1: Pine Seedling Inoculation.

In March of 2009, the two previously unknown Nicaraguan ophiostomatoid species, *L. procerum,* and *L. terebrantis* were placed on 2% MEA and allowed to grow for a period of eight to ten days. Fifty seedlings of each of the tree species were inoculated with one of the selected fungal isolates. After ten weeks the pine seedlings were removed from the soil and the measurements were taken. There was a total of two repetitions resulting in twenty five seedlings of each of the tree species being inoculated at a time.

Methods Continued

Experiment 2: Effects of Oleoresins and Monoterpenes.

Part 1: Direct Contact. Resin was collected from mature slash, loblolly, and longleaf pine from Autugua, Co. Alabama. Along with 4-allylanisole, P-cymene, alpha-pinene, (-)alpha-pinene, (+)alpha-pinene, (-)beta-pinene, and (+)beta-pinene, one milliliter of each of these compounds was evenly spread over Potato Dextrose Agar (PDA) in glass petri dishes. The fungal cultures used in this experiment were two cultures of the previously unknown Nicaraguan *Ohpiostoma spp., Leptographium terebrantis* isolated from a native pine tree, *Leptographium terebrantis* isolated from Nicaragua, *Leptographium profanum* isolated from Nicaragua, and *Leptographium profanum* isolated from a Blackgum. Each dish was sealed with Parafilm and allowed to grow being measure at day 3, day 5, and day 7.

Part 2: Atmospheric. The culture plates for this experiment were plated in a similar fashion as above with the exception that no chemical treatment was used on the plates and the petri dishes were plastic. Two plates of each fungal species (twelve in total) were placed into 3.79 liter paint cans with wire racks. Each paint can contained two milliliters of one chemical treatment placed into a glass petri dish below the wire rack. Each treatment was repeated three times for a total of 36 paint cans (this experiment also had a distilled water control as well as a blank control). The culture plates were allowed to grow for a period of seven days before measurements were taken.

Figure 7: *Pinus oocarpa* in Nicaragua undergoing decline.

Figure 8: Picture of *Dendroctonus approximates.*

Results

Figure 6 shows the percent mortality rate of pine seedlings inoculated from experiment one. *L. terebrantis* which was isolated form a native pine has the highest mortality rate for all three pine species. S3Nic15, which is an isolate of the unknown Nicaraguan *Ophiostoma* spp. shows to have a higher mortality rate than *L.procerum* however, it does not seem to be as pathogenic as either of the *L. terebrantis* isolates.

In experiment two, 4-allylanisole has been shown to be the greatest growth inhibiter two each of the fungal isolates in both the direct contact experiment (Fig. 4) and the atmospheric experiment (Fig.5) followed by P-Cymene. Both of the *L. terebrantis* isolates showed to be the most pathogenic out of all of the isolates. S3Nic15 and P3Nic40, two isolates of the unknown Nicaragua *Ophiostoma* spp., are shown to be more pathogenic than that of either of the *L. profanum* isolates, which were almost inhibited altogether.

Work Cited

Eckhardt, L.G., R.D. Menard, J.D. Ward, A. Sediles.2008. Evaluation of Pine Mortality in the Forests of Nicaragua. Summery.

Sinclair, W. A., H. H. Lyon. 2005. Diseases of Trees and Shrubs. 2nd edition. Cornell University Press. Ithaca, New York. pp.460

Zipfel, R. D., Z. Wilhelm de Beer, K. Jacobs, B. D. Wingfield, M. J. Wingfield. 2006. Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma.* Studies in Mycology. 55: 75-97.