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## ROOT-INHABITING BARK BEETLES (COLEOPTERA: CURCULIONIDAE) AND THEIR FUNGAL ASSOCIATES BREEDING IN DYING LOBLOLLY PINE IN ALABAMA

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Root feeding bark beetles in the genus *Hylastes* (Coleoptera: Curculionidae: Scolytinae) commonly carry ophiostomatoid fungi (Ophiostomatales: Ophiostomataceae) and collectively contribute to root disorders of *Pinus* species around the world (Jacobs & Wingfield 2001). One of the most damaging root disorders is black-stain root disease of conifers in the Western United States, caused by the fungal species *Leptographium wageneri* Kendrick and its primary beetle vector *H. nigrinus* (Mannerheim) (Witcosky et al. 1986). Recently, *Hylastes salebrosus* Eichhoff and *H. tenuis* Eichhoff have been associated with loblolly pine (*Pinus taeda* L.) decline (Eckhardt et al. 2007), which is considered an emerging forest health issue in the southeastern United States (Eckhardt et al. 2010).

Loblolly pine decline (LPD) is a tree disease complex that is characterized by symptoms that include thinning tree crowns, growth reductions (Eckhardt et al. 2007) and premature mortality in localized areas (Brown and McDowell 1968). Evidence suggests the complex is the result of interacting abiotic (Eckhardt & Menard 2008) and biotic (Eckhardt et al. 2007) stress factors. *Hylastes* species vector a variety of root-infecting ophiostomatoid fungi (Klebzig et al. 1991, 1995; Eckhardt et al. 2007; Zanzot et al. 2010) which are pathogenic to loblolly pine (Matusick & Eckhardt 2010; Matusick et al. 2011). *Hylastes* beetles and their associated fungi are thought to collectively contribute to LPD by causing root damage through fungal infection and insect feeding (Eckhardt et al. 2007). Root and lower stem feeding beetle species (Coleoptera: Curculionidae) including *H. salebrosus* and *H. tenuis* are found in increased numbers within LPD-affected stands, compared to healthy stands (Eckhardt et al. 2007, Menard 2007). A recent study has found *Hylastes* to be the most common bark beetle genus observed in loblolly pine stands in central Alabama (Thompson 2011), illustrating their dominance in this ecosystem. Despite their dominance and potential for damage in loblolly pine, the breeding behavior of *Hylastes* species in the southeastern United States is not well understood. In the current study, observational methods were used to investigate the potential for

*Hylastes* breeding in loblolly pine across 3 crown condition classes (healthy, dying, dead). Additionally, the relationship between ophiostomatoid species, their *Hylastes* species vectors, and tree condition were investigated.

Loblolly pine stands across central Alabama have experienced high mortality rates, characterized by scattered stand mortality with a distinct lack of above-ground pests (Brown & McDowell 1968). During an investigation of one stand in May 2008 in central Alabama, dying trees were found to be infested with root-inhabiting beetles and their associated ophiostomatoid fungi. Six trees were selected based on crown condition, including 2 with green, healthy crowns (healthy), 2 with severely chlorotic and thinning crowns (dying), and 2 with red crowns (dead). Two primary lateral roots were randomly selected and excavated to approximately 3 meters from the root collar. Roots were removed from trees and carefully dissected. All insects, including immature stages, were collected from roots and adult beetles were identified to species. Root tissue was obtained from each brood gallery for the isolation of ophiostomatoid fungal species using methods described in Eckhardt et al. (2007). Following surface sterilization, tissue was placed on CSMA (malt extract agar containing 800 mg/L of cycloheximide and 200 mg/l of streptomycin sulfate) selective nutrient agar (Jacobs & Wingfield 2001). Each adult insect was also rolled on CSMA to recover ophiostomatoid fungi from insect bodies (Zanzot et al. 2010). Isolated ophiostomatoid fungal species were identified using morphological characters and established keys. Unknown isolates were sequenced and confirmed as new species by M. Wingfield (Forestry and Agricultural Biotechnology Institute, Pretoria, South Africa).

A total of 157 adult beetles (Coleoptera: Curculionidae) were collected from roots, most from dying trees (Table 1). A majority of the beetles collected were *Hylastes* species, including a total of 113 *H. salebrosus* (all from dying trees) and 34 *H. tenuis* (31 from dying and 3 from dead trees). In addition, *H. salebrosus* larvae and pupae were collected from dying trees. Other adult insect species collected include the black turpentine beetle

TABLE 1. THE TOTAL NUMBER OF BEETLES COLLECTED AND BEETLES COLLECTED FOUND TRANSPORTING OPHIOSTOMATOID FUNGI IN 6 LOBLOLLY PINE TREES REPRESENTING 3 HEALTH CONDITIONS.

Tree Health Class	Tree #	Adult beetles (#)	Beetles vectoring fungi (#)
Healthy <sup>1</sup>	1	0	0
	2	0	0
Dying <sup>2</sup>	3	26	19
	4	125	50
Dead <sup>3</sup>	5	3	3
	6	3	2
Total		157	74

<sup>1</sup>Sample trees with predominantly (>90%) healthy foliage (dark green).

<sup>2</sup>Sample trees with predominantly (>70%) dying foliage (pale green and yellow).

<sup>3</sup>Sample trees lacking most (>90%) foliage, with the remaining being dead (red).

(*Dendroctonus terebrans* (Olivier) (1-dying and 3-dead), and *Xyleborus pubescens* Zimmerman (6-dying trees). This examination is the first to observe and provide evidence for *Hylastes* species breeding in loblolly pine roots and supports previous reports of *Hylastes* species breeding behavior in other, similar ecosystems (Blackman 1941; Stoakley 1968; Witcosky & Hansen 1985).

Adult beetles were commonly found transporting ophiostomatoid fungal species, including 47% of all beetle species, 40% of *H. salebrosus*, and 70% of *H. tenuis*. Collectively, insects transported a total of 5 ophiostomatoid species. *Leptographium terebrantis* was most commonly transported (23% of all beetles). Additional species in order of frequency included, *Grosmannia huntii* (R.C. Rob. Jeffr.) Zipfel, Z. W. de Beer & M. J. Wingfield (*L. huntii* M. J. Wingfield) (19%), *L. procerum* (6%), and *L. serpens* (Goid) M. J. Wingf. (1%). Seven insects transported more than one fungal species. In a similar loblolly pine stand in central Alabama, a large-scale trapping experiment found that *H. salebrosus* and *H. tenuis* transported fungal species at slightly higher rates than those reported here (Eckhardt et al. 2007). With respect to the beetle-fungal association, data from this research supports conclusions made by Zanzot et al. (2010), that ophiostomatoid fungi are likely facultatively associated with *Hylastes* vectors in the southeastern United States.

Ophiostomatoid fungi were predominately isolated from dying and dead trees (Table 2). *Leptographium terebrantis* and *G. huntii* were most frequently isolated including from root tissue and beetles from both dead and dying trees. *Leptographium procerum* was infrequently isolated from dying trees and was the only fungal species

isolated from healthy tree tissue, which supports previous reports (Zanzot 2010). *Leptographium serpens*, which was infrequently isolated from insects, was not isolated from root tissue. Additionally, one undescribed *Ophiostoma* species was infrequently isolated from tissue during sampling.

The findings reported here represent the first observations of *Hylastes* species breeding in roots of loblolly pine. Up until this point, the tissue used for brood development of *Hylastes* species in the southeastern United States has only been hypothesized. These observations have implications on the LPD cycle by confirming dying trees contribute to increasing *Hylastes* populations, suggesting a positive feedback cycle with regards to LPD in severely affected stands. These observations, coupled with previous findings from elsewhere (Witcosky & Hansen 1985) also suggest *Hylastes* species development may be restricted to trees in a dying condition. Additional research is needed to determine the root environmental conditions required for *Hylastes* species development as well as to determine the potential for *Hylastes* species to invade and successfully reproduce in healthy trees, particularly where populations are high.

#### SUMMARY

Root feeding bark beetles in the family Curculionidae and the ophiostomatoid fungi they vector contribute to root disorders of *Pinus* species around the world. In the southeastern United States root feeding beetles in the genus *Hylastes* (Coleoptera: Curculionidae), including *Hylastes salebrosus* and *Hylastes tenuis*, are associated with stands of *Pinus taeda* experiencing premature mortality. Despite this, direct evidence of *Hylastes* species breeding, with their associated ophiostomatoid fungi, in *Pinus taeda* has not been observed until this point. In one *P. taeda* stand experiencing increased mortality in Alabama, roots were excavated and dissected from pines exhibiting a range of crown conditions in order to determine if *Hylastes* breeding and feeding damage could be detected and to investigate the relationship between beetles, ophiostomatoid fungi, and tree crown condition. *Hylastes salebrosus* and *H. tenuis* represented the majority of beetles collected (94%) and were found nearly exclusively in root of trees with dying crowns. Adult beetles and beetle galleries were associated with a host of ophiostomatoid fungi, including *Leptographium terebrantis*, *Grosmannia huntii*, *L. procerum*, and *L. serpens*. These observations suggest roots of dying trees represent a valuable host substrate for expanding *H. salebrosus* and *H. tenuis* populations. A more comprehensive study is required to confirm these findings.

Key Words: galleries, *Hylastes*, loblolly pine decline, ophiostomatoid fungi, root disorders

TABLE 2. NUMBER OF INDEPENDENT ISOLATES OF EACH OF 4 OPHIOSTOMATOID FUNGI ISOLATED FROM ROOT TISSUE (BEETLE GALERIES) AND ADULT BEETLE BODIES, COLLECTED FROM LOBLOLLY PINE TREES WITHIN 3 HEALTH CONDITIONS.

Fungal Species	<sup>1</sup> Dead Trees		<sup>2</sup> Dying Trees		<sup>3</sup> Healthy Trees	
	Tissue	Beetles	Tissue	Beetles	Tissue	Beetles
<i>L. terebrantis</i>	18	3	45	62	0	0
<i>G. huntii</i>	3	2	13	43	0	0
<i>L. procerum</i>	1	0	10	12	9	0
<i>L. serpens</i>	0	0	0	2	0	0

<sup>1</sup>Sample trees lacking most (>90%) foliage, with the remaining being dead (red).

<sup>2</sup>Sample trees with predominantly (>70%) dying foliage (pale green and yellow).

<sup>3</sup>Sample trees with predominantly (>90%) healthy foliage (dark green).

## RESUMEN

Los escarabajos de la corteza en la familia Curculionidae que se alimentan de raíces y los hongos ophiostomatoideos que ellos transmitan contribuyen a los trastornos de las raíces de especies de *Pinus* por todo el mundo. En el sudeste de los Estados Unidos, los escarabajos que alimentan de las raíces que pertenecen al género *Hylastes* (Coleoptera: Curculionidae), incluyendo *Hylastes salebrosus* y *Hylastes tenuis*, son asociados con los lotes de *Pinus taeda* que están experimentando la mortalidad prematura. A pesar de esto, evidencia directa de que *Hylastes* se este reproduciendo junto con los hongos ophiostomatoideos en *Pinus taeda* no se ha observado hasta este momento. En un lote de *P. taeda* en Alabama que esta experimentando una mayor mortalidad, las raíces fueron excavadas y disectadas de pinos que muestran una serie de condiciones de la corona para determinar si la reproducción y el daño causado por la alimentación de *Hylastes* se puede ser detectado e investigado entre la relación de los escarabajos, hongos ophiostomatoideos y el estado de la copa de los árboles. *Hylastes salebrosus* y *H. tenuis* representaba a la mayoría de los escarabajos recolectados (94%) y fueron encontrados casi exclusivamente en las raíces de los árboles con coronas que están muriendo. Los escarabajos adultos y las galerías de los escarabajos fueron asociados con una gran cantidad de hongos de ophiostomatoideos, incluyendo *Grosmannia huntii*, *Leptographium terebrantis*, *L. procerum* y *L. serpens*. Estas observaciones sugieren que las raíces de los árboles que están muriendo representan un substrato valioso para la expansión de poblaciones de *H. salebrosus* y *H. tenuis*. Un estudio más amplio es necesario para confirmar estos hallazgos.

Palabras Clave: galerías, *Hylastes*, disminución de pino loblolly, hongos ophiostomatoideos, trastornos de raíces

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