

# WILD PIGS: INCITING FACTOR IN SOUTHERN PINE DECLINE?

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**Abstract**—During an investigation into southern pine decline at Fort Benning Georgia, the possibility of wild pigs (*Sus scrofa*) as an inciting factor became evident. Their rooting activity caused significant root damage on sites showing symptoms of pine decline. It was thought that perhaps the pigs may be moving around pathogenic fungi during their rooting activity in *Pinus taeda* (loblolly pine) stands. In 2008 and 2010, fungal isolates were obtained from the snouts of wild pigs captured from these stands, as well as, from root-feeding bark beetles and roots sampled 2003-2005. Micromorphology and DNA sequences of the ITS, elongation factor, and beta-tubulin gene regions were employed to identify the fungi recovered. Two new *Ophiostoma* species and a new *Leptographium* species were recovered. This study shows that wild pigs may exacerbate pine decline in this location by predisposing trees to bark beetles by reducing tree vigor when causing wounds for soil-borne or insect vectored pathogen infection and perhaps incidentally vectoring plant or tree pathogens during rooting activity.

## INTRODUCTION

Several reports have indicated that localized forest health problems are increasing in southern pine forests (Eckhardt and others 2007, Hess and others 1999, 2005, Orosina and others 1997). When these events are accompanied by sparse and chlorotic crowns, low annual stemwood growth, and isolation of fungal pathogens from the roots other than *Phytophthora cinnamomi* Rands or *Heterobasidion annosum* (Fr.) Bref., they are commonly referred to as pine decline (Eckhardt and others 2007, Menard 2007, Orosina and others 1997). The etiology of forest declines typically involve complex of biotic and abiotic agents, which either exacerbate or mitigate the extent of tree growth reduction and tree mortality differentially. The decline model developed by Manion (1981) suggested that declines begin with predisposing factors related to host genotype, site or other typically abiotic factors which may be permanent or long term. Inciting factors are short term stressors such as insect defoliation, a frost event, drought or damage to the roots that may cause injury to the tree, which under ideal conditions would not lead to tree death. Predisposing factors reduce the defenses against contributing factors, which are typically biotic agents such as fungi, viruses, or insects that normally do not affect healthy trees, but may infect or infest trees that have been compromised by the predisposing and inciting factors (Manion, 1981).

It has been hypothesized that stress associated with abiotic and biotic factors attract root- and lower

stem-feeding bark beetles (Coleoptera: Curculionidae) to southern pine stands (Eckhardt and others 2007). Infesting insects found associated with declining pines include *Hylastes salebrosus* Eichhoff, *H. tenius* Eichhoff, *Pachylobius picivorus* (Germar), and *Hylobius pales* (Herbst) (Eckhardt and others 2007, Menard 2007, Zanzot and others 2010). *Hylastes* species use the roots of both dead and living conifers for their maturation feeding and breeding activities, rarely causing damage (Milligan 1978). The insects associated with pine decline can also vector root-inhabiting ophiostomatoid fungal associates (Eckhardt and others 2007, Menard 2007, Zanzot 2009).

Wild pigs (*Sus scrofa*) may be an inciting factor in the pine decline complex, as they can be a particularly destructive exotic species in the places of introduction (Wickland 2014). Wild pigs negatively impact almost all aspects of ecosystem function and structure. Their feeding activities significantly disturb soil layers and natural decomposition cycles (Bratton 1975, Lacki and Lancia 1986). Wild pigs are commonly found in pine stands and their feeding activities damage roots (unearthing and removing bark) and could cause stress to trees that may predispose them to bark beetle attack. It is possible that there is a common attraction for both wild pigs and insects. Studies show that root-feeding insects are more abundant on symptomatic pine decline sites and these sites have a higher incidence of ophiostomatoid fungal infection (Eckhardt and others 2007, Menard 2007, Zanzot 2010). Studies suggest that

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trees in stress as a result of abiotic (i.e. drought) and biotic (i.e. fungi) factors are attractive to bark beetle activity (Kelsey and others 2014, Lahr and Sala 2014, Netherer and others 2015, Sopow and others 2015). The same pattern of attraction may play a part in the role of wild pig incidence on symptomatic pine decline sites as well. Pigs may have a palate for the taste of ophiostomatoid infected roots. The feeding activity of insects and wild pigs on ophiostomatoid infected roots presents interesting ecological comparisons. The host is affected similarly by the insect and wild pig as they both are using the same root substrate for feeding and it is possible that both are vectoring fungi in the process.

While both the incidence of pine decline symptoms and wild pig populations appear to be increasing, it is possible that the feeding activities of the wild pigs and the damage they cause, could compound the pine mortality that is occurring. This study was established to determine (1) if there is an association between wild pigs feeding and insect numbers and (2) whether wild pigs are acquiring and transporting the same fungi as the insects through their rooting behavior.

## MATERIALS AND METHODS

### Study Area and Wild Pig Trapping

Wild pig trapping took place 2004-2010 for a variety of studies (Hanson and others 2008, 2009, Holtfeter and others 2008, Jolley and others 2010, Sparklin and others 2009, Williams and others 2011) on approximately 737 km<sup>2</sup> of the Fort Benning military installation which is located in the Midwestern portion of Georgia's Muscogee and Chattahoochee counties that are mid-state on the eastern Alabama border. The predominant land base is Upper Coastal Plain with some Piedmont transition zone along the Fall Line. Fort Benning is planted in longleaf (*Pinus palustris*) and loblolly (*Pinus taeda*), separated by patches of bottomland hardwoods consisting of sweetgum (*Liquidambar styraciflua*), yellow poplar (*Liriodendron tulipifera*), hickory (*Carya* spp.), and oak (*Quercus* sp.). Hanson and others (2008) reported pig densities between 1.2 and 6.1 pigs/km<sup>2</sup> in a similar area of Fort Benning in 2004.

Wild pigs were trapped in corral- and box-style traps that were prebaited with whole corn. Trapping occurred throughout 2008 and 2010, and fungal isolates were collected from wild pigs that were euthanized, and some that were captured and released according to protocols for other studies. A more detailed description of the trapping protocol is described in Williams and others (2011). All animal handling procedures were approved by the Auburn University Animal Care and Use Committee (PRN#2007-1196).

### Isolation of Fungi from Wild Pig Snouts

One hundred wild pigs were trapped and the outer edge of the snout (where soil collects in the hairs) was

swabbed with a sterile Q-tip moistened with sterile water in 2008 and 2010. Swabs were placed in sterile plastic bags (one swab per bag) and placed on ice for transport to the laboratory. Swabs were plated on malt extract agar (MEA, 20 g malt extract, 15 g agar/L) and MEA amended with cycloheximide and streptomycin (CSMA) (Hicks and others 1980). Ophiostomatoid fungi were identified by their characteristic perithecia or conidiophores and transferred to fresh CSMA or MEA. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

### Insect Trapping and Health Assessment

Within the wild pig trapping area were thirty-seven 1/6 acre plots (Forest Health Monitoring protocols, Dunn 1999) that were established in loblolly pine (2003-2005). Sites were predominately loblolly pine with a mix of competing species of pine, including shortleaf (*Pinus eschinata* Mill.) and longleaf as well as hardwood species such as dogwood (*Cornus florida* L.), red maple (*Acer rubrum* L.), water oak (*Quercus nigra* L.), southern red oak (*Quercus falcata* Michx.) and black jack oak (*Quercus marilandica* Muench.). Surface soil type varied from sandy through loam to clay. Topography was generally lower to moderate relief upland ridges with moderate drainages to flat alluvial plains. The location of the plots was determined using the Loblolly Pine Decline Risk Map (Eckhardt 2003) which identified a site as either symptomatic (decline [D]) or asymptomatic (healthy [H]). Each plot was categorized in one of four stand age classes; <10, 10 - 19, 20 - 40 and >40 years. Four replicates of each combination of stand class (D and H, n=2) and age class (n=4) were installed.

At each of these locations, a root health assessment was performed on three dominant or codominant pines nearest to the center plot location. Root sampling was done with the modified two-root excavation method (Otrosina and others, 1997) to examine for root damage (wild pig, fire, insect, mechanical) and fungal presence. Plot disturbance (wild pig, fire, thinning) was also recorded. Root-feeding insects were sampled on subplots using pitfall traps (3 subplots per plot, 192 total pitfall traps) from March to May for a 3 year period (loblolly 2003-2005). Insects were collected on biweekly basis for transport to the laboratory for identification and isolation of associated fungi.

## RESULTS AND DISCUSSION

### Insect Trapping

The total number of root-feeding insects (*Hylastes* spp.) and reproduction weevils (*Hylobius pales* and *Pachylobius picivorus*) captured in pitfall traps increased annually during the 3 years of trapping (1117 in 2003, 1253 in 2004 and 2423 in 2005). Mean insect

numbers were significantly greater on symptomatic pine decline plots than asymptomatic plots for study years 2003 ( $F_{1,30}=4.22$ ,  $p=0.0495$ ) and 2004 ( $F_{1,30}=4.33$ ,  $p=0.0468$ ).

### Isolation of Fungi from Wild Pigs, Insects and Roots

Isolates of two unidentified *Ophiostoma* sp. (Sp. 1 53 percent and Sp. 2 48 percent) and one unidentified *Leptographium* sp. (59 percent), as well as, *Leptographium terebrantis* (68 percent) were recovered from the pig snouts. Isolations also yielded non-staining fungi such as *Aspergillus* spp., *Aureobasidium* spp., *Gliocladium* spp., *Penicillium* spp., and *Trichoderma* spp., along with several bacterial spp. *Leptographium terebrantis*, *L. procerum* and *G. alacris* were recovered from the surface of three species of root-feeding bark beetles [*Dendroctonus terebrans* Olivier (Coleoptera: Curculionidae), *H. salebrosus*, and *H. tenuis*], and two species of root-feeding weevils (*H. pales* and *P. picivorus*), as well as, *Ophiostoma* sp. 1 and the unidentified *Leptographium* sp. found on the wild pig snouts. *Ophiostomatoid* fungi were found in roots from 23 of the 36 plots which included *Leptographium terebrantis*, *G. alacris* and the unidentified *Leptographium* sp. The overall proportion of ophiostomatoid species isolated from all infected roots was greater from roots of trees on symptomatic plots (91 percent) than those from asymptomatic plots (0.08 percent).

These studies indicate that there are a number of fungi, including several species of ophiostomatoid fungi, associated with both snouts of wild pig in addition to the fungi carried on the body of root-feeding bark beetles. The attraction of pigs to pine decline sites may be associated to elevated insect incidence and roots infected by ophiostomatoid fungi that wild pigs actively seek out using their keen sense of smell. Damage to roots by wild pigs can be very extensive and cause root mortality putting host trees under additional stress. Symptomatic loblolly pine sites had 90 percent more wild pig damage compared to the asymptomatic loblolly pine sites. The uprooting activity of pigs while feeding exposes roots to more insect attack and fungal infection compounding host stress and increasing symptoms of pine decline. The implication of wild pigs as possible plant pathogenic fungal vectors may be weak, but pigs do cause significant physical root damage. That damage is a severe biotic stress that increases root-feeding bark beetle numbers ( $F_{1,30}=4.87$ ,  $p=0.0283$ ) and possibly the incidence of ophiostomatoid fungal infection facilitating the spread of pine decline.

### CONCLUSIONS

This preliminary study illustrates two ways in which wild pigs are associated with the pine decline process, including (1) causing damage to roots which has the

potential to attract insects as it stresses the tree and (2) transporting pathogenic fungi. More focused research needs to be completed to determine whether pigs vector fungi between trees.

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