



Correlation between infection by ophiostomatoid fungi and the presence of subterranean termites in loblolly pine (*Pinus taeda* L.) roots

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- Abstract**
- 1 Observations of subterranean termites feeding in pine sapwood containing ophiostomatoid fungi prompted the present study aiming to investigate the effect of infection by *Leptographium* fungi on the probability of encountering subterranean termites in loblolly pine (*Pinus taeda* L.) roots.
 - 2 Root samples were collected from 2350 loblolly pine trees in Alabama, Texas, Louisiana and Mississippi using hand tools. The presence or absence of subterranean termites in the roots was recorded. Samples were plated on malt extract agar and cycloheximide-streptomycin malt agar and incubated to determine which, if any, fungal species were present.
 - 3 The presence of *Leptographium procerum*, *Leptographium terebrantis* or both was significantly associated with an increased presence of subterranean termites in loblolly pine roots.
 - 4 The results obtained in the present study indicate that complex ecological interactions may exist between ophiostomatoid fungi, as carried by root-feeding bark beetles, and subterranean termites.

Keywords Blue-stain fungi, *Leptographium*, loblolly pine roots, ophiostomatoid, *Pinus taeda* L., subterranean termites.

Introduction

Subterranean termites are one of the most ecologically and economically important insect groups and annually account for over \$22 billion in treatment and repair costs to wooden structures worldwide (Rust & Su, 2012). They are primary decomposers of woody materials in forests and also directly regulate ecosystem level processes such as soil nutrient availability and carbon budgets (Ausmus, 1977; Kitchell *et al.*, 1979). These activities, along with their importance as food sources for many species of vertebrate and invertebrate predators, have led to the consensus that termites are keystone taxa in many ecosystems.

The literature is replete with descriptions of termite ecology in tropical and subtropical forest ecosystems (Harris, 1966; Bignell *et al.*, 1997; Black & Okwakol, 1997) but, in general, very little is known about their ecological associations and

influences in temperate forests (Lee & Wood, 1971; Lobry de Brun & Conacher, 1990; Hanula, 1993). Despite the lack of information describing interactions between subterranean termites and other organisms, many fungi are well-documented termite symbionts (Hendee, 1934), with relationships ranging from antagonistic to mutualistic (Martin, 1992). For example, wood infected by the brown-rot fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. can attract and stimulate feeding in some subterranean termites (Esenther *et al.*, 1961). This discovery, along with the identification of the responsible chemical compounds (Smythe *et al.*, 1967; Matsumura *et al.*, 1968, 1969, 1976), led to commercial applications of this fungal metabolite in termite bait stations.

Because decay fungi break down structural components of wood (cellulose, hemicellulose and lignin), it was hypothesized that chemicals produced during this breakdown are the mechanism behind the attraction and feeding preference of subterranean termites (Cornelius *et al.*, 2002). Based on that hypothesis, it became common to assume that only decay fungi might be capable of attracting subterranean termites and

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stimulating feeding preferences within them. For this reason, nondecomposing fungi have been overlooked as symbionts of subterranean termites.

The only example of wood containing nondecay fungi causing a positive feeding response from subterranean termites was discovered recently. Wood wafers from bark beetle-killed southern yellow pines containing blue-stain fungi in the genus *Ophiostoma* were found to elicit feeding preferences from Eastern (Little *et al.*, 2012a) and Formosan subterranean termites (Little *et al.*, 2012b) in laboratory choice feeding assays. Additionally, field stake tests using southern yellow pine inoculated with *Ophiostoma* and *Leptographium* blue-stain fungi were degraded more rapidly than unstained stakes (Little *et al.*, 2013). Ophiostomatoid fungi do not degrade any structural components of wood (Schirp *et al.*, 2003; Valiev *et al.*, 2009) and therefore do not result in losses to wood strength or structural integrity. The mechanism(s) behind subterranean termite feeding preference for blue-stained wood remain under investigation.

Despite laboratory and field assays suggesting that ophiostomatoid fungi can affect subterranean termite feeding responses in lumber, no studies have yet investigated whether this phenomenon occurs in living trees. *Leptographium* blue-stain fungi such as those investigated by Little *et al.* (2013) are primarily vectored to living trees by below-ground feeding bark beetles and weevils, such as *Hylobius* and *Hylastes* spp. (Coleoptera: Curculionidae) (Eckhardt *et al.*, 2004a). *Leptographium* fungi are similar to *Ophiostoma* species, in that they do not decompose structural components of wood and are often vectored to stressed or dying trees by bark beetle symbionts.

In view of the recent discovery of subterranean termite feeding preference for above-ground portions of trees infected with ophiostomatoid fungi (Little *et al.*, 2012a,b, 2013), the present study aimed to investigate whether subterranean termites are correlated with the presence of *Leptographium* fungi in roots of living loblolly pines.

Materials and methods

As part of previous studies conducted to investigate the pathogenicity of *Leptographium* fungi to loblolly pine roots (Eckhardt *et al.*, 2004a,b, 2007), root samples were collected during 2000–2012 from 2350 loblolly pine (*Pinus taeda* L.) trees in Alabama, Texas, Louisiana and Mississippi using hand tools, in accordance with a two-root excavation method modified from Otrosina *et al.* (1997). Root data were collected in 1/16th acre plots in accordance with forest health monitoring protocols (Dunn, 1999). Fifty-percent of plots exhibited pine crown dieback/thinning and foliage chlorosis, whereas the remaining 50% of plots appeared healthy. Sampling plots were almost evenly distributed among the states (Texas had slightly fewer plots) and evenly distributed among military installations, National Forests with up to 30% hardwood mix and private industry monoculture plantations. Six dominant/codominant trees nearest to the plot centre were selected for root excavation. Two lateral root segments > 3 cm in diameter from each of the three trees were excavated with hand tools from opposite sides

of the tree to the approximate crown drip line. Root depth was recorded and lateral roots were examined visually for primary root damage, fine root presence, absence or damage, as well as the presence or absence of subterranean termites. Primary roots were defined as the major lateral roots extending from the base of the tree to the drip line. All remaining roots were categorized as fine roots. During excavation, the presence or absence of subterranean termites, other insects, fire damage, mechanical damage and staining were recorded for each root sample.

The roots were divided into 20-cm-long sections, starting 16 cm from the root collar of each tree and continuing out to the crown dripline, and placed in plastic bags. The number of root sections collected per tree varied depending on the length of the root being sampled. All fine and feeder roots were cut from lateral roots and placed in plastic bags. All root samples were chilled for transport to the laboratory. Roots were stored at 4 °C (approximately 2–3 days) and then examined for the presence of insect damage and fungi, and classified as alive, dead or stained (Klepzig *et al.*, 1991). The root sections from each tree were processed similarly to that described by Otrosina *et al.* (1997) to determine the presence or absence of two species of ophiostomatoid fungi [*Leptographium procerum* (W.B. Kendr.) M.J. Wingf. and *Leptographium terebrantis* Barras and Perry]. Surface sterilization was carried out with a solution consisting of commercial bleach, ethanol and deionized water (10:10:80 v/v/v). One hundred and sixty root pieces were plated (four per plate and 40 plates per sample) from each tree on malt extract agar and cycloheximide-streptomycin malt agar and incubated at 25 °C under fluorescent lighting (460 µmol/m²/s). After 2 weeks, the plates were examined for fungal growth and putative *Leptographium* isolates were identified and recorded for each tree. Data (i.e. the presence/absence of subterranean termites, *L. procerum* or *L. terebrantis*) were pooled for each tree, and subsequent analyses were conducted at tree level.

Data were analyzed using a PROC LOGISTIC procedure in SAS (SAS Institute Inc., 2008) to determine whether the presence of two *Leptographium* spp. had an impact on the likelihood of termite presence or absence in loblolly pine roots. First-order (main effects) interactions and two-way interactions between *L. procerum* and *L. terebrantis* were tested.

Results

Subterranean termites were encountered in only 38.5% of trees when both species of *Leptographium* fungi were absent (the control group) versus 84.7% in trees that were infected with one or both *Leptographium* fungi (Table 1). Subterranean termites were present in 86.1%, 77.5% and 84.7% of trees that had *L. procerum*, *L. terebrantis* or both *L. procerum* and *L. terebrantis* present in root samples, respectively.

The presence of *L. procerum* or *L. terebrantis* in tree roots each had a highly significant positive effect ($\chi^2 = 278.90$, d.f. = 1, $P < 0.0001$ and $\chi^2 = 68.20$, d.f. = 1, $P < 0.0001$, respectively) on the frequency of subterranean termites or termite damage. Both *L. procerum* and *L. terebrantis* were present in 352 (15.0%) of trees sampled and significantly

Table 1 Frequency of encountering subterranean termites in conjunction with the presence of two species of ophiostomatoid fungi [*Leptographium procerum* (W.B. Kendr) M.J. Wingf and *L. terebrantis* Barras and Perry] in root samples from 2350 loblolly pine (*Pinus taeda* L.) trees in Alabama, Texas, Louisiana and Mississippi

Fungus detected	Number of trees	Percentage with termites (%)
Neither	473	38.5
<i>Leptographium procerum</i>	1169	86.1
<i>Leptographium terebrantis</i>	356	77.5
Both	352	84.7

($\chi^2 = 86.09$, d.f. = 1, $P < 0.0001$) increased the frequency of subterranean termites (84.7%) versus trees with unstained roots.

Discussion

Pine mortality in the southeastern U.S.A. that is not caused by primary pests such as the southern pine beetle (*Dendroctonus frontalis* Zimmermann) can be a rather slow and obscure process. Long-term biotic and abiotic stressors such as droughts and site conditions often slowly and inconspicuously increase physiological age of trees until they are susceptible to a host of other pests and pathogens (Franklin *et al.*, 1987; Mueller-Dombois, 1987). In the southeastern U.S.A., the most common predisposing factors are directly related to topsoil erosion or off-site planting of pines in abandoned cotton plantations (Richter *et al.*, 1999).

After tree defences are sufficiently weakened, acute droughts, lightning strikes, improperly managed or unmanaged fires, wounds, mechanized root compaction events, or other biotic and abiotic inciting factors can push the tree across a susceptibility threshold that is often irreversible (Manion, 1991). Humans are often unaware that the process is occurring at this phase because above-ground portions of affected trees often appear healthy.

Conspicuous above-ground signs and symptoms often do not occur until tree mortality is imminent. Above-ground bark beetles, ophiostomatoid fungi, *Monochamus* long-horned beetles and Annosum root rot [caused by *Heterobasidion irregulare* Garbelotto & Orosina (formerly *Heterobasidion annosum*)] (Orosina & Garbelotto, 2010) are all good examples of conspicuous factors that contribute to mortality in Southern pine forests. Even when tree mortality is imminent, below-ground contributing factors such as root-feeding weevils and bark beetles (e.g. *Hylastes* and *Hylobius* spp.) are seldom noticed by the casual observer. However, infestations of root-feeding weevils and bark beetles (and the blue-stain infections they facilitate) often occur long before tree mortality is imminent (Eckhardt *et al.*, 2004a).

Subterranean termites are not generally considered as a contributing factor in pine tree mortality and thus are usually relegated solely to the role of decomposers in forest ecosystems. Observations of subterranean termites feeding in blue-stained portions of trees recently attacked by bark beetles were the first indication that the ecological role of the subterranean termite in pine forests of the southeastern U.S.A. might begin

before actual tree death and decomposition occurs (J. J. Riggins, personal observation). Subsequently, laboratory feeding choice assays utilizing wood infected by bark beetle associated blue-stain fungi elicited subterranean termite feeding preference in both native (Little *et al.*, 2012a) and Formosan (Little *et al.*, 2012b) subterranean termites. The existence of this fungally-mediated biological interaction between termites and bark beetles was further corroborated by field trials using wood stakes infected with various root-feeding weevil and bark beetle blue-stain fungal isolates (Little *et al.*, 2013).

Hylastes and other root feeding weevils utilize roots primarily when they are living or during the very early stages of decomposition (Goheen & Cobb, 1978; Harrington & Cobb, 1983; Witcosky & Hansen, 1985; Day *et al.*, 2004; Eckhardt *et al.*, 2004b). Ophiostomatoid fungi are inoculated into pine root systems when root-feeding weevils and below-ground bark beetles consume fine feeder and lateral roots (Alexander *et al.*, 1988; Wingfield *et al.*, 1988; Klepzig *et al.*, 1991; Nevill & Alexander, 1992a,b; Harrington, 1993; Malloch & Blackwell, 1993; Eckhardt *et al.*, 2007; Menard, 2007). The present study is the first to indicate that the infection of pine tree roots by ophiostomatoid fungi can affect subterranean termite behaviour. It is possible that damage from subterranean termites contributes to ongoing tree health problems in a positive-feedback loop, and could increase tree susceptibility to other contributing factors (such as aboveground bark beetles) by damaging roots and decreasing water and nutrient uptake in affected trees. Further research is needed to investigate the role of ophiostomatoid fungi in mediating interactions between above and below-ground herbivores and the process of pine senescence in the southeastern U.S.A.

In conclusion, the results obtained in the present study indicate that subterranean termites may begin to play a role in temperate pine forest ecology before tree death and decomposition occurs. We propose that complex multitrophic ecological interactions occur between senescing pine trees, their dominate herbivores (above- and below-ground feeding bark beetles) and their dominate invertebrate decomposers (subterranean termites).

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