ROOT DISEASE, LONGLEAF PINE MORTALITY, AND PRESCRIBED BURNING

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Abstract–A study was initiated at the Savannah River Site, New Ellenton, SC, to determine factors involved in decline of longleaf pine associated with prescribed burning. Pretreatment and post-treatment surveys were conducted on all treatment plots. Symptomatic trees were recorded by means of a crown rating system based upon symptom severity. Three years after prescribed burning treatments were initiated, mortality and numbers of symptomatic trees increased in the hot burn plots. Crown symptoms corresponded to tree physiological status determined by cambial sucrose synthase activity. Root pathogenic fungi such as *Leptographium terebrantis*, *L. procerum*, and *Heterobasidion annosum* were widespread throughout the study site, regardless of treatment. The *Leptographium* species were found to be pathogenic based upon inoculation experiments and *H. annosum* was observed to be involved in root infections and mortality. Histological studies indicated a high fine root mortality rate in the hot burn treatment. The decline syndrome on these sites is a complex of interacting factors and involves root pathogens, soil factors, root damage, and physiological dysfunction.

INTRODUCTION

Although the beneficial role of fire is well documented for longleaf pine *(Pinus palustris* Mill.), little is known about fire's biological effects, whether prescribed or wild. We do know that a large portion of the above-ground biomass can be altered or lost without significant mortality of seedlings or adult trees. Saplings of longleaf pines are in continuous flush in height growth and are vulnerable to fire and disease (Allen and Scarbrough 1969). On the other hand, field observations report a high mortality rate in adult longleaf pine that continues several years after prescribed burning. Also, recent studies have indicated certain root-infecting fungi such as *Leptographium* species, other Ophiostomatoid species, and *Heterobasidion annosum* (Fr.) Bref. are associated with declining longleaf pine (Otrosina and others 1995, Otrosina 1998, Otrosina and others 1999). This contrasts with the generally held notion that longleaf pine is resistant or highly tolerant to many diseases and insects that adversely affect other southern pine species (Derr 1966, Mann 1969).

Questions arise as to why, in a tree species adapted to frequent fires, are decline and mortality associated with prescribed burning? This study addresses anatomical, pathological, and physiological processes as they relate to fire intensity and identifies areas needing further investigation.

MATERIALS AND METHODS

The study area was selected on the Savannah River Site in Barnwell County near New Ellenton, SC. A 40-year-old planted stand of longleaf pine was subjected to four burning treatments in a randomized complete block design. Each 2.0-ha treatment plot was replicated four times with unburned check, cool, medium, and hot burn intensities randomly assigned. Four 0.0079-ha subplots were located in each plot starting with one at plot center and three others located 30 m from plot center at 120° intervals starting from due north.

Prior to burning, a 100 percent survey was conducted on all plots to document current mortality and symptomatic trees. Burning took place between January and March 1997. Burn temperature was regulated by monitoring fuel moisture sticks, wind speed, and days since precipitation prior to ignition. Temperature data was obtained from max-min thermometers that were placed between the duff layer and mineral soil interface in four evenly spaced locations on each burn plot. The low and medium intensity burns were head fires while the hot burn was a backing fire tending to move more slowly across the landscape. Fuel data by fuel type were obtained according to Savannah River Forest Station fire crew protocols.

Starting one month post treatment and periodically thereafter, 100 percent surveys were conducted on all plots for three years. We employed a slight modification of a crown rating system used previously for longleaf pine symptoms (Otrosina and others 1999). The present rating system consists of five progressively symptomatic crown classes, differing from the previous system by adding a healthy class (class 0) and defining four symptom classes instead

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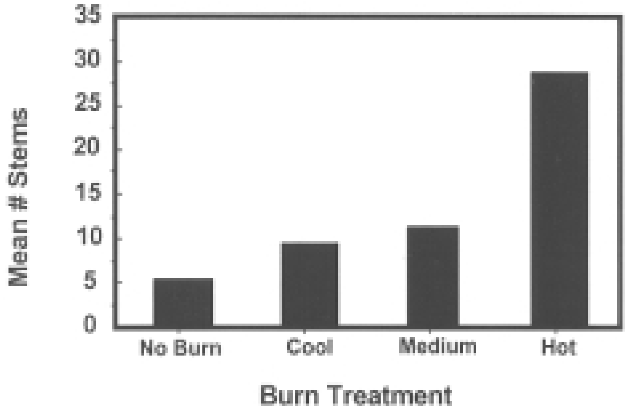

Figure 1a—Mean number of dead longleaf pine stems three years after burn treatments. The hot burn had the largest overall mortality.

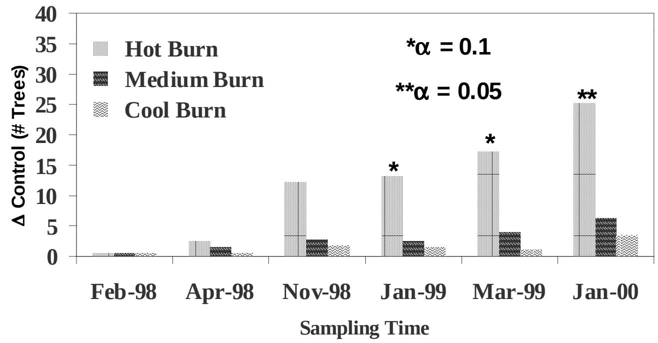

Figure 1b—Mean difference in number of crown symptom class three and four trees between burn treatments and unburned control. A detectable pattern in mortality began to emerge two years after the burn treatments were initiated.

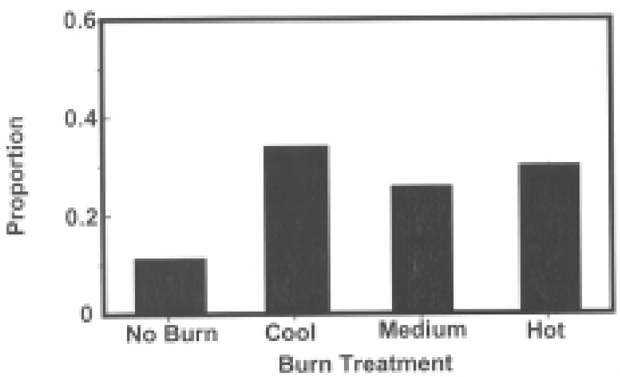

Figure 1c—Mean proportion of trees changing from less severe to more severe crown symptom classes in the three burn treatments and unburned control. All three burn temperatures had a higher proportion of trees with crown class changes than the unburned control.

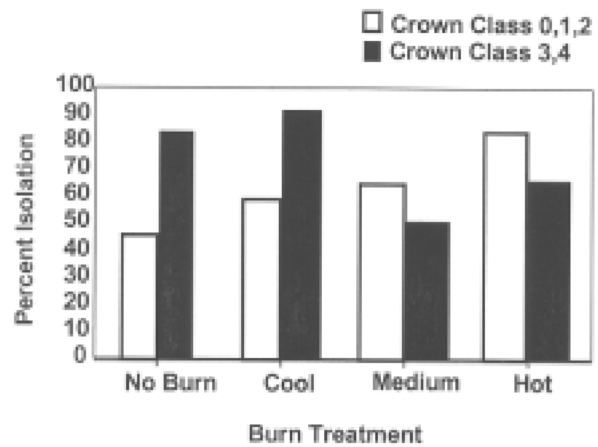

Figure 1d—Percent isolation of *Leptographium* species in woody longleaf pine roots relative to combined (less severe versus more severe) crown symptom classes. Isolation percent is based upon the presence of *Leptographium* species recovered within the total number of trees sampled.

of the three symptom classes used previously. In the present study, we define class 1 trees as those with crowns appearing as slightly off color, although green with less lustrous foliage compared to trees designated as class 0. The remaining three symptom classes are defined as previously reported (Otrosina and others 1999). Mortality and symptomatic trees were tagged and crown symptoms and d.b.h. were recorded.

Randomly selected symptomatic tree woody roots were excavated to approximately 0.5 meters from the root collar. About six root core samples were obtained along the exposed length with a 4-mm diameter increment hammer that penetrated about 2 cm into the xylem. Cores were immediately placed into small plastic bags and then into an ice chest for transport to the laboratory. Core samples were plated onto cycloheximide-amended and unamended 1.25 percent malt extract media, incubated, and evaluated as previously described (Otrosina and others 1999). Pure subcultures were obtained and fungal isolates were identified to genus or species.

Fungal isolates of Ophiostomatoid species obtained from root isolations were used in a pathogenicity study on randomly selected, healthy trees. Isolates of *Leptographium terebrantis, L. procerum, Ophiostoma minus* (used as a positive control), *Leptographium* sp. (resembling *L. serpens*), and an unidentified *Sporothrix* sp. were inoculated onto lower stems (1 m above root collar) and woody roots > 8-cm diameter. Stem bark was shaved to the outer phelloderm with a draw knife or similar blade to allow penetration of a 4-mm-diameter increment hammer to the cambium. Root and stem surfaces were sprayed with 95 percent ethanol prior to wounding. Agar cultures of the isolates were inoculated onto each tree stem and root via 4-mm-diameter agar plugs taken from culture margins. Outer bark retained in the bore of the increment hammer was replaced on the wound. Implements were thoroughly washed in 95 percent ethanol between isolates and inoculation points and each inoculation wound was taped with duct tape and marked for identification.

A stump infection survey was conducted for *Heterobasidion annosum* (Ha) by counting stumps within a 50-m radius circle of each plot center. Within each radial plot, total number of stumps over 10-cm diameter was counted and only those with visible fruiting bodies of Ha were defined as infected. Basidiocarps of Ha that were in good condition were transported to the laboratory for isolation and future study.

During May 2000, five trees from each of the five crown symptom classes were selected at random from hot burn treatment plots to determine stem cambial zone sucrolytic enzymes. A 6-cm by 15-cm section of bark was cut from the stem at breast height to expose cambial tissue. The cambium was scraped with a sharp razor blade and the scraped cambial tissue was then placed in small plastic bags. The bags were immediately submerged in liquid nitrogen contained in a Dewar flask, which served to store the flash frozen cambial tissue until transport to the laboratory for analysis. Analysis of sucrose synthase (SS), ATP-dependent phosphofructokinase (ATP-PFK), and Pyrophosphate-dependent phosphofructokinase (PPi-PFK) was described previously (Otrosina and others 1996).

Within each subplot, a randomly selected tree was used for fine root studies. Four times for each of two years, beginning three weeks after the last burn treatment, soil cores were obtained around the drip line of each selected tree by means of an inertial soil core sampler. Two core samples 6.25 cm in diameter were taken from two depths, 0 to 6 cm and 6 to 12 cm, at opposite positions around the tree. Cored positions were flagged to prevent repeat sampling. Fine roots (< 2-mm diameter) from within organic matter samples (0- to 6-cm depth) were separated from fine roots within mineral soil (6- to 12-cm depth) in the field by screening through a 5-mm mesh screen. Putative root-free soil was retained separately. All samples were placed in an ice chest for transport to the laboratory. Fine roots were oven-dried at 70°C for 24 hr and extracted for ergosterol analysis to estimate living fungal biomass. These procedures have been reported previously (Otrosina and others 1996; Sung and others 1995).

Also, samples of fine roots were taken from the sub-plot trees during March, June, and September of 1997, immediately placed in weak FAA (formalin:acetic acid:alcohol) solution, and were sectioned and stained according to protocols described previously (Walkinshaw and Otrosina 2002). Fine root anatomy was analyzed microscopically and variables such as size and number of starch grains, nuclear condition, tannin accumulation, and root mortality were measured.

Analysis of variance was conducted on tree mortality, crown data, fungal isolation, and fine root variables. The Chi-Square test and Dunnett's treatment versus control test were conducted on data relating to proportions of crown class changes over treatments during the 3-year study period.

Table 1—Proportion of roots in individual trees within the hot burn treatment exhibiting normal anatomy two to six months post burn[1]

Tree	Soil Layer: Organic	Soil Layer: Mineral
	- - Proportion - -	
1	0.22	0.50
2	0.30	0.33
3	0.00	0.10
4	0.20	0.50
5	0.44	0.50
6	0.00	0.10
7	0.40	0.60
8	0.18	0.30
9	0.10	0.00
10	0.30	0.10

[1]Nine to 12 roots were sampled at random in the two soil layers

RESULTS

Post-Burn Observations

Temperatures from the thermometers placed in each treatment plot registered potentially lethal levels (approximately 130^0 to150^0 F.) in the hot burn treatments only. Spot checks of the duff layer revealed a large amount of decomposed organic matter containing fine roots on all treatments. The decomposed organic layer depth in the cool and medium burn treatment plots was indistinguishable from that of the control plots, indicating very little consumption of this organic matter fraction by the fire in these treatments. The hot burn plots had about one half the decomposed organic matter of the control plots (W.J. Otrosina, unpublished data).

Mortality and Root Infecting Fungi

After nearly three years post-treatment, mean cumulative mortality expressed as number of stems was highest in the hot temperature burn treatment (28.75 stems, $p = 0.06$) (figure 1a). The unburned control had the least mortality (5.5 stems) while the cool and medium treatments had mortality intermediate to the hot and control treatments with 11.25 and 9.5 stems, respectively. Numbers of trees with severe symptoms or mortality (crown class 3 or 4 trees) began to increase in the hot burn treatment with respect to the control plots at about two years post-treatment ($\alpha = 0.1$) (figure 1b), based upon Dunnett's treatment versus control test. At three years post-treatment, the number of trees in these symptom classes increased, exceeding control plot symptomatic tree counts by 25 trees ($\alpha = 0.05$). There were significant differences in proportion of trees that changed from less severe to more severe crown classes among all the burn treatments when compared to the control ($p = 0.039$, Chi-Square = 8.36, 3 df; figure 1c).

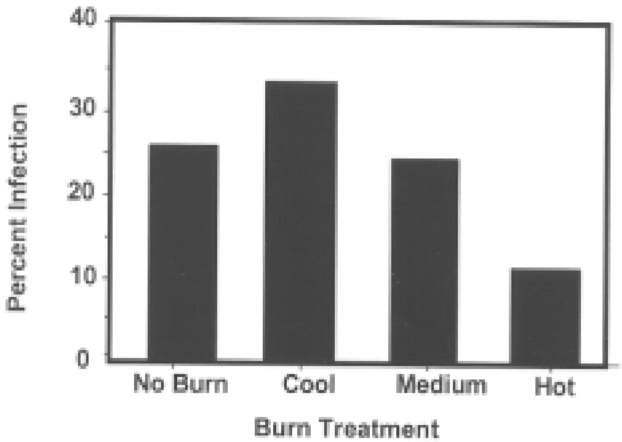

Figure 2—Mean percent of stumps with basidiocarps of *Heterobasidion annosum* within burn treatments. Note the lower percent of stumps with basidiocarps in the hot burn treatment.

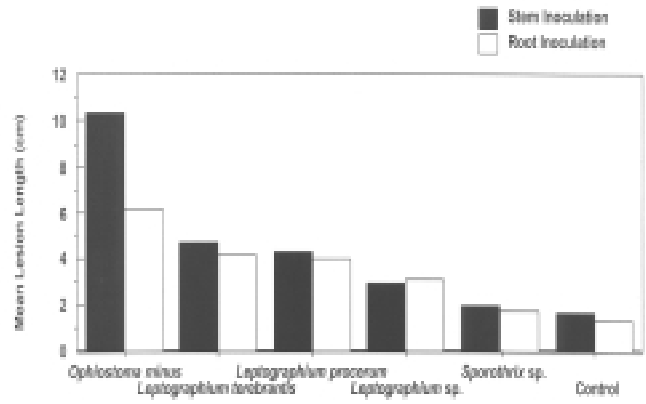

Figure 3—Mean lesion lengths in stems and roots of longleaf pine produced by inoculations with five Ophiostomatoid fungal isolates and a sterile control wound.

Isolations from woody root samples yielded several species of Ophiostomatoid fungi. Among the most common were *Leptographium terebrantis*, *Leptographium procerum*, and a *Sporothrix* sp. These comprised about 80 percent of the isolations from our root samples and no trends were observed in their relative frequencies with respect to burn treatments. The two *Leptographium* species we isolated were widespread throughout the study, regardless of treatment or crown class (figure 1d). On the other hand, *Sporothrix* sp. tended to be isolated more frequently in crown classes 0, 1, and 2.

Heterobasidion annosum Stump Infection

Viable Ha basidiocarps were observed in 7-year-old thinning stumps on our study sites. Eighty-five percent of the basidiocarps were found inside tangential splits in the sapwood caused by thinning equipment. These splits occurred within the outer 8 cm of the stumps and extended downward to the soil line. Infected stumps were widespread throughout the study site and all sampled plots yielded active basidiocarps. The percentage of stumps with active basidiocarps ranged from 7 percent to 51 percent over all treatments. The hot burn treatment had a mean proportion of 0.13 infected stumps, less than the control, cool, and medium burn treatments ((figure 2) ($p = 0.1$)).

Stem and root inoculations

Significant differences in cambial zone lesion length ($\alpha = 0.05$) were found among the fungal isolates tested for pathogenicity (figure 3). *Ophiostoma minus* produced the longest lesions, followed by *L. terebrantis* and *L. procerum*. An unidentified *Leptographium* species, resembling *L. serpens*, also produced a lesion that was significantly longer than the control wound. The lesion produced by an unidentified *Sporothrix* species was slightly longer than the control wound but statistically indistinguishable from it. Roots tended to have significantly smaller lesion lengths than stem inoculations overall ($\alpha = 0.05$).

Stem cambial zone sucrolytic enzymes

Regressions of enzyme specific activities with respect to the five symptom severity classes as independent variables for SS, PPi-PFK, and ATP-PFK indicated decreasing enzyme activities associated with increasing symptom severity (SS = -26.5Z +133, PPi-PFK = -30.3Z+157.8, and ATP-PFK = -10.7Z + 68.7; $p = 0.0001$, $R^2 = 0.53$, 0.42, and 0.44, respectively).

Fungal Biomass Estimates

Ergosterol analysis to estimate live fungal biomass indicated a higher concentration in the soil organic layer root clumps (figure 4) when compared to the other three soil fractions. These organic matter root clumps ranged from 16 to 49 µg/g ergosterol (dry weight basis). Also, these values were consistent over the two-year sampling period. In contrast, root-free soil had the least ergosterol over all sampling intervals. Root clumps in the soil and root free organic matter had intermediate amounts of ergosterol (range 5-15 µg/g dry weight). We did not detect clear treatment effects with respect to ergosterol concentration, although overall values in burn plots tended to be higher in the first year post-treatment than in the second year.

Fine-Root Anatomy and Mortality

Proportion of roots in the hot burn exhibiting normal anatomy two to six months post-treatment is given in table 1. The organic layer root mortality ranged from 56 percent to 100 percent. Root samples collected from the mineral layer had 40 percent to 100 percent mortality. No significant differences were found for root mortality between the two soil fractions, nor was fine root diameter or starch content related to mortality ($r^2 = 0.09$ and $r^2 = 0.30$, respectively). On the other hand, histological analysis of roots from mineral soil indicate a significant relationship between number of cortical cell starch grains and root mortality for the control and cool burn treatments ($r^2 = 0.73$ and 0.75, respectively) (figure 5). We found no relationship between number of cortical cell starch grains and root mortality in the medium and hot burn treatment ($r^2 = 0.02$ and 0.05, respectively).

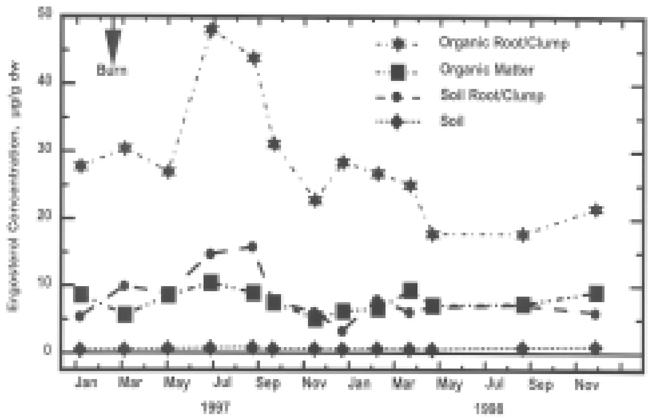

Figure 4—Below-ground distribution of live fungal biomass expressed as mean ergosterol concentrations in four soil or organic matter fractions. Sampling was done periodically over 18 months after beginning of the burn treatments. Symbols represent ergosterol concentrations of a given soil fraction at the specific sample date.

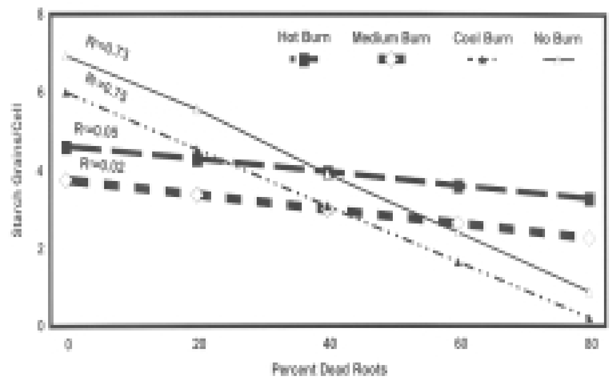

Figure 5—Regression relationship between root death and number of starch grains in fine root cortical cells. An inverse relationship exists between number of starch grains and percent dead roots in the hot and medium burn treatments. No relationship between root death and starch grains was detected in the cool burn and control treatments.

Dead roots often retained their bark cells in tight multicellular layers that resisted tearing when sectioned. When viewed macroscopically, these roots were often confused with undamaged, live ones. Sectioning and staining these roots revealed extensive internal damage. Large numbers of starch grains were trapped within necrotic cells, the cambium was disorganized, and nuclei stained abnormally. Bark formation and persistence of roots in burned plots was normal, as active formation of bark cells occurred in 64 percent of roots from burned plots and 58 percent of roots from unburned plots. Excess tannin accumulated in 65 percent of the roots from the burn plots and in only 12 percent of those from the unburned plots. Hydrolysis of cortical cell contents was limited in the burn plot samples and starch grains were intact. Cell wall structure, the cambium, and nuclei appeared to be preserved by released tannin.

DISCUSSION

The trend toward increasing mortality over time is evident in the hot burn treatment. Mortality onset is delayed in the burn treatments for at least two years, based upon our data (figure 1a). By the third year, clear separation between the hot burn treatment and unburned control plots, and the other burn treatments, is evident. Even the cooler burn treatments, while having less mortality, tended to have more trees progress from less severe to more severe crown symptoms when compared to the control (figure 1b). Thus, mortality cannot be ascribed to direct heat effects such as cambial scorching. Further evidence for indirect effects of the hot burn is the onset of decline symptoms that precede mortality, suggesting physiological and pathological causes. Regarding the physiological basis for the decline syndrome, analysis of SS activity is a quantitative indicator of tree stress (Sung and others 1993, Otrosina and others 1996) and corresponds well to the crown symptom classification we established. This suggests our visual crown evaluations approximate tree physiological status as defined by SS activity.

The mechanism driving this decline and mortality appears very complex. In this study, the term “hot burn” is relative and must be taken in context. In the hot burn treatment, about one half the amount of post-burn, decomposed organic matter containing fine roots still remained. This fact may provide clues toward understanding the mechanism as to why such mortality is triggered by fire. One hypothesis is that infrequent prescribed fire intervals allowed buildup of this decomposed organic matter containing fine roots. These roots are then susceptible to indirect heat effects. Our data on root anatomy show that fine roots can be damaged by heat without being consumed and without showing obvious macroscopic damage. Cortical cell starch grains were intact and their numbers were not associated with root mortality in the hot and medium burn treatment (figure 5). In dead fine roots, we observed limited hydrolysis of cortical cell contents, intact cell wall structure, and abundant intact achromatic nuclei in these burn plot samples, presenting further evidence of rapid cell death as would occur under a lethal pulse of heat. In contrast, there is an inverse relationship between numbers of cortical cell starch grains and root mortality in the mineral soil of the unburned and cool burn treatments (figure 5), indicating a relatively slower physiological process taking place in fine roots where their exposure to high temperatures is limited (Walkinshaw and Otrosina 1999). On the other hand, root death due to direct heat effects in mineral soil is somewhat enigmatic. Soil does not conduct heat efficiently, although steam can conceivably penetrate short distances through soil pores. We observed some interconnections between finer roots in the organic layers and the mineral soil and thus, damage to roots above may effectively cutoff roots in lower soil layers. Also, damage to larger woody lateral roots near the surface can affect more distal fine roots.

We found the highest portion of living fungal biomass in the fine root clumps of the organic layer (figure 4). Because the organic layer fine root clumps are presumed largely comprised of ectomycorrhizal fungi, and some studies indicate fine root production and associated symbiotic fungal biomass may account for two-thirds of the annual biomass production in some forest types (Marshall and Waring 1985), heat damage to this root fraction can result in significant stress.

Another element driving mortality may be facultatively pathogenic fungi. We found Ophiostomatoid species such as *Leptographium terebrantis*, *L. procerum*, and *Sporothrix* sp. to be widespread in woody roots regardless of treatment or crown condition (figure 1d). Because these fungi are adapted to insect dissemination, and little is known about the root feeding bark beetle species that are involved in their spread, critical interactions between insects and these fungi probably occur. Other studies have associated these fungi with insect attack, mortality, or decline symptoms in loblolly pine and longleaf pine stands (Otrosina and others 1997, Otrosina and others 1999). Longleaf pine are generally regarded as resistant or highly tolerant to root disease fungi and reports of Ophiostomatoid fungi attacking woody roots of longleaf pine, other than Otrosina and others (1999), are few. In our inoculation experiment, we demonstrated pathogenicity of these Ophiostomatoid species (figure 3) on longleaf pine and thus established their association with observed decline symptoms. The fact that Ophiostomatoid fungi are widespread begs the question as to their specific role. We recovered these fungi from both asymptomatic trees and declining trees regardless of treatment or crown condition. In asymptomatic trees, we isolated *L. terebrantis*, *L. procerum,* and unidentified *Leptographium* and *Sporothrix* species from both symptomatic roots and roots exhibiting no resinosis or staining, although symptomatic trees tended to have more resinous roots than non-symptomatic trees. Resinous lesions in roots signify an active defense by the tree against the pathogen, diverting energy resources to the infection site. If under stress, this can result in significant loss of growth and maintenance functions and may account for decline symptoms. *L. procerum* can survive in pine woody tissues for some time without causing obvious symptoms (Horner and others 1987) and Bannwart (unpublished MS thesis, University of Georgia Department of Plant Pathology 1998) found that *L. procerum* and *L. terebrantis* can survive in callused stem lesions of longleaf pine seedlings. Also, evidence suggesting the presence of *L. terebrantis*, *L. procerum*, and other Ophiostomatoid species in roots is a stress indicator in longleaf pine and loblolly pine stands has been presented (Otrosina and others, 1997, Otrosina and others 1999). Thus, the occurrence of root infecting *Leptographium* species may contribute to the decline syndrome after an additional stressor, such as fire damaged fine roots, is introduced in an already stressed system. Given these biological circumstances, fungi that are not regarded as pathologically important can cause significant and unexpected damage.

Other root pathogens such as Ha have not been regarded as important in longleaf pine because of its tolerance or resistance to this pathogen. We found the fungus to be widespread in our study, judging by stump infection (figure 2) and by observations of trees infected by Ha throughout our study. The pathogen was an important factor in longleaf pine decline in another area on the Savannah River Site (Otrosina and others 1999). Because Ha infection in longleaf pine is not often reported as a problem, infected trees we observed in this study may be another sign of complex pathological interactions involved. Infected stumps in our study continued to produce basidiocarps at least seven years after the last thinning, although stumps in the hot burn plots had produced less basidiocarps than stumps from all other treatments. Unlike Ophiostomatoid fungi, Ha decomposes woody root tissues and can persist for long periods of time in infected stumps and roots because it is highly adapted to resinous wood (Otrosina and Cobb 1989). Longleaf pine stumps are highly resinous and resist decomposition for a long period of time. When healthy tree root systems contact colonized stump roots or roots from infected living trees, the fungus spreads from tree to tree causing mortality and downed trees from disintegration of structural roots. Once present in a stand, this fungus can become a recalcitrant problem. We found infected stumps as small as 12 cm in diameter, demonstrating the importance of applying borax formulations to freshly cut stump surfaces to prevent infection from airborne Ha basidiospores.

The longleaf pine decline associated with prescribed burning on this study site cannot be attributed to a specific cause. We described a complex of interacting factors implicating fire intensity, fine root damage, *H.* annosum, Ophiostomatoid fungi, and physiological dysfunction. Soil type and stand density are also important components involved in this syndrome that require investigation. Certainly, fire dependent ecosystems should be regarded as exotic ecosystems (Otrosina 1998) when fire reintroduction after a long period of fire suppression is contemplated. Under these circumstances, fire reintroduction must be conducted with caution and consideration must be given to below ground pathological and physiological processes.

ACKNOWLEDGMENTS

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