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Root-infecting fungi associated with a decline of longleaf pine in the southeastern United States

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Abstract

A 35-year-old longleaf pine stand exhibited trees in various stages of decline. A study was conducted to determine root-infecting fungi and other abnormalities associated with varying degrees of crown symptoms. A four-class crown symptom rating system was devised according to ascending symptom severity. *Leptographium procerum* and *L. terebrantis* were significantly associated with increasing crown symptom severity. *Heterobasidion annosum* was also isolated in higher frequency as crown symptoms increased. Also, evidence of insects on roots increased as did amount of resinosis observed. Edaphic and silvicultural factors may interact with these pathogens and insects to pose a pathological limitation on longer-term management objectives. Further research is needed to determine relationships among various edaphic, silvicultural, and biological factors associated with the decline syndrome on this site.

Introduction

Longleaf pine, *Pinus palustris* Mill., is the most important component of a plant community that supports a major proportion of the biological diversity in the coastal plain of the southern United States (Martin et al., 1993). The natural range of longleaf pine includes the Atlantic and Gulf Coastal Plains from southeastern Virginia to eastern Texas and south through the northern two-thirds of Florida at elevations ranging from 0 to 200 m. There is also a mountain province of this species in central Alabama and northwestern Georgia that extends to 600 m elevation. Ultisols are the predominant soil order within the natural range of this tree species, with Typic Paleudults and Plinthic Paleudults being typically associated with natural longleaf pine stands. Deep sandy Entisols such as Quartzipsamments also support longleaf pine extending in elevation from near sea level in Florida through 185 m in Georgia and South Carolina (Boyer, 1990).

Due to factors such as conversion from forest to agriculture or other forest tree species, and past failures in artificial regeneration of this species, the longleaf pine ecosystem has declined from its original (historical) geographic range of 35 million hectares to less than two million hectares (Clark, 1984). At present, there is considerable interest and effort spent in restoration of this species to more of its historical range.

Increased mortality is being observed in certain longleaf pine stands in the 30–45-year-old age class and this mortality often occurs within two years after the administration of prescribed fire (Otrosina, 1998). One puzzling aspect of these observations is that this tree species evolved with periodic fires and depends upon fire for serving natural functions such as seed bed preparation and control of brown spot needle disease caused by *Mycosphaerella dearnessii* Barr (= *Scirrhia acicola* (Dearn.) Siggers). In general, longleaf pine has been characterized as resistant or tolerant to most diseases that affect other southern pine species (Boyer, 1990). These diseases include fusiform rust,

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caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedgc. & N. Hunt) Burdsall & G. Snow, annosum root disease, caused by *Heterobasidion annosum* (Fr.) Bref., and other root diseases. The purported root disease tolerance notwithstanding, root pathogenic fungi such as *Leptographium* species and *Heterobasidion annosum* have been isolated in high frequencies from declining longleaf pine trees on many physiographically appropriate sites; however, their role in causing mortality is unclear (Otrosina et al., 1995; Otrosina, 1998). Coinciding with this decline is a serious lack of information on pathological factors and their relationships to symptom development.

Knowledge of which root-infecting fungi are associated with longleaf pine decline would provide important information relevant to understanding their roles in stand deterioration. Root diseases can thwart management objectives by adversely impacting on stand structure many decades after establishment. Seed production losses and losses in habitat for endangered species such as the red-cockaded woodpecker *(Picoides borealis)* are some potential impacts of root diseases in longleaf pine. Thus, achievement of restoration goals in this species is consequential and at least partly depends upon understanding below-ground pathological dynamics (Otrosina and Garbelotto, 1998). To this end, the objectives of this study are to (1) determine what relationships exist between root pathogenic fungi and above-ground symptoms, (2) determine the root infecting fungi associated with declining longleaf pine, and (3) advance hypotheses regarding the role of these fungi in longleaf pine ecosystems.

Materials and methods

Study site description

A thirty-five-year-old longleaf pine stand with trees in various stages of decline was selected at the Savannah River Natural Resource Management and Research Institute near New Ellenton, South Carolina. The stand had been thinned to approximately 4 m^2/ha basal area five years prior to this study to develop an advanced natural seed orchard. The average height of the dominant and co-dominant trees was 18-22 m tall with diameters at breast height ranging from 30 to 38 cm. Canopy gaps from mortality ranged from 0.1 to 0.3 ha, although symptomatic trees not identified with established gaps occurred randomly throughout the stand. The soil series existing on the study site is Dothan (Typic Kandiudult), characterized by a sandy upper soil layer, a heavier clay layer occurring within 20-30 cm of the surface, and a hardpan at 30–45 cm depth.

Sampling method

After a preliminary survey of the stand, a classification system of crown symptoms was devised based upon progressive severity. Four crown symptom classes were created: (1) healthy, full crown, predominantly dark green foliage, with less than 10% of foliage being off-color (yellow or brown); (2) early decline, visibly reduced foliar density, with 10% to 25% of the foliage greenish-yellow to yellow, possibly with some drooping needles; (3) moderate decline, foliar density 10% to 50% of class 1 trees, 25% to 50% of crown either yellow, yellow-green, brown, or a combination of these, staghorns in the crown evident; and (4) late decline, foliar density less than 50% of class 1 trees, and the crown having greater than 50% yellow or brown foliage along with defoliated branches scattered throughout the crown. Symptom progression on sampled trees over two growing seasons was followed by noting the visual appearance of crowns during periodic site visits.

Every four to six weeks, over two growing seasons, representative trees within each crown symptom class were selected and sampled by means of root excavation. Fifty-two trees were sampled, representing the four crown symptom classes. There were 10, 19, 14, and 9 trees in symptom class 1, 2, 3, and 4, respectively. Two to four lateral roots that were within 25 cm of the soil surface from selected trees of each symptom class were exposed to approximately two meters distal from the root collar. The average diameter of roots sampled ranged from 6.8 to 11.3 cm. After excavation, resinosis, blue-stain, or evidence of insect galleries was recorded. Resinosis was detected on root bark as discolored, resin-soaked patches, often with encrusted soil, or as resin-soaked xylem in sample cores. Evidence of stain was defined as the presence of blue discoloration in the sample core xylem. No attempt to identify insects was made.

Roots were then sampled for presence of root-infecting fungi with an increment hammer designed to remove 5.0 mm diameter cores of woody tissue to a depth of approximately 3 cm. Symptomatic areas along the length of the excavated roots characterized by resinosis, necrosis, or insect attack were sampled preferentially. At least six cores were obtained from individual symptomatic areas along the exposed root length. When no obvious symptomatic areas were encountered on an excavated root, at least six core samples were taken at the root collar, the midpoint of the excavated length, and at the distal (approximately 2 m from the root collar) end of the root length sampled. Individual excavated root lengths sampled ranged from 1.5 to 2.5 m. Samples were placed in polyethylene bags and kept on ice in a cooler until arrival at the laboratory. All samples were stored at 5 °C and processed within 3 days of collection.

Twelve declining saplings ranging in size from the grass stage to 1.0 m in height, and growing near the drip-lines of selected symptomatic trees, were excavated. The tap root and lateral root systems extending 0.5 m from the root collar were collected. Representative samples from these roots were obtained at several locations along their length and stored as described above.

Root cores and root samples from saplings were rinsed in tap water and dipped in 95% ethanol. Segments of tissue approximately 0.5 to 1 .O cm long were plated on to 2% Difco malt extract agar (MEA) or 1.25% MEA amended with 0.2 g/L cycloheximide and 0.2 g/L streptomycin sulfate (Hicks et al., 1980). Plates were incubated at approximately 25 °C on a laboratory bench and evaluated after one week and every two days thereafter for a period of 21 days. Any ophiostomoid fungi observed on cycloheximide amended MEA were transferred onto 2% MEA for purification and subsequent identification. Presence of *Heterobasidion annosum* on unamended MEA was also recorded by observations of its anamorph, *Spiniger meineckellus* (A.J. Olson) Stalpers.

Data analysis

Data on root pathogenic fungal species, presence of root staining, resinosis, and evidence of insect galleries were analyzed on a tree basis (Otrosina et al., 1997), and expressed as a proportion of trees within a symptom class having the particular variable present in at least one root. Since the symptom class scores were discreet and ordinal in scale, the Mantel-Haenszel Chi-Square was used to test for increasing or decreasing trends in the proportions of each variable with respect to symptom class.

Results

Based upon observations of individual trees over two growing seasons, foliar symptoms usually began with thinning crowns, followed by discoloration, defoliation, and death. Discoloration generally began as a slight paling or yellowing of the needles, which became progressively reddish then changed to brown. Trees in early stages of decline (class 2), tended to develop dieback in the terminal ends of branches. In some cases, trees became discolored before noticeable needle thinning had occurred. Drooping foliage and heavy cone crops also characterized latter-stage symptoms. Symptoms of some trees progressed from class 1 to class 2 or from class 2 to class 3 within one month and remained as class 2 or 3 for the two growing seasons. During the course of this study, only 9 symptom class 4 trees were observed on the entire study site.

Ophiostoma piceae (Munch) Syd. & P. Syd., *Leptographiumprocerum* (W.B. Kendr.) M.J. Wingf., and *Leptographium terebrantis* Barras & Perry were the predominant blue-stain fungi isolated from sampled trees. In general, there was a significant trend in the proportion of trees from which at least one of these fungi was isolated with respect to symptom severity (p = 0.001). No relationship was observed between a particular blue-stain fungus and a specific symptom class. Of the symptom class 4 trees, 88.9% yielded one or more of these fungi. The proportion of trees from which *L. procerum* or *L. terebrantis* was isolated revealed a significant increasing trend as symptom severity increased (p = 0.001 and p = 0.008, respectively) (Table 1). No significant trends were found in proportion of trees with *0. piceae* with respect to increasing symptom severity (p = 0.115). *Heterobasidion annosum* tended to be isolated more frequently as crown severity increased (p = 0.075) (Table 1).

The proportion of trees with resinosis in roots increased with symptom severity (p = 0.004). Resinosis in roots was observed in 30% of healthy (symptom class 1) trees. Only one blue-stain fungal species, *0. piceae,* was obtained from a tree with root resinosis in this symptom class. Presence of blue-staining in roots was not associated with symptom severity (p = 0.495) (Table 2).

Proportions of tree roots with insect galleries for symptom classes 1, 2, 3, and 4 (n = 10, 19, 14, and 9) were 0. 10, 0.11, 0.21, and 0.67, respectively, and were significantly associated with progressive crown symptom severity (Chi-Square = 8.69, p = 0.003). All insect galleries were associated with resinous lesions and at

Table 1. The proportion of longleaf pine trees within four progressive crown symptom severity classes having root pathogenic fungi

Symptom class	N	*Leptographium procerum*	*Leptographium terebrantis*	*Ophiostoma piceae*	*Heterobasidion annosum*
		Proportion			
1	10	0.0	0.0	0.10	0.0
2	19	0.26	0.26	0.32	0.16
3	14	0.29	0.36	0.36	0.14
4	9	0.78	0.56	0.44	0.33
Mantel-Haenszel Chi-square		11.46	7.06	2.48	3.16
	P	$p = 0.001$	$p = 0.008$	$p = 0.115$	$p = 0.075$

Table 2. Proportion of longleaf pine trees in four progressive crown, symptom severity classes exhibiting blue-stain and resinosis in roots

Root Condition	Symptom Severity Class 1	2	3	4	Chi-Square	*p*
Resinosis	0.30	0.63	0.64	1.00	8.408	0.004
Stain	0.10	0.05	0.0	0.22	0.466	0.495

least one of the three species of blue-stain fungi, although no relationship was evident between particular fungal species and insect galleries.

Ten of the twelve symptomatic saplings had resinosis associated with some portion of the root system. Of the 12 sapling roots sampled, 11 yielded *L. procerum,* three yielded *L. terebruntis,* and two yielded 0. *piceae.* Two of the 12 saplings were infected with *H. annosum* and *L. procerum.* Blue-stain was evident in roots of only six of the saplings. In one case, excavation revealed that a sapling root infected with *L. procerum* was in contact with but not grafted to a mature sampled tree root also infected with this fungus. None of the saplings sampled had evidence of insect attack.

Discussion

Presence of pathogenic blue-stain fungi in roots of longleaf pine has not been widely reported. This tree species has been considered resistant or highly tolerant to many diseases, including root diseases, that severely affect other pine species in the southern United States. Recent evidence suggests that certain root infecting blue-stain fungi may play a role in the decline of longleaf pine following prescribed burning (Otrosina et al., 1995; Otrosina and Garbelotto, 1998). It is hypothesized that on certain sites, prescribed burning of stands within the 30–45-year-old age class causes stress that, in some as yet unknown way, may predispose tree roots to more rapid colonization by these fungi (Otrosina, 1998). In our study, three species of root infecting blue-stain fungi, *L. terebrantis, L. procerum,* and *O. piceae* are widespread in this longleaf pine seed production area.

The presence of *L. procerum* and *L. terebrantis* in roots increased with increasing above-ground symptom severity while presence of 0. *piceae* was not related to above-ground symptoms (Table 1). 0. *piceae* is regarded as a weak pathogen (Nevill and Alexander, 1992) and may have opportunistically colonized root segments that were debilitated by unknown factors. The pathogenicity of *L. terebrantis* and *L. procerum* on other conifer species has been demonstrated (Klepzig et al., 1991; Nevill et al., 1995; Nevill and Alexander 1992; Rane and Tattar, 1987). This is the first report of these root-infecting fungi associated with above-ground symptoms in longleaf pine.

The fungus *H. annosum,* unlike the blue-stain fungi, is a root-rot fungus that can cause structural instability in conifers in addition to death of root tissue. Its presence on the site is not surprising and it may be a factor in the observed decline. Thinning stumps that are capable of being colonized by the fungus exist on this site, although we did not excavate stump roots to determine the extent of *H. annosum* colonization. No basidiocarps were observed at the root collars of stumps in proximity to sampled trees. Colonized stumps are the primary means of residual tree infection by this fungus (Otrosina and Cobb, 1989). The *H. annosum* in roots of longleaf pine that we found in our study is interesting, since this tree species is reportedly tolerant of annosum root disease (Froelich et al., 1977). Mortality-induced gaps in this seed production area are reminiscent of disease centers characteristic of *H. annosum* (Otrosina and Cobb, 1989). Spreading from distal portions of residual tree roots, where colonized stump root tissues are initially contacted, the fungus moves toward the root collar (Otrosina and Cobb, 1989). Some evidence based on the presence of basidiocarps in longleaf pine thinning stumps seems to indicate the widespread presence of *H. annosum* throughout the range of this tree species (Otrosina, unpublished observations). On the other hand, a high frequency of stumps producing basidiocarps does not necessarily indicate the presence of annosum root disease within a given longleaf pine stand. These observations indicate the need for further research on *H. annosum* infection dynamics in this tree species. Based on the reported tolerance of longleaf pine to annosum root disease (Froelich et al., 1977), we speculate that circumstances such as eroded soils that prevent development of deep root systems, long intervals between prescribed fire, or mechanical damage to residual tree root systems by harvesting equipment may be required for this fungus to cause significant amounts of root disease in this tree species. These conditions seem to be present in our study.

It is possible that *L. terebrantis* and *L. procerum* may be secondary root colonizers of trees with more advanced symptoms and thus play no role in the decline. However, 94% of the root isolations that recovered these two pathogens were associated with resinous lesions. The proportion of trees with resinous roots also increased with increasing symptom severity (Table 2). Presence of resinosis associated with isolation of these fungi implies a host defensive response to infection (Parmeter et al., 1989). Because production of resinous compounds is energetically costly to the tree, these fungi may play an active role in the observed decline syndrome. Resin was observed to infiltrate deep into the root xylem and may also interfere with water conduction (Parmeter et al., 1992).

Also, as symptom severity increased, the proportion of trees with root-feeding insect galleries also increased. Presence of galleries in roots was always associated with resinosis and with the isolation of one or more of the three blue-stain fungal species. Certain root colonizing insects may be vectors of ophiostomoid fungi involved in root diseases of other conifer species (Harrington and Cobb; 1988, Klepzig et al., 1991; Nevill and Alexander, 1992), although little is known about the insects that attack roots of longleaf pine.

L. procerum and the presence of resinosis was associated with the majority of symptomatic seedlings we excavated adjacent to sampled trees. This fungus may pose a threat to subsequent regeneration, as we have observed contacts between a sapling root infected with *L. procerum* and a mature tree root also infected with this fungus.

Root infection by the blue-stain fungi may be indicators of stress from various agents. For example, basal areas greater than approximately 30 m^2/ha have been shown to increase incidence of *L. procerum* and *L. terebrantis* in southern pine beetle *(Dendroctonus frontalis* Zimm.) control plots adjacent to southern pine beetle-attacked plots (Otrosina et al., 1997). A recent study in a 35-year-old longleaf pine stand showed isolation frequencies of blue-stain fungi and *H. annosum* to be higher in prescribed burned plots than in unburned control plots (Otrosina and Garbelotto, 1998). In our study, shallow soils that have a hardpan layer at 30 to 45 cm depth tend to concentrate lateral roots near the surface where damage can occur. Deep, well-developed tap roots characteristic of longleaf pine do not develop on this site, as evidenced by our observations of nearby blown down, 25 m tall trees with an abrupt 30-45 cm root zone below which no tap root or lateral root exists. On this study site, root damage from equipment such as brush cutters, cone harvesting machinery, equipment associated with prescribed burning, as well as prescribed burning itself, may provide susceptible portions of root tissue for insect attraction and fungal colonization. Thirty percent of class 1 trees had resinosis (Table 2) which may indicate abiotic root damage. Due to these factors, a pathological rotation age may be reached that thwarts various longer-term management objectives on these sites.

Considerable circumstantial evidence exists indicating that the decline of longleaf pine on this seed production area may be due to a complex of factors involving root pathogenic fungi, insects, edaphic conditions, and silvicultural practices. Inasmuch as seed production is critical for restoration or reforestation of longleaf pine throughout its range, and because decline problems we have addressed in this study are manifested later in the life of a stand, caution should be exercised when selecting sites for this management goal. Young and apparently healthy stands growing on historically and physiographically correct sites may be selected and managed for seed production only to become unproductive during their peak seed-bearing period. Certainly, eroded, shallow soils ought to be avoided in planning longleaf pine seed production areas. Also, care should be taken to minimize residual tree damage by mechanized equipment. Further research must be conducted that addresses specific causal roles played by various silvicultural, pathological, and edaphic factors on this site as well as other sites where similar decline and mortality in longleaf pine stands are observed.

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