Seedborne Diseases of Southern Pines and Developing Strategies for Their Control

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Abstract-Plant pathogenic fungi such as *Lasiodiplodia theobromae* (the black seed rot fungus) and various *Fusarium* spp., most notably *Fusarium subglutinans* (the pitch canker fungus), are the causes of seedborne diseases in southern pines. Seeds contaminated and infected by pathogenic fungi may cause problems that could adversely affect pine seedling production in nurseries. Recent problems with mortality of longleaf pine seedlings caused by *F subglutinans*, and the association of this fungus with seeds, underscore the importance of developing a better understanding of pathogenic, seedborne fungi and the means to control them. Strategies for the control of various seedborne diseases may differ based on the epidemiology of the diseases, and the biology of the host and pathogen. This paper provides a brief review of seedborne fungal problems that affect southern pine seeds, and discusses established and potential control practices as well as current research efforts.

INTRODUCTION

Pathogenic, seedborne fungi, associated with conifers, can cause seed diseases (Sutherland et al., 1987), as well as pre- and post-emergence damping-off of seedlings (Graham and Linderman 1983; Huang and Kuhlman 1990). Numerous species of fungi are known to be associated with the seeds of southern pines (Anderson 1986; Mason and Van Arsdel 1978; Fraedrich and Miller 1995), but most are probably saprophytes that do not adversely affect seed quality. However, two groups of seedborne fungi are believed to be responsible for seed diseases and seedling disease problems in southern pine orchards and nurseries. These fungal groups include various *Fusarium* spp., such as the pitch canker fungus, *Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas, and Diplodia-like fungi such as *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl.

The detection of seedborne inoculum and the implementation of control practices are important aspects of plant disease management (Irwin 1987). Basic control practices for dealing with seedborne problems include strategies for preventing the establishment of specific fungi with seeds as well as developing remedial treatments to control diseases after establishment of seedborne pathogens. This paper provides a brief review of seedborne fungal problems that affect southern pine seeds, and discusses established and potential control practices as well as current research efforts.

BLACK SEED ROT

Some slash pine (*Pinus elliottii* Engelm. var. *elliottii*) seed orchards in the southern United States have experienced severe losses in seed quality and quantity due to black seed rot. The fungi associated with this disease are *Lasiodiplodia theobromae* (=*Diplodia gossypina*) and possibly *Sphaeropsis sapinea* (Fr.) Dyko & Sutton (=*Diplodiapinea*). Miller and Bramlett

(1979) found that *L. theobromae* was associated with internal seed damage, and poor germination in some slash pine seedlots. Commonly, the embryo and endosperm are destroyed by this fungus in infected seeds. More recent studies have also implicated *S. sapinea* (Fraedrich et al. 1994). In a 1979 survey, Diplodia-like fungi were associated with seeds from 19 of 21 slash pine seed orchards that were examined; a *Sphaeropsis* sp. was also associated with seeds (Anderson et al. 1984). *Lasiodiplodia theobromae* and *S. sapinea* are also known to contaminate and infect the seeds of other pine species such as *P. caribaea* Morelet and *P.oocarpa* Schiede (Rees 1988; Rees and Webber 1988).

Lasiodiplodia theobromae can cause a tip-dieback of loblolly and slash pine seedlings (Rowan 1982). However, no association has been established between seedborne inoculum of this fungus and terminal dieback of seedlings. *Sphaeropsis sapineu* is pathogenic to numerous pine species worldwide and can infect seedlings as well as older trees (Sinclair et al. 1987). This fungus does not appear to cause diseases of pine seedlings or trees indigenous to the southern United States.

Prevention

Studies indicate that black seed rot is primarily a postharvest problem related to the premature collection of cones (Fraedrich et al. 1994). Colonization of slash pine seeds by *L. theobromae* and similar fungi appears to be linked with the time of cone collection and collection practices. In one study, fungus-damaged seeds were not observed at the time that cones were collected from trees (Figure 1; treatment 'NGC/NS'). In contrast, the incidence of fungus-damaged seeds was relatively high in those cones that had been harvested early, and subsequently handled in a manner similar to operational conditions (treatment 'GC/S'). These cones had been dropped from trees, left on the ground for three days, and then stored for five weeks. The incidence of fungus-damaged seeds decreased on later collection dates in the 'GC/S' treatment as cones matured and specific gravities decreased.

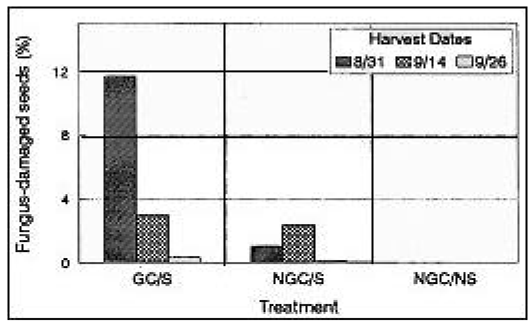

Figure 1. Incidence of fungus-damaged seeds as determined by radiographic evaluation for seeds of slash pine cones collected from four slash pine families on three harvest dates between August 30 and 26 September 1988. Cone treatments included: "no ground contact/no storage" (NGC/NS), "no ground contact/storage" (NGC/S) and "ground contact/storage" (GC/S). The ground contact period was for 3 days; the storage period was for 5 weeks. (Fraedrich et al. 1994)

Guidelines for the harvest of slash pine cones suggest that collections should begin when cone specific gravity is below 0.89 (Wakeley 1954). Slash pine clones and families can vary greatly in their time of maturation (Fraedrich and Spirek 1991; Fraedrich, et al. 1994) and this should be taken into consideration when establishing proper times to harvest cones. Managers of slash pine seed orchards that have unacceptable seed losses due to *L. theobromae* should evaluate the time of harvest with respect to the relative degree of cone maturation of families or clones in an orchard. Slash pine cones are typically dislodged from trees with a mechanical tree shaker in many orchards and subsequently gathered from the ground. These practices appear to be appropriate provided cones are sufficiently mature when harvested.

Remedial control practices

Techniques are currently available to separate fungus-damaged seeds from healthy, viable seeds in order to increase germination of seedlots. One such technique employs a specific gravity table to remove the lighter weight, unsound seeds from seedlots (Karrfalt 1983). This system has been used by several organizations with good results. Another procedure that has shown promise for the separation of viable seeds from filled dead seeds is the IDS-system, and it has been used to remove dead seeds from loblolly (*P. taeda* L.) and slash pine seedlots (McRae 1994). This procedure is based on the differential drying of viable and fi I led dead seeds, and the subsequent separation of these seeds due to their differences in weight and density.

Seedborne fungi such as *L. theobromae* and *S. sapinea* are apparently not related to any major seedlings diseases that occur in southern pine nurseries. Therefore, the use of surface sterilization agents or fungicides as remedial treatments for southern pine seeds infected or contaminated by these fungi does not seem warranted.

DISEASES OF SEEDS AND SEEDLINGS CAUSED BY SEEDBORNE *FUSARIUM SPP.*

Many *Fusarium* spp. can be associated with southern pine seeds (Mason and Van Arsdcl 1978; Pawuk 1978; Fraedrich and Miller 1995) and seeds are regarded as a potential inoculum source for Fusarium related seedling diseases which have occurred in bareroot and container nursery operations (Blakeslee et al.; 1989; Pawuk 1978). In a 1979 survey, *Fusarium* spp. were isolated from seeds of 12 of 21 slash pine seed orchards; in seven of these orchards F subglutinans was isolated from the seeds. However, *Fusarium* spp. are widely distributed, and can be frequently recovered from various sources including air, water and soil samples. Presently, the relationship is not clear between seedborne *Fusarium* spp. and seedling diseases in southern nurseries.

Seedborne inoculum has been suspected to be the source of seedling disease problems in several instances. Root rot and damping-off of container-grown southern pine seedlings in a Louisiana nursery was attributed to *Fusarium* spp. including *F oxysporum* Schlecht., *F solani* (Mart.) Sacc. and *F moniliforme* Sheld. (Pawuk and Barnett 1974; Pawuk 1978). The problem was most prevalent on the longleaf pine (*P. palustris* Mill.) seedlings, although mortality was also observed in loblolly, slash and shortleaf pine (*P. echinata* Mill.) seedlings. The *Fusarium* spp. that were commonly isolated from diseased seedlings were also commonly isolated from the seeds of seedlots sown at the nursery. In another instance, poor germination of longleaf pine seeds and damping-off of seedlings in a North Carolina nursery was caused by *F subglutinans* (Runion and Bruck 1988). Unlike many other *Fusarium* spp. associated with seeds, *F subglutinans* can be highly pathogenic to southern pines. *Fusarium subglutinans* can infect various vegetative and reproductive structures, and the fungus is responsible for pitch canker which is particularly damaging to trees in seed orchards and plantations (Dwinell et al. 1985). In addition, the fungus can infect and damage the cones and seeds of slash and loblolly pines (Miller and Bramlett 1979; Barrows-Broaddus 1990), and mav also contaminate the seedcoat of otherwise healthv seeds. *Fusarium subalutinans* has also caused mortality of slash pine seedlings in Florida nurseries (Barnard and Blakeslee 1980) and seedborne inoculum has been suspected (Blakeslee et al. 1989). Huang and Kuhlman (1990) demonstrated in greenhouse studies that several *Fusarium* spp. could cause pre- and post-emergence damping-off of slash pine seedlings from seedborne inoculum. Isolates of *F subglutinans* caused significant damping-off at 20 and 30 C; however isolates of *F proliferatum* (Matsushima) Nirenberg and *F moniliforme* infected seedlings primarily at the higher temperature.

Recent disease problems of longleaf pine seedlings caused by *F subglutinans* have renewed interest in seedborne fungi and the potential damage they can cause to cones, seeds and developing seedlings. Seed and seedling disease problems caused by *F subglutinans* have been observed at several longleaf pine nurseries and seed orchards in the southern United States. Carey and Kelley (1994) isolated *F subglutinans* consistently from diseased seedlings in a North Carolina container nursery and a bareroot nursery in Alabama. In 1995, mortality of longleaf pine in a Florida nursery, caused by *F subglutinans*, was restricted to seedlings from one particular seed source. Seedlings in adjacent seedbeds produced from other seed sources were healthy and not affected by the disease (Fraedrich, unpublished data). At a Mississippi nursery, longleaf pine seedling mortality due to *F subglutinans* has been observed during 1994, 1995, and 1996. Fusarium subglutinans has been associated with seeds from the longleaf pine seedlots used at this nursery (Fraedrich, unpublished data).

Results of several experiments with longleaf pine seeds used at the Mississippi nursery indicate that *Fusarium spp*., including *F subglutinans*, were primarily associated with seedcoats, and infections of the endosperm and embryos were rare. In an experiment using one longleaf pine seedlot, *Fusarium* spp. were associated with all seeds that were not treated with surface sterilization agents prior to plating on agar media (Figure 2). No attempt was made to identify individual *Fusarium* spp. in this treatment, but it appeared that more than one species frequently grew from individual seeds. *Fusarium* spp. were isolated from 37% of seeds after treatment with a 1% sodium hypochlorite solution for two minutes, and *F. subglutinans* was isolated from 13% of the seeds in this treatment. We isolated *Fusarium* spp. from only 1% of the seeds after treatment with a 30% hydrogen peroxide solution for 55 minutes as described by Barnett (1976).

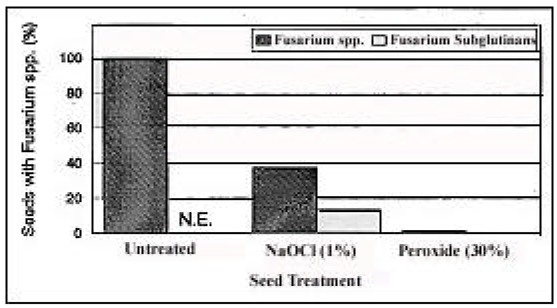

Figure 2. Assessments of *Fusarium* spp. and *F. Subglutinans* associated with seeds of a longleaf pine seedlot that were untreated or treated with surface sterilization agents. Surface sterilization agents were 1% sodium hypochlorite solution (20% Chlorox® for 2 minutes) and hydrogen peroxide (30% for 55 minutes). "N.E." indicated that "No Evaluation" was attempted.

n another experiment using three longleaf pine seedlots, *Fusarium* spp. were isolated from 16-22% of the seeds that had been surfaced sterilized with sodium hypochlorite prior to plating on agar media; *F. subglutinans* was isolated from 2-6% of the seeds (Figure 3). When seedcoats were removed from the sodium hypochlorite-treated seeds, and endosperm and embryo plated on an agar medium, *Fusarium* spp. were isolated from only 0-2% of the seeds. Results of these experiments suggest that *Fusarium* spp. were primarily located in the seedcoats of the longleaf pine seeds and were rarely present in the internal portions of these seeds.

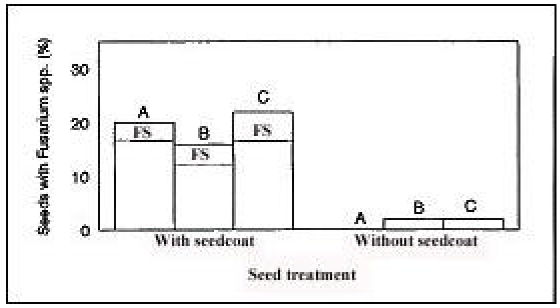

Figure 3. Association of *Fusarium* spp. and *F. subglutinans* with seeds from three (A,B,C) longleaf seedlots. Seeds had been treated with a 1% sodium hypochlorite solution (20% Chlorox® for 2 minutes). "FS" indicates *Fusarium subglutinans*.

Prevention of Seed and Seedling Diseases

Various factors are likely to have an influence on the development of Fusarium-related diseases of seeds and seedlings of southern pines; however, our understanding of the epidemiology of these diseases is presently limited. For instance, susceptibility of some southern pines to pitch canker can vary by pinecone or family (Kelley and Williams 1982; Rockwood et al. 1988), but information is lacking on possible genetic variability in the susceptibility to Fusarium-related seed and seedling diseases. Fertilization also has been linked with pitch canker of slash, loblolly and Virginia (*P. virginiana* Mill.) pines (Fraedrich and Witcher 1982; Wilkerson et al. 1975), but information is not available on possible relationships of fertilization practices in orchards to subsequent seedborne diseases caused by *F subglutinans.*

Fresh wounds are known to provide infection courts for *F subglutinans* (Dwinell et al. 1985), and the fungus is regarded as a wound pathogen of several pine species including slash, loblolly and Virginia pine (Hepting and Roth 1946; Kuhlman 1987). Results of inoculation studies indicate that F subglutinans also infects longleaf pine seedlings through wounds (Fraedrich, unpublished data). Entry of this fungus into slash and loblolly pine cones was dependent on wounds that provided an infection court (Miller and Bramlett 1979; Barrows-Broaddus 1990). Various types of agents may be involved in the wounding of seedlings and reproductive structures. These may include insects, and cone handling practices during cone harvest and processing. Infection of longleaf pine seedlings by *F. subglutinans* is thought to be related to insect damage (Carey and Kelley 1994). Contamination and infection of slash pine seeds by *F solani* has been linked to seedbug damage (Rowan and DeBarr 1974). However, the involvement of insects and other possible causes of wounding in the development of Fusarium-related seedborne diseases requires additional investigation. At present, we have much to team about the seed and seedling diseases of southern pines in order that specific recommendations and practices can be developed to prevent disease outbreaks caused by F. *subglutinans* and other *Fusarium* spp.

Remedial control practices Numerous techniques have been examined with varying degrees of success for increasing seed germination, and eliminating *Fusarium* spp. and other seedborne fungi. These treatments have included fungicides (Runion and Bruck 1988), surface sterilization agents (Barnett 1976; Wenny and Dumroese 1987), and hot water and microwave treatments (James et al. 1988). One treatment for southern pine seeds that seems to provide excellent control of seedborne problems caused by *F subglutinans* and other *Fusarium* spp. is surface sterilization with hydrogen peroxide as described by Barnett (1976). The procedure requires soaking seeds in 30% hydrogen peroxide for varying durations depending on the pine species, and then rinsing seeds thoroughly with water. Campbell (1982) indicated that surface sterilization of longleaf seeds with hydrogen peroxide prior to application of a thiram-endrin-latex repellant could not be recommended because better germinating seedlots could be adversely affected by this combination of seed treatments. Nonetheless, in recent experiments, seed germination of longleaf pine seedlots was improved from 29-49% in untreated controls to about 65% for seeds treated with hydrogen peroxide followed by an application of thiram (Fraedrich and Dwinell, unpublished data). In addition, treatment with hydrogen peroxide has the added benefit of virtually eliminating seedborne *Fusarium* spp. associated with the seedcoat. Seed treatment with hydrogen peroxide to increase seed germination has also been tested operationally by one nursery with apparently good success. The procedure can be costly and potentially hazardous when used at a high concentration for large amounts of seeds. In addition, hydrogen peroxide is not registered as a pesticide, although some believe that it may be used legally to stratify seeds. Studies are needed to better define the concentrations of hydrogen peroxide and soaking times required to disinfest seeds. Further research is needed for the development of additional chemical and non-chemical procedures that could be used to control diseases caused by pathogens associated with southern pine seeds.

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