Seed Diseases and Seedborne Pathogens of North America

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Abstract

Seedborne pathogenic fungi can greatly affect seed quality and cause diseases that impact seedling production in nurseries. Management strategies for the control of various seedborne diseases are 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 conifer seeds and discusses established and potential control practices.

Introduction

Forest-tree seed diseases and diseases related to seedborne pathogens are primarily caused by fungi. Numerous species of fungi are associated with forest tree seeds (Anderson 1986a, Mittal and others 1990), but many are saprophytes that do not adversely affect the performance of seeds sown in nurseries (Mittal and Wang 1987). Losses to seedborne pathogens include reduced seed germination, increased damping-off, and mortality of older seedlings in nursery beds. The effect of seedborne pathogens on seed and seedling production can go unnoticed until extreme germination failures have occurred in seedbeds (Fisher 1941, Epners 1964) or losses have occurred in containers (Campbell and Landis 1990). The extent to which seedborne pathogens cause losses in nursery beds is often difficult to separate from other causes of poor germination, such as damping-off by soilborne fungi. Knowledge of the biology of seedborne pathogens and practices for their management and control can help seed orchard and nursery managers reduce seed and seedling losses.

Types of Seedborne Pathogens

Pathogenic fungi can infect seeds internally and destroy the endosperm and the embryo or contaminate the seeds and affect seedling germination and development. In this paper, seedborne pathogens are defined as any infectious agent carried on the seeds, internally or externally, that has the potential to cause disease in either seeds or the developing plants. A current list of seedborne pathogens of relative importance to forest orchards and nurseries in North America is provided in table 1.

Certain seedborne pathogens primarily cause disease of seeds and have minor effects on other developmental stages of trees. Examples include diseases caused by *Lasiodiplodia theobromae* (Fraedrich and others 1994) and *Caloscypha fulgens* (Epners 1964). *Lasiodiplodia theobromae* is responsible for "black seed rot," which causes destruction of slash pine (*Pinus elliottii var. elliottii*) seeds in the Southern United States (Miller and Bramlett 1979). This fungus also causes shoot dieback of slash and loblolly (*P. taeda*) pine seedlings in nurseries in Georgia and Florida (Rowan 1982), but the shoot dieback has limited effects on production, and it is not certain if seedborne inoculum is a major factor in the disease. *Caloscypha fulgens* causes a seed rot in pine, spruce (*Picea* spp.), and fir (*Abies* spp.) seedlots in Canada and the Northern United States (Sutherland and others 1987). This fungus is particularly important because it can spread from diseased to healthy seeds during stratification and after seeds are sown in nursery beds during cool, moist conditions (Salt 1974).

Other seedborne pathogens can also be responsible for diseases that affect other developmental stages of plants, such as damping-off, shoot dieback, and cankers. Included in this category are *Sirococcus conigenus, Diplodia pinea*, and several *Fusarium* spp.

Sirococcus conigenus, found primarily in the northern latitudes of North America, causes a shoot blight that affects numerous conifer species, including pines, firs, spruces, and hemlock (*Tsuga* spp.) (Sutherland and others 1987). In container nurseries, this pathogen causes diseases of spruce seedlings, with seedborne inoculum thought to be the primary source of infection (Sutherland and others 1981).

Diplodia pinea causes shoot blight and cankers that are devastating to many pines (Sinclair and Lyon 2005). This

Table 1. Seedborne pathogens of North American forest tree species and references.

Pathogen	Host(s)	Disease	Reference
Caloscypha fulgens[1] (Pers.) Boudier	*Abies grandis* [(Dougl.) Lindl.], *Pseudotsuga menziesii* [(Mirb.) Franco], *Picea glauca* [(Moench) Voss], *Picea engelmannii* (Parry), *Picea stichensis* [(Bong.) Carr.], *Pinus contorta* (Dougl.), *Pinus resinosa* (Ait.), *Pinus. sylvestris* (L.), *Pinus strobes* (L.), *Tsuga heterophylla* [(Raf.) Sarg.]	Seed disease	Epners 1964; Salt 1970, 1974; Sutherland 1979
Fusarium spp.	Conifers	Seed disease, cotyledon blight, damping-off	Fisher 1941, Pawuk 1978, James and others 1989, Axelrood and others 1995
Fusarium circinatum Nirenberg and O'Donell (syn. *F. subglutinans* f.sp. *pini)*	*Pinus elliottii* (Engelm.) *var. elliottii, Pinus taeda* (L.), *Pinus palustris* (Mill.), *Pinus radiata* (D. Don)	Seed disease, damping-off, shoot dieback, cankers	Miller and Bramlett 1979, Barrows-Broaddus and Dwinell 1985, Runion and Bruck 1988, Storer and others 1998
Fusarium oxysporum (Schlecht.)	*Pseudotsuga menziesii, Pinus palustris*	Root rot, seed disease, damping-off	Pawuk 1978, Graham and Linderman 1983, Axelrood and others 1995
Fusarium moniliforme var. *moniliforme* (Sheld.)	*Pinus elliottii* var. *elliottii, Pinus taeda, Pseudotsuga menziesii*	Seed disease and damping-off	Mason and Van Arsdel 1978, Huang and Kuhlman 1990, Axelrood and others 1995
Fusarium proliferatum (Matsushima) Nirenberg	*Pinus elliottii* var. *elliottii*	Damping-off	Huang and Kuhlman 1990
Lasiodiplodia theobromae (Pat.) Griff. & Maubl. (syn. *Diplodia gossypina)*	*Pinus elliottii* var. *elliottii*	Seed disease	Miller and Bramlett 1979
Sirococcus conigenus (DC.) P. Cannon & Minter, (syn. *S. strobilinus)*	*Picea sitchensis, P. glauca, P. engelmannii*	Seed disease and top dieback	Sutherland and others 1981
Diplodia pinea (Desmax.) J. Kickx fil., (syn. *Sphaeropsis sapinea)*	*Pinus elliottii* var. *elliottii*	Associated with seed damage	Fraedrich and others 1994
Trichothecium roseum [Link]	*Picea glauca*	Damping-off	Mittal and Wang 1993

[1] *Caloscypha fulgens* is the perfect state of *Geniculodendron pyriforme*.

pathogen is associated with diseased seeds of slash pine (Fraedrich and Miller 1995) and loblolly pine (Fraedrich, unpublished data) and is also a seed disease of some Central American pine species (Rees and Webber 1988). In addition, *D. pinea* has been reported to infect seeds of *P. rigida* Mill. and *P. albicaulis* Engelm. at the Montreal Botanical Gardens, a location outside the natural range of both pine species (Vujanovic and others 2000). *Diplodia pinea* is a periodic problem in some northern nurseries, but inoculum from sources other than seeds is considered more important (Palmer and others 1988). Nonetheless, the association of *D. pinea* with seeds provides a means by which this pathogen may become established in new locations.

Fusarium spp. are widespread in their distribution, and many are associated with seeds of conifer species (Anderson 1986a, Mittal and others 1990). *Fusarium circinatum* (syn. *F. subglutinans* f. sp. *pini*), the pitch canker fungus, is a highly virulent pathogen that can infect reproductive and vegetative stages of many pine species (Dwinell and others 1985). The pitch canker fungus has long been known to be a seedborne pathogen in the Southern United States and, since the late 1980s, as a seedborne contaminant of Monterey pine (*P. radiata*) in California (Dwinell and Fraedrich 2000). The potential transport of this pathogen via infested seedlots is a serious concern nationally and internationally.

Other species of *Fusarium* that can cause seedborne diseases include *F. oxysporum*, *F. moniliforme*, and *F. proliferatum*. The pathogenicity of isolates within these species ranges from highly virulent to nonpathogenic; therefore, the level of contamination by a *Fusarium* sp. does not always correspond to development of seedborne diseases (Pawuk 1978, Graham and Linderman 1983, Axelrood and others 1995). In several studies, damping-off caused by pathogenic isolates of *F. oxysporum*, *F. moniliforme,* and *F. proliferatum* has been shown to increase greatly following heat stress (Huang and Kuhlman 1990, Axelrood and others 1995).

The presence of certain fungi on seeds is often significant because it may indicate problems with the quality of the seedlot due to improper handling and storage of both cones and seeds. Seedborne fungi such as *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., and *Trichoderma* spp. have reduced germination of conifers in some laboratory tests (Fisher 1941, Gibson 1957, Mittal and Wang 1993). However, these fungi were associated with seeds that were damaged (Gibson 1957), of low vigor (Mittal and Wang 1993), or grown in an environment that favors fungal over seedling growth (Fisher 1941). Agarwal and Sinclair (1997) regard many of these fungi as "storage fungi" that may be involved in deterioration of seeds during storage.

Detection of Fungus-Damaged Seeds and Determination of Pathogens Associated With Seeds

The detection of seedborne pathogenic fungi and seed diseases is an important aspect of disease management. Determining the presence of seedborne pathogens allows managers to apply the appropriate controls or modify management practices to avoid the problem in the future.

The presence of diseased seeds in seedlots cannot be reliably detected by visual examination. Radiographic assays of seeds (figure 1) provide an efficient, nondestructive method to determine internal seed damage (Karrfalt 1983). Internal seed contents can be examined by cutting the seed open (figure 2) and looking for mycelium or symptoms of disease (Sutherland and others 1987).

Seedborne pathogens can also be present on seeds without obvious disease symptoms or signs. The presence of pathogenic fungi on seeds is most often determined through laboratory culture and identification. Samples of seeds are placed on various media and the fungi that grow from the seeds are evaluated (Anderson 1986b). Although this technique is widely employed, it is time consuming and may not detect pathogens at low levels. Competitive saprophytic fungi on seeds are an additional problem because they can obscure the presence of a pathogen. For some fungal species, such as *F. oxysporum*, evaluation of isolates from seeds on living seedlings is necessary to determine pathogenicity (Littke 1997).

In recent years, research has been developing more sensitive and less time-consuming techniques to detect pathogens in seedlots. An immunological assay (ELISA test) has been developed to detect *S. conigenus* in spruce seedlots (Mitchell and Sutherland 1986). This type of test is highly specific, less time consuming, and affords greater accuracy because sample size can be greatly increased (Sutherland and others 1987). The development of assays to detect other seedborne pathogens such as *F. circinatum* and *D. pinea* could prove very beneficial. These techniques could be especially useful in seed certification programs where seeds are to be shipped internationally or used in areas outside the known range of specific pathogens.

Pathogen Establishment and Disease Development

Many factors are related to the establishment of pathogens on and inside seeds and the development of seed diseases. Contamination and infection of seeds by pathogens can occur in all phases of seed production. For many diseases,

Figure 1. Radiographs of seeds can be a useful tool for the detection of internal seed problems, including infection by pathogenic fungi (Photo courtesy of Thomas Miller).

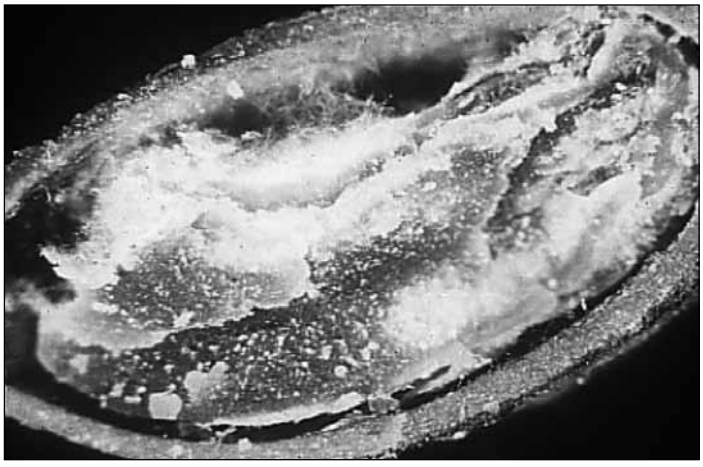

Figure 2. The mycelial growth of seedborne pathogens is often readily detected in seeds with internal infections (photo by Stephen Fraedrich).

however, the relationships between pathogens and seed production are not well understood.

Cone collection practices influence seedborne diseases caused by *L. theobromae* on slash pine (Fraedrich and others 1994) and *C. fulgens* on spruce seeds (Sutherland 1979). These fungi become established on cones when they have been in contact with the ground for extended periods. *Caloscypha fulgens* spreads among seeds in storage or in the field under cold wet conditions (Sutherland and others 1987). Disease development caused by *L. theobromae* is also strongly affected by the degree of cone maturation when cones are shaken from trees. Cones that are collected prematurely (specific gravity >0.89) have a higher incidence of disease than more mature cones with lower specific gravities (Fraedrich and others 1994).

Sirococcus conigenus becomes established in seedlots when older cones are inadvertently included in the cone harvest (Sutherland and others 1981). This seedborne pathogen spreads to seeds before germination and can result in post-germination infection. The conditions that favor this pathogen include high humidity, low light, and cool temperatures ranging from 10 to 20 °C (Sutherland and others 1987).

Several factors have been linked to the development of *Fusarium*-related diseases of seeds and seedlings of conifers; however, our understanding of the epidemiology of these diseases is limited (Kuhlman and others 1982). The method by which *Fusarium* spp. becomes established internally in seeds is still uncertain. Inoculations of strobili with *F. circinatum* during pollination failed to demonstrate that seeds become infected at the time of pollen receptivity (Miller and others 1987). Assessments of seedlots for *F. circinatum* suggest that this pathogen is more likely to be associated with seeds of longleaf pine (*P. palustris*) produced in intensively managed seed orchards than in unmanaged seed production areas (Fraedrich, unpublished data). The use of fertilizers has been suggested as a factor involved in the greater occurrence of *F. circinatum* in orchard seeds. Fertilization has been linked with an increase in pitch canker on slash, loblolly, and Virginia (*P. virginiana* Mill.) pines (Wilkinson and others 1977, Fraedrich and Witcher 1982); however, a direct link between fertilization, *F. circinatum* infection, and seed contamination has not been reported.

Fresh wounds provide infection courts for *F. circinatum* (Miller and Bramlett 1979, Barrows-Broaddus 1990). Various agents can wound reproductive structures, including insects, storm damage, and cone handling (Dwinell and others 1985). Insects can also vector *F. circinatum* (Hoover and others 1996) and have been associated with seed deterioration (Bramlett and others 1977). The levels of seed contamination by *Fusarium* spp. varies by collection date and by orchard (Fraedrich and Miller 1995, Littke 1997). Contamination can also vary by tree clone or family (Kelley and Williams 1982, Rockwood and others 1988, Carey and others 2005), but information is lacking on the regulation of susceptibility to *Fusarium*-related seed and seedling diseases through genetic improvements.

Management and Control of Diseases Caused by Seed and Seedborne Diseases

Strategies for management of seed disease and seedborne pathogens focus on prevention of disease and contamination or on remedial procedures to reduce contamination. The type of problem and the causal agent determine the applicability of various pest management approaches. Generally, no single method will provide complete control of any specific seedborne disease; control is best achieved through an integrated pest management approach (Agarwal and Sinclair 1997). For some seedborne problems, little information is available on the biology of the pathogen and epidemiology of the diseases that they cause. Thus, recommendations for disease prevention and control may not always be readily available.

Cone Collection and Management. Some seed and seedborne diseases are linked to cone collection practices, and modification of practices can help to prevent disease losses. *Sirococcus conigenus* is a problem on spruce seeds only when old cones are included in fresh cone collections (Sutherland and others 1981). The simplest method of controlling this pathogen is to avoid collecting old cones. Yearly collection of spruce cones has probably helped keep seedlots free of *S. conigenus* in Canada (Sutherland and others 1987).

In western North America, *C. fulgens* has been found commonly in seeds from cones collected from the ground and from squirrel caches (Sutherland 1979). In the Southern United States, infection of slash pine seeds by *L. theobromae* is also most prevalent in cones that are collected from the ground (Fraedrich and others 1994). Collecting cones directly from trees eliminates *C. fulgens* from seedlots (Sutherland and Woods 1978) and significantly reduces *L. theobromae* from slash pine seeds (Fraedrich and others

1994). Since slash pine seeds are usually collected by shaking trees and collecting the cones from the ground, managers can reduce *L. theobromae* in the seeds by collecting cones with a specific gravity of less than 0.89. Variation in cone maturation among individual pine clones or families is an important consideration when establishing the appropriate times to collect cones (Zoerb 1969, Fraedrich and others 1994). Seed viability can also decrease when cones are collected in advance of cone maturity, or when cones are stored at incorrect temperatures or seed moisture contents (Barnett 1997). Collection times can be extended for a few weeks for some species, such as loblolly pine. However, seed viability in species such as longleaf pine decreases during cone storage when cones are collected at a specific gravity <0.80; therefore, cones should be collected only when mature and stored for no more than 4 to 5 wk (Barnett 1997).

The effect of cone collection and management practices on the establishment of *Fusarium* spp. with conifer seeds is somewhat less clear than with other fungi such as *C. fulgens* and *L. theobromae*. The association of *F. circinatum* with seeds and cones does vary somewhat among collection times and clones within an orchard (Kuhlman and others 1982, Dwinell and Fraedrich 1997, Carey and others 2005), but the expression of the disease is associated with wounding and has been linked to high levels of fertilization. Therefore, disease incidence is not necessarily correlated with resistance.

Many managers protect pine reproductive structures in orchards from insects with regularly scheduled insecticide sprays, thus minimizing wounding and the presence of possible vectors of *F. circinatum*. Managers can also try to limit wounding due to mechanical damage. When an outbreak of pitch canker does occur in an orchard, managers may be able to reduce seed infestation and loss by avoiding heavily infested trees during cone collection. Carey and others (2005) found a correlation among pitch canker ratings of longleaf pine clones, the percentage of seed infested, and seedling mortality. They concluded that clones exhibiting a high incidence of pitch canker should be removed from seed collections.

There is some evidence that *Fusarium* levels on seeds increase with cone storage (Fraedrich and Miller 1995) and that *Fusarium* spp. infest seeds mostly after seed processing (Littke 1997). Littke (1997) reported that faster cone-drying schedules reduced *Fusarium* levels on seeds of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] following extraction. Faster cone drying may be a benefit when storage is not needed for seeds to mature. Fortunately, when *Fusarium* contaminates seed, more than 90 percent is on the surface and can be controlled by seed treatments (James 1986, Dwinell and Fraedrich 1997, Littke 1997).

Seed Treatments. Various types of seed treatments can control seedborne pathogens. These treatments may include chemical, physical, and mechanical control. Seed treatments have the potential to damage seeds; therefore, seed treatments should be used only when the gain in germination and seedling survival is greater than the potential loss. Chemical treatments, in particular, can be toxic to seeds and should be used with caution (Vaartaja 1964, Runion and others 1991).

Chemical Seed Treatments. Seed treatments currently used in the United States for control of seedborne pathogens are thiram and seed disinfectants. Thiram (tetramethylthiuram disulfide) is commonly used in nurseries as a bird and animal repellent, as well as a fungicide. In particular, thiram has been effective against *F. oxysporum* on ponderosa pine and Douglas-fir seeds (Littke 1997), and against *Fusarium* spp. on longleaf pine seeds (Barnett and Varela 2003). Thiram can have a toxic effect on seeds of many conifer species (Belcher and Carlson 1968, Dobbs 1971, Runion and others 1991), especially at high dosages (Bloomberg and Trelawny 1970). Its benefit as a bird/animal repellent and fungicide, however, often outweighs the relatively small toxic effect on germination under operational conditions (Abbott 1958, Nolte and Barnett 2000). Other fungicides not specifically labeled for use on tree seeds have been tested with some positive results, but so far have not provided significantly better control of fungal infestation or germination than thiram or the disinfectants (Barnett and others 1999, Barnett and McGilvray 2002, Barnett and Varela 2003, Allen and others 2004).

Disinfectants such as sodium hypochlorite (i.e., the active ingredient in bleach), hydrogen peroxide, and hydrogen dioxide (Zero Tol®) can be used to reduce fungal contamination and improve seed germination. Hydrogen peroxide has long been known to eliminate seedborne mycoflora and to stimulate seed germination (Trappe 1961). A 30-percent concentration of hydrogen peroxide can be effective for increasing germination of conifer seeds (Riffle and Springfield 1968, Barnett 1976, Barnett and McGilvray 2002) and can virtually eliminate seedcoat contamination of longleaf pine seeds by *F. circinatum* and

other *Fusarium* spp. (Fraedrich 1997). Concentrations of 3-percent hydrogen peroxide have also been effective for reducing seedborne contamination of conifers by various *Fusarium* spp. (Dumroese and others 1988, Ocamb 1995, Littke 1997, Hoefnagels and Linderman 1999). Poststratification treatments with 3-percent hydrogen peroxide are particularly effective for eliminating seedborne inoculum and maintaining high germination in Douglas-fir seedlots (Dumroese and others 1988).

Prestratification seed treatment with 2-percent sodium hypochlorite also reduces *Fusarium* contamination of Douglas-fir seeds (Dumroese and others 1988) and can increase germination in several Western conifers (Wenny and Dumroese 1987). Pretreatment of seeds briefly with ethyl alcohol can increase the efficacy of sodium hypochlorite in some agricultural crops (Sauer and Burroughs 1986), and this practice may have applications for seeds of some conifers. Hydrogen dioxide (Zero Tol®) is a surface sterilant that is registered as a fungicide for tree seeds. Application of hydrogen dioxide on longleaf pine seed has been found to reduce *Fusarium* contamination without inhibiting germination (Allen and others 2004).

Physical Seed Treatments. Water rinses over 24 to 48 h can be used to reduce seed pathogens and improve germination (Riffle and Springfield 1968, Wenny and Dumroese 1987, Littke 1997). Stratification can increase the presence of fungi in some seedlots and a running water (2 h) imbibition treatment can decrease surface contamination (Axelrood and others 1995). However, water rinses will not eliminate contaminating fungi from seeds (Riffle and Springfield 1968, Littke 1997). Seedlots suspected of being contaminated with a seedborne pathogen should also be treated with a disinfectant or fungicide (Campbell and Landis 1990).

Heat treatments have been used to control certain seedborne pathogens without affecting seed viability. Heat treatments include hot water, aerated steam, and microwave radiation. Heat treatments have been used on seeds of agricultural crops in numerous studies (Agarwal and Sinclair 1997). Microwave hot-water treatments have controlled *Fusarium* spp. on Douglas-fir seeds without significantly affecting germination (James and others 1988). Heat treatments may have potential use as a seed treatment for other conifer seeds but will require additional research.

Mechanical Seed Treatments. Various mechanical methods have been used to remove dead and fungus-damaged seeds from healthy, viable seeds in order to increase germination of seedlots and reduce inoculum of seedborne pathogens. Specific gravity tables can be used to separate fungus-damaged seeds from seedlots (Karrfalt 1983). This system has been used by several organizations with good results. The proper calibration of the specific gravity table for individual seedlots is important to minimize the loss of good seeds while rejecting fungus-damaged seeds. The IDS (Incubation, Drying, Separation) system is another procedure for separating viable from filled-dead seeds (Simak 1984, Karrfalt 1997). The procedure is based on the differential drying of viable and filled dead seeds, and the resulting separation of these seeds according to their differences in weight and density. The IDS system has been used successfully to remove damaged seed from seedlots of various conifers (Donald 1985, Downie and Wang 1992, McRae and others 1994) and *Plantanus* x *acerifolia* (Ait.) Willd. (Falleri and Pacella 1997).

Summary

Compared to seedborne disease problems of agricultural crops, research on seedborne pathogens that affect production of forest-tree species has been very limited in North America. One possible reason is that diseases from seedborne pathogens often go undiagnosed under operational conditions. There are many causes of poor germination, and determining if a seedborne pathogen is a factor can be difficult and time consuming. Testing seed germination is often a first step in determining if a seedborne pathogen is a problem. Confirming the presence of a pathogen usually requires the services of a pathologist.

Some seedborne pathogens and diseases can be avoided by modifications in cone collection practices. Treating seeds with disinfectants and thiram can reduce seedcoat contamination by pathogens and increase germination. Mechanical separation techniques can remove diseased seeds and improve seedlot quality. In some cases, diseases caused by seedborne pathogens are a constant problem, and seed treatments are routinely used by managers. Seed efficiency could be increased through further research on avoiding pathogen establishment and preventing disease development, as well as developing more effective seed treatments.

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