



Keynote Address

Investigations of *Fusarium* Diseases within Inland Pacific Northwest Forest Nurseries

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Abstract – *Fusarium* spp. cause important diseases that limit production of seedlings in forest nurseries worldwide. Several aspects of these diseases have been investigated for many years within Inland Pacific Northwest nurseries to better understand disease etiology, pathogen inoculum sources, and epidemiology. Investigations have also involved improving disease control efforts to limit impacts. Major diseases caused by *Fusarium* spp. include pre- and post-emergence damping-off, root disease, stem cankers, and top blight. The major *Fusarium* pathogen of bareroot nurseries is *F. oxysporum*. It is a common soilborne species with pathogenic and nonpathogenic strains readily isolated from conifer seeds, diseased and healthy seedlings, and nursery soil. Pathogenic strains appear to be genetically distinct from common saprophytic strains. The major *Fusarium* pathogen of container nurseries is *F. proliferatum*. This species is especially adapted for rapid spread throughout greenhouses. Most tested isolates of *F. proliferatum* are highly virulent. Other *Fusarium* species commonly associated with seedling diseases include *F. solani*, *F. acuminatum*, *F. sporotrichioides*, *F. sambucinum*, and *F. avenaceum*. Several other *Fusarium* spp. are encountered infrequently. Most other *Fusarium* spp. are less virulent on conifer seedlings than *F. oxysporum* and *F. proliferatum*. Improved disease control has been obtained by reducing pathogen inoculum on seeds, reused containers, greenhouse environments, and within nursery soil. Biological control has not yet proven as effective as fungicides in reducing disease severity. Pathogens colonizing roots of seedlings are usually replaced by other mycoflora following outplanting on forest sites. Efforts to reduce dependence on pre-plant soil fumigation have been successful in some, but not all bareroot nurseries. Keeping fallow fields free of plants with periodic cultivation for at least one year may often be as effective as fumigation. Conversely, growing cover crops, incorporating organic amendments into soil, and rotating seedling production with *Brassica* green manure crops have usually proven unsuccessful because of stimulation of *Fusarium* populations following addition of organic matter

to soil. Future research efforts should involve using molecular probes to quantify pathogenic *Fusarium* populations to allow better prediction of requirements for implementation of disease controls.

Introduction

Diseases caused by *Fusarium* spp. within forest nurseries have been investigated in North America for nearly a century. However, they still severely impact production of high-quality seedlings at many nurseries. Most previous investigations focused on diseases in bareroot nurseries and very little was known about disease epidemiology or procedures to reduce disease impacts in container nurseries. Therefore, we began investigating diseases associated with *Fusarium* spp. more than 20 years ago. Our goal was to understand disease etiology, sources of pathogen inoculum, and various aspects of disease epidemiology specific to greenhouse environments. This information would lead to developing improved disease control.

Production of container seedlings within greenhouses has greatly increased in western North America during the past two decades. Seedlings can be grown in shorter time periods to provide reforestation stock resulting from changing demands from land managers. Seedling sizes, especially root systems, can be tailored to particular specification requirements. As such, seedling stock may be more adaptable to forest site differences, thereby improving the performance of outplanted seedlings.

Types of Diseases

Fusarium spp. commonly infest conifer seeds, particularly contaminating outer seedcoats (Axelrood and others 1995; James 1985b, 1986a, 1987c, 1999). This inoculum allows rapid colonization of young, succulent seedling tissues upon seed germination which may result in both pre- and post-emergence damping-off (James 1986a, 2004; James and Genz 1981; James and others 1991). Although seedlings are usually susceptible to damping-off for only short time periods, losses can be extensive, particularly if seedlots are extensively contaminated with

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populations of *Fusarium* (Hoefnagels and Linderman 1999; James 1986a; James and others 1991). *Fusarium oxysporum* is the most common conifer seed-contaminating *Fusarium* species; other common seed colonizers include *F. solani*, *F. acuminatum*, and *F. sambucinum* (James 1987c; James and others 1991).

Root diseases caused by *Fusarium* spp. can occur throughout the life of the crop. Within bareroot nurseries, most mortality occurs during the first growing season (James 1987b, 2001b, 2002d), with little or no mortality associated with root disease occurring during the second or subsequent years. With container seedlings, however, the highest root disease mortality often occurs at the end of the growing cycle when seedlings are stressed to stop growing and initiate bud formation (James 1986a; James and Gilligan 1984; James and Perez 1998; James and others 1987). Stresses induced by reduced watering and restriction of nutrients can cause seedlings to begin to show disease symptoms. Many of these seedlings were infected with potential pathogenic *Fusarium* early in their growth cycle (James and Gilligan 1988a; James and others 1987) but disease did not appear because seedlings were provided with plenty of water and optimum fertilization. As seedlings become stressed, colonizing *Fusarium*, which previously acted like non-pathogenic root endophytes, begin to induce tissue necrosis and disease symptoms subsequently appear.

Fusarium spp. can potentially cause stem cankers, particularly in dense bareroot seedbeds within coastal nurseries where high humidity prevails (Hansen and Hamm 1988; James 1986c, 2003b). These cankers are either produced low on the stem, just above the ground line, or higher in the seedling near foliage. Incidence and severity of stem cankers vary from year to year and may be related to weather influences as well as level of soilborne inoculum (Hansen and Hamm 1988; Hansen and others 1990; James 2003b).

Top blight of container seedlings associated with attack by *Fusarium* is usually rare. Ponderosa pine (*Pinus ponderosa*) is the most susceptible conifer species to this type of damage (James 1992a, 2003a), probably because seedcoats may persistently remain attached to cotyledons. Seedborne inoculum may result in cotyledon infection with subsequent colonization of stem and root tissues resulting in seedling mortality.

Major *Fusarium* Pathogens _____

Fungi in the *Fusarium oxysporum* species complex are the most important *Fusarium* pathogens in bareroot forest nurseries (James 2004; James and others 1991, 1996b, 2000). These organisms also occur on container seedlings, particularly as a result of seed contamination (Dumroese and others 1988; James 1987c; James and Genz 1981). Organisms classified as *F. oxysporum* may actually comprise several genetically distinct species (Gordon and Martyn 1997; Kistler 1997). Pathogenic and nonpathogenic strains are common within forest nurseries and usually appear very similar morphologically. Pathogenicity tests have indicated a wide range of potential virulence on conifer seedlings under controlled conditions (James and others 2000). Genetic markers may be useful in separating pathogenic from nonpathogenic isolates (Donaldson and others 1995; Gordon and Martyn 1997; Stewart and others 2004).

Isolates of *F. oxysporum* commonly reside in nursery soil and can remain viable for prolonged periods because of production of long-lived chlamydospores and sclerotia (James and others 1991; Nelson and others 1983). These fungi are excellent colonizers of soil organic matter, regardless of its source (Hansen and others 1990; James 1988b, 2002a; James and others 1996b, 2004a, 2004b, 2004c). When susceptible seedling crops are sown in infested soil, seedlings can become readily infected following stimulation of chlamydospores by host root exudates (James and others 1991; Schippers and others 1981). Once infected, seedlings can exhibit root disease symptoms, probably due to infection by virulent isolates. However, some infected seedlings may remain infected but not become diseased (James and Gilligan 1988b). In this case, *F. oxysporum* acts more like a nonpathogenic root endophyte than a true pathogen. Pathogenic isolates usually induce cortical cell necrosis resulting in root decay (James 2004; James and others 1991), and may readily colonize most root and stem tissues. Seedling mortality occurs when decayed root systems no longer function.

In container seedlings, the most important *Fusarium* pathogen is usually *F. proliferatum* (James 1997a; James and others 1995a, 1997). This *Fusarium* species is taxonomically placed in the section *Liseola*, which includes other important pathogens such as *F. circinatum*, cause of pitch canker of pines (Gordon and others 2001). *Fusarium proliferatum* is not commonly found on conifer seeds, nor is it a very common nursery soil inhabitant (James 1987c, 1997a, 1998a, 1998b, 2002b). This species does not

produce chlamydospores that would allow long-term survival (James 1997a; Nelson and others 1983). However, it is an excellent colonizer of organic matter and may colonize a wide range of plants without inducing disease symptoms (James 1997a; James and Gilligan 1988a; Nelson and others 1983). This species spreads rapidly throughout greenhouses, primarily from microconidia that are readily produced in chains (James 1997a; Nelson and others 1983). Roots of container seedlings may become infected by *F. proliferatum* when seedlings are relatively young (James 1997a; James and others 1987). However, they may or may not exhibit disease symptoms. Production of disease symptoms is primarily related to level of seedling stress (James and others 1990a). Most tested isolates of *F. proliferatum* exhibited high virulence (James and others 1986, 1989, 1997) under controlled pathogenicity tests that utilize a perlite-cornmeal-potato dextrose agar inoculum (James 1996; Miles and Wilcox 1984). Therefore, these fungi are capable of causing serious losses to conifer seedling crops grown in greenhouses. In some cases, entire crops have been lost to this pathogen, resulting in extensive economic consequences. However, healthy appearing seedlings can have roots that are extensively colonized by *F. proliferatum* by the end of the growth cycle (James and Gilligan 1988a). When seedlings with *Fusarium* root colonization are outplanted on forest sites, *Fusarium* spp. gradually declines on roots systems and are usually replaced of other mycoflora (Dumroese and others 1993a; Smith 1967).

Several other *Fusarium* spp. are encountered in forest nurseries. Those most commonly isolated include *F. solani*, *F. acuminatum*, *F. sporotrichioides*, *F. sambucinum*, and *F. avenaceum*. Several of these are routinely isolated from roots of diseased or healthy appearing seedlings (James and Gilligan 1988a, 1988b; James and others 1991), nursery soil (James 1984, 2002b, 2002c), peat-based container growing media (James 1985a, 2005), conifer seeds (Dumroese and others 1988; James 1987b), and the inner walls of reused styrofoam or plastic containers (Dumroese and others 1995; James 1989b, 1992b, 2001a). Tests to evaluate potential virulence of some of these species indicated that they are mostly saprophytes, although selected isolates may be capable of eliciting disease on conifer seedlings (James 2000b; James and Perez 1999, 2000). It is possible that some of these other *Fusaria* contribute to disease when associated with more aggressive pathogenic species such as *F. oxysporum* and *F. proliferatum*.

Fusarium Disease Management

Successful management of *Fusarium* diseases in forest nurseries requires emphasis on prevention (James and others 1990a, 1995b). Most approaches are designed to reduce inoculum of potential pathogens within and near seedling growing areas. In addition, it is important to maintain the vigor of nursery seedlings by proper manipulation of water and fertilizer (James 1997b; James and others 1990a). If pathogen inoculum is reduced to a minimum and seedlings are kept vigorous, *Fusarium* disease losses are usually small.

Seedborne inoculum can be greatly reduced by treating seeds with running water rinses or surface sterilization chemicals (James 1985a, 1986a, 1987a; James and others 1996a; Lock and others 1975). This type of treatment may be preferable prior to stratification because *Fusarium* spp. are known to spread throughout seedlots during prolonged stratification (Axelrod and others 1995; James 1987a, 1987c; Kliejunas 1985). Some chemical seed treatments are routinely used for particular conifer species (Sutherland and van Eerden 1980; Wenny and Dumroese 1987). However, care must be taken to ensure that chemicals do not adversely affect seed germination or emergence of young germinants (Dumroese and others 1988; James 1986a; James and others 1995b; Pawuk 1979). The most effective and least potentially harmful treatment is exposing seeds to running water rinses for at least 48 hours during which seeds are periodically agitated (James 1985b, 1987a; James and others 1996a). This water treatment greatly reduces fungi, including pathogens, which commonly contaminate seedcoats.

Container seedlings are usually produced within either plastic or styrofoam containers. Because these containers are costly, several seedling crops must be grown within individual containers. As a result, potential pathogenic fungi, especially *Fusarium* spp., often contaminate the inner walls of reused containers (James 1989a, 2001a; James and others 1988). This inoculum presents an important potential hazard to subsequent seedling crops. Procedures for cleaning reused containers have changed over the years. Initially, containers were either washed superficially to remove most residual organic matter or steam treated. These treatments, however, were ineffective in significantly reducing populations of potential pathogens. Chemical treatments such as sodium hypochlorite (James and Sears 1990), sodium metabisulfite (Dumroese and others 1993b), or coating containers with copper solutions (Dumroese and others 1995) were more effective, but

were hazardous to use or resulted in chemical disposal problems. Immersion in hot water (at least 68°C for 30 seconds) became the most widely used, effective container treatment (Dumroese and others 2002; James 1982b; James and Eggleston 1997; James and Woollen 1989). However, high costs of energy required to maintain sufficient water temperatures, stimulated investigations into effective alternatives. Tests of radio frequency waves (James and Trent 2001), dry heat (James and Trent 2002), and large capacity steam rooms (Trent and others 2005) indicated that alternatives to hot water immersion are not only effective, but may be less expensive.

Cleaning greenhouse interiors (walls, floors, benches) with surface sterilants between seedling crops is necessary to reduce inoculum of potential pathogens, including *Fusarium* spp., which tend to accumulate within greenhouses during typical crop cycles (James 2004; James and others 1990a, 1995b). In addition, if greenhouse floors are not made of concrete, weeds growing within houses may harbor important pathogens (James and Gilligan 1984; James and others 1990a) and should be periodically removed. Sanitation procedures, such as periodically removing diseased plants, also helps reduce disease impacts (James 2004).

Soil is a major source of potential pathogen inoculum in forest nurseries. Several *Fusarium* spp. are particularly well adapted to maintain high populations in nursery soil. The most effective and widely used technique for reducing soil pathogens is pre-plant soil fumigation with non-specific biocide chemicals (Hansen and others 1990; Hildebrand and others 2004; James 1989a; James and Beall 1999; James and Ziedler 2004; James and others 1990b, 1996b). Such treatments are very expensive. Although potential pathogens are killed by soil fumigation, all other microorganisms, including beneficial and potential biocontrol fungi and bacteria, are killed as well. Therefore, once a nursery begins soil fumigation, growers usually have to repeat treatments prior to sowing each subsequent seedling crop (James 1989a; James and Beall 2000).

Biological control has potential for reducing *Fusarium* disease losses in forest nurseries. Several commercial biocontrol products are available with proven efficacy against *Fusarium* (James and others 1993). Unfortunately, most tests of fungal (Dumroese and others 1996; James 200a; Mousseaux and others 1998) and bacterial (Dumroese and others 1998) preparations in controlling diseases have so far been disappointing. This may be because tested

formulations were developed for specific agricultural crops rather than for use in forest nurseries. Perhaps biocontrol formulations of effective pathogen competitors that routinely occur in forest nurseries, such as nonpathogenic isolates of *F. oxysporum* (James 2000a), may be more effective.

Most growers still rely heavily on applications of chemical fungicides to control many nursery diseases, including those caused by *Fusarium* spp. For some parts of the seedling growing cycle, such as the damping-off stage, chemical treatments are usually quite effective. However, to control root diseases of older seedlings, drenching soil or container media with fungicides has generally been ineffective (James 1984, 1988, 1998a; Shrimpton and Williams 1989). Incorporating chemicals into growing media prior to sowing container seedlings has also been ineffective (Dumroese and others 1990). Although *Fusarium* spp. are generally sensitive to particular fungicides, such as thiophanate methyl, treatments may not result in sufficient chemical concentrations reaching infected roots. In addition, most fungicides are good at preventing diseases rather than acting therapeutically (James 1988) so that treating seedlings already extensively colonized by potential pathogens has little value.

Alternatives to Pre-Plant Soil Fumigation

As indicated above, pre-plant soil fumigation has become widely used in many bareroot nurseries in the United States. Traditionally, the chemical of choice for fumigation is a mixture of methyl bromide (67%) and chloropicrin (33%). However, methyl bromide was recently identified as an important contributor to the destruction of stratospheric ozone (Hildebrand and others 2004; James and others 1994). As a result, it was programmed for reduction and eventual elimination as a soil fumigant and other applications worldwide. Although the deadline for use of methyl bromide in the United States was January 2005, exemptions have been allowed for industries for which no other viable alternatives exist. Some forest nurseries have applied for these exemptions.

When we first learned of the phaseout and eventual elimination of methyl bromide as a soil fumigant, we began a series of trials at different bareroot nurseries to evaluate potential alternatives. Our goal was primarily to find effective alternatives to chemical soil fumigation altogether rather than finding a replacement chemical fumigant. We found that fallowing fields for a least one year (preferably 2-3

years) with periodic cultivation to keep soil aerated, remove weeds, and expose propagules of potential pathogens can be as effective as chemical soil fumigation (Hildebrand and others 2004; James and Ziedler 2004; James and others 1996b). Fallowing is especially effective at nurseries with well-drained loam soils containing limited amounts of clay (James and Beall 2000). The beneficial effects of fallowing, however, can be eliminated by incorporating organic matter into soil, either as a direct amendment or incorporating a green manure crop (James 1998b; James and others 1996b). *Fusarium* populations greatly increase within soils to which high amounts of organic matter have been added (Hansen and others 1990; Hildebrand and others 2004). This effect occurs even if the green manure crop is a *Brassica* spp. (James and others 1996b, 2004a, 2004bg, 2004c), which is grown and incorporated into soil because residues produce methyl isothiocyanate, the active ingredient in some chemical soil fumigants such as dazomet (Smolinska and others 2003). Apparently, the amount of added organic matter overrides the potential toxicity to resident pathogens afforded by *Brassica* tissues.

Our investigations indicated that some nurseries can effectively replace methyl bromide by prolonged fallowing of fields or substituting with another chemical fumigant, such as dazomet (James and others 1990b, 1996b). However, other nurseries have yet to have effective alternatives to methyl bromide and will probably use this chemical as long as the law allows. One additional alternative is conversion of most seedling production to containers, which has been used effectively throughout much of Canada.

Future Directions

Over the century we have learned a great deal about *Fusarium* diseases in forest nurseries. Losses to these fungi are now usually much less in both bareroot and container nurseries than previously. However, these diseases are usually still present at some level in most seedling crops and disease losses may become unacceptable on occasion. When new inexperienced growers are employed at nurseries or when new container facilities are installed, initial disease losses may be quite high. As growers become more experienced, they learn how to prevent diseases more effectively and losses from *Fusarium* usually decline over time.

Plant pathology entered the era of molecular biology several years ago. As a result, many plant diseases are often routinely diagnosed using molecular probes that can be developed for specific strains of particular pathogens (Gordon and Martyn 1997; Kistler 1997;

Summerell and others 2003). Preliminary work with *F. oxysporum* in forest nurseries (Donaldson and others 1995; Stewart and others 2004) indicates that these tools have great potential application. If the pathogenic strains of this important fungus can be easily identified and quantified in nurseries, growers can more accurately predict expected disease levels and make appropriate adjustments to implementing control measures. Identifying saprophytic strains that are genetically different from pathogens opens the possibility of using these strains as more reliable biological control agents because they behave similarly, i.e., occupy the same niches, to pathogens.

More emphasis on cultural manipulations rather than chemical pesticides will be needed in the future. Many standard chemical fungicides used at high levels in the past are either currently or will shortly be unavailable in forest nurseries. Environmental concerns, such as worker exposure and groundwater contamination, are important considerations for the future of nursery disease management. More benign, but efficacious, treatments will be required in the future. Biological control has an important part to play in the future of nursery disease management. Evaluating new commercial products as they become available will be important.

As nursery growers reach the end of their careers, it is important that their replacements become knowledgeable about disease management. Successful disease management often evolves over time at particular nurseries and much of this important information can be lost to new growers. Continued support for nursery pathology is an important way to ensure that disease management information remains available to growers in federal, state, tribal, and private nurseries. This “extension” role is very important for agencies mandated to provide pathology assistance. Hopefully, nursery pathology assistance will continue to receive high priority.

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