Fusarium Species—a British Columbia Perspective in Forest Seedling Production

Michael Peterson

MICHAEL PETERSON
President and Principal Scientist
Applied Forest Science Limited
4417 Bennett Road
Victoria, British Columbia V9C 3Y3
Tel: 250.478.8358
E-mail: michael.peterson@afslimited.ca

Peterson M. 2008. *Fusarium* species—a British Columbia perspective in forest seedling production. In: Dumroese RK, Riley LE, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2007. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-57:109-125. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p057.html

ABSTRACT
This review provides a brief biological outline of some species in the genus *Fusarium* and how these can be implicated as seedborne organisms leading to conifer seed and seedling losses in British Columbia. *Fusarium* spp. are implicated with pre- and post-emergence damping-off, seedling wilt, late damping-off, root rot, and seedling mortality after outplanting. Current understanding of *Fusarium* spp., with regard to cone and seed pest management in British Columbia, is outlined. Shortfalls that still exist and how these might be addressed with the development of a vision for better understanding this group of fungi and a mission statement of how this might be achieved are presented.

KEYWORDS
diseases, pest management,
seedborne diseases, damping-off

The Genus *Fusarium*: an Overview

Members of the genus *Fusarium* are among the most important plant pathogens in the world. *Fusarium* spp. are a widespread cosmopolitan group of fungi that commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders. Fungi in this genus cause a huge range of diseases on a wide range of host plants. The fungus can be soil-, air-, and waterborne, or carried in or on plant residue or seeds, and can be recovered from any part of a plant: roots, shoots, flowers, fruits, cones, and seeds (Summerell and others 2003).

Summerell and others (2003) point out that *Fusarium* taxonomy has been plagued by changing species concepts, with as few as 9 to over 1,000 species being recognized by different taxonomists during the past 100 years. Differing opinions on species identification has stabilized since the 1980s following publications by Gerlach and Nirenberg (1982) and Nelson and others (1983) who defined widely accepted morphological species concepts. Since that time, however, the application of biological (Leslie 2001) and phylogenetic (Nirenberg and O'Donnell 1998) species concepts to new, as well as existing, strain collections has resulted in further splitting of many of the previously described species. If changing

these taxonomic designations were only rare, or of limited economic importance, they could be viewed as being merely pedantic. However, many of these species can be important. For example, *F. andiyazi* and *F. thapsinum* are major pathogens of sorghum that differ from one another but had previously been grouped as *F. moniliforme* (Leslie 2001; Marasas and others 2001). Further description of taxonomy is well beyond the scope of this note. However, its complexity and the recognized difficulty of rapidly identifying cultures to species (Summerell and others 2003) has meant that research and development of cone and seed pest management associated with *Fusarium* spp. in British Columbia (BC) has generally been limited to genus.

A taxonomic treatment for *Fusarium* is presented for completeness:

KINGDOM: Mycetae (fungi); DIVISION: Eumycota; SUBDIVISION: Deuteromycotina (the imperfect fungi); CLASS: Hyphomycetes; ORDER: Hyphales (Moniliales); GENUS: *Fusarium.*

Fusarium spp. are grouped in the subdivision Deuteromycotina, which encompasses the imperfect (asexual) fungi. Nelson and others (1983) point out that the perfect (sexual) states of *Fusarium* are generally unfamiliar to many people working with these fungi. Plant pathologists most often deal with the imperfect states, as the perfect states often have little to do with the disease problem under study. Some of the most successful *Fusarium*, for example, *F. oxysporum* and *F. culmorum*, appear to have lost their sexual ability and have adopted other methods of facilitating genetic adaptations (Booth 1981).

General Characteristics

Due to the great variability within this genus, it is one of the most difficult of all fungal groups to distinguish taxonomically (Alexopoulus and Mims 1979). Conidia (asexual spores) are hyaline and can be divided into 3 groups: macroconidia, microconidia, and chlamydospores. Macroconidia are several-celled, crescent or canoe-shaped spores. Their ends vary in that some species produce sharply pointed macroconidia, while others produce spores with rounder ends. The shapes of these spores are used to differentiate morphologically between species (Toussoun and Nelson 1968). Most *Fusarium* produce their macroconidia on sporodochia, which are cushion-shaped fruiting structures covered with conidiophores (simple or branched hyphae bearing conidia) (Figures 1 and 2). Macro-conidia can also be found, however, throughout the aerial mycelium. Microconidia are 1- or 2-celled, ovoid or oblong, and borne singly or in chains. These spores are found scattered throughout the aerial mycelium. The 1- or 2-celled microconidia are usually smaller than the macroconidia. Macroconida and microconidia are produced from phialides (a type of conidiogenous cell). Chlamydospores are round, 1- or 2-celled, thick-walled spores produced terminally or intercalary on older mycelium (Agrios 1988). Chlamydospores generally function as resting spores, having the ability to survive adverse conditions and enable the fungus to regenerate when favorable conditions for growth are reencountered. This is illustrated by a disease triangle (Figure 3). In the presence of a suitable host (for example, seedling) and pathogen (for example, *Fusarium* chlamydospore), disease of the host will progress when the environment favors spore germination and infection over time.

Disease Cycle

Fusarium are soil inhabitants that overwinter between crops in infected plant debris as mycelia and in 3 spore forms. As chlamydospores, *Fusarium* can remain in the soil for long time periods. Mycelium can infect healthy plant tissue in the same manner as spores do. Healthy plants can become infected through their root tips; either directly, through wounds, or at the point of formation of lateral roots (Agrios 1988). The fungus can grow as mycelium through the root cortex intercellularly, ultimately advancing to the vascular tissue. As the mycelium continues to grow, usually up toward, and into the stem, it branches

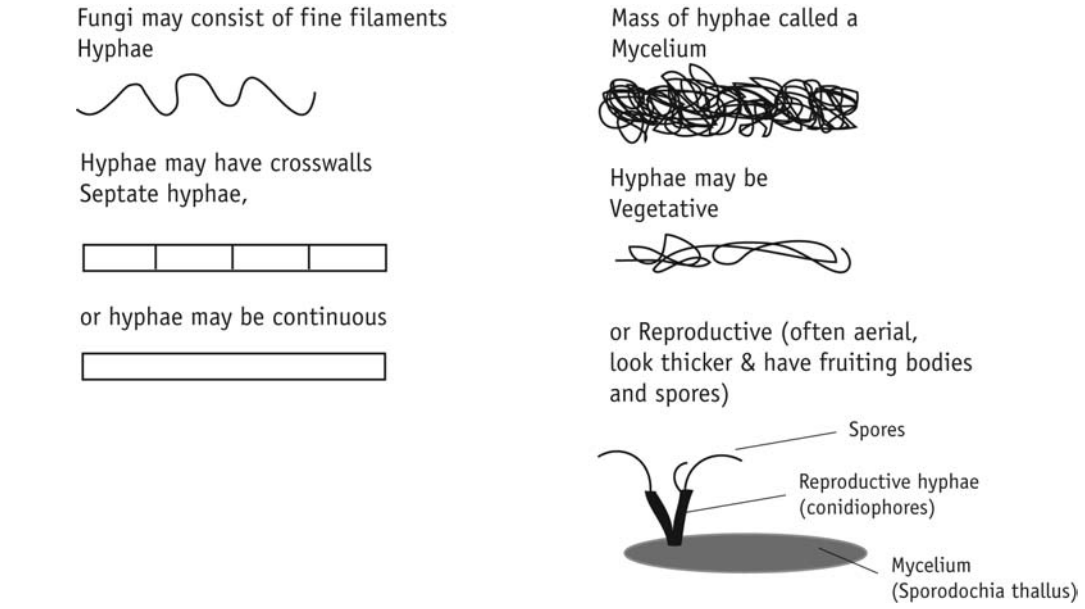

Figure 1. Structure of some fungi associated with disease (fungi imperfecti).

and produces microconida. The proliferation of fungal growth in a plant's vascular tissue can eventually cause the plant to wilt and die. Conifer seedlings are especially susceptible to this pathogenic modality when subjected to drought stress and high transpirational demands. The fungus can continue to grow on the decaying tissue, where it can sporulate profusely, visibly presenting salmon to coral-pink colored sporo-dochia on the lower portion of seedling stems. At this point, the spores can be spread to other plants or areas by wind, water, or through the movement of seedlings themselves (Agrios 1988).

Types of Disease

In addition to vascular wilting, *Fusarium* can infect other plant parts close to the soil to induce root and stem rots. When seeds become contaminated or seedlings are infected with *Fusarium*, damping-off may occur. The *Fusarium* that cause vascular wilts can be spread in soil, dust, and irrigation water. Wind, rain, nursery equipment, and decaying plant tissue can also help to spread the fungus. Additionally, *Fusarium* can enter nurseries as seed contaminants, be carried over from previous years within surface cracks on dirty growing containers, or within attached extraneous root fragments.

Forest seedling nurseries represent artificial growing environments. In BC, seeds are sown in soilless, peat-based growing media in Styrofoam™ (Styroblock™) containers. Styro-block™ containers generally reside on benches over concrete or gravel. Seed germination and the early part of the growing cycle take place in polyethylene covered greenhouses where temperature, light, and moisture are closely controlled, and nutrients are applied through overhead irrigation.

Fusarium are considered natural soil inhabitants and readily isolated from agricultural and forest soils. Understanding *Fusarium*-caused diseases in forest seedling container nurseries, however, requires the recognition that in this growing environment, *Fusarium* are *introduced* pests. They are introduced to the container nursery via seeds, water, and wind, or on old containers or dirty equipment. *Fusarium* can lead to seed and seedling losses in several ways:

1) seedborne contamination;
2) pre- and post-emergence damping-off;
3) seedling wilt;

4) late damping-off;
5) seedling root rot;
6) seedling mortality after outplanting.

Seedborne Contamination

Seedborne fungi are defined as those "that are dispersed in association with some kind of dispersal units of the host (that is, seeds)" (Ingold 1953). This definition includes all seed types and all associated microfungi, and is the one adopted with reference to conifer seedborne fungi in BC. Some authors classify fungi as being either seedborne or seed-transmitted (Thomsen and Schmidt 1999). They define seedborne fungi to include all fungal types contaminating the surface of seeds or infecting seed tissues. Seed-transmitted fungi are those that cause no infection of a seed itself, but infect seedlings in the nursery or field (Neergaard 1979). It must be remembered that not all seedborne fungi are pathogenic, and they may include symbionts actually beneficial to the plant (Mallone and Muskett 1997). With regard to seed transmission of fusaria, we are more interested in it as a seedborne pathogen than a seedborne disease. Seedborne pathogens (as opposed to diseases) are defined here as organisms, whether on or in seeds, which may or may not cause infections and symptoms on the seeds. Some seedborne pathogens may actively infect seeds, and may or may not cause symptoms on the seeds. Seedborne pathogens associated with conifer seeds may inhabit the external or internal tissues of seeds. Seedborne diseases occur on seedlings as a result of pathogens carried in or on the seeds. Evidence shows that *Fusarium* rarely exist within conifer seeds (Peterson 2007).

Seeds harbouring fungi can be described as being either contaminated or infected. Contamination is used to denote the occurrence of a pathogen as either spores or mycelium on the surface of seeds. Contamination may be entirely superficial, where spores or mycelium are usually retained in small cracks or fissures in the seedcoat. Infection refers to the penetration of seeds

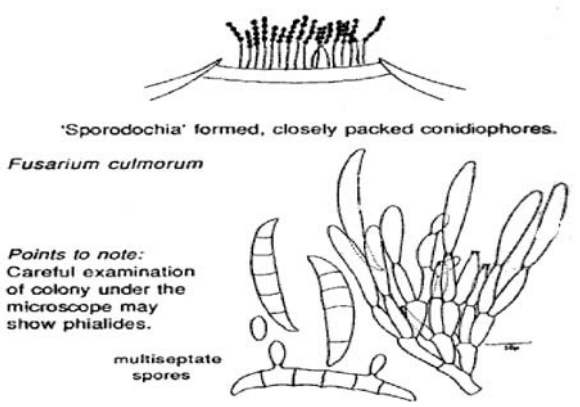

Figure 2. *Fusarium culmorum* showing sporodochia, macroconidia, and conidiophores.

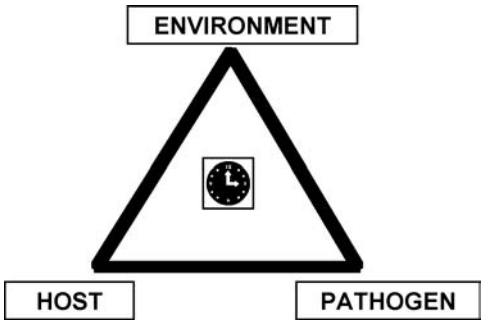

Figure 3. Disease triangle indicates 3 conditions that must be maintained over time for any disease to progress.

by an organism followed by the establishment of a relationship (that is, saprophytic or parasitic) within the seeds. Once established, such a relationship can give rise to outward hyphal growth from within the seeds, which becomes apparent upon penetrating the seed surface. While this hyphal growth can appear as a contaminant, it is indicative of the presence of an infection deeper within the seeds. In certain situations, it is possible to disinfest seeds that are only superficially contaminated. Surface disinfestations of infected seeds are of little value, as an internal relationship between the seeds and fungi will still exist. One of the easiest ways to eliminate or reduce seedborne contamination is through the use of running water during imbibition, followed by a post-strat-

ification rinse with running water (Kolotelo and others 2001). Disinfestation in this manner can reduce the incidence of seedborne *Fusarium* by reducing what has previously been observed as the tendency for contamination to actually increase during stratification.

Seedborne contamination may occur through indirect routes, such as via cone parts to the ovary and ovule tissues, or through direct routes when seeds contact contaminated soil and water. Dirty equipment in a processing facility may also contaminate seeds during interim storage, processing, or seed treatment for stratification. As spores can be released throughout the year, at almost any time in the general lifecycle of major BC conifer seedlings, seeds are exposed to contamination over a wide range of conditions. Examination of tree seed samples from over 2600 seedlots stored at the BC Ministry of Forests and Range (MoFR) Tree Seed Centre has indicated the frequency of seedborne *Fusarium* to be the same on seeds originating from seed orchards and those taken from natural stands (Peterson 2000). Spores freed from soil or grasses within and around seed orchards may be spread by irrigation sprinklers. This could be exacerbated by the use of sprinklers to control pollination in the spring. Indirect contamination through cone parts to the ovary and ovule tissues such as this could similarly occur in wild stands via rainfall. Fungal inoculum (for example, spores) reaching maturing cones on trees is thought to be one way that seeds can become contaminated and *Fusarium* become seedborne. Seeds and cone parts harbouring the fungi can contaminate processing facility equipment, contributing to further contamination of otherwise clean seeds. Regardless of the initial source, seedborne *Fusarium* can intensify throughout a contaminated seedlot during seed stratification.

Pre- and Post-Emergence Damping-off

Pre-emergence damping-off is characterized by seeds failing to germinate, or rotting of emerging shoots or radicals with the associated seedling losses. Damage and losses here are usually confined to individually contaminated seeds. Symptoms of post-emergence damping-off include stem rotting at the groundline and subsequent toppling of the seedling shoot. Post-emergence damping-off results in damage and loss of infected germinants after the stems rot. However, the disease can also spread by spores produced on the infected stems, which can then infect adjacent seedlings and cause further losses.

Seedling Wilt

Conifer seedlings, especially Douglas-fir (*Pseudotsuga menziesii*), are susceptible to seedling wilt caused by *Fusarium* when fungal growth proliferates in the plant's vascular tissue. This condition is often encountered when cool and overcast weather in the late spring or early summer is followed by a sudden clear warming trend. Seedlings that may otherwise have been tolerating a compromised vascular system are then subjected to drought stress induced by sudden high transpirational demands. The avoidance or reversal of these conditions (for example, increased irrigation) may either prevent or reverse the symptoms and minimize any subsequent damage and loss.

Late Damping-off

Late damping-off, also sometimes called Fusarium top blight, is often a progression from the intensification of seedling wilt. Symptoms include needle chlorosis, browning, and desiccation with a hook or crooked-shaped leader tip. Fusarium top blight following wilt damage will not necessarily lead to seedling losses if the trees are promptly treated with a systemic fungicide. When seedling death results, that is, late damping-off, the fungus may continue to grow on the decaying tissue where it can sporulate profusely, visibly presenting salmon to coral-pink colored sporodochia on the lower portion of seedling stems.

Seedling Root Rot

The symptoms of seedling wilt and late damping-off can also be indicative of Fusarium root rot, which is further characterized by blackened,

thin and wispy roots with little sign of actively growing root tips. The root cortex often easily strips away, leaving an exposed root stele. Fusarium root rot does not necessarily lead to seedling losses if the damage to the root system is limited. Root rot often occurs later in the growing season or can also occur if seedlings with infected roots are mishandled after leaving cold storage. Fusaria are natural rhizosphere inhabitants, and healthy, unstressed seedlings can survive well in their presence. The avoidance of stresses to the plants will limit damage and losses caused by Fusarium root rot.

Seedling Mortality after Outplanting

Fusarium are commonly found on conifer seedling roots and in the root zone throughout the growing media plug. In BC, the presence of *Fusarium* on seedling roots in the absence of any disease symptoms is generally not sufficient grounds to reject seedlings scheduled for outplanting. However, as seedlings commonly have *Fusarium* on or around their roots, it is important that proper handling care is taken so that any fungi present do not become aggressively pathogenic. Seedlings scheduled for outplanting must never be allowed to remain in boxes or in conditions where they can become overheated and the roots remain warm and moist for prolonged periods. Under such conditions, *Fusarium* can rapidly spread from seedling to seedling, as well as intensify within the roots of infected seedlings. When outplanted following these conditions, seedlings can quickly succumb to planting shock and, if exposed to a subsequent heat or drought stress, will often die.

Cone and Seed Pest Management: Research to Date

Tree species occurring in BC that are affected by seedborne *Fusarium*, in decreasing order of frequency as indicated from fungal assays, include: Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*), western larch (*Larix occidentalis*), western white pine (*Pinus monticola*), western redcedar (*Thuja plicata*), ponderosa pine (*Pinus ponderosa*), grand fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), subalpine fir (*Abies lasiocarpa*), sitka spruce (*Picea sitchensis*), yellow cedar (*Chamaecyparis nootkatensis*), noble fir (*Abies procera*), amabilis fir (*Abies amabilis*), interior spruce (*Picea glauca* and *P. engelmannii*, mountain hemlock (*Tsuga mertensiana*), and interior lodgepole pine (*Pinus contorta* var *latifolia*) (Kolotelo and others 2001).

It was indicated earlier that, within the context of the forest conifer container nursery system, *Fusarium* can be viewed as introduced pests. Species of *Fusarium* that are part of this disease complex can be introduced via air, water, on greenhouse equipment, on contaminated plant parts, as well as on seeds. Research and development of cone and seed pest management practices to reduce the negative effects of *Fusarium* on conifer seedling production have focused on all of the previously discussed aspects of this disease complex. Each of these areas of investigation is summarized here.

Seedborne Contamination

Initial Contamination

Several species of *Fusarium*, that is, *F. sporotrichoides*, *F. acuminatum, F. avenaceum* and *F. culmorum* have been isolated from Douglas-fir seeds (Mallams 2004). *Fusarium solani* and *F. oxysporum* are 2 other *Fusarium* species that Mallams (2004) notes have been isolated from diseased seedlings in fields at the J Herbert Stone Nursery, Central Point, Oregon. However, as she did not isolate these species from seeds, Mallams (2004) suggested that these infections occurred during or after sowing.

Although *F. acuminatum* and *F. avenaceum* commonly colonize conifer seeds, James (2000) found most *F. acuminatum* isolates he studied were not pathogenic to Douglas-fir. Other studies by James (1985a, 1993) found *F. acuminatum* and *F. avenaceum* both associated with pre- and post-emergence damping-off of conifers, and he suggests they were the result of seedborne inocula.

Several different species of *Fusarium* can cause root rot of container seedlings, with the major source of inocula being the seeds (Landis and

others 1990). Seedborne *Fusarium* are usually responsible for pre-emergence damping-off, but can also lead to post-emergence damping-off as well as *Fusarium* root rot and shoot blight, in this order of importance.

A sound understanding of 2 important seedborne fungi, *Caloscypha fulgens* and *Sirococcus conigenus*, has led to management guidelines to reduce their incidence on seeds, thus lowering outplant mortality attributable to their occurrence. A similar understanding of infection routes for seedborne *Fusarium* does not exist, and establishing this remains an essential first step to developing guidelines for reducing its incidence. As a seedborne contaminant (that is, carried on seeds) or seed infection, *Fusarium* can attack roots and be implicated as a wilt following outplanting. Observations during the winter of 2003/04 by Applied Forest Science Limited (AFS), as part of their seedling diagnostic and adjudication services for the BC MoFR and private forest companies, indicate that *Fusarium* have the ability to grow systemically within the vascular system of 2-year-old seedlings (Peterson 2004a). As a seedborne disease (that is, actively attacking seeds), *Fusarium* can be responsible for pre-emergence damping-off where seeds fail to germinate. To function as a seedborne contaminant as well as a disease suggests more than one infection route. Direct infection of angiosperm seeds occurs as systemic invasion via mother plant tissues to the seed embryo, whereas indirect infection and contamination can occur via the stigma to the seed embryo or via the flower/fruit to parts of the ovary and ovule tissues (Maude 1996). Direct infection via mother plant tissues is common for biotrophic fungi, which are parasitic in nature and dependent upon the survival of their host. Maude (1996) states, however, that this form of seed infection is less likely to occur within nectotrophic fungi which degrade tissues as they advance, with the exception of wilt fungi, including Fusarium, which invade vascular tissue. As a wilt fungus invading vascular tissues, *F. oxysporum* has been shown to infect seeds via the xylem of the mother plant (Baker 1948). *Fusarium moniliforme, F. oxysporum*, and *F. scirpi* have been isolated from vascular bundles from all parts of cotton plants, including bolls and seeds (Rudolph and Harris 1945). *Fusarium moniliforme* has been shown to invade corn seeds through vascular tissues of the stalk (Kingsland and Wernham 1962), while systemic infection in sweet corn plants by *F. moniliforme* and *F. oxysporum* has been shown to occur with hyphae of each species growing in intercellular spaces (Lawrence and others 1981). Mycelia of *F. oxysporum* f. sp. *carthami* have been observed in receptacles of safflower heads where hyphae traversed through the abscission zone of the cypsil and were associated with, but not limited to, the xylem (Klisiewicz 1963). Finally, mycelium has been observed to be inter- and intracellular, and also seen in the vascular elements of the seedcoat and cotyledons in seeds of Fabaceae (Sharma 1992).

Littke (1996) speculated that seed association with this pathogen originates from aerial deposition on developing cones. He deduced that likely routes of subsequent seed contamination exist as a physical transfer from exterior cone parts (bracts and scales) to seedcoat surfaces during seed development. AFS Limited routinely isolates *Fusarium* from seed surfaces during fungal assay testing for the BC MoFR Tree Seed Centre, confirming that *Fusarium* inoculum exists on the seedcoats of many conifer species. Littke's hypothesis requires airborne *Fusarium* spore inoculum to be present in the vicinity of receptive cones during pollination. Data collected by AFS Limited, as one of 24 locations across Canada over the past 10 years under the auspices of the Weather Network/MetroMedia and calculated by Aerobiology Research Laboratories, Ontario, showed airborne spore densities in the Victoria region to closely mimic those of conifer pollen density during the spring of 2003 (Peterson 2003). That airborne spore inoculum can occur during times of pollination in the vicinity of Douglas-fir seed orchards supports Littke and could explain the presence of inoculum on seed surfaces.

Research by Peterson (2007) indicates that seedborne *Fusarium* on several conifer species does not likely occur as internal infections, but is limited to seedcoat contamination. These observations appear to indicate that seedborne fungi in some conifer species do not occur systemically, and more likely occurs following Littke's (1996) hypothesis. How seeds become initially contaminated remains uncertain, and more research is needed to precisely define how and when this occurs prior to developing pest management guidelines to minimize this occurrence.

Spread and Intensification of Initial Seed Contamination

Regardless of the initial source, infested seedlots can potentially cross contaminate those that are uninfested, as well as intensify within themselves during imbibition and stratification. Cross-contamination between seedlots can occur wherever mutual seedlot contact exists through shared seed handling equipment. This can occur when unsanitized cone sacks are reused between cone harvests, or inoculum can potentially be transferred from seed handling equipment during various stages of the seed extraction process. Kolotelo and Peterson (2006) found some trends with regard to the presence of *Fusarium* on cones, debris, and seeds at various stages during cone and seed processing. They found processing stages incorporating agitation to indicate increases in contamination. Kilning appeared to decrease contamination on seed and cone scales despite peak kilning temperatures of 40 °C (104 °F) not totally eliminating the fungal contaminant. Their overall finding was that initial seed contamination levels may not be indicative of final seedlot contamination. This was emphasized by the fact that, despite BC interior Douglas-fir having a very low incidence of seedborne *Fusarium*, some of the associated debris had significant amounts of contamination. Also, although initial levels on cone scales, seeds, and debris in some seedlots were high to moderate, the final contamination levels were very low and below what are considered to be a concern. Their observations indicated trends only that were difficult to substantiate statistically, indicating this to be an area in need of further investigation.

The potential for conifer seeds to become contaminated with *Fusarium* makes testing for its presence a viable first step for managing it as a seedborne organism, thus allowing specific seedlots to be targeted for special treatment. The ability to spread or intensify within a seedlot, the fact that some tree species are more susceptible to the effects of the fungi, as well as the fact that some species represent a higher potential monetary loss, are also reasons that seeds are tested for contamination. A matrix established by the BC MoFR Tree Seed Centre outlining the seed fungal testing priorities for the 3 important seedborne fungi in BC has been developed. The priorities for *Fusarium* testing are such that subalpine fir, coastal Douglas-fir, western larch, western white pine, and ponderosa pine are all rated high; amabilis fir, grand fir, western redcedar, interior Douglas-fir, western hemlock, Sitka spruce, interior spruce, and Sitka x interior spruce hybrid are rated medium; and coastal and interior lodgepole pine, and yellow cedar are rated low priority (Kolotelo and others 2001).

Past sampling has indicated average levels of *Fusarium* on seeds to be typically less than 2.5%, with a moderate degree of variation within seedlots (Kolotelo and others 2001). Not all species of *Fusarium* are pathogenic; those that are pathogenic are often weakly so. In addition, past studies to detect seedborne *Fusarium* in BC were often limited to genus. Thus, when routine fungal assays of seeds in BC were adopted, it was elected to detect levels within any seedlot at a relatively conservative level of 5%. To detect levels of 5% with a 95% degree of confidence requires a sample size of 500 seeds per seedlot for each seedlot tested. Samples are not adjusted for seedlot size, but sampling intensity is adjusted according to the ISTA (1999) standards. The laboratory methods used to test seedborne *Fusarium* are outlined in Peterson (2007), and testing for its presence

provides useful information for nursery growers.

The results of fungal assays are available for each seedlot tested on the Seed Planning and Registry Information System (SPAR), in seedlot detail reports from SPAR, as well as on the sowing request label sent to growers with each batch of seeds. Knowing the percentage of contaminated seeds within a seedlot provides growers, as well as others handling the seeds, with the option of taking steps to minimize their impact on seedling germination and growth. Historical records indicate contamination levels of greater than 5% within any seedlot to be significant for disease potential, and growers target seedlots with levels higher than this. The main strategy for levels above this are aimed at minimizing its ability to spread within a seedlot.

Seed orchard seeds appear to be affected by *Fusarium* at the same rate as seeds collected from natural stands (Peterson 2000). Current knowledge still does not provide a clear understanding of how cones become contaminated. More control is available, however, when collecting in seed orchards compared to natural stands, and Kolotelo and others (2001) point out 3 things that can be done when making these collections to prevent further spread of the fungus. First, cones should be collected during dry weather whenever possible. Second, cones should be stored in new, or steam- or hot water-sterilized sacks to prevent contamination from previous year's collections. Informal investigations conducted by AFS Limited for the BC MoFR have shown that cone sacks can become contaminated, and the sacks themselves, especially when wet, will readily trap airborne inoculum (Peterson 2004b). Third, filled sacks should be stored following the general recommendations for all species described in Portlock (1996).

The ability of *Fusarium*-contaminated seeds to intensify within seedlots during imbibition and stratification, as well as the management practices to reduce this phenomenon, are extensively reviewed in Kolotelo and others (2001). Seedborne *Fusarium* primarily exist as contaminants and do not readily infect the seed interior during storage. Infection of an emerging shoot and/or radical may occur in the germination phase, but Kolotelo and others (2001) point out that early stages of seed colonization are primarily dependent on abiotic factors, such as environmental water availability and temperature, rather than seed moisture content. Strategies to reduce the exposure of contaminated seeds to environmental conditions conducive to fungal growth can help prevent any intensification of seedborne *Fusarium* within a seedlot.

Three strategies to minimize losses from seedborne pathogens are: 1) eliminating or reducing initial inoculum; 2) slowing the rate of pathogen spread; and 3) shortening the time seeds are exposed to the pathogen (Berger 1977). It is valuable to view these strategies in the context of a disease triangle with the seeds as host, *Fusarium* the *pathogen*, and seed handling (from cone collection, through extraction, storage, sowing, germination, and seedcoat loss) as the environment, representing each corner, respectively. Sanitation encompasses cone collection procedures, seed orchard management and seed processing, falling into Berger's (1977) first category. Kolotelo and others (2001) relate seed treatments and storage to the second, and stratification and germination procedures to the third category. The first category is quite well understood, as presented by Eremko and others (1989), Leadem and others (1990), and Portlock (1996). Some questions still remain, however, with regard to when *Fusarium* become seedborne on seeds produced in orchards (Peterson 2007). Kolotelo and others (2001) present a good summary of collection methods to minimize seedborne disease, as well as discussions of seed processing. Initial research toward the potential for seedborne *Fusarium* to spread during seed processing has been started, but some questions still remain in this area (Kolotelo and Peterson 2006). Long term seed storage in BC generally takes place at -18 °C (0 °F), and between 4.9% and 9.9% moisture content, neither measure being conducive to fungal growth. Therefore

storage itself does not present a significant threat to spread or intensification.

Research in BC with regard to cone and seed pest management and seedborne *Fusarium* within Berger's (1977) third category to minimize loss to seedborne disease, that is, stratification and germination, has concentrated on treating tested seedlots having significant contamination by cleaning seed surfaces. Most research in this area has aimed at reducing seedcoat infestations (James 1985b; Axelrood and others 1995). And the importance of this research is emphasized by the findings that infestation levels of can increase significantly during seed imbibition and stratification (Axelrood and others 1995; Hoefnagels and Linderman 1999). Likewise, dry seed levels (< 1%) of *Fusarium*, below what is considered a potential disease threat (> 5%), can substantially increase during stratification to as high as 10% (Neumann 1996). Neumann investigated potential external sources that may have contributed to these increases, for example, airborne inoculum in the drying room and the soaking mesh and/or tanks. It was ultimately deemed, however, that as the observed "bulking up" of seedborne *Fusarium* occurred during the first 6 hours of imbibition, a faster water flow over the seeds during this time might be a simple cultural control to prevent this escalation. Further studies by Neumann (1997) concluded that simple cleaning of soaking tanks between seedlots could reduce inoculum and the potential for cross-contamination.

Applying fungicides directly to seedcoats to control seedborne *Fusarium* has been investigated. However, it is difficult to find fungicides that meet the many requirements necessary for their safe and effective application (Bennett and others 1991). These range from being suitably efficacious under different climatic conditions, being non-phytotoxic, being residue-free, as well as non-toxic to humans and wildlife. Earlier research findings of the negative effects of fungicides on seed germination, as well as variable efficacy and handling difficulties have all led to their reduced usage (Lock and Sutherland 1975; Lamontagne and Wang 1976; Wenny and Dumroese 1987).

The use of running water to imbibe seeds followed by a post-stratification running water rinse is the simplest strategy to reduce the intensification of seedborne contamination (Kolotelo and others 2001). Running water treatments appear to reduce the incidence of post-stratification seedborne *Fusarium* (James 1985b; Dumroese and others 1988; Axelrood and others 1995). The method used at the BC MoFR Tree Seed Centre is to imbibe seeds in mesh bags in a tank of running water for 24 to 48 hours. However, this requires significant water resources. Kolotelo and others (2001) suggest complete water changes every 4 to 8 hours will have similar effects. This is likely due to the response noted by Neumann (1996), that the first 6 hours of imbibition is critical for what she termed "bulking up" of seedborne *Fusarium.* Seed imbibition at the BC MoFR Tree Seed Centre generally involves soaking several sowing requests of differing seedlots and conifer species in the same tanks for a running water soak. However, Neumann (1995) identified a potential for cross-contamination between low- and highly-infested Douglas-fir and western larch seedlots when soaked together, and these are now soaked in individual tanks at the BC MoFR Tree Seed Centre.

Very effective chemical seed sanitation can be achieved using hydrogen peroxide. Differences in concentration and treatment duration, stratification timing, and conifer species tolerance exist, and an excellent summary of hydrogen peroxide seed treatment is presented in tabular form in Kolotelo and others (2001). It is worth noting that for 12 conifer species, 4 hydrogen peroxide concentrations, up to 10 exposure durations, and for both pre- and post-stratification treatments, no reductions in germination are indicated and neither were any increases in fungal contamination. In BC, the recommended hydrogen peroxide technique is to treat post-stratification seeds by immersing them in a 3% hydrogen peroxide solution, at 3:1 solution to seed volume ratio, for 30 minutes to 4 hours followed by a running water

rinse. The potential for reducing fungal levels on seeds with hydrogen peroxide clearly exists. However, some *Abies* species do not respond consistently, and for this reason Kolotelo and others (2001) suggest more research and operational studies are needed to address this.

Pre- and Post-emergence Damping-off

Pre-emergence Damping-off

Seedborne *Fusarium* are most often responsible for pre-emergence damping-off, that is, seeds that become infected and fail to germinate. Technically, the seed contents do not become infected prior to their being exposed to the environmental conditions of moisture and temperature that allow seedcoat inoculum to germinate. If these conditions are present while actual seed germination is slow to initiate, the seed contents may become infected and rot. However, what commonly happens below ground is that the beginnings of a radical and shoot will emerge and can become infected by any seedcoat inoculum present, with the result that no sign of a germinating seedling will appear above the soil line. Pre-emergence damping-off refers to both of the above situations. Given the appropriate conditions, pre-emergence damping-off might still occur in the absence of seedborne *Fusarium*. This can happen if sufficient inoculum is present in the growing media, most often encountered when dirty growing containers carry over inoculum from the previous year. Neumann (1993) did not find planting mix or water to be a source of inoculum in a 2-year study of seedborne *Fusarium* and root colonization of container-grown Douglas-fir, but she did suggest that other sources of inoculum likely came from wooden pallets. Axelrood and Peters (1993) found 50% of the cavities in operationally sanitized Styro-block™ containers to contain infested root fragments. They also found 60% of the growing cavities to be contaminated with *Fusarium* on their surfaces. *Fusarium* have also been found on the wooden pallets used to support growing containers, as well as on plant debris beneath these pallets (Neumann and Axelrood 1992).

Greenhouse sanitation, including floors, benching, pallets, and Styroblock™ containers, will all reduce levels of inoculum in the immediate vicinity of germinating seeds. However, these strategies are targeted more to reduce risk to seedlings in the post-emergence environment. Aside from employing the strategies outlined in the previous section to reduce the ability for *Fusarium* to intensify on contaminated seeds during imbibition and stratification, some other methods can be employed to reduce pre-emergence damping-off. For seedlots with a greater than 5% incidence of contamination, it is recommended that greenhouse temperatures be optimized to encourage rapid germination. This will often reduce the incidence of pre-emergence damping-off and promote rapid shedding of the seedcoat.

Post-emergence Damping-off

Young germinant or seedling root infections, resulting from roots growing in close proximity to germinating chlamydospores, can lead to stem rotting at the groundline, which typifies post-emergence damping-off. Seedborne *Fusarium* can also be responsible for post-emergence damping-off when the seeds germinate. However, for reasons such as a slow-to-shed seedcoat, for example, inoculum contacting and infecting the emergent tissues will often cause the new shoot to rot at the goundline. Young germinants rotting at the goundline and breaking or falling over at this point typify symptoms of post-emergence damping-off. The strategy of encouraging rapid loss of the seedcoat will reduce the time any contamination on the seedcoat surface is likely to be in contact with the germinating needles and stem, and can reduce losses here. It is also important during this growth phase to irrigate early in the day to encourage rapid drying of seedling foliage, which will also help reduce the spread of spore inoculum.

Sanitation of older Styroblock™ containers can significantly extend the useful life of these growing containers by reducing pathogen inoculum associated with old rough surfaces and associated extra-

neous root material from past years use. Peterson (1990) achieved significant reductions in levels on old Styroblock™ containers using a variety of sanitation techniques, and developed these into a set of practical guidelines for the sanitation of nursery seedling containers using either heat or chemical methods (Peterson 1991). The adoption of many of these guidelines is commonly used to extend container life while reducing the presence of inoculum in the container seedling root zone.

Seedling Wilt

Following germination and subsequent seedling growth, it is important to reduce stress on the plants. Seedlings can tolerate low levels of *Fusarium* on their roots. However, heat or drought stress can impair the seedling's ability to transport water and nutrients, especially if fungi have entered the roots and xylem tissues. Seedlings that continue to grow and become infected by either chlamydospores, introduced air- or water-borne spore inoculum, or from infected root fragments or dirty container surfaces, can be influenced by heat or drought stress leading to top blight or wilting. Top blight or wilt, also sometimes called late damping-off, often shows symptoms of needle chlorosis, browning, and desiccation with a hook or crooked-shaped leader tip.

Top blight and wilt damage will not necessarily lead to seedling losses if the trees are promptly treated with a systemic fungicide. In Canada, Senator® 70WP is registered for use on container greenhouse conifers for controlling *Fusarium*, and can be applied at 14-day intervals to provide systemic control. Also, as this damage is often initiated by a sudden heat or drought stress in the presence of the pathogen, the avoidance or reversal of these conditions can either prevent or reverse the symptoms and minimize any subsequent damage and loss. Sutherland and others (1989) point out that *Fusarium* can enter seedling roots early in a growing season, with disease development being delayed until the seedlings become stressed for moisture and nutrients.

The potential for beneficial soil or growing media amendment should be mentioned here, as the use of artificial media is particularly suited to this. Suppressive growing media can be created by introducing beneficial organisms, or by using media components that suppress disease organisms. An excellent review of this technology is presented by Linderman (1986). One soil-inhabiting fungus, that is, *Trichoderma harzianum*, is actively antagonistic to *Fusarium*, as it competes with the pathogen for substrate. *T. harzianum* is the active ingredient in the biological fungicide RootShield®, and it is best applied as a greenhouse and nursery soil amendment early in the growing season.

Seedling wilt caused by *Fusarium* usually only affects young germinants. Resistance of conifers to wilt diseases develops with ageing, and plant resistance to wilt pathogens is known to depend on the synthesis rate of phenolics, with free and bound phenolics preventing or retarding disease development. Shein and others (2003) treated Scots pine (*Pinus sylvestris*) seedlings with virulent spore suspensions of *F. sporotrichiella* and deduced conifer seedling resistance to wilt diseases to be correlated with the synthesis rate and accumulation of insoluble phenolic polymers. The ability to resist wilt was higher in plants unable to synthesize these polymers as they accumulated with age.

Late Damping-off

If left unchecked, seedling wilt can progress as foliage becomes chlorotic to brown, severe needle necrosis occurs, needles drop, and the seedling dies. Seedlings at this point usually become crooked at the leader and appear to die from the tip down. Little can be done to reverse the disease at this point. It is important, however, to remove affected seedlings, as they can lead to increased spore inoculum in the greenhouse. Left in containers, infected seedlings often develop salmon-pink sporodocia that produce and release conidia that can be splashed from irrigation water to infect adjacent seedlings. Not only is it important

to remove any infected seedlings at this stage, but attention must be paid to seedling growth. Seedlings usually present the above described symptoms during their rapid growth phase. This is characterized by accelerated tissue growth and expansion, and is generally considered the time when seedlings are most succulent and susceptible to infection. Dennis and Trotter (1995) point out that environmental and cultural manipulation during the rapid growth phase must concentrate on providing the seedling with select growing conditions in order to accentuate its growth potential. They also emphasize that seedling environment and culture have a significant impact on whether disease develops or not, pointing out that disease-causing fungi can infect seedlings at an early stage of development, and then remain latent and cause disease later in the growing season when plants become stressed.

Seedling Root Rot

Fusarium root rot, characterized by blackened, thin and wispy roots with little sign of actively growing root tips, is often the final stage of a disease continuum that may have begun with the seeds, or at least at the time of sowing. Not all species of *Fusarium* are pathogenic (James and others 1989), and many of those that are pathogenic are weakly so. However, Neumann (1993) points out a very important adaptive characteristic that contributes to its persistent ability as a pathogen, and that is that *Fusarium* are often facultative parasites well adapted for survival in either dormant (chlamydospores) or saprophytic states (Bruehl 1987). Saprophytic survival in container nursery settings occurs when dead root fragments, carried over on old containers, have been colonized by saprophytic *Fusarium* following parasitic colonization during the previous year. Thus, although often a weak pathogen that is tolerated in a stress-free environment, as a facultative parasite it persists in seedling containers, alternating between saprophytic and more aggressively pathogenic phases while the environment corner of the disease triangle changes as seedlings develop. Root rot often occurs later in the growing season, or can also occur if seedlings with infected roots are mishandled after leaving cold storage. *Fusarium* root rot does not necessarily lead to seedling losses if the damage to the root system is limited.

Seedling Mortality after Outplanting

Seedlings with minor amounts of *Fusarium* on their root surfaces, or low levels of root infection, often readily survive being outplanted when handled properly and not exposed to severe planting shock. However, when infested seedlings remain in storage or shipping containers under warm, moist conditions for extended periods prior to planting, the disease can rapidly develop into root rot, severely jeopardizing seedling survival. In British Columbia, the presence of *Fusarium* on seedling roots in the absence of any disease symptoms is generally not sufficient grounds to reject seedlings scheduled for outplanting. In fact, Axelrood and others (1998) concluded that *Fusarium* are probably of little consequence with regard to the mortality of seedlings on reforestation sites after they were unable to find a significant difference between seedling infections and root colonization. However, the mean age of the outplanted and naturally regenerated seedlings examined was 5.6 and 4.7 years, respectively, and they did not take into account seedling mortality that may have arisen immediately after outplanting. Thus, their results perhaps speak more for the long-term than for what might happen in the short-term, when planting shock may have a role in initial survival.

Fusarium can be isolated from visually healthy nursery-grown conifer seedlings (Bloomberg 1966; James 1986; Kope and others 1996). Because of this, Axelrood and others (1998) point out that the recovery of *Fusarium* from the roots of nursery-grown conifers does not necessarily indicate a disease situation. Instead, they state that this can be indicative of a potential for disease to develop following outplanting if conducive environmental (refer to environment corner of disease triangle) conditions are present. Container seedlings commonly have *Fusarium* on

or around their roots (Landis 1976; Graham and Linderman 1983; James 1985a), and it is therefore important that care is taken so any fungi present does not become aggressively pathogenic. Seedlings scheduled for outplanting must never be allowed to remain in boxes, or in conditions where they can become overheated and the roots remain warm and moist for prolonged periods. Under such conditions, *Fusarium* can rapidly spread from seedling to seedling, as well as intensify within the roots of infected seedlings. When outplanted following these conditions, seedlings can quickly succumb to planting shock and, if exposed to a subsequent heat or drought stress, will often die.

Cone and Seed Pest Management: Vision for the Future

Understanding the disease biology of the major fungal pathogens of forest nursery conifer seedlings in BC has been an important step toward developing pest management plans to eliminate or minimize their impact on cone production and seed handling, as well as forest nursery seedling production and increased seedling survival after outplanting on reforestation sites.

Fungi in the genus *Fusarium* can negatively affect the reforestation value chain from the time of cone and seed production, through seed handling and processing, as well as during the course of nursery operations, to successful survival of outplanted seedlings. Increased understanding of *Fusarium* host-pathogen interactions throughout many aspects of conifer seedling production in BC is desirable.

A vision of increased seed and seedling survival from cone and seed production to outplanting at reforestation sites is attainable through better understanding of the disease mechanisms associated with these fungi, and will lead to the development of more effective cone and seed pest management plans.

Increased understanding is needed of the following *Fusarium* host-pathogen interactions of conifer seedling production in BC:

1) Seedborne contamination:
   a) How seeds become contaminated is still not clear;
   b) It remains unclear how seed contamination may be exacerbated during cone and seed processing.

2) Pre- and post-emergence damping-off:
   a) Develop better Standard Operating Procedures (SOP) to eliminate *Fusarium* as introduced greenhouse pests, that is, benching, pallets, and container sanitation;
   b) Improve seedcoat disinfestations procedures.

3) Seedling wilt:
   a) Improve SOP to reduce risks to sudden heat or drought stress induced transpirational demands.

4) Late damping-off:
   a) Improved understanding of 2 and 3 will help resolve this.

5) Seedling root rot:
   a) Improved understanding of all steps 1, 2, 3 and 4 will reduce losses to root rot;
   b) Improved handling practices during storage and especially post-storage will reduce losses to root rot.

6) Seedling mortality after outplanting:
   a) Plantation failure usually occurs when *Fusarium* have survived through all the components of the reforestation value chain described above, and a satisfactory environmental component of the disease triangle is met at the reforestation site. Improved understanding of value chain steps 1 through 5 could lower the incidence at the pathogen corner of the disease triangle to below what is necessary to cause significant losses at the reforestation site.

Cone and Seed Pest Management: Mission Statement

For the vision of increased seed and seedling survival from cone and seed production to outplanting at reforestation sites to be attainable, better understanding of the disease mechanisms associated with *Fusarium* and seedling production are needed. Many of these mechanisms are understood individually, perhaps the most important being the fact that the fungi are facultative parasites. As such, it has the ability to move in and out of a pathogenic or saprophytic relationship with its host, depending, in part, on the conditions at the environment corner of the disease triangle. The ability to survive as a saprophyte on tissues it has previously colonized as a parasite allows some fusaria to enter the reforestation value chain as a seedborne contaminant and still pose a threat to seedling survival many months later at the reforestation site. Better understanding of the key components of this value chain and the interactions between host, pathogen, and environment will allow the development of cone and seed pest management plans so that interventions can be made to break these disease triangle connections where possible.

To achieve the vision of increased seed and seedling survival from cone and seed production to outplanting at reforestation sites requires building on the current state of knowledge with regard to *Fusarium* as cone and seed pests. Specifically, better understanding is needed as to how seeds become contaminated; how seed contamination may be exacerbated during cone and seed processing needs to be examined; and what improvements if any, can be made to seedcoat disinfestation procedures.

References

Agrios GN. 1988. Plant pathology. 3rd ed. San Diego (CA): Academic Press, Inc. 803 pp.

Alexopoulos CJ, Mims CW. 1979. Introductory mycology. Toronto (ON): John Wiley and Sons.

Axelrood PE, Peters R. 1993. Influence of nursery cultural practices on *Cylindrocarpon* and *Fusarium* root rot infection of Douglas-fir. Victoria (BC): British Columbia Ministry of Forests, Major Service Contract # 08384.

Axelrood PE, Neumann M, Trotter D, Radley R, Shrimpton G, Dennis J. 1995. Seedborne *Fusarium* on Douglas-fir: pathogenicity and seed stratification method to decrease *Fusarium* contamination. New Forests 9:35-51.

Axelrood PE, Chapman WK, Seifert KA, Trotter DB, Shrimpton G. 1998. *Cylindrocarpon* and *Fusarium* root colonization of Douglas-fir seedlings from British Columbia reforestation sites. Canadian Journal of Forest Research 28:1198-1206.

Baker KF. 1948. *Fusarium* wilt of garden stock (*Matthiola incana)*. Phytopathology 38:399-403.

Bennett MA, Callan NW, Fritz VA. 1991. Seed treatments for disease control. HortTechnology 1:84-87.

Berger RD. 1977. Application of epidemiological principles to achieve plant disease control. Annual Review of Phytopathology 15:165-183.

Bloomberg WJ. 1966. The occurrence of endophytic fungi in Douglas-fir seedlings and seed. Canadian Journal of Botany 44:413-420.

Booth C. 1981. Perfect states (teleomorphs) of *Fusarium* species. In: Nelson PE, Toussoun TA, Cook RJ, editors. *Fusarium*: diseases, biology and taxonomy. University Park (PA): Pennsylvania State University Press. p 446-452.

Bruehl GW. 1987. Soilborne plant pathogens. New York (NY): MacMillan Publishing Company. 368 pp.

Dennis J, Trotter D. 1995. Life on the edge of the curve or the current status of root rots in coastal Douglas-fir seedlings. In: Kooistra CM, editor. Proceedings of the 1995, 1996, 1997 Forest Nursery Association of British Columbia Annual Meetings. Victoria (BC): Forest Nursery Association of British Columbia.

Dumroese RK, James RL, Wenny DL, Gilligan CJ. 1988. Douglas-fir seed treatments: effects on seed germination and seedborne organisms. In: Landis TD, technical coordinator. Proceedings, combined meeting of the Western Forest Nursery Associations; 1988 Aug 8-11; Vernon, British Columbia. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. General Technical Report RM-167. 6 p.

Eremko RD, Edwards DGW, Wallinger D. 1989. A guide to collecting cones of British Columbia conifers. Victoria (BC): Forestry Canada and BC Ministry of Forests. FRDA Report Number 055.

Graham JH, Linderman RG. 1983. Pathogenic seedborne *Fusarium oxysporum* from Douglas-fir. Plant Disease 67:323-325.

Gerlach W, Nirenberg HI. 1982. The genus *Fusarium* — a pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft, Berlin-Dahlem 209:1-406.

Hoefnagels MH, Linderman RG. 1999. Biological suppression of seedborne *Fusarium* spp. during cold stratification of Douglas-fir seeds. Plant Disease 83(9):845-852.

Ingold CT. 1953. Dispersal in fungi. London (United Kingdom): Oxford University Press.

[ISTA] International Seed Testing Association. 1999. International rules for seed testing. Seed Science and Technology. Supplement 27.

James RL. 1985a. Studies of *Fusarium* associated with containerized conifer seedling diseases: (2). Diseases of western larch, Douglas-fir, grand fir, subalpine fir, and ponderosa pine seedlings at the USDA Forest Service Nursery, Coeur d Alene, Idaho. Missoula (MT): USDA Forest Service, Northern Region. Forest Health Protection Report 85-12. 7 p.

James RL. 1985b. Diseases of conifer seedlings caused by seedborne *Fusarium* species. In: Shearer RC, technical coordinator. Proceedings of a symposium on conifer tree seed in the Inland Mountain West. Missoula (MT): USDA Forest Service, Intermountain Research Station. General Technical Report INT-203. p 267-217.

James RL. 1986. Mortality of containerized western larch seedlings at the Champion Timberlands Nursery, Plains, Montana. Missoula (MT): USDA Forest Service, Northern Region. Forest Pest Management Report Number 86-16.

James RL. 1993. *Fusarium* species associated with post-emergence damping-off and root disease of young container-grown Douglas-fir seedlings USDA Forest Service Nursery, Coeur d Alene, Idaho. Missoula (MT): USDA Forest Service, Northern Region. Nursery Disease Notes #129. 5 p.

James RL. 2000. Pathogenic characteristics of *Fusarium acuminatum* isolated from inland Pacific Northwest nurseries. Missoula (MT): USDA Forest Service, Northern Region. Forest Health Protection Report 00-16. 8 p.

James RL, Dumroese RK, Gilligan CJ, Wenny DL. 1989. Pathogenicity of *Fusarium* isolates grown from Douglas-fir seed and container-grown seedlings. Moscow (ID): University of Idaho College of Forestry, Wildlife and Range Sciences. Bulletin Number 52. 10 p.

Kingsland GC, Wernham CC. 1962. Etiology of stalk rot of corn in Pennsylvania. Phytopathology 52:519-523.

Klisiewicz JM. 1963. Wilt-incitant *F. oxysporum* f. sp. *carthami* present in seed from infected safflower. Phytopathology 53:1046-1049.

Kolotelo D, Peterson M. 2006. *Fusarium* spp. Trends in conifer cone and seed processing (CSP) [PowerPoint presentation]. Presentation at the IUFRO Tree Seed Symposium; 2006 July 18-21; Fredericton, New Brunswick.

Kolotelo D, Van Steenis E, Peterson M, Bennett R, Trotter D, Dennis J. 2001. Seed handling guidebook. Victoria (BC): British Columbia Ministry of Forests, Tree Improvement Branch.

Kope HH, Axelrood PE, Sutherland JR, Reddy MS. 1996. Prevalence and incidence of the root-inhabiting fungi, *Fusarium*, *Cylindrocarpon* and *Pythium* on container-grown Douglas-fir and spruce seedlings in British Columbia. New Forests 12:55-67.

Lamontange Y, Wang BSE. 1976. Germination of polyram treated white spruce seeds from various provenances. Tree Planters' Notes 27(1):5-6, 22.

Landis TD. 1976. *Fusarium* root disease of container-grown tree seedlings. Lakewood (CO): USDA Forest Service, Rocky Mountain Region. Forest Insect and Disease Management, Biological Evaluation R2-76-16. 7 p.

Landis TD, Tinus RW, MacDonald SE, Barnett JP. 1990. The container tree nursery manual. Vol. 5. The biological component: nursery pests and mycorrhizae. Washington (DC): USDA Forest Service. Agriculture Handbook 674.

Lawrence EB, Nelson PE, Ayers JE. 1981. Histopathology of sweet corn seed and plants infected with *Fusarium moniliforme* and *F. oxysporum*. Phytopathology 67:1461-1468.

Leadem CL, Eremko RD. Davis IH. 1990. Seed biology, collections and post-harvest handling. In: Lavender DP, Parish R, Johnson CM, Montgomery G, Vyse A, Willis RA, Winston D, editors. Regenerating British Columbia s forests. Vancouver (BC): University of British Columbia Press. p 193-205.

Leslie JF. 2001. Population genetics level problems in the *Gibberella fujikuroi* species complex. In: Summerell BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW, editors. *Fusarium*: Paul E Nelson Memorial Symposium. St Paul (MN): American Phytopathological Society. p 113-121.

Linderman RG. 1986. Managing rhizosphere microorganisms in the production of horticultural crops. HortScience 21(6):1299-1302.

Littke W. 1996. Seed pathogens and seed treatments. In: Landis TD, South DB, technical coordinators. National proceedings, forest and conservation nursery associations. Portland (OR): USDA Forest Service, Pacific Northwest Research Station. General Technical Report PNW-GTR-389. p 187-191.

Lock W, Sutherland JR. 1975. Fungicide treatment of seeds for damping-off control in B.C. forest nurseries. Tree Planters' Notes 26(3):16-18.

Mallams KM. 2004. Fungal contaminants on selected conifer seed, J. Herbert Stone Nursery. Central Point (OR): Southwest Oregon Forest Insect and Disease Service Center. SWOFIDSC-04-01.

Mallone JP, Muskett AE. 1997. Seed-borne fungi. Description of 77 fungus species. In: Sheppard JW, editor. Ottawa (ON): Agriculture and Agri-Food Canada. Published by the International Seed Testing Association, Zurich, Switzerland.

Marasas WFO, Rheeder JP, Lamprecht SC, Zeller KA, Leslie JF. 2001. *Fusarium andiyazi* sp. nov., a new species from sorghum. Mycologia 93:1203-1210.

Maude RB. 1996. Seedborne diseases and their control. Principles and practice. Wallingford (United Kingdom): CAB International. 280 p.

Neergaard P. 1979. Seed pathology. London (United Kingdom): MacMillan Press.

Nelson PE, Tousoun TA, Marasas WFO. 1983. *Fusarium* species: an illustrated manual for identification. University Park (PA): Pennsylvania State University.

Neumann M. 1993. Seedborne *Fusarium* and root colonization of container-grown Douglas-fir seedlings [MSc thesis]. Vancouver (BC): University of British Columbia.

Neumann M. 1995. Cross-contamination of conifer seed by *Fusarium* and the running water soak. Surrey (BC): British Columbia Ministry of Forests, Tree Seed Centre. Report 18.

Neumann M. 1996. Seedborne *Fusarium* and seed preparation at the MOF Tree Seed Centre. Surrey (BC): British Columbia Ministry of Forests, Tree Seed Centre.

Neumann M. 1997. Sanitation methods for conifer seeds, soaking tanks and screens to control seed-borne *Fusarium*. Surrey (BC): British Columbia Ministry of Forests, Extension Service and Tree Seed Centre. Contract Report.

Neumann M, Axelrood P. 1992. Assessment of *Fusarium* inoculum sources at Surrey Nursery. Surrey (BC): British Columbia Ministry of Forests, Tree Seed Centre. Minor Service Contract Number 63393. 10 p.

Nirenberg HI, O Donnell K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia 90:434-458.

Peterson M. 1990. Sanitation of styroblocks to control algae and seedling root rot fungi. Victoria (BC): Forestry Canada and British Columbia Ministry of Forests. FRDA Report 140.

Peterson M. 1991. Guidelines for the sanitation of nursery seedling containers. Victoria (BC): British Columbia Ministry of Forests, Silviculture Branch. Supplement to FRDA 140.

Peterson M. 2000. Seed-borne *Fusarium* on seeds collected from seed orchards and natural stands. Surrey (BC): British Columbia Ministry of Forests, Nursery Extension Service. Seed and Seedling Extention Topics 12(1):13-15.

Peterson M. 2003. [Unpublished data]. Located at: Applied Forest Science Limited, Victoria, British Columbia.

Peterson M. 2004a. [Unpublished data]. Located at: Applied Forest Science Limited, Victoria, British Columbia.

Peterson M. 2004b. [Unpublished data]. Located at: Applied Forest Science Limited, Victoria, British Columbia.

Peterson M. 2007. *Fusarium* infection routes. Victoria (BC): Applied Forest Science Limited. Contract report to the Forest Genetics Council of BC.

Portlock FT, compiler. 1996. A field guide to collecting cones of British Columbia conifers. Victoria (BC): Canadian Forest Service and British Columbia Ministry of Forests.

Rudolph BA, Harris GJ. 1945. The invasion of the internal structure of cotton seed by certain Fusaria. Phytopathology 35:542-546.

Sharma MR. 1992. Mycoflora of soybean seeds and their pathological effects [PhD dissertation]. Jaipur (India): University of Rajasthan.

Shein IV, Shibistova OB, Zrazhevskaya GK, Astrakhantseva, Polyakova GG. 2003. The content of phenolic compounds and the activity of key enzymes in their synthesis in Scots pine hypocotyls infected with *Fusarium*. Russian Journal of Plant Physiology 50(4):516-521.

Summerell BA, Salleh B, Leslie JF. 2003. A utilitarian approach to *Fusarium* identification. Plant Disease 87:2.

Sutherland JR, Shrimpton GW, Sturrock RN. 1989. Diseases and insects in British Columbia forest seedling nurseries. Victoria (BC): British Columbia Ministry of Forests and Forestry Canada. FRDA Report 065.

Thomsen K, Schmidt L. 1999. Control of fungi during seed procurement. Humblebaek (Denmark): Danida Forest Seed Centre. Technical Note Number 53.

Toussoun TA, Nelson PE. 1968. A pictorial guide to the identification of *Fusarium* species according to the taxonomic system of Snyder and Hansen. University Park (PA): The Pennsylvania State University Press. 51 p.

Wenny DL, Dumroese RK. 1987. Germination of conifer seeds surface sterilized with bleach. Tree Planters' Notes 38(3):18-21.