

## *Fusarium* species

cone & seed pest management &

British Columbia conifers:

Status of investigative research to date

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## **1.0 Introduction**

This note provides a brief biological outline of some species in the genus *Fusarium* and how these can be implicated as seed-borne organisms leading to conifer seed and seedling losses in British Columbia (BC). *Fusarium* species are implicated with pre- and post-emergence damping off, seedling wilt, late damping off, root rot and outplant seedling failure. Current understanding of *Fusarium* species, with regard to cone and seed pest management in BC 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.

## **2.0 The genus *Fusarium*: An overview**

Members of the genus *Fusarium* are among the most important plant pathogens in the world. *Fusarium* species 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 water-borne or carried in or on plant residue or seeds, and can be recovered from any part of a plant from roots, shoots, flowers and or cones as well as seeds (Summerell et al. 2003).

Summerell et al. (2003) point out that *Fusarium* taxonomy has been plagued by changing species concepts, with as few as nine, to over 1000 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 et al. (1983) who defined widely accepted morphological species

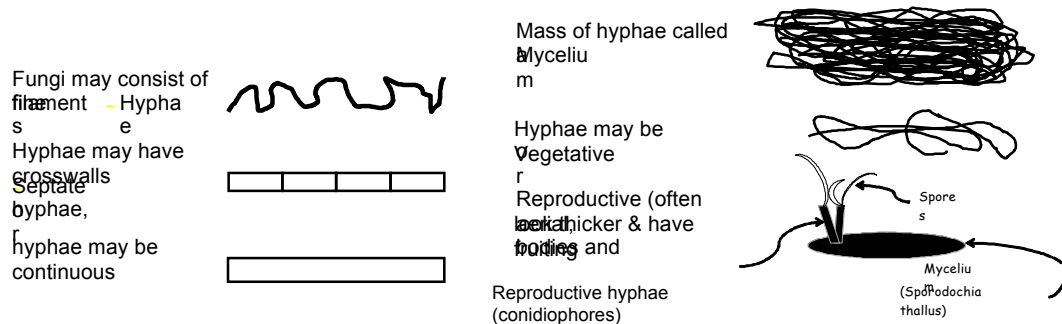
concepts. Since that time however, the application of biological (Leslie, 2001) and phylogenetic (Nirenberg and O'Donnell, 1998) species concepts to new as well as existing strain collections has resulted in further splitting of many of the previously described species. If changing these taxonomic designations were only rare, or of limited economic importance, they could be viewed as being merely pedantic. However, many of these species can be important, e.g. *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 et al. 2001). Further description of *Fusarium* taxonomy is well beyond the scope of this note however, its complexity and the recognized difficulty of rapidly identifying cultures to species (Summerell et al. 2003) has meant that research and development of cone and seed pest management associated with *Fusarium* in 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* species are grouped in the subdivision Deuteromycotina which encompasses the imperfect (asexual) fungi. Nelson et al. (1983) point out that the perfect (sexual) states of *Fusarium* species 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* species, e.g. *F. oxysporum* and *F. culmorum* appear to have lost their sexual ability and have adopted other methods of facilitating genetic adaptations (Booth, 1981).

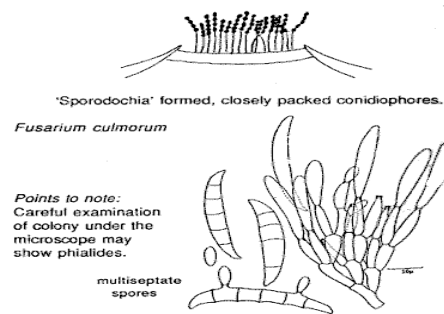
### 3.0 General Characteristics

Due to the great variability within this genus, it is one of the most difficult of all fungal groups to distinguish taxonomically (Alexopoulos and Mims, 1979). Conidia (asexual spores) are hyaline and can be divided into three groups: macroconidia, microconidia, and chlamydospores. Macroconidia are several-celled, crescent or canoe-shaped spores. Microconidia are one- or two-celled, ovoid or oblong, and borne singly or in chains. Chlamydospores are round, one- or two-celled, thick-walled spores produced terminally or intercalary on older mycelium (Agrios, 1988).



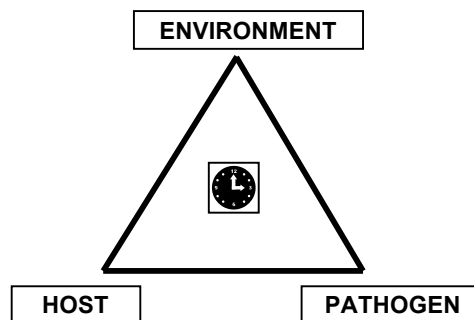
Structure of some fungi associated with disease (Fungi imperfecti)

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). The majority of *Fusarium* species produce their macroconidia on sporodochia, cushion-shaped fruiting structures covered with conidiophores (simple or branched hyphae bearing conidia). However, macroconidia can also be found throughout the aerial mycelium. Microconidia are one or two-celled, ovoid or oblong, and borne singly or in chains. These spores are found scattered throughout the aerial mycelium. The one- or two-celled microconidia are usually smaller than the macroconidia. Both macroconidia and microconidia are produced from phialides (a type of conidiogenous cell). Chlamydospores are round, one- or two-celled, thick-walled spores produced terminally or intercalary on older mycelium (Agrios, 1988).



*Fusarium culmorum* showing sporodochia, macroconidia and conidiophores.

Chlamydospores generally function as resting spores, having the ability to survive adverse conditions and enable the fungus to regenerate when favourable conditions for growth are reencountered. This is illustrated by a disease triangle. In the presence of a suitable host (e.g. seedling) and pathogen (e.g. *Fusarium* chlamydospore), disease of the host will progress when the environment favours spore germination and infection over time.



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

#### 4.0 Disease Cycle

*Fusarium* is a soil inhabitant which overwinters between crops in infected plant debris as mycelium and in its three 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 and produces microconidia. 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 coloured sporodochia 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).

## **5.0 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 seed becomes contaminated or seedlings are infected with *Fusarium*, damping-off may occur. The *Fusarium* species 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 a seed contaminant, 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 soil-less, peat based growing media in Styrofoam

(Styroblock®) containers. Styroblocks® generally reside on benches over concrete or gravel. Seed germination and the early part of the growing cycle takes place in polyethylene covered greenhouses where temperature, light and moisture are closely controlled and nutrients are applied through overhead irrigation.

*Fusarium* species 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* is an *introduced* pest. Introduced to the container nursery via seed, water, and wind or on old containers or dirty equipment, *Fusarium* can lead to seed and seedling losses in several ways:

1. Seed-borne contamination
2. Pre- and post-emergence damping-off
3. Seedling wilt
4. Late damping-off
5. Seedling root rot
6. Outplant seedling failure

### 5.1 Seed-borne contamination

Seed-borne fungi are defined as those “that are dispersed in association with some kind of dispersal units of the host (i.e. seeds)” (Ingold 1953). This definition includes all seed types and all associated microfungi and is the one adopted with reference to conifer seed-borne fungi in BC. Some authors classify fungi as being either seed-borne or seed-transmitted (Thomsen and Schmidt 1999). They define seed-borne 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 seed-borne 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 seed-borne pathogen than a seed-borne disease. Seed-borne pathogens (as opposed to diseases) are defined here as organisms, which whether on or in seeds, may or may not cause infections and symptoms on the seeds. Some seed-borne pathogens may actively infect seeds, and may or may not cause symptoms on the seeds. Seed-borne pathogens associated with conifer seeds may inhabit the external or internal tissues of seeds. Seed-borne diseases occur on seedlings as a result of pathogens carried in or on the seed. Evidence shows that *Fusarium* rarely exists within conifer seed (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 seed coat. Infection refers to the penetration of seeds by an organism followed by the establishment of a relationship (i.e., 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 seed is of little value as an internal relationship between the seed and fungus will still exist. One of the easiest ways to eliminate or reduce seed-borne

contamination is through the use of running water during imbibition, followed by a post-stratification rinse with running water (Kolotelo et al. 2001). Disinfestation in this manner can reduce the incidence of seed-borne *Fusarium* by reducing what has previously been observed as the tendency for contamination to actually increase during stratification.

Seed-borne 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 seed during interim storage, processing, or seed pre-treatment for stratification. As *Fusarium* 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 seed-borne *Fusarium* to be the same on seeds originating from seed orchards and those taken from natural stands (Peterson 2000). *Fusarium* 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 (e.g. spores) reaching maturing cones on trees, is thought to be one way that seed can become contaminated and *Fusarium* becomes seed-borne. Seeds and cone parts harbouring *Fusarium* can contaminate processing facility equipment, contributing to further contamination of otherwise clean seeds. Regardless of the initial source, seed-borne *Fusarium* can intensify throughout a contaminated seedlot during seed stratification following imbibition.

### 5.2 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 causing further losses.

### 5.3 Seedling wilt

Conifer seedlings, especially Douglas-fir, 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 (e.g. increased irrigation) may either prevent or reverse the symptoms and minimize any subsequent damage and loss.

### 5.4 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, i.e. late damping-off, the fungus may continue to grow on the decaying tissue where it can sporulate profusely, visibly presenting salmon to coral pink-coloured sporodochia on the lower portion of seedling stems.

### 5.5 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 *Fusarium* 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.

### 5.6 Outplant seedling failure

*Fusarium* is 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 *Fusarium* 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.

## **6.0 Cone and seed pest management: *Fusarium* research to date**

Tree species occurring in BC that are affected by seed-borne *Fusarium* species in decreasing order of frequency as indicated from fungal assays, include: Douglas-fir (*Pseudotsuga menziessi* (Mirb.) Franco var. *menziessi*), western larch (*Larix occidentalis* Nutt.), western white pine (*Pinus monticola* Dougl. Ex D. Don), western red cedar (*Thuja plicata* Donn ex D. Don), Ponderosa pine (*Pinus ponderosa* Dougl. ex P. & C. Laws.), grand fir (*Abies grandis* (Dougl. ex D. Don) Lindl.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), sitka spruce (*Picea sitchensis* (Bong.) Carriere), Yellow cedar (*Chamaecyparis nootkatensis* (D. Don) Spach), Noble fir (*Abies procera* Rehd.), Amabilis fir (*Abies amabilis* (Dougl. ex Loud.) Dougl. ex J. Forbes), interior spruce (*Picea glauca* (Moench) Voss and *P. engelmannii* Parry ex Engelm.), mountain hemlock (*Tsuga mertensiana* (Bong.) Carriere), and interior Lodgepole pine (*Pinus contorta* Dougl. ex Loud. Var *latifolia* Engelm.) (Kolotelo et al. 2001).

It was indicated earlier that within the context of the forest conifer container-nursery system, *Fusarium* can be viewed as an introduced

pest. 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 seed. 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.

## 6.1 Seed-borne contamination

### 6.1.1 Initial contamination

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

*Fusarium acuminatum* and *F. avenaceum* commonly colonize conifer seed, however James (2000) found the majority of *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 seed-borne inocula.

Several different species of *Fusarium* can cause root rot of container seedlings, with the major source of inocula being the seeds (Landis et al. 1990). Seed-borne *Fusarium* is 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 two important seed-borne fungi, *Caloscypha fulgens* and *Sirococcus conigenus*, has led to management guidelines to reduce their incidence on seed, thus lowering outplant mortality attributable to their occurrence. A similar understanding of infection routes for seed-borne *Fusarium* does not exist and establishing this remains an essential first step to developing guidelines for reducing its incidence. As a seed-borne contaminant (i.e. carried on seed) 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* has an ability to grow systemically within the vascular system of two year old seedlings (Peterson, 2004a. *unpublished data*). As a seed-borne disease (i.e. actively attacking seed), *Fusarium* can be responsible for pre-emergence damping-off where seeds fail to germinate. To function as a seed-borne contaminant as well as a disease suggests more than one infection route for *Fusarium*. 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. However, Maude (1996) states 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 spp.* which invade vascular tissue. As a wilt fungus invading vascular tissues, *Fusarium oxysporum* has been shown to infect seed 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 seed 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 et al. 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 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 seed coat 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 seed coat surfaces during seed development. AFS Limited routinely isolates *Fusarium* spp. from seed surfaces during fungal assay testing for the BC MoFR Tree Seed Centre, confirming that *Fusarium* inoculum exists on the seed coats 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 *Fusarium* spore densities in the Victoria region to closely mimic those of conifer pollen density during the spring of 2003 (Peterson, 2003. *unpublished data*). That airborne *Fusarium* spore inoculum can occur during times of pollination in the

vicinity of Douglas-fir seed orchards supports Littke and could explain the presence of *Fusarium* inoculum on seed surfaces.

Research by Peterson (2007) indicates that seed-borne *Fusarium* on several conifer species does not likely occur as internal infections but is limited to seedcoat contamination. These observations appear to indicate that seed-borne *Fusarium* in some conifer species does not occur systemically and more likely occurs following Littke's (1996) hypothesis. How seed becomes initially contaminated with *Fusarium* 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.

#### **6.1.2 Spread and intensification of initial *Fusarium* seed contamination**

Regardless of the initial source, *Fusarium* 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 *Fusarium* 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* species on cones, debris and seed at various stages during cone and seed processing. They found processing stages incorporating agitation to indicate increases in *Fusarium* contamination. Kilning appeared to decrease *Fusarium* contamination on seed and cone scales despite peak kilning temperatures of 40°C not totally eliminating the fungal contaminant. Their overall finding was that initial seed

contamination levels may not be indicative of final seedlot *Fusarium* contamination. This was emphasized by the fact that despite BC interior Douglas-fir having a very low incidence of seed-borne *Fusarium*, some of the associated debris had significant amounts of *Fusarium* contamination. Also, although initial *Fusarium* levels on cone scales, seed 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 seed to become contaminated with *Fusarium* makes testing for its presence a viable first step for managing it as a seed-borne organism, thus allowing specific seedlots to be targeted for special treatment. *Fusarium*'s ability to spread or intensify within a seedlot, that some tree species are more susceptible to *Fusarium*'s effects, as well as the fact that some species represent a higher potential monetary loss, are also reasons that seeds are tested for *Fusarium* contamination. A matrix established by the BC MoFR Tree Seed Centre outlining the seed fungal testing priorities for the three important seed-borne fungi in BC has been developed. The priorities for *Fusarium* testing is such that the tree species; Bl, Fdc, Lw, Pw and Py are all rated high; Ba, Bg, Cw, Fdi, Hw, Ss, Sx and Sxs are rated medium; and Plc, Pli and Yc are rated low priority (Kolotelo et al., 2001).

Past sampling has indicated average levels of *Fusarium* on seed to be typically less than 2.5% with a moderate degree of variation within seedlots (Kolotelo et al., 2001). Not all species of *Fusarium* are pathogenic and those that are, are often weakly so. Also past studies to detect seed-borne *Fusarium* in BC were often limited to genus. Thus,

when routine fungal assays of seed in BC were adopted, it was elected to detect *Fusarium* 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 International Seed Testing Association (1999) standards. The laboratory methods used to test seed-borne *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 seed 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 *Fusarium* disease potential, and growers target seedlots with levels higher than this. The main strategy for *Fusarium* levels above this are aimed at minimizing its ability to spread within a seedlot.

Seed orchard seed appears to be affected by *Fusarium* at the same rate as seed collected from natural stands (Peterson, 2000). Current knowledge still does not provide a clear understanding of how cones become contaminated with *Fusarium* however, more control is available when collecting in seed orchards compared to natural stands, and Kolotelo et al. (2001) point out three 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 with *Fusarium* and the sacks themselves, especially when wet will readily trap airborne *Fusarium* inoculum (Peterson, 2004b *unpublished data*). Finally, filled sacks should be stored following the general recommendations for all species described in Portlock (1996).

The ability of *Fusarium* contaminated seed to intensify within seedlots during imbibition and stratification, as well as the management practices to reduce this phenomenon are extensively reviewed in Kolotelo et al. (2001). Seed-borne *Fusarium* primarily exists as a contaminant and does not readily infect the seed interior during storage. Infection of an emerging shoot and/or radical may occur in the germination phase but Kolotelo et al. (2001) point out that early stages of seed colonization by *Fusarium* 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 seed to environmental conditions conducive to fungal growth can help prevent any intensification of seed-borne *Fusarium* within a seedlot.

Three strategies to minimize losses from seed-borne pathogens are: 1) eliminating or reducing initial inoculum, 2) slowing the rate of pathogen spread and 3) shortening the time seed is exposed to the pathogen (Berger, 1977). It is valuable to view these strategies in the context of a disease triangle with the seed as *host*, *Fusarium* the *pathogen* and seed handling (from cone collection, through extraction, storage, sowing, germination and seed coat 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 et al. (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 et al. (1989), Portlock (1996) and Leadem et al. (1990). However, some questions still remain with regard to when *Fusarium* becomes seed-borne on seed orchard produced seed (Peterson, 2007). Kolotelo et al. (2001) present a good summary of collection methods to minimize seed-borne disease as well as discussions of seed processing. Initial research toward the potential for seed-borne *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  $-18^{\circ}\text{C}$  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 the spread or intensification of seed-borne *Fusarium*.

Research in BC with regard to cone and seed pest management and seed-borne *Fusarium* within Berger's (1977) third category to minimize loss to seed-borne disease, i.e. 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 seed coat *Fusarium* infestations (Axelrood et al. 1995; James 1985b). And the importance of this research is emphasized by the findings that infestation levels of *Fusarium* can increase significantly during seed imbibition and stratification (Axelrood et al. 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 of *Fusarium* that may have contributed to these increases, e. g. airborne inoculum in the drying room and the soaking mesh and/or tanks. However, it was ultimately deemed that as the observed “bulking up” of seed-borne *Fusarium* occurred during the first 6 hours of imbibition, a faster water flow over the seed 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 *Fusarium* inoculum and the potential for cross contamination.

The use of fungicides applied directly to seedcoats to control seed-borne *Fusarium* has been investigated, however it is difficult to find fungicides that meet the many requirements necessary for their safe and effective application (Bennett et al. 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 (Wenny and Dumroese, 1987; Lock and Sutherland, 1975; Lamontagne and Wang, 1976).

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 seed-borne *Fusarium* contamination (Kolotelo et al., 2001). Running water treatments appear to reduce the incidence of post-stratification seed-borne *Fusarium* (Axelrood et al., 1995; James, 1985b; Dumroese et al., 1988). The method used at the BC MoFR Tree Seed Centre is to imbibe seed in mesh bags in a tank of running water for 24-48 hours, however this requires significant water resources. Kolotelo et

al. (2001) suggests complete water changes every 4-8 hours will have similar effects and 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 seed-borne *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 et al. (2001, pp. 66). 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 seed by immersing it 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 levels of *Fusarium* on seed with hydrogen peroxide clearly exists, however some *Abies* species do not respond consistently, and for this reason Kolotelo et al. (2001) suggest more research and operational studies are needed to address this.

## 6.2 Pre- and post-emergence damping-off

### 6.2.1 Pre-emergence damping-off

Seed-borne *Fusarium* is most often responsible for pre-emergence damping-off, i.e. seed that becomes infected and fails 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 seed-coat *Fusarium* 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 seed-coat 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 seed-borne *Fusarium*. This can happen if sufficient *Fusarium* 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 *Fusarium* inoculum in a two year study of seed-borne *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 styroblocks to contain *Fusarium*-infested root fragments and they also found 60% of the growing cavities to be contaminated with *Fusarium* on their surfaces. *Fusarium* has 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 styroblocks will all reduce levels of *Fusarium* inoculum in the immediate vicinity of germinating seed but 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 seed 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 *Fusarium* 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.

#### **6.2.2 Post-emergence damping-off**

Young germinant or seedling root infections, resulting from roots growing in close proximity to germinating chlamydo spores can lead to stem rotting at the groundline, which typifies post-emergence damping-off. Seed-borne *Fusarium* can also be responsible for post-emergence damping-off when the seed germinates but for reasons such as a slow to shed seed-coat for example, inoculum contacting and infecting the emergent tissues will often cause the new shoot to rot at the groundline. Young germinants rotting at the groundline 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 *Fusarium* 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 *Fusarium* spore inoculum.

Sanitation of older Styroblocks® can significantly extend the useful life of these growing containers by reducing pathogen inoculum associated with old rough surfaces and associated extraneous root material from past year's use. Peterson (1990) achieved significant reductions in *Fusarium* levels on old Styroblocks® 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 Styroblock® life while reducing the presence of *Fusarium* inoculum in the container seedling root zone.

### 6.3 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 *Fusarium* has entered the roots and xylem tissues. Seedlings that continue to grow and become infected by either *Fusarium* 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 *Fusarium* top blight or wilting. *Fusarium* 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.

*Fusarium* 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 *et al.* (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, i.e. *Trichoderma harzianum* is actively antagonistic to *Fusarium* as it competes with the pathogen for substrate. *Trichoderma 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 *et al.* (2003) treated Scots pine seedlings with virulent spore suspensions of *Fusarium sporotrichiella* and deduced that conifer seedling resistance to wilt diseases to be correlated with the synthesis rate and accumulation of insoluble phenolic polymers. The ability to resist *Fusarium* wilt was higher in

plants unable to synthesize these polymers as they accumulated with age.

#### 6.4 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, however, it is important to remove affected seedlings as they can lead to increased spore inoculum in the greenhouse. Left in the container cavities, 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 *Fusarium* 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 (*Fusarium*) 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.

#### 6.5 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 seed or at least at the time of sowing. Not all species of *Fusarium* are pathogenic (James et. al. 1989) and many of those that are, are weak pathogens. However, Neumann (1993) points out a very important adaptive characteristic of *Fusarium* that contributes to its persistent ability as a pathogen and that is that *Fusarium* is often a facultative parasite 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 *Fusarium* 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.

### 6.6 Outplant seedling failure

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 *Fusarium* 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 et al. (1998) concluded that *Fusarium* species 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 *Fusarium* infections and root colonization. However, the mean age of the planted and naturally regenerated seedlings Axelrood et al. (1998) examined were 5.6 and 4.7 years respectively and they did not take into account seedling mortality that may have arisen immediately after planting. 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 et al., 1996). Because of this, Axelrood et al. (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-grown seedlings commonly have *Fusarium* on or around their roots (Graham and Linderman, 1983; James, 1985a; Landis, 1976) and it is therefore important that care is taken so any *Fusarium* 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.

## **7.0 Cone and seed pest management: *Fusarium* species 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 outplant seedling survival 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. Seed-borne contamination
  - a. How seed becomes contaminated with *Fusarium* 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 an introduced greenhouse pest, i.e. benching, pallets and Styroblock® 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. Outplant seedling failure
  - a. Outplant failure usually occurs when *Fusarium* has 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 of *Fusarium* at the pathogen corner of the disease triangle to below what is necessary to cause significant losses at the reforestation site

## **8.0 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 *Fusarium* is a facultative parasite. 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 seed-borne 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* species as cone and seed pests, specifically: better understanding is needed as to how seed becomes contaminated with *Fusarium*; 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.

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