

# Seed Sanitation

Nurseries in BC are increasingly aware of the importance of sanitation in combating pathogens that have repeatedly caused significant stock losses. While there can be a large microflora associated with seeds, their effects on seed viability and seedling production are not well understood (Richardson 1996; Mittal and Wang 1993). Therefore, it is often critical to reduce the sources and degree of pathogen inoculum on seeds. Disinfesting seed can control some seedborne fungi. The first fungus understood to be seedborne was *Tilletia caries*, responsible for causing bunt or covered smut of wheat (Tull 1733). Tull recorded observations of farmers whose wheat seed that had been salvaged from the ocean was free of smut, concluding that the brining action of the salt water disinfested the wheat seed. Early in the drive to guarantee high quality seed, organizations like ISTA recognized the need to better characterize seedborne pathogens. Latest estimates document approximately 1300 seedborne organisms capable of being pathogenic. Most are fungi; seedborne fungi have been reported on 305 host genera in 96 plant families (Richardson 1996). In addition to pathogens, many saprophytic fungi can infect seeds when they are harvested and stored under sub-optimal conditions. They sometimes reduce seed viability and produce mycotoxins (e.g., aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*).

Some seedborne fungi can spread to clean seeds when they are stored with contaminated or infected seeds in a moist environment at temperatures conducive to fungal growth. These temperatures vary for fungal species but in general range between 15–22°C. In some of these situations, control measures can be as simple as avoiding favourable conditions for fungal growth. Where conditions conducive to fungal growth and spread cannot be avoided, more aggressive measures can be taken to reduce inoculum levels. In BC, most efforts have been concentrated on assaying seedlots for *Caloscypha*, *Sirococcus*, and *Fusarium*. When seedborne *Caloscypha fulgens* and species of *Fusarium* begin to infect or contaminate seeds in a seedlot, steps should be taken to minimize the impact of these organisms (5% or more for *Caloscypha* and *Fusarium* or 1% for *Sirococcus*).

Strategies for *Fusarium* and *Caloscypha* control are aimed at minimizing the ability of each pathogen to spread within a seedlot.

The methods used to control the impact of *Sirococcus* infection in a seedlot are designed to eliminate the organism. Seeds infected with *Sirococcus* usually develop *Sirococcus* blight, where primary needles on germinants die from the base upwards. The importance of this seed-borne disease lies in its ability to kill seedlings via secondary spread of inoculum from infected germinants.

Research into seedborne pathogens indicated that early stages of fungal colonization of seeds are dependent primarily on abiotic factors such as water availability and temperature (Ramos et al. 1997). Because most of these fungi contaminate only seed coat surfaces, they depend more on environmental relative humidity than seed moisture content (Thomsen and Schmidt 1999). Successful pathogens exploit the best opportunities to infect seed. For example, they may be transported along with seeds of the host plant, capable of causing infection at the earliest opportunity (Richardson 1996).

*Strategies for Fusarium and Caloscypha control are aimed at minimizing the ability of each pathogen to spread within a seedlot, while methods to control Sirococcus infection are designed to eliminate the organism*

Any proposed strategy to curtail the effects of seedborne pathogens must be practical. There is little value in telling a seed processor or nursery grower that seeds are infected/infested unless it is possible to provide assay

information on the effects of the infection on seedling requests and whether or not any treatment or strategy is available. In addition, there is also an ongoing need for knowledge of the disease process and how to make fungal assay results meaningful tools of what will happen to seeds once they are sown.

There are three general strategies to minimize losses from seedborne pathogens (Berger 1977). These are eliminating or reducing initial inoculum, slowing the rate of pathogen spread, and shortening the exposure time of the seed to the pathogen. Cone collection protocols, seed orchard management, and seed processing fall into the first category and are operationally referred to as sanitation. Seed treatments and storage relate closely to the second strategy, while stratification and germination procedures are encompassed by the third. Seed treatments fall into three basic categories: *seed protection* (e.g., chemical, biological, physiological

procedures that protect the seed/seedlings from pathogens); seed *disinfestation* (e.g., removal of seed coat pathogens); and seed *disinfection* (e.g., eliminate pathogens inside the seed) (Bennett et al. 1991). Ideally any treatment should reduce or eliminate the effects of the pathogen but not adversely affect seed germination.

Littke (1996) indicated that an understanding of the source and development of seedborne fungi was needed to reduce losses and realize gains in the nursery. He surmised that the most likely modes of seed contamination are physical transfer from the exterior of the cone to the seed coat during seed development, cone storage, and seed cleaning; cross contamination with "dirty" seedlots during seed processing, imbibition, stratification, and sowing; and contact with soil during storage or collection from squirrel caches. Overall, he concluded that 90% of seedborne inoculum resides on the seed coat and that cone-borne fungi are the most likely source of infestation. Cone handling and seed storage protocols that enhance drying and sanitation can reduce fungal contamination levels. Seed treatments (e.g., hydrogen peroxide, bleach, or bromine) are effective only in reducing levels of infestation and can have both positive and negative effects on germination performance (Littke 1996).

## Seed Cleaning Techniques

Campbell and Landis (1990) developed a strategy for dealing with seedborne diseases by determining the degree of possible risk to a nursery manager and attaching appropriate actions to minimize their effects on container nursery production. The general methods listed for treating seeds were surface cleaning or washing with running water; surface disinfecting seed coats with heat or chemical disinfectants; and coating seeds with fungicides (e.g., thiram or captan). They advocated a sequential approach to managing seed diseases. The steps are:

1. Determine if seed treatments are warranted. Treatments should be considered if the seeds fit any two of the following criteria: a) seedlings are to be grown in containers; b) the seedlot has a history of problems; c) the seeds are from a high risk conifer species; and d) the seedlot is of high value (e.g., seed orchard seedlots).
2. Assay the seedlot and identify the pathogens. Most seedlots do not possess significant levels of pathogens. Fungal assay results are present on sowing request labels (Figure 33, page 31) and on SPAR.
3. Treat assayed seedlots with significant contamination by cleansing seed surfaces (e.g., running water soaks and disinfecting seed surfaces with bleach or hydrogen peroxide, for example).
4. Surface dry seeds during extended stratification.

This section of the guide will primarily concentrate on the third step.

Once the decision has been made that there is a risk to seeds, procedures are needed to reduce the potential for seedborne pathogens to adversely affect seed viability and seedling survival and health. For the most part, nearly all techniques reduce seed coat infestations. Unfortunately, most of these seed cleaning techniques have little effect on seedborne *Caloscypha* and *Sirococcus*. For these two pathogens recommendations include reducing stratification periods if possible, sowing the seedlot as soon as possible after stratification, providing optimal germination conditions, and reducing the number of seeds per cavity by seedlot upgrading techniques (Landis et al. 1990; Sutherland et al. 1989).

Most seed treatment research has focused on reducing seed coat infestations of *Fusarium* spp. (Axelrood et al. 1995; James 1985). Presence of *Fusarium* on seeds and/or seedlings may be an unsatisfactory indicator of disease potential (James et al. 1989a, 1987). Causal relationships are difficult to pinpoint and some researchers question the true effects of seed treatments. Under operational conditions, any technique that can reduce the *Fusarium* disease potential helps reduce possible risks of disease. This does not preclude the notion that micro-environmental conditions may still be favourable to disease expression, but the degree of damage may be lessened with the reduced number of potential infection vectors. Research has shown that the relation of contamination level and subsequent disease may vary greatly among seedlots (James 1985). For Douglas-fir and ponderosa pine, James (1987a) found that most of the seedlots he tested had less than 10% seedborne contamination with *Fusarium*. Although infestation levels appear low, they may be sufficient to cause widespread disease. This may be related to recent observations that infestation levels of *Fusarium* spp. can increase greatly during seed imbibition and stratification (Axelrood et al. 1995; Hoefnagels and Linderman 1999). Neumann (1996, 1997) found that *Fusarium* levels on seedlots of Douglas-fir, spruce, western larch, and true firs increased substantially during stratification even on seedlots with low (<1%) dry seed pathogen levels. Numerous pathogenicity assays on conifer seeds, particularly Douglas-fir, have demonstrated a high

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species were retrieved from asymptomatic seedlings suggesting that environmental conditions play a key role in disease expression (Motta et al. 1996).

Numerous methods have been suggested or evaluated in an effort to reduce or eliminate seedborne contamination. One method has been to apply fungicides directly to the seed coats (Bennett et al. 1991). A second method has involved rinsing seed in running water for 24–48 hours to wash off fungal spores (James 1987b; James and Genz 1981). A third method recommends soaking seed for a determined time period in sterilants (e.g., sodium hypochlorite, ethanol, or hydrogen peroxide) to disinfect seed coats (Dumroese et al. 1988). These and other methods such as biological control will be explored in greater detail.

### Running Water

#### Background

One of the easiest recommended strategies is running water imbibition coupled with a post-stratification rinse with running water. From a disease management perspective, the practical benefit of running water versus standing water soaks is the removal of fungal inoculum from infested seed coats (James and Genz 1981; James 1987a). In most studied seedlots, *Fusarium* levels have been observed to increase during stratification though inoculum levels are less in running water compared to standing water soaks. The result of running water treatment is an overall reduction in post-stratification seedborne levels of *Fusarium* (Axelrood et al. 1995; James 1985; Dumroese et al. 1988; Neumann, 1993).

#### Methodology

At the BC Ministry of Forests Tree Seed Centre, the method has been to pre-treat sowing requests in a mesh bag in a tank of running water for 24–48 hours. Unfortunately, this type of treatment requires extensive water resources, which may be limited at some facilities. It is suggested that even systematic water exchanges are sufficient to conserve water and reduce the amount of seed coat infestations. Complete water exchanges every 4–8 hours will have similar benefits. The addition of supplemental aeration between exchanges is recommended. The associated water movement helps to

degree of virulence for a majority of the isolates of selected *Fusarium* species (Axelrood et al. 1995; James et al. 1989a, 1989b, 1987). It must be emphasized that many of these pathogenic *Fusarium*

dislodge seedcoat fungi while maintaining an even distribution of oxygen within the water column.

The seeds making up a sowing request should be placed in a running water volume at least three times the seed volume within a mesh or porous container to allow for sufficient coverage of seed surfaces. The temperature of the water should not be higher than 25°C (Kaufmann and Eckard 1977). After stratification, some studies have recommended at least an additional 48 hour rinse (Dumroese et al. 1988). This may have the effect of removing fungal inoculum on the seed coats that was established during stratification. **Figures 76 and 77** (a and b) demonstrate the effect of running water on removing some of the seedborne fungal mycelium on heavily infested seeds of *Abies lasiocarpa* and *Picea glauca*.

It is the general practice at the Tree Seed Centre to soak together sowing requests of most seedlots and conifer species, such as interior spruce and lodgepole pine, in large tanks for the running water soak period.

However, certain species such as Douglas-fir and western larch, and seedlots with critical levels of fungi are now imbibed in individual running water tanks (see **Figure 78**, page 65). Some operational factors may contribute to increases in *Fusarium* inoculum such as cross-contamination from infested to uninfested seedlots and sowing request size.

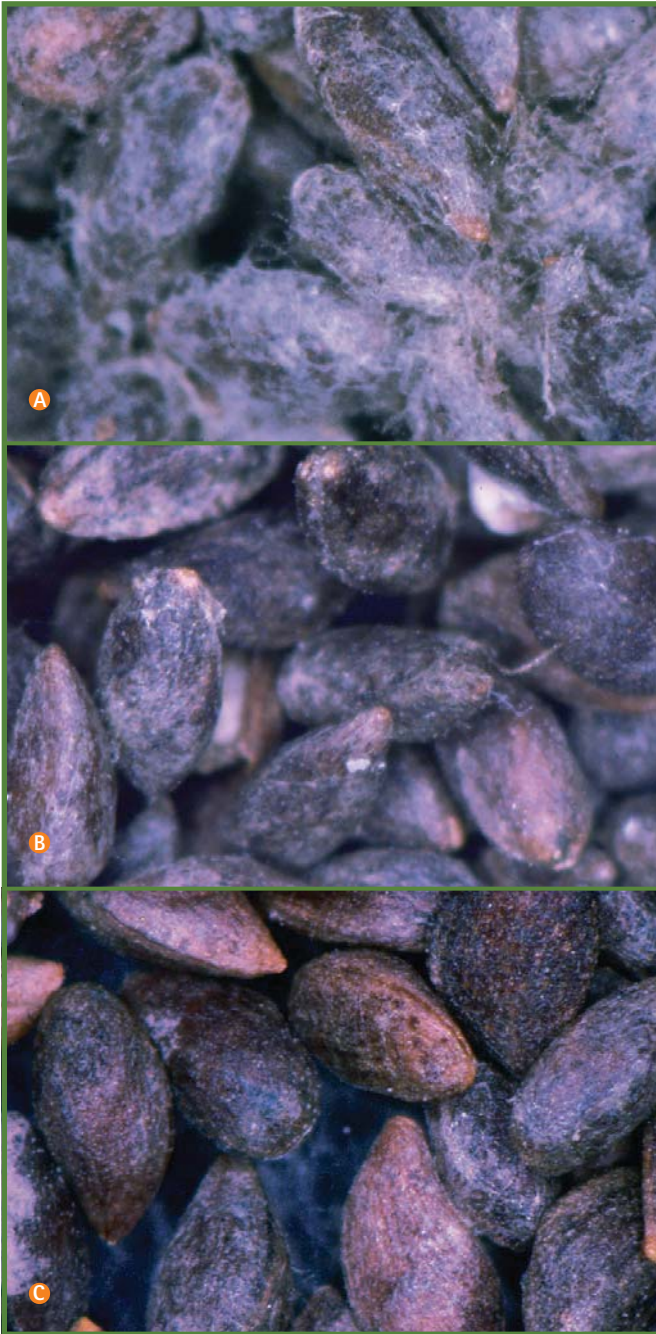
Neumann (1995) identified the potential for cross-contamination problems between low and highly infested seedlots of Douglas-fir and western larch when several seedlots are soaked together (see **Figure 79**, page 65). In 1996, she found significant differences in *Fusarium* levels between seeds sampled from the centre and edges of seed bags from three sizes of Douglas-fir requests (100 g, 250 g, and 500 g) though no significant trend was found.

### Hydrogen Peroxide

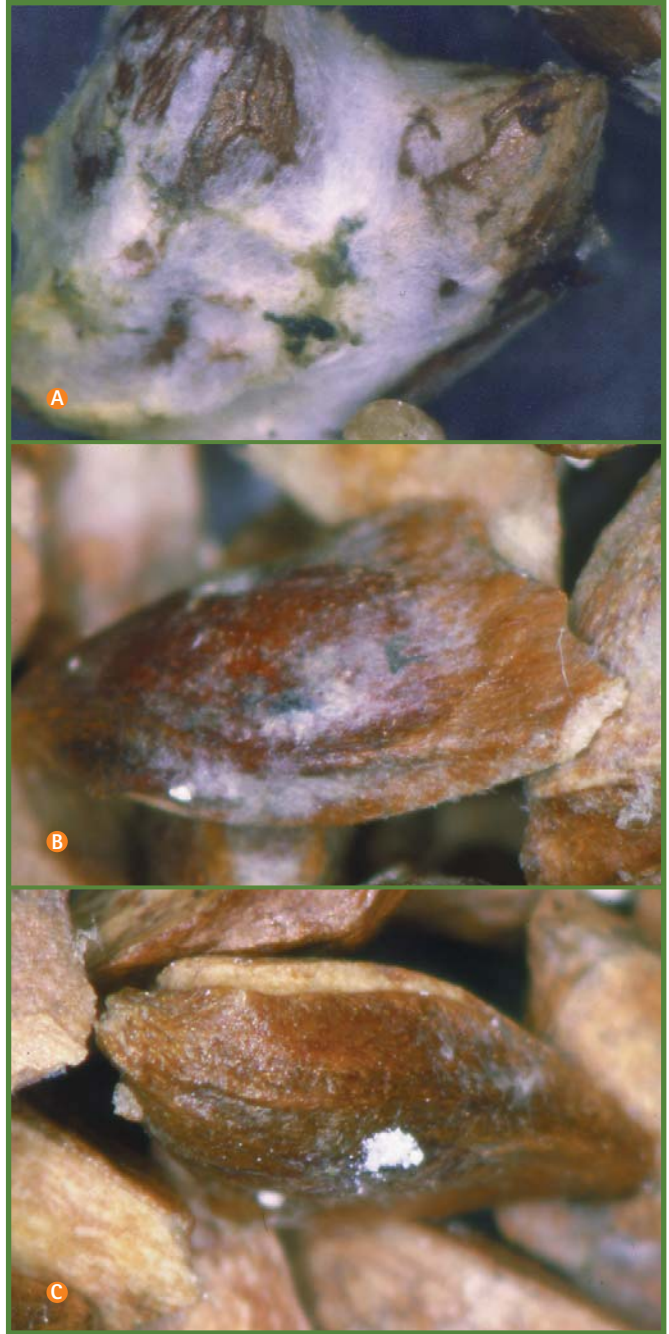
#### Background

By far, the most common chemical seed sanitation treatment is hydrogen peroxide. Substantial differences in concentration, duration of treatment, stratification timing, and conifer species tolerance have been reported by a variety of researchers. A summary of published hydrogen peroxide treatment results is listed in **Table 10** (page 66).

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**Figure 76** Seedborne fungi on spruce seeds a) prior to and b) after a post-stratification running water rinse. c) The effect of a post-stratification soak in a 3% hydrogen peroxide solution for 4 hours on seedborne fungi.



**Figure 77** *Caloscypha* on *Abies lasiocarpa* seeds a) prior to and b) after a post-stratification running water rinse. c) The effect of a post-stratification soak in a 3% hydrogen peroxide solution for 4 hours on seedborne fungi.

Most of these studies result in two general treatment categories: 30% hydrogen peroxide for under 1 hour; or a 3% solution for over 4–8 hours, both followed by a 1–48 hour rinse with fresh running water (see **Figures 76c** and **77c**). Both treatments can be applied prior to imbibition, post-imbibition and prior to stratification or post-stratification. Almost all studies have shown these treatments to be effective at reducing *Fusarium* levels on conifer seeds while

enhancing cumulative germination. Ching (1960) suggested that enhanced germination may be the result of hydrogen peroxide accelerating respiration.

#### Technique

In BC, we recommend that post-stratification seed should be immersed in a 3% hydrogen peroxide solution at a 3:1 solution to seed volume ratio for 0.5–4 hours followed by a running water rinse. This recommendation is based



**Figure 78** A large single- and a multi-chamber soaking tank used operationally at the BC Ministry of Forests Tree Seed Centre.



**Figure 79** Large running water tank used to soak a number of different conifer species seed requests. Tank in the foreground has been left open to show sowing requests; tank in the background is covered to ensure uniform imbibition.

on previous studies and the following observations:

- 1) the characteristic "bulking up" of fungi during stratification can be significantly reduced at this time;
- 2) fully imbibed stratified seeds are less likely to uptake the chemical;
- 3) a 3% hydrogen peroxide solution is easily obtained and poses substantially less occupational risk to workers;
- and 4) the treatment can be carried out at any facility with a minimum of equipment just prior to sowing.

The potential for reducing the levels of seedborne *Fusarium* with hydrogen peroxide shows good promise though certain inconsistencies still remain. In particular, *Abies* species do not respond consistently and sometimes unfavorably to hydrogen peroxide treatments. More research and operational studies are needed that address these ongoing issues.

## Bleach

Chemical treatment can be deployed to reduce seedborne *Fusarium* inoculum (Campbell and Landis 1990). Sodium hypochlorite or "bleach" has been used for many years as a seed coat sanitation treatment. It is readily available, easy to use and inexpensive at the concentrations generally recommended for seed cleaning. Rates range from 1–5.25% sodium hypochlorite for 2–10 minutes (James et al. 1987; Thomsen and Schmidt 1999; Mittal and Wang 1993; James and Genz 1981; Dumroese et al. 1988; Fraedrich 1996). Most of these treatments were applied prior to stratification. Studies by Trotter (1990) and Axelrood (1990; unpubl.)<sup>6</sup> found inconsistent results with 2.1% sodium hypochlorite for

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10 minutes when applied as a post-stratification treatment to Douglas-fir seedlots. As a pre-stratification treatment, other researchers have found this particular treatment to provide excellent reductions in seedborne infestations of *Fusarium* with increases in cumulative germination percentages on a variety of pine species and Douglas-fir seedlots (Dumroese et al. 1988; Wenny and Dumroese 1987). Wenny and Dumroese (1987) stress that this treatment should not be used on seeds of the true firs, larch, and spruces.

## Other Techniques

The aforementioned treatments are based on most research efforts to reduce effects of seedborne pathogens on conifer seeds but other techniques have or are being proposed that similarly address this issue. Two of these techniques, ethanol and fungicides, may be detrimental to seed viability and have therefore not been used extensively to reduce seedborne fungi.

## Ethanol

Ethanol is commonly found in pathology and medical laboratories to sterilize tools and equipment and is used extensively in the administration of vaccination programs to animals and humans. There are few studies using ethanol as a conifer seed treatment. Typical concentrations/exposure times have been in the 70–75% range for three minutes to 95% for 10 seconds (Dumroese et al. 1988; James et al. 1989a; Trotter 1992, unpubl.<sup>7</sup>). Each of these studies has been on Douglas-fir seedlots and resulted in reduced germination, inconsistent fungal assays across replicates,

<sup>6</sup> P.E. Axelrood, unpublished data, 1990, BC Research, Vancouver, BC.

<sup>7</sup> D. Trotter, unpublished data, 1992, BC Min. For., Extension Services, Surrey, BC.

**Table 10** Summary of hydrogen peroxide treatments to conifer seeds by species

Conifer species	H <sub>2</sub> O <sub>2</sub> (%) concentration	Duration (hr)	Timing	Results	Reference
<i>Abies amabilis</i> and <i>A. grandis</i>	3, 15, 30	0.5–48	Pre-strat, post-strat	No effects on germination. Poor fungal reductions.	Edwards and Sutherland 1979
<i>Abies lasiocarpa</i>	1, 3	1–16	Post-strat	Variability in germination. Moderate fungal reductions.	Neumann 1997
<i>Larix occidentalis</i>	1, 3	1–16	Post-strat	Increase in germination. Significant fungal reductions.	Neumann 1997
<i>Pinus ponderosa</i>	3	5	Pre-strat	Increase in germination. Significant fungal reductions.	James and Genz 1981
<i>Pseudotsuga menziesii</i>	1	12–48	Pre-strat	Increase in germination.	Ching 1960
<i>Pseudotsuga menziesii</i>	3, 30	0.5–8	Post-strat	Variable germination. Significant fungal reductions.	Neumann 1993
<i>Pseudotsuga menziesii</i>	1, 3	1–16	Post-strat	Increase in germination. Significant fungal reductions.	Neumann 1997
<i>Pseudotsuga menziesii</i>	30	0.67	Pre-strat, post-strat	Significant fungal reductions.	Hoefnagels and Linderman 1999
<i>Pseudotsuga menziesii</i>	3	5	Pre-strat, post-strat	Variable germination. Significant fungal reductions.	Dumroese et al. 1988
<i>Picea abies</i>	30	1	Pre-strat	Moderate fungal reductions.	Motta et al. 1996
<i>Pinus taeda</i> , <i>P. elliotii</i> , <i>P. palustris</i> , <i>P. echinata</i>	3, 30	4–48 and 0.25–3	Pre-strat	Increase in germination. Significant fungal reductions.	Barnett 1976
<i>Pinus palustris</i>	30	0.92	Pre-strat	Increase in germination. Significant fungal reductions.	Fraedrich 1996

and observations of physical damage to seed coats. Unlike other treatments, ethanol may be readily imbibed by the seed resulting in tissue damage and lower germination. Therefore, ethanol is not a recommended treatment.

## Fungicides

Fungicides have long been used on vegetable and agronomic seedlots to provide protection for general diseases such as seed rot, damping-off, wilt, and root rots. Several active ingredients are combined with other chemical constituents to produce a variety of formulations, like dusts, wettable powders, emulsifiable concentrates, and flowables (Bennett et al. 1991). The basic requirements for a seed-treatment fungicide are: 1) effectiveness under different climatic conditions; 2) non-phytotoxic; 3) safe to operators and wildlife; 4) leave no harmful residues; 5) compatible with other seed treatments; and 6) low price (Agarwal and Sinclair 1997). It is difficult to find fungicides that have all these traits, particularly when most seed-treatment fungicides are phytotoxic at label rates (Bennett et al. 1991; Thomsen and Schmidt 1999). Commonly used fungicides on conifer seeds are captan, ethazole, and thiram but their negative effects on germination and variable efficacy have reduced their usage (Wenny and Dumroese 1987; Lock and Sutherland 1975; Lamontagne and Wang 1976). As broad-based chemicals they maybe effective against a variety of fungi including beneficial and antagonistic organisms. The use of these pathogen-antagonistic organisms as seed treatments is discussed later.

For *Sirococcus* and *Caloscypha* infected seedlots, a fungicide may be the only viable chemical treatment. Systemic fungicides may be absorbed by seeds and reach internal seedborne fungi (Thomsen and Schmidt 1999). Such fungicides may also provide a level of protection to the developing embryo through early germination phases. In a larger context, the long-term viability of fungicide treatments may be limited because regulatory agencies, environmental groups, worker safety organizations, and governments encourage alternative methods. Additional research in this area is warranted.

## Hot Water

A possible seed treatment that may provide a viable alternative to traditional chemicals is hot water. Such treatments have been used widely in agriculture to control seedborne pathogens while maintaining high levels of germination. Treatments typically range from 50–60°C for 5–60 minutes but exact temperatures vary with different seeds and associated pathogen species. (Thomsen and Schmidt 1999; Agarwal and Sinclair 1997). Erdey et al. (1997) found that *Fusarium moniliforme* Sheldon in maize was reduced by 85% with a treatment of hot water for 15 minutes at 55°C.

For conifer seeds, James et al. (1988) used microwaves to heat the water and assessed its effects on seedborne fungi of Douglas-fir. Their results indicated a thermal window of 43 and 55.5°C for between 60 and 90 seconds for efficacy on *Fusarium* and *Trichoderma* while maintaining high seed viability. Further work was suggested to determine the response of other conifer species, optimal sowing request size, other fluids (e.g., vegetable oils), and strategies to maintain antagonistic organisms (Dumroese et al. 1988).

## Biological Control Micro-organisms

The use of naturally occurring micro-organisms to inhibit the effects of pathogens is a technique gaining popularity, particularly because of problems and restrictions of chemical use. These organisms are selected for their ability to persist long enough on seeds and developing radicles to compete with pathogens and reduce or prevent disease development. Biocontrol organisms used against soilborne pathogens include both bacteria or fungi. *Bacillus*, *Pseudomonas*, and *Enterobacter* spp. represent some of the bacterial genera currently being investigated for their biocontrol properties. Axelrood et al. (1993) isolated several *Pseudomonas* spp. that significantly reduced *F. oxysporum* in growth room assays on Douglas-fir. Early results suggested that the bacteria may promote seedling survival and root growth. As a seed treatment, *Pseudomonas chlororaphis* reduced the bulking up of *Fusarium* during stratification of Douglas-fir seeds (Hoefnagels and Linderman 1999). Additional study found that the greatest reduction in post-stratification levels of *Fusarium* were achieved when seeds received a pre-stratification treatment of hydrogen peroxide followed by exposure to a solution of live *P. chloroaphis* cells.

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Of the fungi used for biocontrol of soilborne pathogens, various *Trichoderma* spp. have received much attention. *Trichoderma* spp. are commonly found associated with seeds but their role as possible seed coat antagonists is not well understood. *Trichoderma* spp. are saprophytic fungi that can utilize many different food sources including seeds. The effectiveness of *Trichoderma* lies in a combination of competition for nutrients, production of anti-fungal metabolites, and mycoparasitism (Quarles 1993). Non-pathogenic isolates of various *Fusarium* spp. (particularly *F. oxysporum*) may also be used as biocontrol agents. Studies have shown that these isolates can compete with pathogenic strains for nutrients

and infection sites while conferring some enhanced resistance in the host (Mandee 1996). Numerous non-pathogenic *Fusarium* isolates have been found on Douglas-fir seedlings (Axelrood et al. 1995; James et al. 1989b) suggesting the possibility that these isolates may confer some level of protection to conifer seeds and seedlings.

For biocontrol organisms to be successful commercial seed treatments, they must meet a variety of conditions: 1) they must be harmless to the seeds, seedling roots, and people; 2) they must demonstrate efficacy under different environmental conditions; 3) they should be active during germination and then be able to colonize developing plants roots; 4) appropriate fungal structures need to be available at high levels; and 5) these structures must withstand drying and storage (Jensen 1996; Taylor and Harman 1990). Improved delivery systems (e.g., pelleting, film coats) for these biocontrol agents are needed (Taylor et al. 1998).

## Seed Equipment Sanitation

### Background

The elimination or reduction of initial inoculum sources is one component of a strategy that can help to minimize subsequent losses due to seedborne pathogens (Berger 1977). Seed handling has been identified as one of the prime avenues of seed contamination (Littke 1996). Mittal and Wang (1993) found that the incidence of fungi on pine and spruce seeds was low at cone harvest, but increased during air drying of cones as well as the cone and seed processing operations. It is paramount that only clean equipment and containers be used to avoid possible cross contamination among seedlots (Thomsen and Schmidt 1999).

Cone and seed processing and sowing procedures offer many opportunities where fungi may infest seeds. Not only may seeds be brought into contact with contaminated equipment but also clean seeds may be in close physical contact with contaminated seeds.

### Techniques

In BC, all processing equipment and areas are cleaned between seedlot batches using vacuuming, sweeping, and air hosing methods. Liquid separation tanks are rinsed with water and wiped. A preliminary study reviewing each stage of the seed extraction process at the BC Ministry of Forests Tree Seed Centre found little or no *Fusarium* on the final seed product of a single seedlot (Neumann 1993). It was noted that the presence of *Fusarium* in other seedlots warranted a larger survey of the seed processing phase. In a follow-up study on seed preparation for stratification, Neumann (1996) found that pathogen inoculum from the ambient air and drying room screens were very low, but that the running water soak tanks and soaking screens used for imbibition had significant *Fusarium* inoculum. Based on subsequent trials, Neumann (1997) recommended that seed soaking tanks should be cleaned using an Ivory® dishwashing soap and hot water scrub treatment during the seed preparation season and that the tank bottoms in particular should be cleaned and rinsed thoroughly. In addition, welding points were shown to harbour high *Fusarium* levels. Limiting the size and number of welds will limit the crevices in which fungi can reside. Optimally, the cleaning protocol should be carried out weekly but bi-weekly cleanings are adequate to prevent the build-up

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of *Fusarium* inoculum. An alternative is to fill the tanks with a 3% hydrogen peroxide solution with sufficient volume to soak the bottom of the tanks. The seed soaking screens should be cleaned bi-weekly using a 3–4 hour soak in a 0.5% bleach and buffer solution followed by a 30 minute water rinse.

Overall, sanitation should be an integral part of every step in the seed handling process. All work surfaces should be cleaned and wiped with domestic cleaners or low concentration sterilants (e.g., 0.5% bleach solution). In addition, debris must be discarded from work areas and all tools and instruments that are used directly on seeds disinfected before and after use (Thomsen and Schmidt 1999).