Seed Fungi

Fungi have been associated with seed and have evolved mechanisms for seed-borne transmission of diseases for more than 130 million years (Buller 1950). This long association between fungi and seeds is important because it indicates that, for certain pathogens, there has been a long period of evolutionary development leading to sophisticated host-pathogen relationships. Understanding these complex relationships is essential, as it is often difficult to isolate different causes of disease when control measures are developed and implemented (Maude 1996). The introduction of "exotic" pathogens in the absence of co-evolutionary development between host and pathogen can lead to significant and unchallenged damage to the host. This is especially important in intensively managed agricultural and forestry operations where seeds encounter new fungi while being handled in unnatural environments where they have not had time to evolve any natural defense mechanisms.

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 we will adopt. 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 to a seed itself but infect seedlings in the nursery or field (Neergaard 1979). For the purposes of this guide, we are most interested in seed-borne pathogens and seed-borne diseases. 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. 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 seeds, susceptibility of the host plant, and suitable environmental conditions.

Seed-borne fungi are dispersed in association with some kind of dispersal units of the host (i.e., seeds)

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. 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 may be possible to **disinfest** seeds that are only superficially contaminated. Once seeds have become infected by a fungus, it cannot be **disinfected** in this manner and control becomes more difficult.

Contamination is used to denote the occurrence of a pathogen as either spores or mycelium on the surface of seeds

Seed Contamination/ Infection Routes

Seeds can become infected directly by a **systemic** invasion via mother plant tissues to the seed embryo (**Figure 18a**). Infection occurs this way through the **xylem** tissues of the mother plant to the embryo. This manner of seed infection is confined primarily to viruses and bacteria. However, some fungi infect seeds this way as well. A more common fungal infection sequence to the seed embryo is indirect, via the plant stigma or with conifers, as fungal hyphae grow from spores caught within receptive cones at or near the time of pollination, infecting the embryo (**Figure 18b**). Infection of seeds in this manner occurs only when the release and dispersal of fungal spores coincides with pollination. A species of *Botrytis* responsible for anther mould of red clover infects seeds in this manner (Silow 1934). In the interior of BC, cones of spruces can become infected in this manner by inland spruce cone rust (*Chrysomyxa pirolata* Wint.). Species of *Fusarium* may infect and become seed-borne in this way also.

Other common indirect infection routes in seeds are those of infection via flower or fruit parts to the ovary and ovule tissues or direct contact between seeds and

Infection refers to the penetration of seeds by an organism followed by the establishment of a relationship

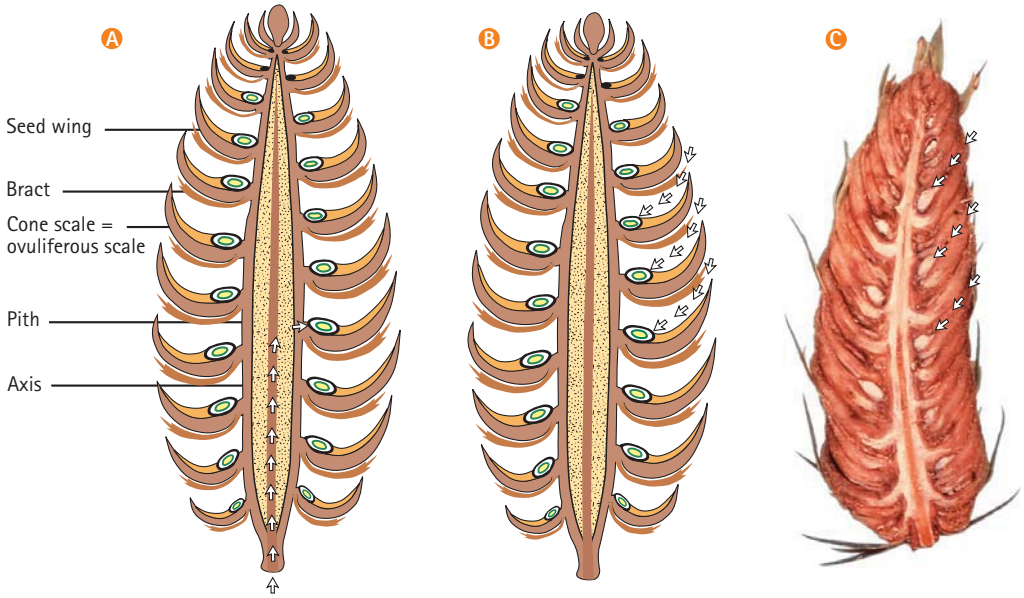


Figure 18 Direct and indirect infection routes (arrowed): a) direct invasion via the xylem of mother plant tissues; b) indirect invasion by spores at time of pollination; and c) indirect penetration of mature cone.

contaminated soil or water (Figure 18c). The so-called "seed" or "cold" fungus, *Caloscypha fulgens*, as well as *Sirococcus conigenus* become seed-borne in this manner. These latter two infection or contamination methods are the most common for conifer seeds. Seeds within cones can become contaminated when contacted by air-borne spores, air-borne water droplets containing spores, or by contacting contaminated soil directly. This can occur when cones are open and receptive during pollination or later when cones are mature and closed (Figure 18b, c). In each of these situations, seeds become contaminated as spores become lodged within small cracks and irregularities on their surface. Infection then follows as the spores germinate, penetrate, and invade the seeds. Seeds contacting contaminated soil may themselves become contaminated in a similar manner. Infection may also occur as spores or mycelium in the soil contact and penetrate the seed surface.

Pathogenic and Non-pathogenic Seed-borne Fungi

Many fungi are routinely found on conifer seeds, some of which are potentially pathogenic given the right environmental conditions, while others are relatively harmless. Also, some fungi may be pathogenic on seeds of low quality (i.e., damaged or immature), but cause no damage on healthy, vigorous seeds. Seed-borne organisms and for our purposes, fungi, are not all pathogenic nor do they pose a threat to seeds or subsequent seedling health. In fact, some fungi may be **symbionts** which are beneficial to the plant (Mallone and Muskett 1997). This is important because although the appearance of mould or fungal hyphae growing from seeds may indicate good conditions for fungal growth in general—a reflection of sub-optimal storage—it does not necessarily indicate the seeds to be inferior. Some saprophytic fungal species may produce toxic substances that control certain active pathogens (Mallone and Muskett 1997). A species of *Trichoderma*, known to produce antagonistic toxins, is used as a seed dressing to control damping-off due to seed- and soil-borne *Fusarium* species.

In BC, three fungal genera found on conifer seeds are of special importance to seed and seedling health. Species of *Fusarium* contaminate seeds and are responsible for damping-off of seedlings and potentially lead to *Fusarium* root rot and possibly *Fusarium* shoot blight. Both *Caloscypha* and *Sirococcus* infect seeds. *Caloscypha* is responsible for killing seeds while *Sirococcus* can kill the resulting germinants, and spread by spores to further infect and kill adjacent seedlings. These three important seed-borne fungi as well as other commonly occurring fungi found on BC conifer seeds are listed in Table 2 in order of decreasing seriousness to seed and seedling health.

Fusarium species

Species of fungi belonging to the genus *Fusarium* are responsible for both pre- and post-emergence **damping-off** and can be implicated with root rot and shoot blight of conifer seedlings (Bloomberg 1971; Bloomberg 1981; Nelson et al. 1981). *Fusarium* is primarily spread by spores borne by either air, water, soil, or seeds. Soil-borne *Fusarium*, which can overwinter as **chlamydospores** in soil or be introduced as spores by either air or water, is primarily a concern in bareroot nurseries. However, similar mechanisms to those encountered in natural soils occur in container settings where contaminated container growing media are encountered or when *Fusarium* spores are introduced by air or water (**Figure 19**). In these situations *Fusarium*-contaminated growing media can result in infected seedling roots leading in most cases to post-emergence damping-off, *Fusarium* root rot, or shoot blight, in this order of importance. Seed-borne *Fusarium* can lead to any of these results but is most often responsible for pre-emergence damping-off.

Life history

When and how conifer seeds become contaminated with *Fusarium* remains unclear. *Fusaria* are a ubiquitous group of fungi with spore **inoculum** present in the environment throughout the year. General disease cycles of pre- and post-emergence damping-off as well as *Fusarium* root rot and shoot blight illustrate possible times throughout the year when spores might be released and become available as contaminants (**Figure 19**). 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 can also contaminate seeds during interim storage, cone and seed processing, seed stratification, or at the nursery from contaminated sowing equipment, growing containers, or pallets. As *Fusarium* spores can be released throughout the year, at almost any time in the general life cycle of major BC commercial 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 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 released from soil or grasses within and around seed orchards may be spread by irrigation sprinklers. This problem could be compounded 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. 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 spread throughout a contaminated seedlot during the period of imbibition prior to seed stratification. A general understanding of *Fusarium* disease cycles and potential times of contamination and spread presents opportunities for intervention during the pre- and post-collection phases of the seed handling system.

Fusarium is primarily spread by spores borne by either air, water, soil, or seeds

Table 2 Commonly occurring fungi found on BC conifer seeds in order of *decreasing* significance to seeds and seedling health

Seed-borne fungi	Fungus source and effects on seeds or seedlings
Fusarium spp.[a]	soil-, air-, and water-borne damping-off fungus
Caloscypha fulgens[a]	soil-borne pathogen kills and mummifies seeds
Sirococcus conigenus[a]	water-splash, air-borne from seedlings of infected seeds
Cylindrocarpon destructans	soil-borne saprophyte and weak parasite
Alternaria spp.	air-borne saprophyte and weak parasite
Phoma glomerata	soil-borne blight fungus
Phomopsis spp.	water-borne blight fungus
Botrytis cinerea	air-borne saprophyte and weak parasite
Trichoderma viride	soil-borne fungal antagonist
Penicillin spp.	air-borne saprophyte
Mucor spp.	water-borne saprophyte

[a] Seed-borne fungi of greatest concern, whose presence is routinely tested for in BC.

A

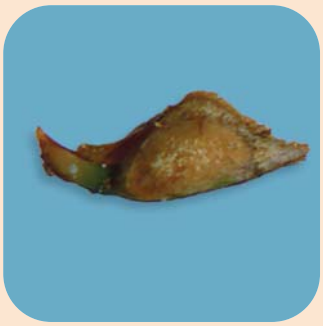
Pathogen overwinters or survives other adverse conditions as chlamydospores in small pieces of roots or organic matter in the soil.

B

In spring, spores contaminating seed or in proximity to germinants or young seedlings, germinate and infect host.

C

Post-emergence damping-off:
Stem rotting at the groundline and subsequent toppling of shoot; seedlings are susceptible up to 6 weeks after emergence; disease spreads by mycelium or spores.



D

Pre-emergence damping-off:
Rots seeds or germinants; Disease spreads by mycelium or spores.

Fusarium root rot:
In early summer chlamydospores germinate and infect seedling roots that grow nearby. Later in season when seedlings are larger, more crowded and stressed, fungus ramifies and destroys root system.

Fusarium top blight:
Needle chlorosis, browning and desiccation with hook-shaped leader tip. Fruiting structures on stem tissue can release spores to contaminate and infect other tissues.

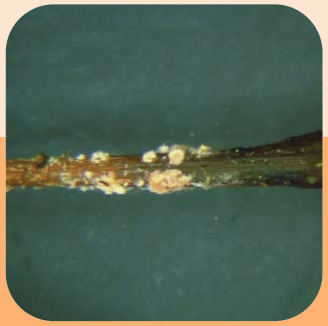


Figure 19
General life cycle for *Fusarium* spp. (airborne spores in water droplets released at points A, B, C, or D can contaminate seeds).

...the frequency of seed-borne Fusarium *is the same on seeds originating from seed orchards and those taken from natural stands*

Sources of contamination

Probable sources of seed contamination by species of *Fusarium* are indicated in **Figure 20**. Seeds may become initially contaminated with *Fusarium* both prior to entering the seed handling system as well as at several points within the system. Seed orchard seeds, as well as those originating from wild stand collections, are susceptible. Regardless of the initial source, the incidence of *Fusarium* can intensify within a contaminated seedlot depending on how the seeds are handled.

Caloscypha fulgens

The common names "seed" or "cold" fungus attached to this pathogen refer to its seed-borne nature as well as its ability to spread from diseased to healthy seeds during conditions of cold, such as stratification. This fungus was first reported in Ontario bareroot nurseries where it caused damping-off of fall-sown pine seeds (Epners 1964). It was next identified as a pathogen in Britain on Sitka spruce seeds that had been imported from North America (Salt 1971). Salt (1971) described and named the fungus *Geniculodendron pyriforme* Salt. This is the asexual form of the fungus and its sexual or "perfect" state was labeled *Caloscypha fulgens* in 1978 (Paden et al. 1978). The fungus was subsequently isolated from stored seeds in BC (Sutherland 1979), Oregon, and Washington (Harvey 1980). This pathogen becomes seed-borne when cones contact forest duff or litter where *C. fulgens* lives.

Life history

The seed fungus inhabits forest duff. It can infect cones through direct contact with forest soil during collection from wild stands (**Figure 21**). In spring, usually after snowmelt, *C. fulgens* growing in the forest duff produces cup-shaped, orange fruiting bodies called **ascocarps**. Ascocarps produce sexual spores called **ascospores** while non-sexual components of the fungus can lead to the formation of **conidiophores** that produce asexual spores called **conidia**. Neither sexual nor asexual spores appear to play any role in seed infection but rather are responsible for disseminating the fungus. Seeds in cones become infected with *Caloscypha* when they contact mycelium or hyphal threads of the fungus growing in the duff. Incidence of diseased seeds is dependent on several factors, the primary one being the length of time cones remain in contact with the ground during cool moist conditions. Sutherland et al. (1989) point out that the optimum temperature for growth of the seed fungus is 20°C. However, appreciable growth occurs even at 1 or 2°C. The pathogen can spread among contaminated seeds during stratification where conditions are commonly cool and moist. Because infection within a seedlot can intensify in this manner, detection of low levels of infection is important. Avoiding prolonged cool, moist conditions such as those encountered during stratification, is central to management of the disease. Most species require stratification for optimal germination and one must critically examine the trade-off between decreased disease incidence and decreased germination capacity before abandoning stratification. However, *C. fulgens* infects and kills seeds, with their contents becoming hard and mummified rather than rotten, as is the normal result of damping-off. *Caloscypha fulgens* does not infect emerging seedlings once the seeds have germinated.

Sources of contamination

Caloscypha infects seeds after cones contact the forest duff in areas where the fungus occurs. This largely limits the occurrence of seed-borne *Caloscypha* to seeds originating from wild stand collections. The initial source of *Caloscypha* is encountered both prior to and just after seeds enter the seed handling system; however, infection can spread to healthy seeds at several points within the system (**Figure 22**).

Sirococcus conigenus

Sirococcus conigenus causes a shoot blight of over 19 coniferous species in North America, Europe, and Asia (Hamelin 1986). The disease is particularly severe in BC forest nurseries where it mainly affects spruce, lodgepole pine (Illingworth 1973; Sutherland and Van Eerden 1980; Sutherland et al. 1981; Sutherland et al. 1982), and western hemlock (Funk 1972). Recent unpublished data indicates *S. conigenus* also has a large impact on ponderosa pine (*Pinus ponderosa* Dougl. Ex P.&C. Laws) (J. Dennis, pers. comm., May, 2000). In BC where the disease is known to be seed-borne on spruces, *S. conigenus* has recently been observed on western larch seeds (Peterson 1998, unpubl.[2]).

Life history

Sirococcus conigenus is confirmed to be seed-borne on spruce and can create disease centres which develop from infected germinants originating from infected seeds (**Figure 23**). Fully developed seeds in cones become infected. However, the mode of infection is unknown. The fungus produces spores in fruiting structures called **pycnidia**. Water plays a significant role in spore dissemination and it is likely rain-splashed spores that infect cones. Disease also results as air- and mist-borne

[2] M.J. Peterson, unpublished data, 1998, BC Min. For., Tree Seed Centre, Surrey, BC.

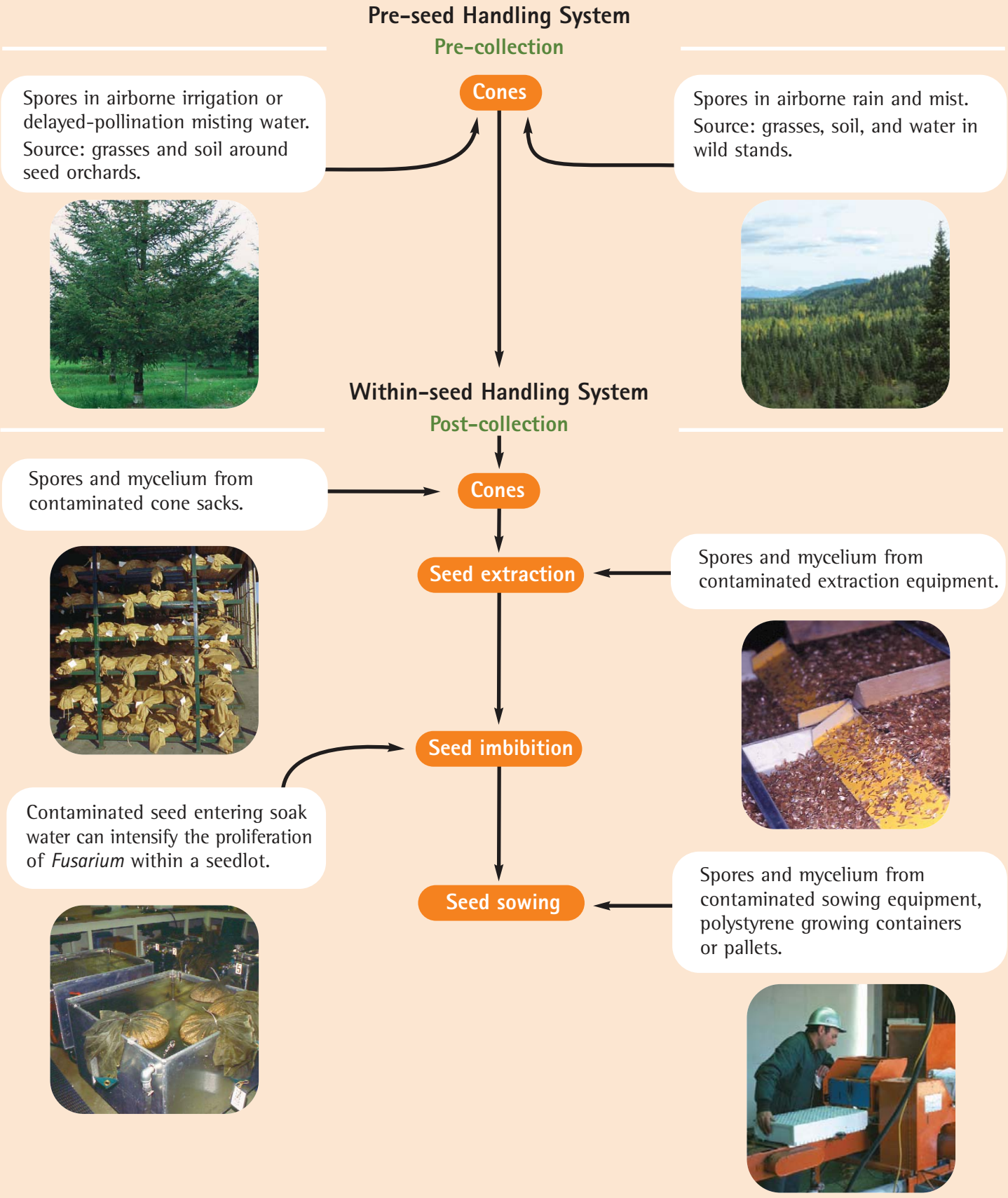


Figure 20
Potential sources of contamination by species of *Fusarium* prior to and within the seed handling system.

Conidiophores in forest duff produce **asexual** spores called conidia, disseminate fungus.

Mycelium

Fruiting bodies called ascocarps in forest duff produce **sexual** spores in the spring, disseminate fungus.

Mycelium in forest duff, infects seed in cones contacting forest floor.

Pathogen spreads among seed within a contaminated seedlot during and following stratification as well as in cool, moist container cavities.

Figure 21
Life cycle for the 'seed' or 'cold' fungus, *Caloscypha fulgens*.

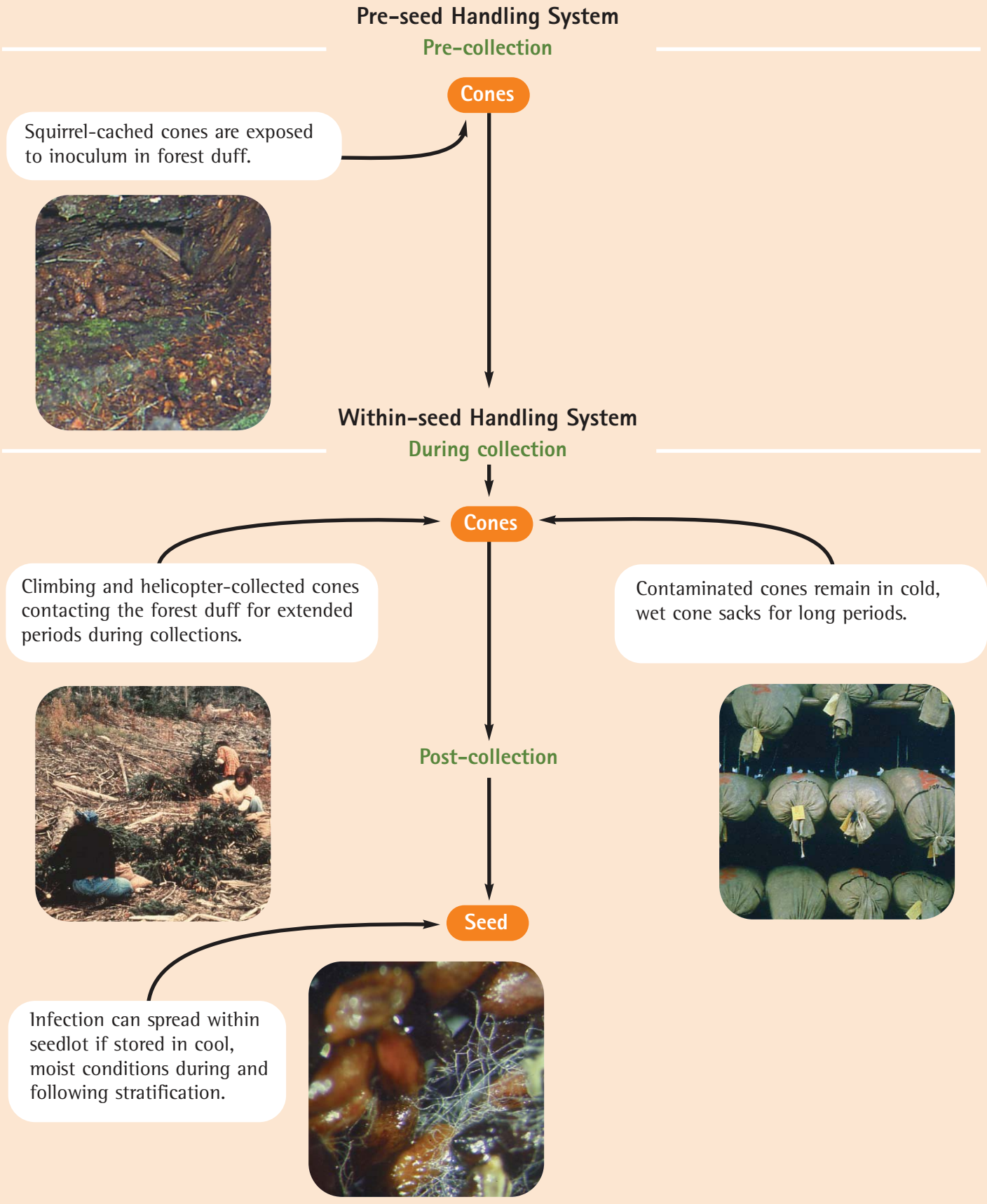


Figure 22
Potential sources of contamination by *Caloscypha fulgens* prior to and within the seed handling system.

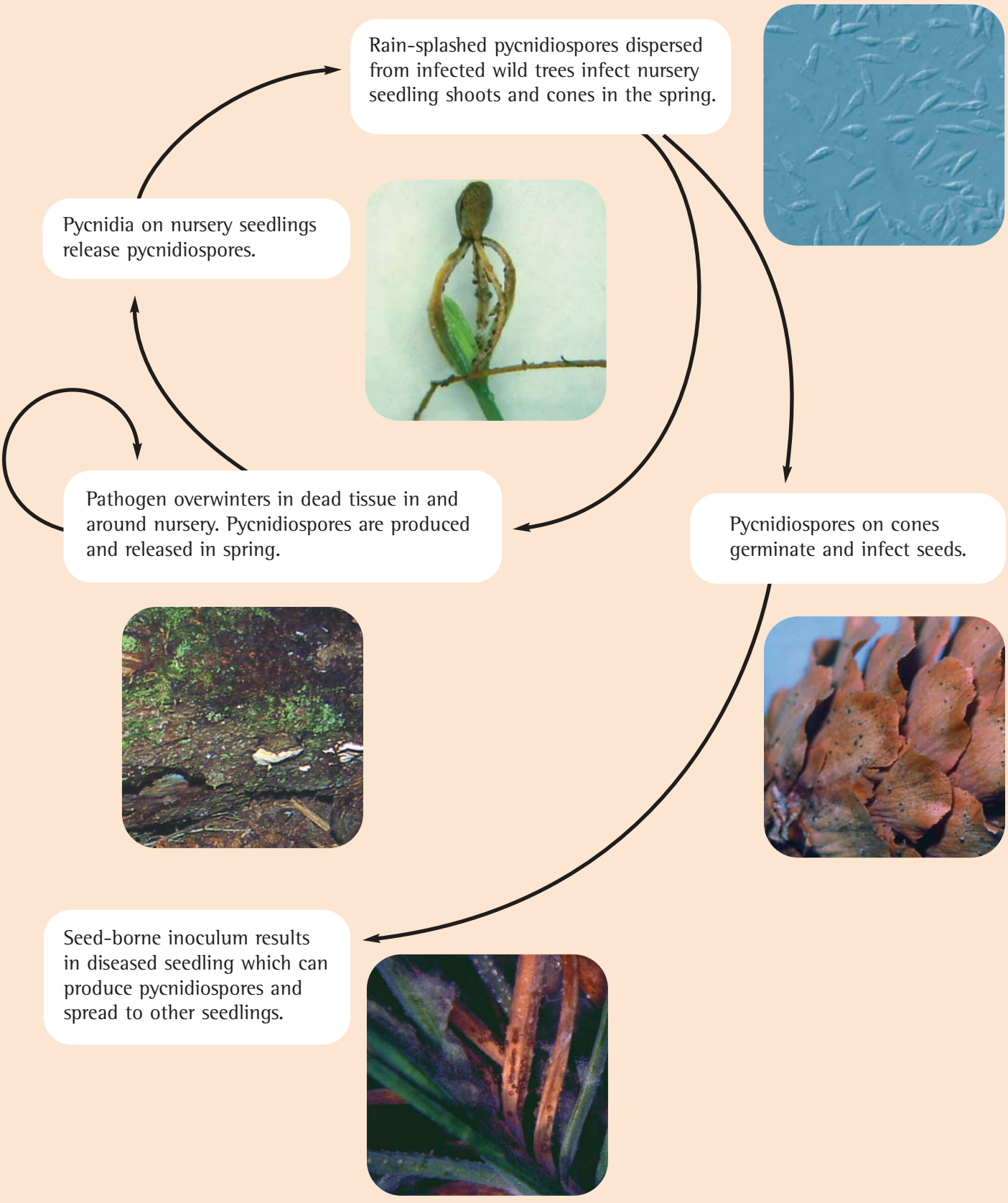


Figure 23
Life cycle for *Sirococcus* blight caused by the fungus *Sirococcus conigenus*.

spores enter from outside nurseries and although this is usually secondary to seed-borne sources, it is implicated with the appearance of *Sirococcus* blight on lodgepole pine seedlings (Peterson 1996). The disease is favoured by frequent or prolonged periods of below-average temperatures and wet, cloudy weather. Spruce seeds are the primary species assayed for this fungus both for its ability to kill spruce seedlings and also for the ability of infected spruce seeds to become a potential inoculum source for lodgepole pine seedlings.

Fully developed seeds in cones become infected with Sirococcus conigenus. *However, the mode of infection is unknown*

Infection on spruce seedlings occurs most readily between temperatures of 16 and 21°C, in the presence of moisture, and under low light conditions (Wall and Magasi 1976). Diseased seedlings exhibit a tip dieback as well as stem and branch cankers in the current years growth. Foliage distal to the infection becomes yellow and dies. Smaller seedlings are killed from multiple infections, while larger stock become forked and misshapen. Nursery losses are due to seedling death as well as from culling misshapen stock. In BC, the most common form of damage on container seedlings is the death of the primary needles from the base upwards. *Sirococcus conigenus* is seed-borne on spruce (Sutherland et al. 1981) and unlike most other seed-borne diseases, seedlings can germinate from these seeds. Pycnidia may then form on needles of the infected germinant (**Figure 23**). As spores are released and dispersal progresses, the disease can spread outward from a single seedling to infect others. This allows the disease to spread from container cavities sown with single or multiple seeds, to other seedlings in the nursery. It is this ability to spread to other seedlings from a single infected seed that makes early detection of the presence of seed-borne *Sirococcus* especially important.

Sources of contamination

Rain-splashed *Sirococcus* spores result in infection of cones. Seeds in contaminated cones become infected at this point, prior to entering the seed handling system (**Figure 24**). This usually occurs in wild stands, although seed orchard trees are not immune to infection. Exposure to additional *Sirococcus* inoculum occurs within and beyond the seed handling system with seeds as well as seedlings being susceptible.

It is this ability to spread to other seedlings from a single infected seed that makes early detection of the presence of seed-borne Sirococcus *especially important*

It is important to cull seedlings infected with *Sirococcus* and burn them to destroy any fruiting bodies on the dead foliage, which can otherwise continue to release spores. Dead seedlings should not be left to overwinter as pycnidia can form and release spores again in the spring. Finally, as spores can spread some distance via rain splash and mist, whenever possible, lodgepole pine seedlings should not be grown downwind of spruce crops known to have high *Sirococcus* infection levels.

Not all species of conifers are affected by each of the three major seed-borne fungi outlined above. Most seeds are susceptible to *Fusarium* contamination, fewer species become infected with the cold fungus and fewer still by *S. conigenus*. The conifer species on which each of the above seed-borne fungi has been found to occur are shown in **Table 3.**

Laboratory Testing for Seed-borne Pathogens

Establishing Testing Priorities

The potential for conifer seeds to become contaminated or infected with species of *Fusarium*, *Caloscypha fulgens*, or *Sirococcus conigenus* makes testing for their presence a viable first step for managing these seed-borne fungi. Different species are susceptible to contamination or infection from each pathogen in varying degrees. Facts such as the ability of any of the fungi to spread within a seedlot, ways in which the seeds are collected, and the fact that some tree seeds are not affected by any of these fungi, result in some species being more susceptible than others. Also, if attacked, seeds from some species represent a higher potential monetary loss. For these reasons, seeds are tested for fungal pathogens in order of priority based on each species' potential to become contaminated or infected by each pathogen. Past testing has indicated the frequency with which individual species have been contaminated or infected by each species of fungi (**Figures 25, 26, 27** [page 29]) and these data, combined with known information about each fungus' life history, the value of the seeds, and incidence of disease occurring on seedlings, are used in deciding priorities for testing. Seed-borne fungi can also influence seedling health with tree species most susceptible to seed-borne fungi also tending to be most affected by seedling disease (**Figures 28, 29** [page 29]). A synthesis of this conifer and fungal information is presented in a matrix of seed testing priorities (**Table 4**).

Seed Testing Methods

In BC, screening tests called fungal assays are routinely conducted for the presence of contaminated or infected seeds within

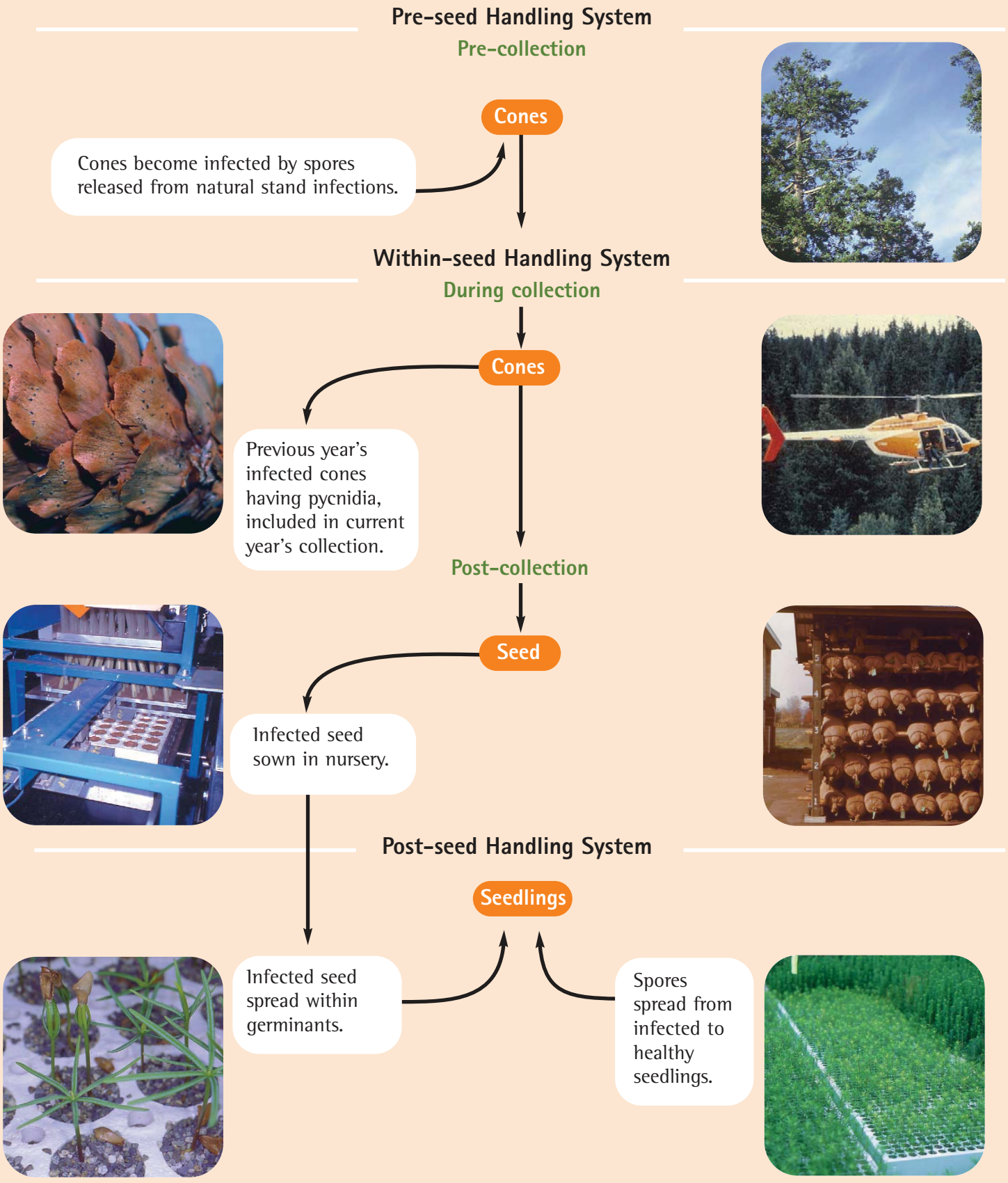


Figure 24
Potential sources of contamination by *Sirococcus conigenus* prior to, within, and after leaving the seed handling system.

Table 3 Tree species affected by seed-borne *Fusarium* spp., *Caloscypha fulgens*, and *Sirococcus conigenus* in decreasing order of frequency as indicated through fungal assays

	Fusarium spp.	*Caloscypha fulgens*	*Sirococcus conigenus*
Affected tree species	Interior Douglas-fir	Sitka x interior spruce hybrid	Sitka x interior spruce hybrid
	Western larch	Grand fir	Western larch
	Western white pine	Subalpine fir	Sitka spruce
	Western redcedar	Interior spruce	Interior spruce
	Ponderosa pine	Sitka spruce	Western hemlock
	Coastal Douglas-fir	Western white pine	
	Sitka x interior spruce hybrid	Noble fir	
	Grand fir	Amabilis fir	
	Western hemlock	Interior Douglas-fir	
	Subalpine fir	Western hemlock	
	Sitka spruce	Coastal Douglas-fir	
	Yellow-cedar		
	Noble fir		
	Amabilis fir		
	Interior spruce		
	Mountain hemlock		
	Interior lodgepole pine		

seedlots stored at the Tree Seed Centre. The assays are carried out following a strict set of protocols, providing confidence in the results from year to year (repeatability) as well as between testing agencies and laboratory personnel. Specific assay protocols are described and rationales are provided for each test.

Before the fungal assay protocols were established, studies looking at suspect seedlots were conducted on dry unstratified seeds. For this reason as well as the potential for *Fusarium* or *Caloscypha* to spread within seedlots during and following stratification, assays are conducted on dry unstratified seeds. This also simplifies seed handling and reduces variability in seed condition prior to testing. Seeds are withdrawn from the Tree Seed Centre and stored at 4°C after which testing is carried out as expeditiously as possible.

Past testing combined with known information about each fungus' life history, the value of the seeds, and incidence of disease occurring on seedlings, are used in deciding priorities for testing

Derivation of Seed Sample Size

Small samples of seed from specific seedlots can be assayed to determine the rate of contamination or infection for an entire seedlot. It is important that random, representative samples of appropriate size are chosen to allow these inferences to be made with what is considered an acceptable degree of certainty. Sample size is determined based on several factors including the level of contamination to be detected, the expected variation within the sample, and the amount of risk associated with our inferred estimate for an entire seedlot. Knowledge of each organism's life history and some historical information regarding its frequency of occurrence is also used to help establish the size of sample to be assayed. Sample sizes given are not adjusted for seedlot size, but sampling intensity is, according to ISTA (1999) standards.

Table 4 Seed testing priority[a] for fungal pathogens by conifer species

Tree species	Fungal species		
	Caloscypha fulgens	*Fusarium* spp.	*Sirococcus conigenus*
Ba	**H**	M	L
Bg	M	M	L
Bl	**H**	**H**	L
Cw	L	M	L
Fdc	L	**H**	L
Fdi	L	M	L
Hw	L	M	L
Lw	L	**H**	M
Plc	L	L	L
Pli	L	L	L
Pw	M	**H**	L
Py	L	**H**	L
Ss	**H**	M	**H**
Sx	M	M	**H**
Sxs	M	M	**H**
Yc	L	L	L

[a] L = low priority, M = medium priority, **H** = high priority.

Average levels of *Fusarium* are typically less than 2.5% (Figure 25). Past sampling also indicates a moderate degree of variation within seedlots. Occasionally seedlots having contamination levels higher than this are encountered, but these are rare. All species of *Fusarium* are not pathogenic and those that are, are often only weakly so. Therefore, it is currently desirable to detect *Fusarium* levels within any seedlot at a relatively conservative level of 5%. With this knowledge and the desire to detect levels of *Fusarium* with a 95% degree of confidence, it is necessary to sample 500 seeds per seedlot.

Assays for *Caloscypha* have shown average infection levels to be about 3% (Figure 26) but the amount of variation between species is higher than for *Fusarium*. With this in mind, detecting levels of *Caloscypha* contamination of 5% but with a greater allowance for variation around this estimate, has indicated a sample size of 250 seeds to be sufficient.

Seed-borne *Siroccocus* has the ability to spread systematically within an infected germinant and spread via spores to infect adjacent seedlings. *Sirococcus* is primarily seed-borne on spruce seeds but can spread to other seedlings. Assays for *Siroccocus* have shown average infection levels to be less than 1% (Figure 27). Therefore, it is desirable to detect low levels of infection within a seedlot. We want to detect *Sirococcus* at infection levels of 1% within a seedlot, which requires a sample size of 1500 seeds.

Laboratory Assay Methods

Fusarium species

Seeds become contaminated by species of *Fusarium*, through spores or mycelium trapped in irregularities on the seed surface. Therefore, seeds used in *Fusarium* assays should not be sterilized as these contaminants could potentially be eliminated. The assay is performed by plating 500 seeds (Figure 30) (20–25 seeds per petri dish) on Komada's selective medium (Komada 1975). This medium is selective for *Fusarium* spp. and is modified to have a pH 4.0 and no fungicide (benomyl) is added. The seeds are then incubated at 24/18°C with a 14/10 hour day/night photo period. Seeds are examined for *Fusarium* at 5 and 10 days after plating. Cultures are examined macroscopically for characteristic hyphae as well as pigmentation on the selective media. The presence of *Fusarium* is confirmed by microscopic examination (Figure 31). The sole appearance of **microconidia** is not diagnostic and demands further incubation of any cultures until banana- or canoe-shaped, **macroconidia** can be seen (Figure 32a). Identification of the fungus to the genus *Fusarium* is confirmed by macroconidia shape. Percent

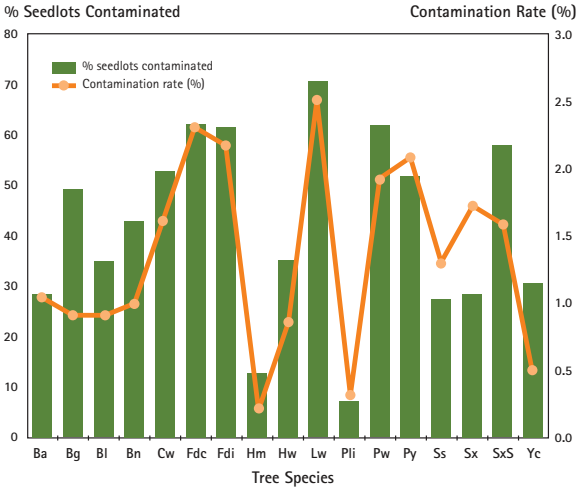


Figure 25 Proportion of tested seedlots showing evidence of being contaminated with *Fusarium* spp. and the mean contamination level (1992–2001).

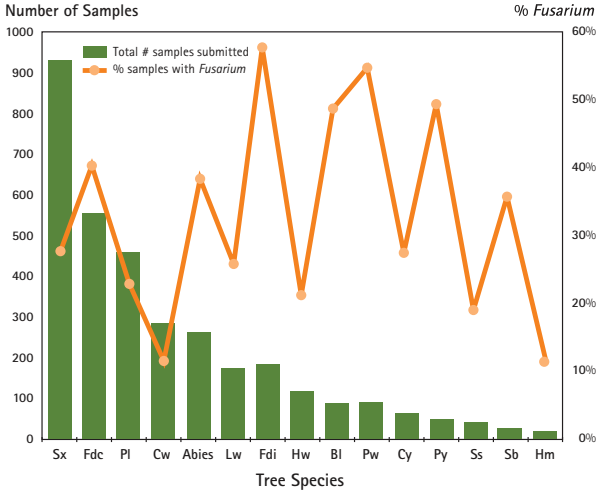


Figure 28 Total number of samples submitted and percentage of samples contaminated with *Fusarium* received at the Canadian Forest Service Health Clinic (1985–2001).

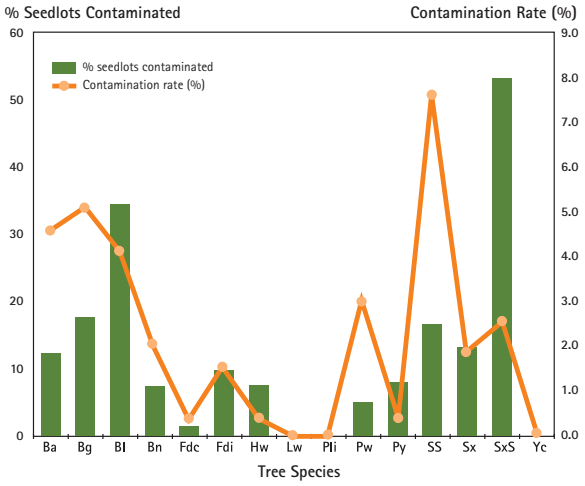


Figure 26 Proportion of tested seedlots showing evidence of being contaminated with *Caloscypha fulgens* and the mean contamination level (1992–2001).

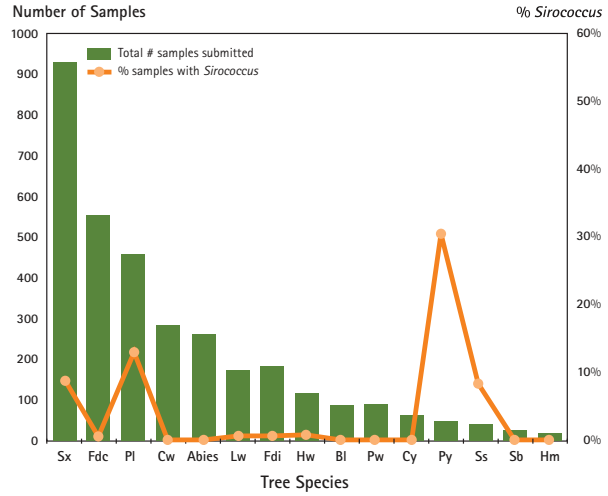


Figure 29 Total number of samples submitted and percentage of samples contaminated with *Sirococcus* received at the Canadian Forest Service Health Clinic (1985–2001).

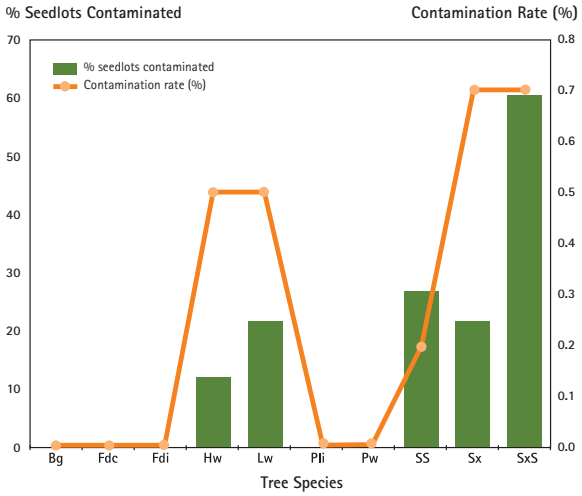


Figure 27 Proportion of tested seedlots showing evidence of being contaminated with *Sirococcus conigenus* and the mean contamination level (1992–2001).

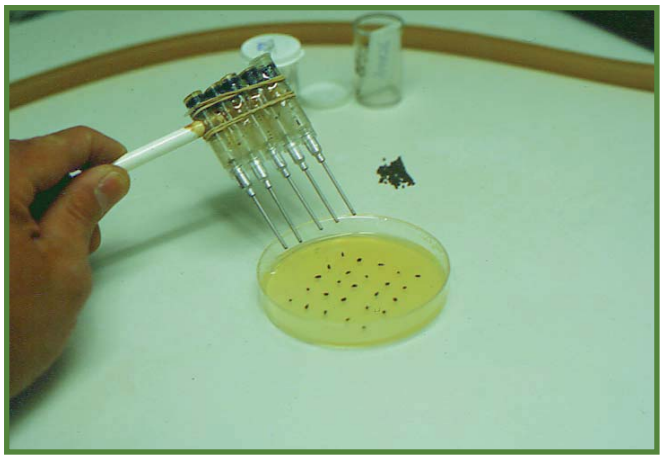


Figure 30 Seeds are placed on culture medium in Petri plates under sterile conditions to determine the presence of potential seed-borne fungi.

Figure 31 Positive identification of potential seed-borne fungi is made following microscopic examination.

contamination of seeds for the assayed seedlot is then calculated. 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). Species of *Fusarium* are highly variable and can undergo morphological changes in culture. For species identification, all cultures must be grown from a single spore (Nelson et al. 1983). Identification is based primarily on macroconidial and conidiophore morphology and presence of absence of other spore types. Such identification can be time consuming and extremely costly; therefore we identify seed-borne *Fusarium* to the genus level only.

Caloscypha fulgens

Detection of *Caloscypha fulgens* within seeds requires the elimination of all surface contaminants on the seeds. These assays can be conducted by first soaking 250 seeds for 30 minutes in a glass container in a 30% solution of hydrogen peroxide (H_2O_2) at three times the volume of the seeds. The seeds should next be rinsed three times with sterile distilled water and surface dried on a paper towel. The seeds should be plated on 2% water agar and incubated at 15°C (light unimportant). Once every three days thereafter, the plates should be examined for blue or indigo-coloured, verrucose (warty) hyphae that branch at right angles (**Figure 32b**), characteristic of *Caloscypha fulgens*.

Sirococcus conigenus

Seed-borne *Sirococcus conigenus* also results in an infection and as for the cold fungus assay, it is necessary to surface sterilize the seeds. Similarly, 1500 seeds should be soaked for 30 minutes in a 30% solution of hydrogen peroxide (H_2O_2) at three times the volume of the seeds. The seeds should next be rinsed three times with sterile distilled water and surface dried on a paper towel. The seeds should be plated on 2% water agar and incubated at 20°C with 8 h light (900 lux) per day. Following this, the seeds should be examined for *S. conigenus* three days after plating and twice weekly for up to three weeks. Cultures are then checked for fruiting bodies (pycnidia) and two-celled, spindle-shaped spores (**Figure 32c**). *Sirococcus conigenus* is slow to form fruiting bodies. Therefore, it is important to transfer fungi that have not produced pycnidia or been identified as *S. conigenus* to separate plates so they do not contaminate other seeds. These are encouraged to fruit so they can be either identified or discounted not to be *S. conigenus*.

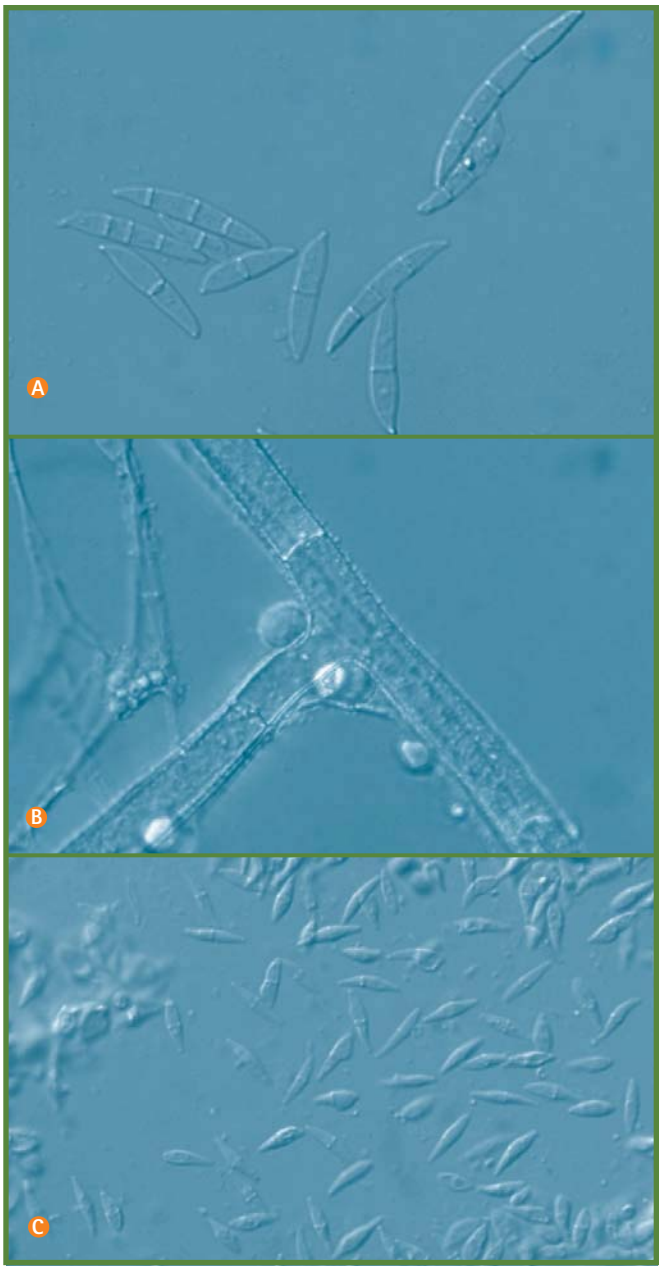


Figure 32 a) *Fusarium* macroconidia are characterized to be "canoe" or "banana-shaped." b) *Caloscypha* hyphae typically branch at right angles. c) *Sirococcus* spores are spindle shaped and two celled.

Interpretation of Assay Results

Testing for the presence of seed-borne pathogens provides useful information for nursery growers. As levels of contamination or infection within a seedlot rise, the potential to negatively affect seedling germination and growth becomes significant. Knowing the percentage of infected or contaminated seeds within any seedlot provides growers the option of taking steps to minimize their impact on seedling germination and growth. The level where the presence of seed-borne pathogens becomes significant is based on our understanding of each organism's life cycle as well as past records of its frequency of occurrence. Remember that thresholds for indicating significant levels of infected or contaminated seeds are guides only. Decisions regarding seed assay results must be tempered with other factors such as the economic value of specific seedlots and the unique environment (nursery) at which they are grown. While unlikely, very low levels of seed-borne contamination or infection do have a potential of spreading within a seedlot. However, potential risk to a seedlot increases as a threshold of infected or contaminated seeds is approached.

...thresholds for indicating significant levels of infected or contaminated seeds are guides only

Historical assay records indicate contamination or infection levels of 5% or greater within any seedlot to be significant for either *Fusarium* or *Caloscypha*. As seed-borne *S. conigenus* can become systemic in resulting germinants and spread via spores to infect adjacent seedlings, infection levels as low as 1% are significant.

When seed-borne *C. 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 (see "Seed Sanitation" chapter). The main strategies used for *Fusarium* and *Caloscypha* are aimed at minimizing the ability of each pathogen to spread within a seedlot. The main methods used to control the impact of *Sirococcus* infection in a seedlot are designed to eliminate the organism.

The results of fungal assays are available for each seedlot on the Seed Planning and Registry Information System (SPAR) under the seedlot test screen. If you are unfamiliar with SPAR and require access or training, contact the BC Ministry of Forests Tree Improvement Branch. For those familiar with SPAR, you can access seedlot tests by simply typing "v slt" on the command line and you will be prompted to enter a seedlot number. Otherwise, you can follow the menus from choice #1 = Seed/Cutting Lot Queries and then choice #2 = Seed Lot Query and, once a seedlot number is entered, press shift-F1 to get to seedlot tests.

The fungal assay results are also available in seedlot detail reports from SPAR. Fungal assay information is also forwarded on the sowing request label that is sent with each batch of seeds (**Figure 33**). This seedlot has been tested for *Caloscypha* (CAL) and *Fusarium* (FUS), but not for *Sirococcus*. This reflects the priorities indicated in **Table 4**. The level of *Caloscypha* is significant, but no *Fusarium* was found on the seeds from this seedlot.

Figure 33 A sowing request label illustrating presentation of fungal assay results.

...contamination levels of 5% or greater within any seedlot may be significant for either Fusarium *or* Caloscypha. S. conigenus *can infect adjacent seedlings and infection levels as low as 1% are significant*