PATHOLOGY OF CONIFER SEED AND SEEDLINGS ON NATURAL AND DISTURBED FOREST FLOOR SEEDBEDS IN THE ENGELMANN SPRUCE - SUBALPINE FIR ZONE OF THE INTERIOR OF BRITISH COLUMBIA

by

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Abstract

Natural regeneration in spruce-dominated stands is seldom satisfactory in British Columbia. The present study was undertaken to identify pathogenic fungi residing in seedbeds in the ESSF and able to infect seed and seedlings of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), and to estimate the frequency of infection of naturally shed seed and seedling by such fungi in various natural and disturbed seedbeds. This was determined by examining viability of conifer seed over-wintered on various natural and disturbed forest floor seedbeds in natural stands, isolating and determining any potential pathogenic fungi from ungerminated seed. A paralleled experiment was conducted in partially cut and clear-cut stands to evaluate the effect of silvicultural practices on the impact of such pathogenic fungi in the ESSF.

The study was conducted at the Sicamous Creek research forest in the ESSFwc2 subzone. Seedbed treatments in natural stands consisted of i) undisturbed moss type forest floors; ii) undisturbed litter type forest floor; iii) and iv) removal of litter layer from i) and ii); v) removal of the whole forest floor; vi) mixing mineral soil with surface and organic layers; and vii) moss covered rotten wood. In disturbed stands, only two seedbeds (i and v above) were employed. In addition, a study of conifer seedling emergence, and first-year survival and a survey of pathogenic soil-borne fungi were conducted on these seedbeds in natural and disturbed stands.

Engelmann spruce and subalpine fir seed over-wintered on natural forest floor seedbeds showed very poor seed survival. This was attributed to the presence of some pathogenic fungi in surface and organic layers which invaded and killed seed before snow melt. Average germination was 13% for Engelmann spruce and 12% for subalpine fir. Two major pathogenic fungi, an as yet unidentified black mold fungus and *Rhizoctonia* were responsible for the poor germination. Regression analysis showed that there was a significant linear relationship between frequency of the two pathogens and the viability of seed over-wintered on various seedbeds. Another pathogenic pathogen, *Caloscypha fulgens* (Persoon) Boudier was also present on the undisturbed forest floor seedbeds in the ESSF and caused severe loss of Engelmann spruce seed under laboratory conditions. These pathogenic fungi reside mainly in litter and organic layers (L+F+H) of the forest floor. Exposure of mineral soil by removing surface and organic layers or scalping forest floor seedbeds was the best way to reduce the population of these pathogens and improved both Engelmann spruce and subalpine fir seed germination. Average germination on MIN was 57.3% for Engelmann spruce and 41.8% for subalpine fir, respectively. Removing only the litter layer significantly improves Engelmann spruce but not subalpine fir seed germination. Low seed viability and high frequency of pathogenic fungi also occurred on untreated forest floor seedbed in partially cut and clear-cut stands. Seedbed preparation such as the scalping forest floor is needed to reduce frequency of pathogens and to improve seed germination.

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Chapter I. Introduction

Forest sites in the Engelmann spruce - subalpine fir (ESSF) zone in British Columbia have been harvested, planted and managed under an even-aged clearcutting silvicultural systems for the past twenty years. In the last few years, there has been an increasing interest in silvicultural systems other than clearcutting. The reasons for the new interest is that public pressure is mounting to promote alternatives to the efficient but ugly practice of clearcutting. Alternative logging practices in the future may well keep disturbance to a smaller scale than presently occurs with progressive or large patch clearcutting (Weetman and Vyse 1990), or may address both the biological and the integrated resource management issues associated with forest management to reduce increasing conflict of extensive clearcutting with forest values other than timber such as visual quality and wildlife habitat (Jull *et al.* 1996). Natural regeneration, while poorly understood, may provide a solution in high elevation conifer forests after harvesting (Butt and Vyse 1992). All those have led a need for better understanding of the process involved in natural regeneration.

1.1 Forest Regeneration in the Engelmann Spruce-Subalpine Fir Zone of British Columbia

The Engelmann spruce - subalpine fir zone is one of the most extensive forest zones in British Columbia, covering 13.3 million ha or 14% of the province's land area. It occurs at elevations of 1200 to 2100 m in the southwest, from 1500 to 2300 m in the southeast, and from 900 to 1700 m in the northern part of the zone, below alpine tundra and above the Interior Cedar-Hemlock, Montane Spruce, or Sub-Boreal spruce zones. The ESSF zone is dominated by stands of Engelmann spruce (*Picea engelmannii* Parry), and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). The third major commercial tree species in the zone is lodgepole pine (*Pinus contorta* Dougl.). The ESSF zone, which also has one of the province's most severe climates for forest growth, is dominated by a cold, moist and snowy continental climate with long, cold and snowy winters, and short, cool summers (Coupe *et al.*. 1991). The snow free period, particularly at higher elevations within the zone and on north aspects, can be as little as 110 days. Frost free periods are very short, with frosts possible at almost any time during the growing season. Reforestation is often slow and can be difficult resulting from the short, growing seasons and harsh climate associated with this subalpine zone.

1.1.1 History of regeneration in the ESSF, British Columbia

Until the early 1970's, when the interior spruce planting program expanded dramatically, there was almost total reliance on natural regeneration to regenerate interior spruce in British Columbia (Weetman and Vyse 1990). In the 1940's, large scale logging expanded from low elevation to the ESSF zone around Prince George. Early logging in spruce-fir stands involved selective removal of the best spruce trees to a diameter limit of 25 or 30 cm. However, very few spruce seedlings were establishing in the understory. Instead, canopy openings filled in mainly with subalpine fir (Coate *et al.* 1994).

Throughout the 1950's and 1960's, single tree selection and alternate strip cutting were introduced as alternative approaches to selective removal. Single tree selection did not successfully induce spruce regeneration. Average stocking of spruce on plots established by Glew (1963) was 2.7 to 6.3%, with the vast majority of spruce seedlings occurring on skid roads. In alternate strip cutting (50 to 100 m wide), a blade scarification method was introduced in cut strips to provide a mineral soil seedbed for natural regeneration. It was generally regarded as successful in achieving adequate spruce regeneration (Glew 1963; Gilmour and Konishi 1965), although some problems were encountered in wet and brushy areas (Arlidge 1967). However, only a small fraction of the strip-logged areas was scarified, and the second pass to remove "the leave strips" was rarely carried out. Consequently, large areas were left in poor condition with low spruce stocking and numerous clumps of slow-growing residual subalpine fir interspersed with deciduous trees and shrubs. Strip cutting, like the earlier forms of partial cutting, also created optimum conditions for the build-up of spruce beetle populations in shaded slash and windthrow (Dyer and Taylor 1971).

By the early 1970's, with the newly-introduced technology in pulp mills, diameter-limit logging and strip cutting had been replaced by large-scale clearcutting, the system that prevails today. There was an almost complete shift to artificial regeneration because of very poor natural regeneration on the large clear-cut openings. It proved difficult to produce high-quality stock that was well-adapted to out planting conditions and did not suffer an extended period of planting check. The result was many large areas of clear-cut openings remained not satisfactorily restocked (NSR) (British Columbia Ministry of Forests 1990). Artificial regeneration is increasingly successful in prepared forest sites, but problems related to frost and snow damage, over-winter mortality, and vegetation competition have led to renewed attempts at natural regeneration, hoping that natural regeneration may solve these problems.

Coate *et al.* (1994) listed the advantages and disadvantages of natural regeneration as: i) avoids regeneration cost and achieves management priorities, if successful; ii) reduces risk and damage from unusual weather, pest outbreaks or other catastrophic events; iii) maintains genetic diversity in regeneration forest and assures that regeneration is locally adapted; and iv) may produce better stem form and wood quality. However, lack of control over ecological factors governing germination and seedling establishment, slower-growing, uneven stocking, and potentially increasing cost for site preparation to assure adequate germination are considerations in choosing natural regeneration.

Current estimates are that only 20% of spruce-dominated stands will become satisfactorily stocked through natural regeneration following clearcutting (Kuhnke 1989), much of the regeneration on these areas is made up of species other than spruce. A series of problem analyses carried out to determine the causes of regeneration failure in the biogeoclimatic zone and subzones of interior British Columbia confirms the low rate of regeneration success in cutovers left for natural regeneration (Scagel 1987; Butt 1988; Beaudry and McCulloch 1989). Biological factors affecting successful natural regeneration of Engelmann spruce are discussed in next section.

1.1.2 Biological factors

Major problems of spruce natural regeneration are: i) low seed germination; and ii) severe loss of first-year seedling in natural forest sites (Roe and Schmidt 1964; Ronco 1970; Alexander 1984). The germination rate of natural shed Engelmann spruce seed in natural forest sites is 0 to 11%. The highest rate, occurring on sites which have been scarified with exposure mineral soil after partial cutting or clearcutting and are shaded in summer is less than 28% (Boyd and Deitschman 1969; Ronco 1970; Alexander 1984). It is estimated that an average of 665 (range 60 to 2,066) sound spruce seeds is required to produce one first-year seedling. Most spruce seedling mortality (usually about 50% or more of total mortality) occurs during the first growing season (Alexander 1984), even though the loss can be substantial during each of the first 5 years following germination (Roe and Schmidt 1964; Alexander 1984). An average of 6,800 spruce seeds (range 926 to 20,809) is required to produce one established seedling (a seedling more than 5 years old is considered established and will likely live) (Alexander 1987).

The biological requirements for successful natural regeneration of Engelmann spruce have been intensively studied in North America and include: i) an adequate supply of viable seed; ii) a favorable seedbed for germination; and iii) a suitable microenvironment for subsequent survival and growth of the seedling (Rowe 1955; Roe *et al.*. 1970; Lees 1972; Alexander 1987). Factors affecting seed supply and microenvironment for seedling survival and growth have been fairly well studied.

Seed supply. There are three main factors affecting seed supply: i) seed crop production; ii) seed dispersal; and iii) predispersal and post-dispersal seed loss. Seed production is one of the most important factors limiting the success of natural regeneration. Large variability in the amount of seed produced from year to year means that natural regeneration cannot be expected every year (Alexander 1987). Good spruce seed crops in British Columbia occur on average only once every 4 to 12 years (Konishi 1985). Where environmental conditions for seed dispersal and seedling success are exceptionally favorable, a poor or moderate crop may be all that is required to achieve restocking (Noble and Ronco 1978). Most spruce seed is dispersed from early September through the end of October, but some seed may fall throughout the winter. The distance that viable seeds are dispersed is an important factor limiting successful natural regeneration of Engelmann spruce in clear-cut openings. Fifty to seventy percent of seeds fall within 30 m of the windward stand edge, 95% within 100 to

120 m, and few or none beyond approximately 200 m (Noble and Ronco 1978; McCaughey and Schmidt 1987). Predispersal and post-dispersal seed loss is a very important factor affecting adequate seed supply. Major predispersal seed loss in Colorado resulted from insects and small mammals (Alexander 1987). Total insect-caused loss in Engelmann spruce averaged 28% of the total seed produced during a four-year period. Pine squirrels are major consumers of spruce and fir cones and seeds, as evidenced by the large caches common to spruce-fir forests. Once seeds are on the ground, rodents are the most important source of seed losses (Smith 1955; Dobbs 1972; Alexander 1974; Johnson and Fryer 1996). Mice and voles are important seed predators and they are the major reason why Engelmann spruce does not have a persistent seed bank (Johnson and Fryer 1996).

Factors affecting initial survival and establishment. Aspect, shade, disease and animal damage are considered the major factors affecting initial survival and establishment of Engelmann spruce. Aspect greatly affects initial seedling survival and establishment through its influence on temperature and moisture (Alexander 1987). Survival of spruce seedlings on the Fraser Experimental Forest was much higher on north than south aspects, and shade is essential on south slopes to protect spruce germinants from heat injury and desiccation, even when sites are scarified (Noble and Ronco 1978; Alexander 1984). Disease damage of Engelmann spruce seedling is caused by damping-off fungi such as *Fusarium* spp. and snow mold fungus, e.g. *Herpotrichia nigra* Hartig. Newly-germinated seedlings killed by damping-off are a common cause of post-emergence mortality on moist seedbeds (Ronco 1967; Noble and Alexander 1977; Alexander 1984). Damping-off was responsible for 17% of the first-year seedling mortality in central Colorado on both mulched and unmulched mineral soil seedbeds in a year when the growing season was particularly wet (Ronco 1967). The snowmold fungus (*Herpotrichia nigra* Harig) occasionally damages or kills both natural and planted seedlings (Ronco 1967, 1970; Noble and Alexander 1977; Alexander 1984). Several animals kill or damage spruce seedlings, e.g. 15 to 20% of the total mortality on a long-term study on the Fraser Experimental Forest resulted from the clipping of cotyledons on newly-germinated seedlings by gray-headed juncos (*Junco caniceps*) (Noble and Shepherd 1973). Established seedlings can be debarked and killed by montane voles (Ronco 1967).

Seedbed conditions. Seedbed conditions are considered as the most important factor affecting seed germination, seedling survival and in turn the regeneration of Engelmann spruce. Most research concludes that Engelmann spruce seed germination has seedbed preference (Griffith 1931; Place 1955; Smith 1955; Rowe 1955; Day 1964; Knapp and Smith 1982; Geier-Hayes 1987; and Harvey *et al.* 1987). Decayed wood is a suitable seedbed for Engelmann spruce germination in undisturbed stands. Numerous authors have observed that Engelmann spruce seedlings occur on decayed wood in a larger proportion than would be expected from the area occupied by the seedbed (Rowe 1955; Lees 1972; Knapp and Smith 1982). Spruce seed germinates very poorly in the litter and organic layers (Griffith 1931; Smith 1955; Alexander 1984; Cheng and Igarashi 1990). A long exposure of Engelmann spruce seed to a duff seedbed under a blanket of snow is lethal to the seed (Daniel and Glatzel 1966). Removal of the top layer of soil or scarified soil surface during logging could stimulate seed germination and the first-year seedling survival (Alexander 1984; Cheng and Igarashi 1990; Johnson and Fryer 1996). Burned seedbed is also good for Engelmann spruce germination (Smith 1955; Woodward 1987; Johnson and Fryer 1996). These studies indicate that there are some unknown factors in the litter and the organic soil layer which inhibit seed germination on undisturbed Engelmann spruce and subalpine fir forest floors. Many researchers explain that limited spruce seed germination and germinant survival in litter, moss and humus result from drought in these seedbeds (Boyd and Deitschman 1969; Novel and Alexander 1977; Noble and Ronco 1978; Alexander 1984; Baseman 1989; Butt 1990; Butt and Vyse 1992). However, the poor regeneration on moist sites in the ESSF can not be fully interpreted as the result of low moisture in the forest soils. Johnson and Fryer (1996) noticed a dramatic loss in seed viability under litter from 92% in October to 8% by the next August. Moreover, Daniel and Schmidt (1972) reported that Engelmann spruce and subalpine fir germination increased to 77.3% and 20.8% respectively, on treated Engelmann spruce 0-horizon with powdered 75% captan, compared to only 3.5% and 1.3% germination respectively for Engelmann spruce and subalpine fir on untreated 0-horizon. Results from the studies of Johnson and Fryer (1996), and Daniel and Schmidt (1972) indicate that some biotic agents, perhaps pathogenic fungi living in the forest floor litter and the organic layers of soil are responsible for the low seed germination on natural forest stands in the ESSF.

1.2 Pathogenic Fungi Involved in The Failure of Natural Regeneration in The ESSF, Interior of British Columbia

Pathogenic fungi can affect natural regeneration of conifers by severely damaging or killing cones and seeds before dispersal (cone pathogens), seeds and new germinants on site before or after emergence (soil-borne pathogens), young seedlings and young stands after establishment (root, stem and needle pathogens).

1.2.1 Fungal pathogens affecting spruce cones in the ESSF, B.C.

Cone fungi affect spruce natural regeneration by destroying or reducing seed supply.

Inland spruce cone rust, *Chrysomyxa pirolata* Wint. and coastal spruce cone rust, *C. monesis* Wint. are the only pathogens that consistently cause serious losses of spruce cones in British Columbia (Sutherland *et al.* 1987). Although it occurs throughout the province, *Chrysomyxa* sp. only cause major damage in the interior where Engelmann spruce (*Picea engelmannii*) and white spruce (*Picea glauca*) are found (Sutherland and Hunt 1991). Diseased cones weigh significantly less. Usually no seed form in diseased cones, but even then it is produced, cone malformation and resinous hinder seed dispersal (Sutherland *et al.* 1987). Severe damage can cause total loss of seeds.

1.2.2 Soil-borne fungi killing seed

Some soil-borne pathogenic fungi can affect seeds and seedlings on the forest floors in the ESSF. *Caloscypha fulgens* (Pers.) Boud. (imperfect stage: *Geniculodendron pyriforme* Salt) is known as the seed or cold fungus infects seeds when coniferous seeds and cones fall on the forest floor. It also kills seeds during seed stratification and in cool, moist seedbeds or container growing media (Epners 1964; Sutherland and Wood 1978; Sutherland 1979; Harvey 1980; Wicklow-Howard 1980; Diamadis *et al.* 1983; Egger and Paden 1986). In Japan *Racodium therryanum* can cause similar seed mortality both in the forest floor and bareroot nursery soils (Sato and Ota 1960; Hayashi and Endo 1975; Igarashi and Cheng 1988; and Cheng and Igarashi 1990). These pathogens may some times be the major factor causing natural regeneration failure in the ESSF.

Caloscypha fulgens is a common inhabitant in coniferous litter in forest floors in North America and Europe (Epners 1964; Salt 1974; Sutherland and Woods 1978; Sutherland 1979; Harvey 1980; Wicklow-Howard 1980; Diamadis *et al.* 1983; Egger and

Paden 1986). As a pathogenic fungus infecting embryo and endosperm, *C. fulgens* causes up to 50% loss or mortality of planted Engelmann spruce seed in forest floors in western United States (Wicklow-Howard 1980). The fungus lives in coniferous litter as hyphae (Diamadis and Minter 1983; Egger and Paden 1986; Sutherland *et al.* 1987). Infection occurs mainly when cones come in contact with litter. Mycelium of the fungus forms cushions on the surface of the seed, then penetrates through holes made beneath the cushions (Woods *et al.* 1982). It can stay in dead seeds for 3 years and still retain pathogenic ability (Sutherland *et al.* 1987). It can even remain viable on the forest floor after fire (Egger and Paden 1986). Cones in contact with the forest floor under cool, moist conditions have a great probability of infection (Sutherland 1981). Small mammals, such as squirrels can disseminate the fungus and high disease incidence occurs in seedlots cached by squirrels (Sullivan *et al.*. 1984). *Caloscypha fulgens* is only isolated from seeds collected from the forest floor (Sutherland and Woods 1978) and it can spread from infested seed to healthy seed during seed stratification. This fungus is often found on stored spruce seed in British Columbia (Sutherland 1979; Dennis *et al.* 1993). The highest percentage of infection in stored seed recorded was on white spruce with 22.4% (Sutherland 1979). It severely damages stored and sown seeds in bareroot nurseries in Ontario (Epners 1964), Oregon and Washington (Harvey 1980), and British Columbia (Sutherland 1979).

The dark, snow-blight fungus, *Racodium therryanum* is the most important factor inhibiting natural germination of Yezo spruce (*Picea jezoensis* Carr.), Todo-fir (*Abies sachalinensis* Mast.) and Japanese larch (*Larix kaempferi* Carr.) seeds in natural forest sites in Hokkaido, Japan (Sato and Ota 1960; Hayashi and Endo 1975; Igarashi and Cheng 1988; Cheng and Igarashi 1990). This fungus was first reported causing pre-emergence damping-off of coniferous seedlings in forest nurseries in Northern Japan (Sato *et al.* 1960; Sato 1964), and it was later shown to cause heavy loss of conifer seed both in nurseries and on regeneration sites. The fungus lives in the litter and the organic soil layer of coniferous forest floors and attacks conifer seeds under the snow at 0 to 5^0C and 92 to 100% relative humidity, causing severe seed rot (Hayashi and Endo 1975; Cheng 1989; Cheng and Igarashi 1990). Yezo spruce seed germination rate decreased by 66 to 100% (Cheng and Igarashi 1990). Japanese larch seed germination was only 7 to 13% on an undisturbed organic layer compared to 60 to 63% on soil from which the organic top had been removed (Igaryashi and Cheng 1988).

Racodium therryanum or a related pathogen may be present in snow-covered forest floors of the ESSF zone in British Columbia because there are some similarities between Hokkaido, Japan and the interior of British Columbia. For example, the long and heavy snow in winter in the ESSF zone of British Columbia is similar to that in Hokkaido, and there is the same natural regeneration problem of seed germination failure on the undisturbed forest floor. Perhaps the same or similar pathogenic fungi are present in the litter and organic layers of forest sites in the ESSF of British Columbia.

1.2.3 Pathogens of germinating seed and young seedlings

Germinants before emerging from soil and young seedlings within a few month after emerging out of soil on site might be killed by damping-off fungi, such as species of *Fusarium*, *Rhizoctonia* or *Cylindrocarpon*. These fungi were found in the forest floor and mineral soil of spruce stands (Mittal and Wang 1987; Widden and Parkinson 1973). When the condition is suitable for the fungus infection, larger seedlings may also be damaged by pathogenic fungi such as *Herpotrichia juniperi*.

Mittal and Wang (1987) isolated 13 species of fungi from white spruce cones and seeds which had been left on the forest floor for 15 days in Algonoquin Park, Ontario. Among them, *Alternaria alternata* (Fr.) Keissl., *Cladosporium cladosporoides* (Fres.) de Vries, *Fusarium sporotrichioides* Sherb, *Rhizopus nigricans* Ehrenb., *Trichothecium roseum* Link, and *T. vivide* Pers. caused damping-off of white spruce seedlings (Mittal and Wang 1993). Although most of these pathogens were found on cones and seeds collected directly from branches, the frequency was very much higher in cones collected from the ground, suggesting that cones and seed became contaminated from the forest floor (Mittal and Wang 1987).

Widen and Parkinson (1973) isolated 38 fungi from many layers of forest soil in a lodgepole pine-white spruce stand in Kananaskis valley, in the Rocky Mountains of southern Alberta. Some of them, *Fusarium* spp., *Cladosporium herbarum* (Pers.) Link, *Cylindrocarpon destructans* (Zins.) Scholten, *Sclerotium* sp. , *Alternaria* spp, and *Botrytis cinerea* Pers. are considered to be weakly pathogenic to spruce seeds (Urosevic 1961; Mittal and Wang 1993). *Fusarium* spp., *Cylindrocarpon destructans* and *Sclerotium* spp. were only isolated from mineral soils and could be potential inoculum of pre- or post-damping-off disease in mineral soil seedbeds.

Damping-off (*Fusarium* spp.) caused 17% of the first-year Engelmann spruce seedling mortality on both mulched and unmulched mineral soil seedbeds in the ESSF of central Colorado (Ronco 1967; Nobel and Alexander 1977). *Cylindrocarpon destructans* is a common inhabitant of coniferous forest floors (Kowalski 1980; Schonhar 1987; Phillips and

Burdekin 1992). It infects germinants or weakened young seedlings on site in Europe (Kowalski 1980; Schonhar 1987). In British Columbia, this fungus is found on stored coniferous seed (Dennis *et al.* 1993), and is a pathogen in both bareroot and container nurseries causing root rot of Douglas-fir, Engelmann spruce and white spruce seedlings (Sutherland *et al.* 1989). Whether or not these weakly-pathogenic fungi damage seeds and seedlings of Engelmann spruce in natural and disturbed seedbeds in the ESSF is unknown.

Brown felt blight fungus, *Herpotrichia juniperi* (Duby) Petr. (= *Herpotrichia nigra* R. Hartig.), has been reported on subalpine and amabilis fir, Engelmann, Sitka and white spruce, and other conifer trees (Allen *et al.* 1996). It has been reported to damage or kill both natural and planted seedlings in regeneration sites in Alberta, the USA, and Europe (Schonhar 1987; Hiratsuka 1987; Alexander 1984; Larios *et al.* 1988; Phillips and Burdekin 1992). Losses caused by this pathogen are most severe when seedlings remain under the snow too long, as in years of heavy snowfall or when weather retards snow melt in the spring, or in depressions where snow normally accumulated and melts slowly (Philips and Burdekin 1992). Brown felt blight was responsible for 5% of the mortality on north aspects on the Fraser Experimental Forest, Colorado, USA (Alexander 1987).

1.3 Research Status of Seed and Seedling Diseases in Natural Regenerated Sites in the ESSF, Interior of British Columbia

Although much research work has been done elsewhere, we know little about the role of pathogenic fungi in natural regeneration of Engelmann spruce in British Columbia. Almost all of the studies on conifer seed and seedling pathology have been conducted in the context of bareroot and container nurseries. Seed and seedling diseases in natural and disturbed forest floor have received little attention in British Columbia. The reason for the lack of attention to these diseases is their ephemeral nature. Disease losses in forest nurseries are easy to recognize in seedbeds or in containers by the absence of seedlings and residues of diseased seedlings. In natural forest sites, seed that falls on the forest floor and dies because of invasion by pathogens is never noticed and diseased seedlings may disappear within weeks of germination. Thus, very serious losses of seedlings to pathogens may escape attention unless a special effort is made to detect such losses.

In summary, studies conducted in the ESSF and the SBS (The Sub-Boreal Spruce Biogeoclimatic zone) in British Columbia and similar habitats elsewhere suggest that fungi pathogenic to seed or young seedlings may reside in the forest floor of high elevation, spruce dominated forests. These pathogens may play a significant role in the failure of natural regeneration following disturbance. This study was designed to: i) identify the pathogenic fungi residing in seed beds in the ESSF which are able to infect seed and seedlings of Engelmann spruce and subalpine fir; ii) estimate the frequency of infection of naturally shed seed and of seedling by such fungi in various natural and disturbed seed beds; and iii) evaluate the impact of such pathogenic fungi on silvicultural practices in the ESSF.

Chapter II. Regeneration of Engelmann Spruce and Subalpine Fir in Natural and Disturbed Forest Floor Seedbeds in Sicamous Creek Research Forests

2.1 Introduction to The Sicamous Creek Silvicultural Systems Project

The goal of the Sicamous Creek Silvicultural Systems Project (SCSSP) is "to provide the forestry community with information on the ecology of high-elevation forests in the southern Interior. This project will study the responses of the most common ecosystems to a wide range of disturbance levels created by tree and stand removal and subsequent site preparation for regeneration. With this information, operational foresters should be able to regenerate the common forest types. If the project is successful, a much wider range of silvicultural systems will be in use in the high-elevation forests of the southern Interior by the year 2000. Alternatives to clear-cutting will be routinely prescribed with the confidence that wood supply targets can be met with minimal damage to forested ecosystems" (Vyse 1997). The Sicamous Creek forest is located in the Salmon Arm Forest District, south of the north fork of Sicamous Creek and north of Mount Mara, near the town of Sicamous (Vyse 1997). The research site is located in the Engelmann spruce - subalpine fir wet and cold biogeoclimatic subzone (ESSFwc2). The Sicamous area receives about 1000 mm of precipitation annually. The snowpack accumulates to about 2.5 m, depending on aspect and elevation, and generally melts by mid- to late June. The mean annual temperature is 1^{0}C, and the continuous frost-free period amounts to less than 40 days between mid-July and mid-August (Lloyd and Inselberg 1997).

The forest cover type is predominantly an old-growth subalpine fir - Engelmann spruce stand. The standing volume of living timber was estimated at 264 m^3/ha based on standard cruise procedures. Of this volume, approximately 35% was spruce and 65% subalpine fir.

The trees at Sicamous Creek forest are young. Seventy-eight percent of the spruce and fir (>4 cm dbh) are less than 150 years old. The remaining 22% of the fir range from 150 to 340 years old. Six percent of subalpine fir are over 300 years old. Seventeen percent of the spruce are over 300 years old. There is no spruce in the 150 to 200 age class. Standing dead trees comprise 20% of the mapped trees. These trees have been dead from 1 to 60 years (Parish 1997).

The main shrubs are *Rhododendron albiflorum* Hook, *Vaccinium membranaceum* Dougl. ex Hook., and *Vaccinium ovalifolium* Sm. in Rees (Lloyd and Inselberg 1997). The herb layer is dominated by *Valeriana sitchensis* Bong., *Arnica latifolia* Bong., *Gymnocarpium dryopteris* (Hofm.) Newman, *Tiarella unifoliata* Hook., *and Streptopus roseus* Michx. (Lloyd and Inselberg 1997).

Soils on the site are predominantly sandy loam textured Orthic Humo-ferric Podzols. These soils occur on the ESSFwc2/01 and 04 site series. Forest floors are thin throughout the site. Humus forms are predominantly Hemimors. Forest floors contain 10 to 33% decayed wood, and also contain an intermittent, thin charcoal layer at the mineral soil-forest floor interface. Details of site and soil characteristics at the Sicamous Creek forest were described by Hope (1997) and are summarized in Table 1.

Table 1. Site and soil characteristics at the Sicamous Creek *

	Site series unit		
	ESSFwc2/01	ESSFwc2/04	ESSFwc2/06
Slope position regime	midslope	Upper, rocky slopes	Lower and seepage areas
Soil moisture regime	4 (mesic-subhygric)	3-2 (submesic)	5 (subhygric)
Soil nutrient regime	poor	poor	medium
Soil classification	Orthic Humo-ferric Podzols	Orthic Humo-ferric Podzols	fluvial veneer over till
Soil texture	sandy loam	sandy loam-loamy sand	silt loam over sandy loam
Humus form Thickness (range in cm)	Hemimor 4.0 (1.5-9)	Hemimor 3.6 (1-12)	Mor and Moder 5.0 (1-14)
Soil drainage	well	well to rapid	imperfect (to poor)

* From Hope (1997).

The silvicultural systems in the Sicamous Creek forest consisted of partial cutting, clearcutting and no canopy removal. Canopy opening size was the main treatment effect in the design, aimed to provide opportunities to study logging practices, regeneration, and also to assess the effects on the spatial distribution of such ecosystem features as ground vegetation, snow accumulation and melt, and wildlife habitat structures. Micro site disturbance was selected as the secondary treatment since considerable experimental evidence suggests that some degree of forest floor disturbance will significantly improve regeneration success. The five main treatments are: i) Control: No removal.

ii) Single tree selection (Partially cut) : 33% of the volume was removed over 30-ha area on the first pass by cutting every fifth tree (no marking) and marked skid trails. This treatment is the same as a single-tree selection cut, but with no attempt to "improve the stand."

iii) 0.1 ha clear-cut: 33% of the volume was removed over a 30-ha area by cutting approximately 60, 0.1-ha openings, each about 31 m square, with 60 m between centers and with skid trails linking all groups.

iv) 1.0 ha clear-cut: 33% of the volume was removed over a 30-ha area by cutting nine groups of 1-ha, each about 100 m square, with 200 m between centers and skid trails linking all groups.

v) 10 ha clear-cut: 33% of the volume was removed over a 30-ha area by cutting one 10-ha opening approximately 330 m square.

Three replicates of each canopy treatment were arranged in a randomized block design. Buffer strips (>80 m) of mature forest were left around each treatment to reduce interactions. See the Sicamous Creek silvicultural systems treatment units in Figure 2.

In each treatment, about 33% of the volume were removed in the first pass. The remaining volume will not be removed until adequate regeneration is established in the first openings, concerns about water quality and quantity in Sicamous Creek are resolved, and experiments are completed in the initial openings. A nested plot design will be used to assess the effect of four canopy opening sizes and 12 site preparation/regeneration strategies (Vyse 1997).

Thirty-five studies concerned with nutrition, soil productivity; regeneration, forest health, and wildlife are currently underway at the SCSSP Site. This study decided here focuses on effects of soil-borne and seed-borne pathogenic fungi on seed survival, germination and seedling establishment on disturbed and natural seedbeds. The experimental plots were mainly set up along D-transact line (Figure 1), located in A2, A3, B2, B3, C2, and C3. It covers undisturbed and disturbed seedbeds in natural stands, and scalped and unscalped seedbeds in selective cutting and 1- ha clearcutting sites, from bottom to top of the mountain. The 10-ha clearcut treatment was not included in the present experiment because natural seedfall in this size of clearcutting is too low to rely on natural regeneration. Our work does not apply to sites on which plantation will be practical.

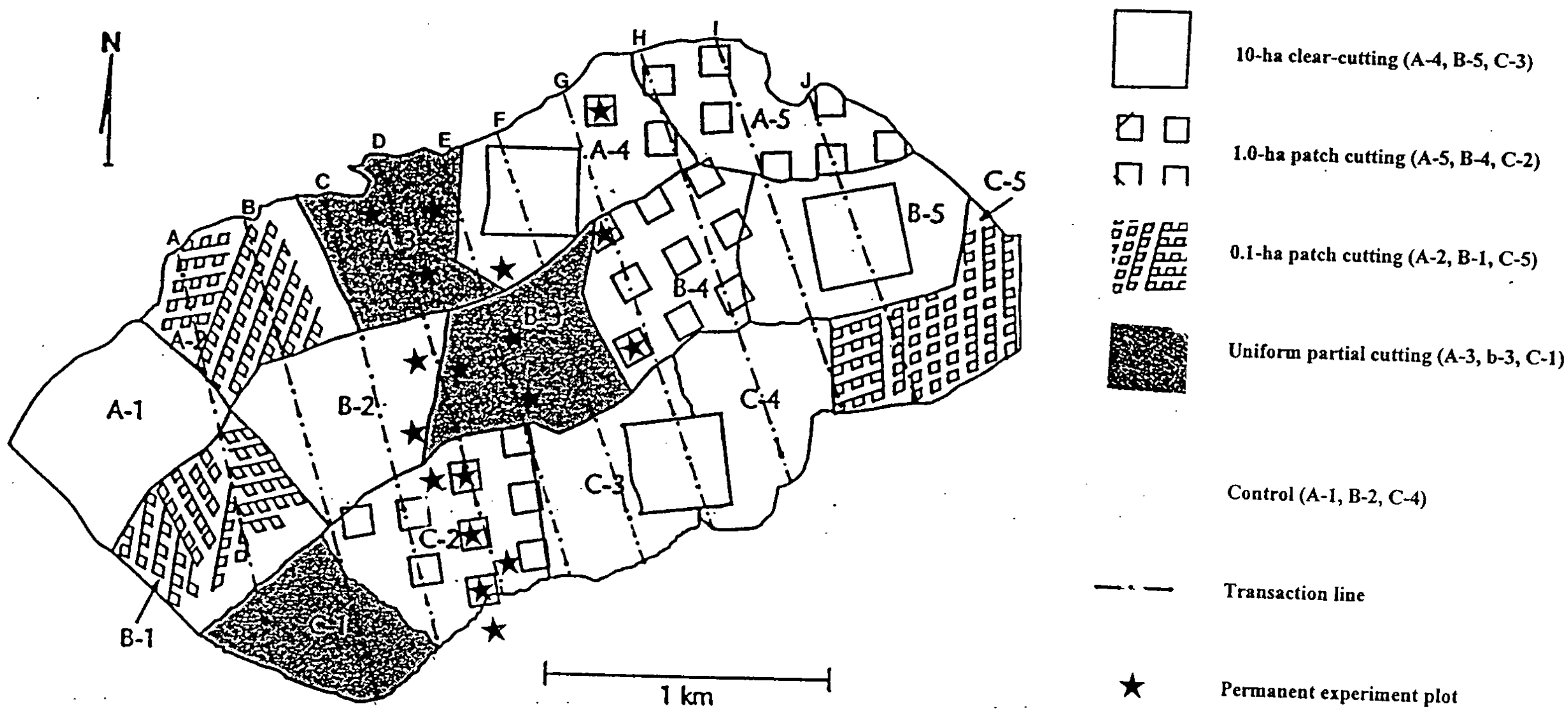


Figure 1. Map of the Sicamous Creek Silvicultural Systems Project treatment units and the location of permanent sample plots for seed pathology study

2.2 Materials and Methods

The major purpose of this study was to determine the role of pathogenic fungi in the survival, germination and development of Engelmann spruce and subalpine fir seed and seedlings on forest floor seedbeds in natural and disturbed forest stands at the Sicamous Creek Research site. Three separate investigations were undertaken to:

i) survey the forest floor seedbed types and natural regeneration on these seedbeds in natural forest stands in the ESSF wc2 subzone;

ii) study Engelmann spruce and subalpine fir seed survival by sowing on variously treated and natural forest floor seedbeds in disturbed and natural stands in the fall and recovering that seed at snow melt the next spring;

iii) study spruce and fir seedling development on the experimental seedbeds to record the emergence and survival of young seedlings.

2.2.1 Survey of forest floor seedbed types and natural regeneration on the seedbeds in natural and disturbed stands

A survey of forest floor seedbed types and natural regeneration on the seedbeds in natural and disturbed stands was conducted to describe the natural regeneration pattern in the ESSF as related to seedbed type.

From June 16 to June 25, 1995, a pre-survey was conducted at the Sicamous Creek site. Based on that pre-survey, we categorized four types forest floor seedbeds. They were: i) moss-type seedbed: forest floor covered with mosses and litter (MOS); ii) litter-type seedbed: forest floor covered with litter (LIT); iii) rotten logs covered with mosses (LOG); and iv) areas not suitable for seedlings (ANSFS): large rocks and living basal area, and sound, bare down woody material (Table 2).

Eighteen, 20 m^2 4 X 5 m plots were randomly placed in undisturbed stands along the D-transaction line from the bottom to top of the mountain for seedbed type and naturally regenerated seedling survey. Within each plot, the area of each seedbed type was determined and the Engelmann spruce and subalpine fir seedlings (<5 years old) on each of seedbed type were counted. Estimates of percentage of seedbed type and number of seedlings per square meter and per ha were calculated with simple random sampling SRS methods (Cochran 1977; Thompson 1992). Chi-square goodness-fit analysis was used to test differences between seedbeds (Blaisdell 1993).

Table 2. Forest floor seedbed types and their descriptions.

Seedbed	Descriptions
LOG	Rotten logs covered with mosses. Rotten stumps covered with moss layer are also counted as in this type of seedbed.
MOS	Forest floor with surface layer of mosses and litter, about 2-5 cm, often found in open area covered with *Rhododendron* spp. and other vegetation.
LIT	Forest floor covered with layer of 2 - 15 cm litter, often under shade of dense conifer trees, moss and herb largely absent.
ANSFS	Areas on which conifer seedlings can not grow, including areas such as occupied by rocks and living tree basal area.

2.2.2 Engelmann spruce and subalpine fir seed survival, germination and seedling development on seedbeds in natural and disturbed stands

This is the main experiment in this project. Seedbags placed on various seedbeds in the fall were retrieved at snow melt the following spring. Seed viability and the presence of pathogens in dead seed were determined. Two main experiments were conducted. The first determined the effect of seedbed type, all within undisturbed stands while the second looked at the effect of three silvilcutural treatments, clear-cut, partially cut and uncut control on survival of seed, germinant and seedling, and on frequency of soil-borne pathogenic fungi. Two types of seedbed were employed in the second experiment, replicated in all three types of stands.

Experimental plot layout and seedbed treatment. For the seedbed treatment study, a total of 48, 1-m^2 plots were selected and set up in natural stands, including six rotten-log (LOG) plots, 18 moss-type (MOS) plots and 18 litter-type (LIT) plots. Of the 18 moss-type and 18 litter-type seedbed plots, six plots were kept as controls, and three plots of each type had all the organic material removed (MIN); in another three of each, the whole forest floor was mixed with the mineral soil to a depth of 30 cm and in the remaining six of each type, the litter layer and all living plants were removed without disturbing the fermentation and humus layer (Table 3). Each 1-m^2 plot was divided into four, 0.5 X 0.5m subplots which were assigned to one of the following treated seed sources: i) subalpine fir seeds over-wintered on the site; ii) subalpine fir control - no seeds sown; iii) Engelmann spruce seeds over-wintered on the site; iv) Engelmann spruce control - no seeds sown. Thus, there were seven types of seedbeds, two species and six replications of each for a total of 84 treatment and 84 control subplots in the seedbed treatment study.

Table 3 . Experiment design I: seedbed treatment in natural stands. *

Factor	Type	Levels	Values
Species	fixed	Two	1. *Picea engelmannii* 2. *Abies lasiocarpa*
Seedbed	fixed	Seven	1. MOS: Undisturbed forest floor covered with moss and litter, Control 1; 2. LIT: Undisturbed forest floor covered with litter (Mor), Control 2; 3. LOG: Rotten logs covered with mosses, Control 3; 4. MOSR: Surface and hemidecomposited moss and litter layer was removed from MOS seedbed and organic soil was exposed Treatment 1; 5. LITR: Surface and hemidecomposited litter layer was removed from LIT seedbed and organic soil was exposed, Treatment 2; 6. MIN: All organic and duff layers were removed, and mineral soil was exposed, Treatment 3. 7. MIX: Surface and organic layers were mixed with mineral soil, Treatment 4.
Replication		Six	

* Total degrees of freedom = 2 (species) X 7 (seedbeds) X 6 (replicates) = 84.

To determine the effects of silvicultural systems, 12, 1 m^2 plots in six 1.0-ha clear-cut sites and 12, 1 m^2 plots in six partially cut stands were randomly laid out. Of these, six were on scalped forest floor seedbed with exposed mineral soil (MIN) and six were on unscalped seedbed (MOS). The 12 parallel plots in the undisturbed stands described above served as controls. Each 1 m^2 plot was divided into four subplots and the treatments were the same as in the seedbed treatment study. There were two species, two seedbed types, six replications and three silvicultural systems stands (natural, 1-ha clear-cuts and partially cut) for a total of 72 subplots in the study (Table 4). Statistical analyses for the two experiments were conducted with MINTAB for Windows.

Table 4. Experiment design II: silvicultural systems effects *

Factor	Type	Levels	Values
Species	Fixed	Two	1. Picea engelmannii; 2. Abies lasiocarpa.
Operation	Fixed	Three	1. Natural stands; 2. Partially cut; 3. One-ha clear-cut.
Seedbed	Fixed	Two	1. MOS: Undisturbed forest floor covered with surface layer of mosses and litter; 2. MIN: exposed mineral soil after site scarification or top layer removal.
Replication		Six	

* Total degree of freedom = 2 (species) X 3 (operation) X 2 (seedbeds) X 6 (replicates) = 72.

Seed sources and seed treatment: Engelmann spruce and subalpine fir seeds were provided by the Tree Seed Center (TSC), British Columbia Ministry of Forests, in late August, 1995. These seeds were collected in 1978 and 1985 and had been stored at the Center. Seed collection information and germination test results provided by TSC are listed in Table 5.

Each of the seedlots was divided into equal portions. One portion was placed on forest floor seedbeds in nylon mesh bags and over-wintered on site. The other portion of the seed was stored the pathology laboratory at 0^0 C till February, 1996 and then soaked for 24 hr for spruce and 48 hr for subalpine fir, stratified for 56 days and placed at room temperature in wet petri-dishes for germination test and seed-borne pathogenic fungi assay at late of June, 1996.

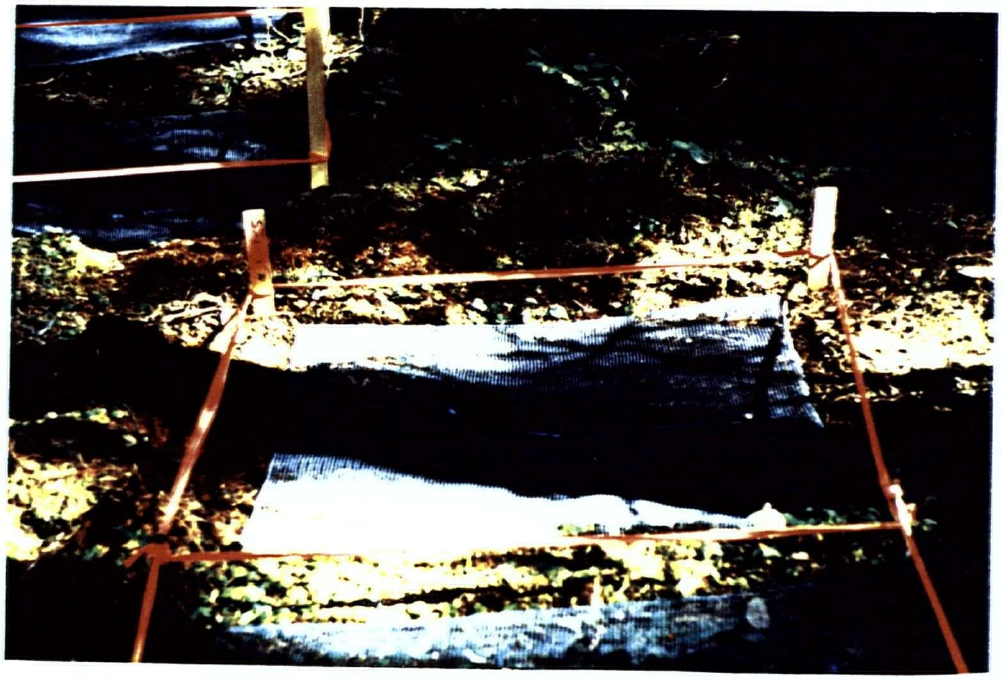
Table 5. Seed source information and germination results*

Species	Seedlot Number	Collection Date and Method	Location	Elevation m	Germination ** Date	%
Engelmann spruce	29751	1985 08 30 Not available	Apex Mt.	1450	1993 05 26	96
Subalpine fir	03717	1978 08 03 Felled tree	Vale Mt.	838	1994 05 18	77

* Information provided by Tree Seed Centre (TSC), Forest Services, Ministry of Forests, British Columbia;
** Using ISTA rules.

Three hundred, unstratified Engelmann spruce and 150 subalpine fir seeds each were put into each of nylon mesh bags, i.e. 152 bags and 45,600 seeds of Engelmann spruce and 152 bags and 22,600 seeds of subalpine fir. From September 13 to 15, 1995, the seedbags were covered with 0.5 cm layer of material taken from the seedbed surface. A 1.25 cm wire screen was used to cover the plot to prevent rodent feeding (Figure 3). The first snow fell on October 3. The seedbags were recovered from June 17 to 28, 1996 before snow melt.

Viability of seed over-wintered on the site was originally intended to be determined by a germination test in the laboratory. Half of the seed was brought back to the lab while the other half was to be resown in the subplot from which it was recovered. When seedbags were retrieved in mid June, 1996, some seeds of both Engelmann spruce and subalpine fir in the bags had germinated. Consequently, the experiment was redesigned. In each recovered seedbag, the numbers of ungerminated seeds, long germinants (radicles > two times seed length), short germinants (radicles between two and one half seed length), and starters (radicles visible but less than half seed length) were counted. Starters and ungerminated seeds were wrapped with wet paper towers in plastic lunch bags and brought back to laboratory for further germination test and pathogen bioassay. Total germination for a particular seed bag was estimated as the number of germinants and starters at seed retrieval plus an estimate of the number of viable seeds that had not yet germinated, all expressed as a percentage of seed sown. Viability of ungerminated seed was determined by a germination test in the lab. Germination test in the lab was conducted by placing ungerminated seeds on wet paper at room temperature (about 20^0 C) for 3 weeks.



A. Experimental plot layout

B. Seed bags were placed on treated forest floor seedbed

Figure 2. Experimental plot layout (A) and seed bags placing on treated seedbeds (B) in natural forest stands.

Resown ungerminated seeds and germinants plus starters: About 20% ungerminated seeds were resown on each seedbed as well as all starters for study of emergence and seedling development. An average of 50 seed (range 30 to 70) were resown per subplot.

2.2.3 Observation of seedling development in disturbed and natural stands

The purpose of this experiment was to study the loss of young seedling on various seedbed types in natural and disturbed stands during seedling development. Observation of seedling development was done in two ways. The first was to determine survival of naturally-regenerated seedlings on rotten logs in natural and disturbed stands and to examine harvesting pattern effect on survival of naturally-regenerated seedlings in disturbed stands since most of the young seedlings were supposedly lost during this period. Only seedlings less than 6 years-old were used in this part of study. The second set of observation was done to determine survival of new germinants during the first growing season.

Development of naturally regenerated seedling on rotten logs covered with mosses. Naturally regenerated seedlings on rotten logs with mosses (LOG) in disturbed and natural stands were labeled in late June, 1995, by placing a plastic tag at each seedling. Two hundred and sixteen Engelmann spruce seedlings (92 in natural stands, 109 in selective cut stands and 15 in clear-cut stands) and 232 subalpine fir seedlings (102 in natural stands, 97 in selective cut stands and 33 in clear-cut stands) were labeled and checked every 10 days until early September. Seedling survival in each stand type was calculated as the percentage of seedlings alive at the end of growing season divided by the total labeled seedlings in the stand.

Development of new germinants during the first growing season. The emerging and surviving seedlings in each subplot were recorded every 10 days until the end of growing season, i.e. August 28, 1996. To distinguish germinants originating from the seed sown intentionally and from naturally shed seed, the number of seedlings of each species in the control subplot were designated as the control of the treatment. The data for the number of seedlings in subplots were adjusted by deducting the average number of seedlings from controls. Percentage emergence was calculated as the adjusted number of emerged seedlings divided by total seeds sown. Emergence of germinants in each subplot was calculated as the adjusted number of emerged seedling divided by the sum of sown germinants (SSG). Balanced ANOVA with MINITAB was used to analyze the emergence difference for seedbed and stand treatments. Duncan's multiple range test was used to determine the significance of mean differences.

Survival was calculated as the number of seedlings surviving to the end of growing season divided by the adjusted number of current-year-emerged germinants. Since no germinants or only a few emerged on some seedbeds, statistical tests were not possible. Survival was expressed as percentage calculated by the sum of survived germinants from the six replications of the same type of

2.3 Results

2.3.1 Natural regeneration of Engelmann spruce and subalpine fir on forest floor seedbeds in undisturbed stands

Seedbed types in undisturbed spruce-fir type stands

Table 6 gives the percentage of surface area each of the four major seedbed occupies. In natural spruce-fir type stands, seedbed of forest floor covered with moss, or moss-type seedbed (MOS) was the most common seedbed type, averaging 72.5%. This type of seedbed is often seen as small openings filled with shrubs, of which *Rhododendron albiflorum* is the most common. The second seedbed type was rotten logs covered with moss (LOG), average 14.9%. Forest floor with pure litter on the surface layer or litter-type seedbed (LIT) is only accounted for 4.2%, and occurred in the more heavily shaded areas.

Table 6. Percentage of seedbed types at the Sicamous Creek forest *

Groups	Rotten log (LOG)	Moss-type (MOS)	Litter-type (LIT)	Others
%	14.9	72.5	4.2	8.3
Variance	27.4	55.4	17.6	16.9
Confidence Interval a=0.05	12.4, 17.5	69.0, 76.1	2.2, 6.2	6.4, 10.3

* Based on 18, 20-m^2 sample plots.

Naturally-regenerated seedlings on seedbeds in natural and disturbed stands

Natural regeneration of Engelmann spruce in undisturbed stands was restricted to rotten logs covered with mosses (LOG), while subalpine fir occurred mainly on the same type of seedbed (Table 7). A t-test for difference showed that subalpine fir was significantly more common than Engelmann spruce on the rotten log seedbed (Table 8). A few subalpine fir seedlings were found on moss-type forest floor seedbed, or MOS (average 0.16 seedling per square meter, or 1161 seedlings of *A. lasiocarpa* per ha), which accounted for 6.5% of all naturally regenerated seedlings in natural stands. The total seedlings (age 1 to 5 years old) in the ESSF wc2 is about 17849 per ha (Table 7).

Table 7. Naturally-regenerated Engelmann spruce (Se) and subalpine fir (Bl) seedlings on seedbeds in undisturbed stands

Seedbed	%	Number of seedlings / m^2			Number of seedlings / ha			
		Se	Bl	Total	Se	Bl	Total	%
LOG	14.94	3.66	7.50	11.17	5474	11214	16688	93.50
MOS	72.55	0	0.16	0.16	0	1161	1161	6.50
LIT	4.17	0	0	0	0	0	0	0.00
OTHERS	8.33	0	0	0	0	0	0	0.00
Total	100	3.66	7.66	11.33	5474	12375	17849	
%					30.7	69.3		100

Table 8. Student t-test: Paired two samples for means of Engelmann spruce and subalpine fir seedlings on LOG seedbeds in undisturbed stands

	Engelmann spruce	Subalpine fir
Mean of seedling / m^2	7.5	3.7
Variance	46.0	12.79
Observation	18	
Pearson Correlation	0.588	
df	17	
t Stat (Alpha = 0.01)	2.963*	
P (T< =t)two-tail)	0.009	

* Significant difference with two-tail test at a=0.01

The t-test showed that there was significantly more (p=0.01) subalpine fir than Engelmann spruce seedlings on LOG seedbed in undisturbed stands (Table 8).

In disturbed stands, naturally-regenerated seedlings were restricted to the rotten log seedbed (LOG). No conifer seedlings were found on the untreated, forest floor seedbeds. The density of Engelmann spruce seedlings on LOG was significantly different from that in natural stands. In the 1-ha clear-cuts, there were an average 1.8 spruce seedlings/m^2, half that of natural stands. In partially-cut stands, there were 5.6 spruce seedlings/m^2, three times more than in the 1-ha clear-cuts and 52% more than in natural stands (Table 9). Subalpine fir followed the same trend, i.e. the number of seedlings/m^2 was higher in partially cut stands and lower in clear-cut sites compared with that in natural stands. However, these differences were not statistically significant (Table 10).

Table 9. Mean of naturally regenerated Engelmann spruce and subalpine fir seedlings on LOG seedbeds in disturbed and natural stands

Stand	Engelmann spruce / m^2	Subalpine fir / m^2	Total / m^2
1.0 hectare clear-cut	1.8	5.9	7.7
Partially Cut	5.6	8.1	13.7
Natural	3.7	7.5	11.2

Table 10. Analysis of variance for naturally regenerated conifer seedlings on LOG seedbed in disturbed and natural stands

Source	SS	df	MS	F	P-value	F crit
Stands	57.479	2	28.740	1.664	0.206	3.316
Species	116.503	1	116.503	6.745 *	0.014	4.17
Interaction	5.132	2	2.566	0.149	0.863	3.316
Within	518.140	30	17.271			
Total	697.256	35				

* Statistically significant

Survival of conifer seedlings on LOG seedbed in natural and disturbed stands

Survival of Engelmann spruce and subalpine fir seedlings on LOG seedbed was surveyed from 1995 to 1996. Survival of Engelmann spruce seedlings on LOG seedbed in natural stands, selective cutting and one-ha clear-cuts were 91.3, 68.8 and 43.8%, respectively. Seedling mortality in disturbed stands was significantly higher than in natural stands. A Chi-square test showed that survival of seedlings on LOG in partially cut sites and in clearcut sites was significantly lower than that in natural stands. Also mortality in clear-cut stands was significantly higher than in partially cut stands (Table 11).

Survival of subalpine fir seedlings on LOG seedbed in natural and partially cut and clear-cuts stands was 87.6, 85.6 and 42.4%, respectively. Seedling mortality increased significantly in clear-cut sites compared with that in undisturbed and partially cut stands (Table 11). A Chi-square test showed that there was no effect of selective cutting on subalpine fir seedlings survival on LOG seedbed compared with that in natural stands (Table 12).

Table 11. Survival of Engelmann spruce and subalpine fir seedlings on LOG seedbed in disturbed and natural stands in the ESSF*

Species	Stand	Number of seedlings		Survival (%)
		Survived	Total	
Engelmann spruce	Natural	84	92	91.3 a
	Partial Cut	77	112	68.8 b
	Clear-cut	7	13	43.8 c
Subalpine fir	Natural	85	97	87.6 a
	Partial Cut	83	97	85.6 a
	Clear-cut	14	33	42.4 c

* Seedlings were labeled in summer of 1995 and the last observation date was September 2, 1996

Table 12. Chi-square test for naturally-regenerated Engelmann spruce and subalpine fir seedlings on LOG seedbeds in disturbed and natural stands.

Species	Stand Treatment	df	X^2 value	X^2 Critical value
Engelmann Spruce	Natural Partially cut Clear-cut	2	$X^2 = 19.09$ *	$X^2_{.05} = 5.991$
	Natural Partially cut	1	$X^2 = 15.45$ *	$X^2_{.05} = 3.841$
	Partially cut Clear-cut	1	$X^2 = 32.51$ *	$X^2_{.05} = 3.841$
Subalpine fir	Natural Partially cut Clear-cut	2	$X^2 = 34.75$ *	$X^2_{.05} = 5.991$
	Natural Partially cut	1	$X^2 = 0.178^{ns}$	$X^2_{.05} = 3.841$

* Survival of naturally regenerated conifer seedlings on LOG is significant different among stands.
ns Not significantly different at a= 0.05.

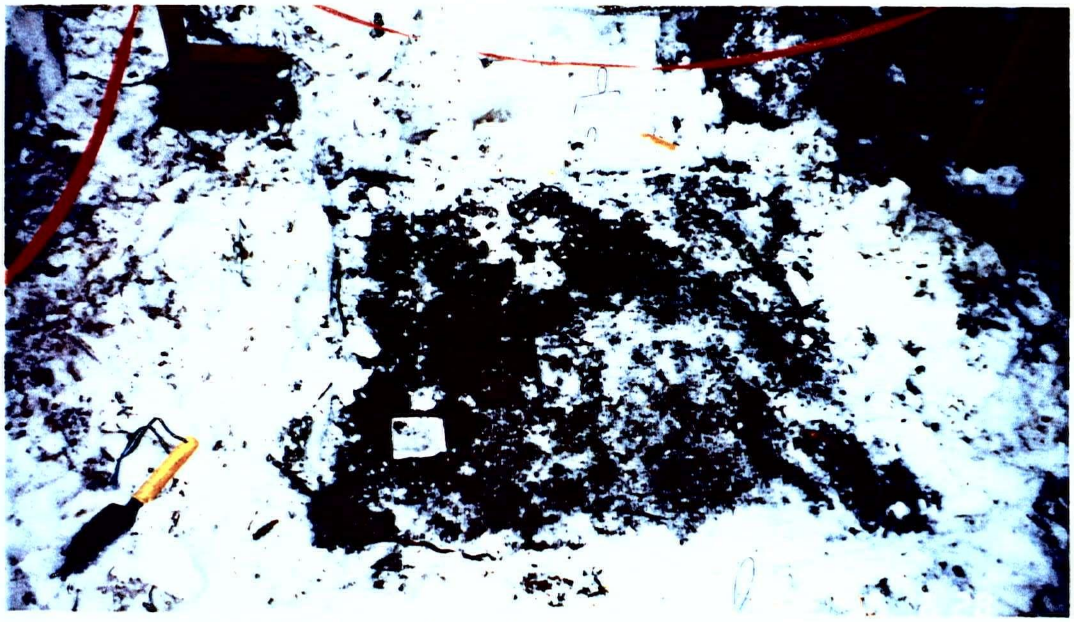
2.3.2 Survival of Engelmann spruce and subalpine fir seed over-wintered on seedbeds in natural and disturbed stands

Over-winter survival of Engelmann spruce and subalpine fir seeds was examined by determining seed germination. On June 16, 1996, the first day the seedbags were checked under snow, some seeds of both species had already germinated (Figure 3). Radicals of some subalpine fir were as long as 4 cm. At that time, snow cover was still complete in the Sicamous Creek Research Forest, with average 15 to 30 cm of snow. In some areas, snow was more than 50 cm deep. Over-wintered Engelmann spruce and subalpine fir seed germination was examined on site and later (before or after snow melt). Seed which had germinated under snow before retrieval (before snow melt) was counted as site germination. Some seeds germinated later, within 15 days under moist conditions in the laboratory after retrieval. These germinants were counted as late germination. The sum of site germination and late germination was designated as total germination, or over-winter survival. The highest total germination (over-winter survival) was 96% for Engelmann spruce and 62% for subalpine fir.

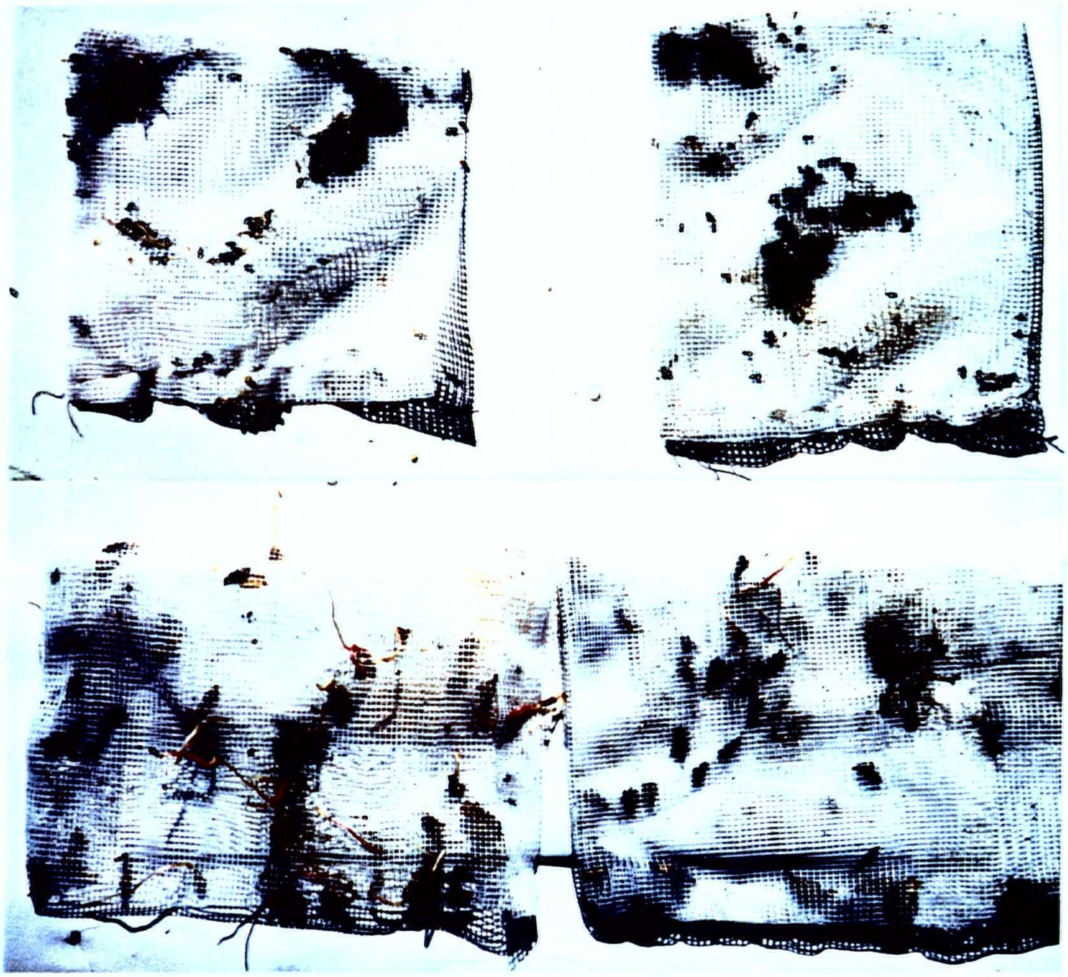
Seed survival on seedbeds in natural stands. On undisturbed forest floor seedbeds in natural stands, the survival of both Engelmann spruce and subalpine fir was very poor. Total Engelmann spruce germination was 12.2% on litter-type seedbed (LIT), 13.9% on moss-type seedbeds (MOS), and 37.9% on rotten log seedbed (LOG), respectively. Survival on forest floor seedbeds was only about one third, and significantly lower than on rotten log seedbed (Table 13).

The total germination of subalpine fir was 11.8% on litter-type seedbed 12.3% on moss-type seedbed, and 23.1% on rotten log seedbed. The survival of subalpine fir on forest floor seedbeds was about a half that on rotten log seedbeds, but not statistically different (Table 13). The low survival of Engelmann spruce and subalpine fir on the undisturbed forest floor seedbed in natural stands is one of the major reasons for poor natural regeneration in undisturbed stands in the ESSF.

Site germination for Engelmann spruce on undisturbed seedbeds was relatively lower than that of subalpine fir on undisturbed stands. Site germination for Engelmann spruce was 39.4, 63.7 and 29.2% PTG (percentage out of total germination) on MOS, LIT and LOG seedbeds, respectively (Table 13), and for subalpine fir was 77.2, 83.5 and 89.3% PTG (Table 14).



A. Retrieving seed bags before snow melt



B. Engelmann spruce (upper) and subalpine fir (bottom) seeds germinated in seedbags on mineral soil (left), and litter (right) seedbed before snow melt

Figure 3. Conifer seed germinated before snow melt

Table 13. Engelmann spruce seed survival on treated and undisturbed seedbeds in natural stands

Seedbed Treatment		N	Total Germination (%)	Site Germination		Late Germination***	
				Mean	PTG (%)**	Mean	PTG (%)
Moss-type	(MOS)	6	13.9 c*	5.5	39.4	8.4	60.6
Litter-type	(LIT)	6	12.2 c	7.8	63.7	4.4	36.3
Rotten Log	(LOG)	6	37.9 ab	11.1	29.2	26.8	70.8
Moss Layer Removal	(MOSR)	6	42.7 ab	21.2	49.8	21.5	50.2
Litter Layer Removal	(LITR)	6	38.4 ab	25.6	66.6	12.8	33.4
Forest floor removal	(MIN)	6	57.3 a	29.0	50.7	28.3	49.3
Mixing forest floor with mineral soils	(MIX)	6	30.8 bc	16.0	51.9	14.8	48.1

* Seedbeds with same letter are not statistically different from each other.

** PTG --- Percentage out of total germination.

*** Seed germinated within 14 days after recovery.

Table 14. Subalpine fir seed survival on treated and undisturbed seedbeds in natural stands

Seedbed Treatment		N	Total Germination (%)	Site Germination		Late Germination	
				Mean	PTG (%)**	Mean	PTG (%)
Moss-type	(MOS)	6	12.3 b*	10.3	83.5	2.0	16.5
Litter-type	(LIT)	6	11.8 b	9.1	77.2	2.7	22.8
Rotten Log	(LOG)	6	23.1 b	20.6	89.3	2.5	10.7
Moss Layer Removal	(MOSR)	6	19.1 b	16.2	84.9	2.9	15.1
Litter Layer Removal	(LITR)	6	24.3 ab	21.2	87.0	3.	13.0
Forest floor removal	(MIN)	6	41.8 a	36.3	86.9	5.5	13.1
Mixing forest floor with mineral soils	(MIX)	6	23.4 ab	20.0	85.2	3.4	14.8

* Seedbeds with same letter are not statistically different from each other.

** PTG --- Percentage out of total germination.

Seedbed treatment had a significant effect on seed survival. Analysis of variance showed that there was a significant difference in seed survival between the two species and among seedbeds for each species. Overall, seed survival of Engelmann spruce was higher than that of subalpine fir. Survival of Engelmann spruce and subalpine fir seed over-wintered on all treated seedbeds in natural stands were higher than on undisturbed moss-type and litter-type forest floor seedbeds (Table 15).

Table 15. Analysis of Variance for effect of seedbed treatment on Engelmann spruce and subalpine fir seed survival in natural stands

Source	df	SS	MS	F	P
Species	1	2566.7	2566.7	9.40**	0.003
Seedbed	6	11629.7	1938.3	7.10**	< 0.001
Species X Seedbed	6	1247.3	207.9	0.76 ns	0.603
Error	70	19107.2	273.0		
Total	83	34550.9			

** Significant at a = 0.01 level;
ns Not significant at a = 0.05 level

Engelmann spruce seed survival on treated seedbeds was significantly higher than on untreated litter (LIT) and moss-type (MOS) forest floor seedbeds except that the treated seedbed with mixed soils (MIX) was not significantly different from any seedbed except MIN. The highest survival, 57.3%, occurred on the seedbed which had all layers of soil above mineral soil removed (MIN). Survival was also significantly improved by surface layer removal (MOSR and LITR). The multiple range test results showed that survival rank of Engelmann spruce seed on treated and original seedbeds trends was (from the lowest to the highest):

LIT / MOS => MIX => LOG / LITR / MOSR => MIN.

There was no significant differences between MIN and MOSR, LITR and LOG (Table 13). A possible interpretation of these results is that some agents which kill Engelmann spruce seed during the winter, live mainly in the surface layer of the forest floor although they also occur at lower frequency throughout the forest floor. Removing surface layer significantly improved survival of Engelmann spruce seed.

Survival of Subalpine fir seed on treated seedbeds in natural stands was only significantly improved on the mineral soil seedbed (MIN) with the highest total germination of 41.8%. Survival on other treated seedbeds, surface layer removal (MOSR and LITR) and the mixed (MIX) was higher than, but not significantly different from the untreated forest floor seedbeds (MOS and LIT) (Table 14).

Effects of silvicultural systems on survival of conifer seed on MOS and MIN seedbeds

In the experiment dealing with the effect of silvicultural systems, there was significant seedbed preference for Engelmann spruce and subalpine fir seed survival. Engelmann spruce seed survival was significantly higher than subalpine fir and there was a silvicultural systems effect on Engelmann spruce survival but not on subalpine fir (Table 16).

Table 16. Analysis of variance for effects of silvicultural systems on Engelmann spruce and subalpine fir seed survival on MOS and MIN seedbeds

Source	df	SS	MS	F	P
Stands	2	809.1	404.5	1.49 ns	0.23
Species	1	1332.0	1332.0	4.92 *	0.03
Seedbed	1	25565.7	25565.7	94.37 **	< 0.001
Stand X Species	2	2666.7	1333.3	4.92 *	0.01
Stand X Seedbed	2	482.3	241.2	0.89 ns	0.42
Species X Seedbed	1	253.0	253.0	0.93 ns	0.34
Stand X Species X Seedbed	2	258.1	129.1	0.48 ns	0.42
Error	60	16254	270.9		
Total	71	47621.8			

* Significant at a = 0.05.
ns Not significantly different.

Engelmann spruce had significantly higher survival than subalpine fir, especially in 1-ha patch cuts stands. Total germination was 66.3% on mineral soil seedbed (MIN) and 28.3% on moss-type forest floor seedbed (MOS) for Engelmann spruce, in contrast, only 35.5% on MIN and 10.7% on MOS for subalpine fir (Table 17).

Seedbed treatment of scarification significantly improved both Engelmann spruce and subalpine fir seed survival. Both species had significantly higher germination rates on scarified seedbed (MIN) than on forest floor seedbed (MOS) in the three stands (Table 17).

Table 17. Engelmann spruce and subalpine fir seed survival on MOS and MIN seedbeds in partially cut, clear-cut and natural stands

Stand	Seedbed	Germination (%)	
		Engelmann spruce	Subalpine fir
Partially cut	MOS	2.7 d	7.4 c
	MIN	45.7 b	53.5 a
Clear-cut	MOS	28.3 c	10.7 c
	MIN	66.3 a	36.9 b
Natural	MOS	13.9 cd	12.3 c
	MIN	57.3 ab	41.8 ab

Harvesting pattern significantly affected Engelmann spruce seed survival. The multiple comparison test showed that germination on both MIN and MOS seedbeds in 1-ha clear-cut stands was significantly higher than that on same type of seedbeds in partially cut stands. However, germination on each type of seedbed in the two disturbed stands was not significantly different from that in natural stands (Table 17).

2.3.3 Emergence of Engelmann spruce and subalpine fir on seedbeds in natural and disturbed stands

Emergence is defined as the process that starts when germination is complete (radicle = twice seed length) and finishes when the cotyledons are fully expanded. It involves both radicle and hypocotyl elongation. In natural forest floor seedbeds, cotyledons of some germinants will not fully open until the end of the growing season or next year. Consequently, the germinants which were visible on the surface of seedbed with their seed coat attached were counted as having emerged. Percentage of emergence is thus based on the percentage of emerged germinants from sown, live seeds.

The purpose of this study was to understand the loss during emergence. During emergence, young germinants of conifers are easily damaged as the result of their lower tolerance to harsh environment and pest attack. During this period radicles are easily infected by certain soil-borne pathogens. The estimate of the difference between the number of live seeds sown in the soil and the number of emerged germinants would be the loss of germinants during emergence.

Time of Emergence. At Sicamous Creek, emergence for both Engelmann spruce and subalpine fir in natural stands started 2 to 3 weeks after snow melt and lasted throughout the growing season, from late June to late August. Emergence at 3 weeks after snow melt in natural stands was 84% complete for Engelmann spruce and 53.9% for subalpine fir (Table 18).

In 1996 in disturbed stands, germinants of Engelmann spruce and subalpine fir began to emerge 10 days after snow melt on the most suitable seedbed (scalped seedbed) in partially cut stands. The time and percentage of emergence for Engelmann spruce, but not for subalpine fir, were nearly the same as in natural stands. For the latter, emergence in the 1-ha clear-cut was complete 3 weeks after sowing, while in the natural stand emergence continued throughout the growing season (Table 18).

Table 18. Time and percentage of emerged Engelmann spruce and subalpine fir in disturbed and natural stands in the ESSFwc2, 1996

			Seedling survival (%)				
Species	Stand	N*	June 28	July 18	July 28	Aug 18	Aug 28
Engelmann spruce	Natural	262	0.0	84.0	93.9	98.1	100
	Partial cut	142	0.0	86.6	97.9	100	100
	1-ha Clear-cut	56	0.0	91.1	92.9	94.6	100
Subalpine fir	Natural	26	0.0	53.9	69.2	80.8	100
	Partial cut	28	0.0	82.1	96.4	96.4	100
	1-ha Clear-cut	19	0.0	100	100	100	100

* Total number of emerged germinants on experimental plots in the stand during 1996 growing season

Emergence on treated and undisturbed seedbeds in natural stands. Generally, emergence of both Engelmann spruce and subalpine fir in natural stands in the ESSF was very low. Average emergence was 30% for Engelmann spruce and 10.7% for subalpine fir (Table 19). Percentage of emerged Engelmann spruce seed was significantly higher than that of subalpine fir.

Table 19. Emergence of Engelmann spruce and subalpine fir in natural stands

Species	N*	Emergence (%)**
Engelmann spruce	42	27.0
Subalpine fir	42	10.7

* Number of samples from 7 types of seedbed and each seedbed type with 6 replications in the natural stand.
** Mean of emergence from the 42 samples.

Analysis of variance showed that there was a significant emergence difference between seedbeds in natural stands (Table 20). On undisturbed seedbeds in the ESSF, emergence of both Engelmann spruce and subalpine fir was very low. There was no emergence for the two species on undisturbed litter-type seedbed (LIT). Compared with litter-type seedbed, emergence on moss-type seedbed (MOS) and rotten-log seedbed (LOG) was higher (Table 20). However, there was no statistically significant difference emergence among any of the undisturbed seedbeds in natural stands.

Table 20. Analysis of variance for effect of seedbed treatment on emergence of conifer germinant on treated and undisturbed seedbeds in natural stands

Source	df	SS	MS	F	P
Species	1	5525.1	5525.1	8.31 *	0.005
Seedbed	6	22819.2	3803.2	5.72 *	< 0.001
Species X Seedbed	6	3876.6	646.1	0.97 ns	0.451
Error	70	46544.0	665.1		
Total	83	78774.9			

Seedbed treatments showed various effects on emergence of both Engelmann spruce and subalpine fir (Table 21). The mixing soil treatment (MIX) provided the highest emergence for Engelmann spruce (67.3%) which was significantly higher than the other treatments and controls except for the removal of soil layers above mineral soil (MIN). Emergence on MIN was also significantly higher than that on the litter-type seedbed, both the treated and undisturbed. Removal of litter- or moss-layer did not significantly improve in Engelmann spruce emergence (Table 21).

The pattern of emergence for subalpine fir was similar to that for Engelmann spruce, except that the overall average was lower. Also, exposed mineral soil (MIN) gave an unexpected low emergence (Table 21).

Table 21. Emergence of Engelmann spruce and subalpine fir on treated and undisturbed seedbeds in natural stands

Seedbed	Emergence (%)	
	Engelmann spruce	Subalpine fir
LOG	28.7 bc*	16.7 ab
MOS	19.1 bc	12.8 ab
LIT	0.0 c	0.0 b
MOSR	22.7 bc	0.0 b
LITR	4.1 c	0.0 b
MIN	46.8 ab	7.7 ab
MIX	67.3 a	37.5 a

* Seedbeds with same letter are not significantly different from each other

Effects of silvicultural systems on emergence of Engelmann spruce and subalpine fir

Analysis of variance showed that there were significant differences in emergence by stands, species and seedbeds, and also with the seedbed X stand and seedbed X species interactions (Table 22).

Table 22. Analysis of variance for effects of silvicultural systems on emergence of Engelmann spruce and subalpine fir on MIN and MOS seedbeds

Source	df	SS	MS	F	P
Stand	2	6270.2	3135.3	4.10 *	0.021
Species	1	7203.5	7203.5	9.41 **	0.003
Seedbed	1	111822.5	11822.5	15.45 **	< 0.001
Stand X Species	2	2495.5	1247.7	1.63 ns	0.204
Stand X Seedbed	2	10538.8	5269.4	6.88 **	0.002
Species X Seedbed	1	5444.7	5444.7	7.11 **	0.010
Stand X Species X Seedbed	2	169.2	84.6	0.11 ns	0.896
Error	60	45921.9	765.4		
Total	71	89866.8			

There was a significant harvesting pattern effect on conifer emergence of both Engelmann spruce and subalpine fir in the ESSF. Compared with the emergence (21.2%) in undisturbed stands, average emergence was higher in partially cuts (35.9%) and lower in clear-cuts (13.4%). The multiple comparison test showed that emergence in partially cut was significantly higher than that in clear-cuts. Emergence in neither partially cut nor clear-cuts was significantly different from that in undisturbed stands (Table 23).

Table 23. Overall emergence of conifers in partially cut, clear-cut and natural stands

Stand	N	Emergence (%)*
Partially cut	24	35.9 a
Clear-cuts	24	13.4 b
Natural	24	21.2 ab

* Emergence (%): mean of emergence percentage of Engelmann spruce and subalpine fir on both MIN and MOS in each stands.

Emergence of Engelmann spruce varied with both seedbeds and stands. Generally, emergence on scalped seedbeds (MIN) was higher than on unscalped seedbeds (MOS). The best conditions for Engelmann spruce emergence occurred on scarified seedbeds in partially cut stands. Percentage emergence on MIN seedbeds in partially cut stands was 93.6%, which was significantly higher than that on scarified seedbeds (MIN) and unscalped seedbeds (MOS) in both natural or clear-cuts stands, or on MOS in partially cut stands (Table 23). Emergence in clear cut stands was only 27% on MIN seedbeds. On unscalped seedbeds (MOS) in partially cut stands, emergence was also poor, only 12%.

For subalpine fir, there was no significant emergence difference among all the three stands and scalping significantly improved the percentage of emergence in partially cut stands but not in the other two stands (Table 24). The highest percentage of emergence, 38.1%, occurred on the scalped seedbed (MIN) in partially cut stands and was only significantly higher than that on the unscalped seedbed (MOS) in the same stands. No significant improvement in emergence was obtained by seedbed preparation (scalping) in clear-cut stands. A notable phenomenon was that there was no emergence of subalpine fir on unscalped forest floor seedbed (MOS) in the partial and the clear-cut stands.

Table 24. Emergence of Engelmann spruce and subalpine fir on MOS and MIN seedbeds in disturbed and natural stands

Stand	Seedbed	Emergence	
		Engelmann spruce	Subalpine fir
Partial cutting	MOS	12.0 c	0.0 b
	MIN	93.6 a	38.1 a
Clear-cut	MOS	4.8 c	0.0 b
	MIN	27.0 bc	7.0 ab
Natural	MOS	19.1 bc	12.8 ab
	MIN	46.8 b	7.7 ab

2.3.4 Survival of emerged first-year Engelmann spruce and subalpine fir seedlings on seedbeds in natural and disturbed stands

Survival is defined here as the number of seedling living by the end of the growing season in 1996 as a percentage of the number of seedling that emerged during the growing season.

Survival data was only available for those treatments and sites where seedlings emerged. On some seedbeds in natural and disturbed stands, no seedlings emerged, therefore seedling survival could not be calculated.

Survival of current-year seedling on seedbeds in natural stands. Table 25 shows the survival of Engelmann spruce and subalpine fir seedlings on treated and undisturbed seedbeds in natural stands. For Engelmann spruce, the best seedbed for survival under natural conditions was rotten logs (LOG), and the worst survival was on the litter-layer removal seedbeds (LITR). Seedling survival of subalpine fir was higher than Engelmann spruce on the MIN seedbeds, nearly the same on MIX, but lower on LOG (Table 25).

Table 25. Engelmann spruce and subalpine fir current-year seedling survival on treated and undisturbed seedbeds in natural stands

Seedbed	Engelmann spruce			Subalpine fir		
	Emergence	Survival		Emergence	Survival	
	N	N	%	N	N	%
LOG	33	33	100.0	12	3	22.2
MOS	21	11	52.0	1	1	100
LIT	0	nv	nv	0	nv	nv
MOSR	72	54	74.6	0	nv	nv
LITR	27	1	3.7	0	nv	nv
MIN	85	38	44.8	17	15	83.9
MIX	91	62	68.0	33	23	69.8

nv Data are not available for calculation

A Chi-square tests showed that there was a significant difference in Engelmann spruce current-year seedling survival among treated and undisturbed seedbeds in natural stands. Further comparison showed that the difference resulted from the higher survival on LOG and lower on LITR. When the LOG and LITR survival data were excluded, there was no significant difference in survival among the remaining four treated and undisturbed seedbed (Table 26). Seedbed treatment did not significantly improve subalpine fir seedling survival (Table 26).

Table 26. Chi-square test for effects of seedbed treatment on conifer current-year seedling survival in natural stands

Species	Seedbed[1]	df	X^2	X^2 Critical value
Engelmann spruce	A	5	30.03*	11.07
	B	4	6.98 ^{ns}	9.49
Subalpine fir	C	3	4.52 ^{ns}	7.81

[1] A. Total six types of seedbed, LOG, MOS, MOSR, LITR, MIN, and MIX.
B. LITR was excluded from A.
C. Total four types of seedbed, LOG, MOS, MIN and MIX.

Effects of silvicultural systems on current-year seedling survival. Seedling survival in disturbed stands was significantly better for Engelmann spruce but not for subalpine fir, compared to in natural stands (Table 27). Chi-square test results showed that survival on MIN in both partially cut and 1-ha clear-cut stands was significantly higher than in natural stands (Table 28). There was no significant difference in survival of subalpine fir between disturbed and natural stands.

Table 27. Seedling survival of Engelmann spruce and subalpine fir on MOS and MIN seedbeds in disturbed and natural stands

		Engelmann spruce			Subalpine fir		
Stand	Seedbed	Emergence	Survival		Emergence	Survival	
		N	N	%	N	N	%
Partial cut	MOS	5	4	83.3	0	nv	nv
	MIN	122	99	80.6	104	80	76.9
Clear-cut	MOS	8	7	80.0	0	nv	nv
	MIN	68	48	70.4	15	4	27.3
Natural	MOS	21	11	52.0	1	1	100
	MIN	85	38	44.8	17	15	83.9

nv Data was not available for calculation.

Table 28. Chi-square tests for effects of silvicultural systems on Engelmann spruce and subalpine fir current-year seedling survival on MIN and MOS seedbeds

Species	Seedbed[1]	df	X^2	X^2 Crit.
Engelmann spruce	A	5	11.38*	11.07
	B	4	2.39 ns	9.49
Subalpine fir	C	2	5.18 ns	5.99

[1] A. Both types of seedbeds, MOS and MIN in the three stands were included.
B. MIN in natural stands was excluded from A.
C. Only MIN seedbeds in the three types of stands were counted.

2.3.5 Summary of conifer seed, germinant and first-year seedling survival on seedbeds in disturbed and natural stands

The survival of Engelmann spruce and subalpine fir seed, germinant and first-year seedlings on natural and disturbed seedbeds in the ESSF is summarized in Tables 29 and 30. Survival of Engelmann spruce from seed to first-year seedling on forest floor seedbeds in natural stands was very low. No Engelmann spruce seedlings survived on litter-type seedbeds and only 1.4% of sown live seed on moss-type seedbeds in the first growing season. More than 85% of seed lost viability before germination. The rest of the loss was result of the failure of emergence of seed that survived the winter. Seedbed treatment with exposing mineral soil (MIN) effectively reduced the total loss and the number of seeds needed to produce would be close to that on LOG. In disturbed stands, seedbed preparation was needed to reduce the total loss and fewer estimated seed was needed to produce one surviving seedling in the first growing season (Table 29).

For subalpine fir, the pattern of the first-year seedling survival from seed in natural stands was as the same as that of Engelmann spruce except the overall survival was less. In disturbed stands, scalping seedbeds also effectively reduced the total loss in partially cut stands, but not in the clear-cuts (Table 30).

Table 29. Summary of Engelmann spruce seed, germinant and first-year seedling survival on seedbeds in disturbed and natural stands in the ESSF

Stand	Seedbed	Viable Seed % A*	Emerged Germinants % B	First-year seedling % C	Survived (%) from seed: Germinant D	Survived (%) from seed: Seedling E	Number of seed required to produce a surviving seedling in the first growing season F
Natural	LOG	37.9	28.7	100	10.9	10.9	9
	MOS	13.9	19.1	52.0	2.7	1.38	72
	LIT	12.2	0.0	---	0.0	0.0	---
	MOSR	42.7	22.7	74.6	9.7	7.2	14
	LITR	38.4	4.1	3.7	1.6	0.1	1000
	MIN	57.3	46.8	44.8	26.8	12.0	8
	MIX	30.8	67.3	68.0	20.7	14.1	7
Partially cut	MOS	2.7	12.0	88.3	0.3	0.3	333
	MIN	45.7	93.6	80.6	42.8	34.5	3
Clear-cut	MOS	28.3	4.8	80.0	1.36	1.1	91
	MIN	66.3	27.0	70.4	17.9	12.6	8

* A = Percentage of viable seed over-wintered on site.
B = Percentage of survived germinant from sown live seed.
C = Percentage of survived seedling from emerged germinants.
D = Percentage of survived germinant from seed before sowing = A X B.
E = Percentage of survived seedling from seed before sowing = A X BX C.
F = Number of seed required to produce each survived first-year seedling = 1/E.
--- Data were not available for calculation.

Table 30. Summary of subalpine fir seed, germinant and first-year seedling survival on seedbeds in disturbed and natural stands in the ESSF

Stand	Seedbed	Viable Seed	Emerged Germinants	First-year seedling	Survived (%) from seed		Number of seed required to produce a surviving seedling in the first growing season
					Germinant	Seedling	
		% A	% B	% C	D	E	F
Natural	LOG	23.1	16.7	22.2	3.9	0.9	111
	MOS	12.3	12.8	11.1*	1.6	0.2	500
	LIT	11.8	0.0	---	0.0	0.0	---
	MOSR	19.1	0.0	---	0.0	0.0	---
	LITR	24.3	0.0	---	0.0	0.0	---
	MIN	41.8	7.7	83.9	32.2	2.7	37
	MIX	23.4	37.5	69.8	8.8	6.1	16
Partially cut	MOS	7.4	0.0	---	0.0	0.0	---
	MIN	53.5	38.1	76.9	20.4	15.7	6
Clear-cut	MOS	10.7	0.0	---	0.0	0.0	---
	MIN	36.9	7.0	27.3	2.6	0.7	142

2.4 Discussion

At Sicamous Creek and in the ESSF generally, rotten logs covered with moss constitute the major seedbed on which Engelmann spruce and subalpine fir naturally regenerate in old, undisturbed stands. Conversely, forest floor seedbed is "lethal" to Engelmann spruce and very few subalpine fir seedlings grow on it. These results agree with previous studies by Griffith (1931), Smith (1955), Day (1964), Daniel and Glatzel (1966), Knapp and Smith (1982), Alexander (1984), Geier-Hayes (1987), and Jonson and Fryer (1996). Almost all naturally regenerated Engelmann spruce saplings and young trees were found growing on or nearby nurse logs or rotted stumps covered with moss during the surveys. In natural conditions, rotted logs and stumps covered with mosses can provide moisture for Engelmann spruce and subalpine fir seedling growth and rooting, and might be pathogen-free thus avoiding seed and seedling loss caused by diseases. However, this type of seedbed is disturbed and the ecological relationship between the host and nursing seedbed are destroyed by large scale clearcutting. In large post-logging openings, temperature, wind, and direct radiation are much higher and relative humidity is lower (Alexander 1987). The moss layer as well as the rotten wood of rotted logs quickly dries out upon exposure, killing young Engelmann spruce and subalpine fir seedlings growing on it.

Engelmann spruce seedlings were not found on the forest floor covered with mosses and litter, moss-type (MOS) or forest floor with litter, litter-type (LIT), but there were a few subalpine seedlings on the forest floor seedbeds. This indicates that there is a factor or some factors in the forest floor that is lethal to Engelmann spruce and nearly always lethal to subalpine fir. The absence of Engelmann spruce seedlings on the forest floor may be due to one of following reasons or their combination: i) lack of seed supply and seed loss from rodents; ii) germination failure; and iii) failure of emergence or establishment. The number of spruce seedlings on rotten logs indicates that the seed supply is sufficient. Hence the failure must be due to either rodents, or ii), or iii), or their combination.

In natural seedbeds, germination on forest floor seedbeds, MOS and LIT was very low for both spruce and fir. On rotten logs, germination for spruce was 37.9%, which was significantly higher than that on forest floor seedbeds (MOS and LIT), and for fir 23.1% which was double, though not significantly different from that on MOS and LIT. The low germination on LIT and MOS for both species suggests that there are some seed-damaging agents in these seedbeds that are not present or common on rotten logs.

We suspected that the damaging agents were fungal seed pathogens residing in the litter layer or the whole forest floor, or the both and that these pathogens invade and kill seed before germination or germinants before emergence during the first winter. If our suspicions are true, treatment by removing both surface and the F+H layers of the forest floor would reduce or eliminate damage by these pathogens. To find out in which layer of soil (L, and/or F and /or H) the majority of seed pathogens reside, the effect of removing either the L or the L, F+H on survival and germination was determined. Two patterns of seed germination rank on original and treated seedbeds are expected from this experiment to prove our hypotheses:

i) if the majority of pathogens live in litter layers only, then, on seedbeds in natural stands, the pattern of germination would be:

Pattern A: MOS / LIT < MOSR / LITR = MIN

ii) if the majority of pathogens live throughout forest floor, the pattern of germination on treated and natural seedbeds would be:

Pattern B: MOS / LIT = MOSR / LITR < MIN

The germination pattern for Engelmann spruce follows pattern A. The highest germination occurred on MIN and the lowest on the forest floor seedbeds, LIT and MOS; litter- and moss-layer removal significantly improved seed germination. Germination on MIX was neither significantly different from that on LIT and MOS, nor from that MOSR and LITR. Most pathogens, or damage agents of Engelmann spruce seed inhabit the litter layer, on both types of litter and moss/litter seedbeds. Our interpretation assumes that there is a significant loss of seed viability on all soils, attributable to several unspecified factors, which reduces germination and emergence to about half. The concern in this study is with the further low of viability found on some but not all seedbeds.

The germination pattern for subalpine fir is close to pattern B. Although the highest germination was on MIN and the lowest on LIT/MOS, only germination on MIN was significantly different from that on LIT/MOS. Other seedbed treatments did not significantly improve seed survival in natural stands. These results suggest that pathogens, or damage agents of subalpine fir seed reside throughout the forest floor.

The silvicultural system used did not significantly affect seed germination. Engelmann spruce germinated best on mineral soil seedbeds in partially cut stands.

A surprising result from this study was that many of the viable Engelmann spruce and subalpine fir seed had already germinated before snow melt. An average 50% (range 29 to 67%) of Engelmann spruce seed on seedbeds in natural and 85% (range 77 to 89%) of Subalpine fir seed germinated before snow melt.

It is usually assumed that, at higher elevations, conifer seed shed in the fall lies dormant on the seedbed under snow during the winter and starts to germinate shortly after snow melt the following spring or summer (Alexander, 1987). Wang (1996) found that the temperature at surface of forest floor seedbed under deep snow (> 30 cm) in the ESSF near Prince George was usually -4 to 0^0 C. We believe that at the Sicamous the temperature of the seedbed at the soil surface was at or slightly above the freezing point for the whole or almost the whole period of snow cover from October to mid June. On the surface of rotten logs, some distance above the forest floor, we would expect temperatures to have been somewhat lower since these locations were within the snowpack. That might explain why on LOG, and for spruce, pre-retrieval germination as a percentage of viable seed was lower than on forest floor seedbeds.

Generally, emergence of live seed on seedbeds in natural stands for both Engelmann spruce and subalpine fir was very low, only 27% for Engelmann spruce and 10.7% for subalpine fir. Emergence for the two species was mainly affected by seedbed type. Litter-type seedbed is the worst seedbed for emergence of the two species, no emergence occurred. Rotten log seedbed was the best undisturbed seedbed in natural stands in the ESSF, with emergence of 28.7% for spruce and 16.7% for fir. Mixed soil (MIX) is the only treatment which can significantly improve Engelmann spruce emergence on both moss-type and litter-type floor seedbeds in natural stands. On litter-type seedbeds, removing all layers above the mineral soil can also significantly increase spruce emergence. However, emergence of subalpine fir can be significantly improved only on litter-type seedbeds by a mixing-soil treatment. No subalpine fir emerged on litter- or moss-layer removed seedbeds (LITR and MOSR). These results suggest that in natural stands, the failure of emergence on forest floor seedbeds could be a further reason for the lack of seedlings on forest floor seedbeds for both Engelmann spruce and subalpine fir.

In disturbed stands, emergence of Engelmann spruce was affected by the combination of harvesting pattern and seedbed preparation. Emergence on scalped seedbed (MIN) in partially cut stands was as high as 94%, but there was no significant improvement in clear-cut stands. For subalpine fir, there was no harvesting pattern effect on emergence. Seedbed preparation significantly improved emergence only in partially cut stands. This suggests that seedbed preparation, such as scalping will improve both emergence and germination for Engelmann spruce, and survival of subalpine fir in partially cut stands.

Seedling survival of Engelmann spruce varied with seedbed type in natural stands during the first growing season. Rotten logs were the best seedbed for Engelmann spruce survival and litter-type forest floor (LIT/LITR) was the worst. Generally, there was no significant effect of seedbed treatment on survival for both Engelmann spruce and subalpine fir and disturbance by harvesting did not significantly improve survival of both Engelmann spruce and subalpine fir.

Loss of conifers from seed sowing to the end of first growing season was very high on forest floor seedbeds in natural stands. Engelmann spruce and subalpine fir barely survived on forest floor seedbeds (LIT/MOS) in the first growing season. It was estimated that 72 spruce seed and 541 fir seeds were needed to produce one first-year seedling on the moss-type seedbeds. Since seed loss from rodents was deliberately avoided in this study, the actual number would be much higher.

The poor survival of both Engelmann spruce and subalpine fir by the end of first growing season on forest floor seedbeds in natural stands was about equally due to loss of seed viability before snow melt and failure of emergence of seed that did survive the winter. More than 85% of sown seed lost their viability before germination. Almost all the rest live-seeds were lost during the emergence except 2.7% of Engelmann spruce and 1.6% of subalpine fir seed surviving on moss-type seedbed (MOS). This could explain why it is hard to find Engelmann spruce and subalpine fir seedlings on forest floor seedbeds in natural stands in the ESSF. Natural regeneration of Engelmann spruce in the ESSF is best following partially cutting and site preparation, such as scalping.

Chapter III. Conifer Seed and Seedling diseases in the ESSFwc2, Interior of British Columbia

3.1 Materials and Methods

The purpose of this study was to: i) identify the species of pathogenic fungi which invade and kill seed on forest floor seedbeds in natural and disturbed stands in the ESSF, ii) evaluate their frequency by seedbed type, and iii) test their effects on conifer seed germination. Special attention was paid to some pathogenic fungi, such as *Caloscypha*, *Racdium* or *Racdium*-like fungi, *Fusarium* and *Cylindrocarpon*. To achieve that goal, a series of experiments were undertaken from 1995 to 1997. These experiments were: i) seed-trapping fungi from natural and disturbed seedbeds; an assay of soil-borne fungi; ii) frequency analysis of putative pathogenic fungi on natural and disturbed forest floor seedbeds and their relationship with conifer seed germination on site; and iii) pathogenicity test of some fungi on Engelmann spruce and subalpine fir seed. In addition, a *Caloscypha* fruiting body (apothecium) survey and other seedling damage agent surveys in natural stands were conducted to obtain further information about conifer seed and seedling diseases in the ESSF.

3.1.1 Soil-borne fungi and their frequency on natural and disturbed seedbeds in natural stands

Experimental Design. Experiments were conducted in both natural stands and disturbed stands to assay soil-borne pathogens from conifer seed on seedbeds.

<u>Frequency of soil-borne fungi from natural and treated seedbeds in natural stands</u> In this experiment Engelmann spruce and subalpine fir seeds were placed on seven different

seedbeds including three types of undisturbed seedbeds and four treated seedbeds, with six replications for each type of seedbed, all in natural forest stands (Table 3).

Silvicultural systems effect on frequency of some pathogenic fungi on seedbeds This experiment was similar to the experiment of silvicultural systems effect in Chapter 2. Two species, three stand types including natural stands, partial cutting and 1-ha clear-cut stands, and two types of seedbeds were utilized in this experiment.

Methods and Procedures. Undisturbed and treated seedbeds were selected and prepared in the summer of 1995. Seedbags with 300 spruce seeds or 150 subalpine fir seeds per bag were placed on seedbeds in mid-September, 1995. The first snow fall in 1995 was on October 3. Seedbags were recovered between June 17 and June 28, 1996, when the seedbeds were still covered with snow. Ungerminated seed from each seedbag was brought to the laboratory, where 20% of the seeds were used to determine their germination and the rest were for pathogen isolation. Details of plot layout and seedbed treatments are described in Chapter 2.

Isolation of Fungi. The ungerminated seeds from each seedbag available for fungal isolation were divided into four equal portions, keeping seeds in each of the six seedbags from each type of seedbed separate. There were averages of 35 seeds of Engelmann spruce and 20 seeds of subalpine fir in each portion (from one bag) or about 210 Engelmann spruce seeds and 120 subalpine fir seeds from each type of seedbed. Thus, from the natural stand, 5880 spruce seed (7 seedbeds X 4 isolation media X 6 replications X 35 seeds) and 3360 subalpine fir seed (7 seedbeds X 4 isolation media X 6 replications X 20 seeds) were examined in this part of the study. From each of the 1-ha clearcut and the partially cut stand, 1680 spruce seed (2 seedbeds X 4 media X 6 replications X 35 seeds) and 960 subalpine fir seed (2 seedbeds X 4 media X 6 replications X 20 seeds) were examined in this part of the study.

Four media were used to isolate specific pathogenic fungi from the seeds. Seeds were cleaned with tap water, surface sterilized (surface sterilization method for each medium is described in the details of isolation procedure below), placed on the media, left in incubators at various temperatures for a period of time until fungal colonies could be identified using a light microscope. Fifteen spruce seeds or 10 subalpine fir seeds were placed in each 9 cm diameter petri-dish.

The details of isolation procedure: To isolate *Caloscypha fulgens,* one portion of cleaned seeds of Engelmann spruce and subalpine fir was surface sterilized in a 30% hydrogen peroxide for 30 minutes and washed three times with sterilized distilled water. Seeds were put on plates with 2% water agar (WA) medium, and incubated at 15^0 C for 2-3 weeks (Sutherland and Woods 1978). The seeds with *Caloscypha fulgens* or other fungi were counted and the frequency of each fungus was calculated as the number of infected seeds expressed as a percentage of the total number of seeds placed on the medium.

To isolate *Racodium* or similar fungi, cleaned seeds were surface sterilized in 10% bleach solution for 5 minutes, then washed three times with sterilized, distilled water. Seeds were put on potato sucrose agar (PSA) medium and incubated at 5^0 C for 3 to 4 weeks (Sato *et al.*1960; Cheng 1989). The seeds with each isolate were counted and expressed as a percentage of tested seeds.

To isolate species of *Cylindrocarpon* and *Fusarium*, seeds were placed directly on Komado medium (KMB) and incubated at room temperature for 5 to 10 days (Singleton *et al.* 1992).

To isolate other fungi, the last portion of seeds was surface sterilized in 10% bleach solution for 5 minutes and washed three times with sterilized distilled water, then the seeds were put on potato dextrose agar (PDA) medium at 20^0 C for 3 to 7 days. Cultures were examined once a week for up to 3 weeks.

Fungal identification. Identification was based on cultural characteristics and morphology of the fungi. Most of the isolates were identified to genus due to limited time and knowledge. Several references by Barnett and Hunter (1987), Barron (1968), Carmichael *et al.* (1968), Domsch *et al.* (1980), Sutherland et al. (1987) and Watanabe (1994) were used for general identification.

Data Analysis. Calculation of frequency of putative fungi. Frequency of a putative pathogenic fungus is the estimate of the number of seeds in the seedbag infected by that fungus expressed as a percentage of the total seeds in the bag. An infected seed is defined as seed from which a putative pathogen was isolated and which did not germinate on the culture medium. The frequency of each isolate was therefore calculated as the followings:

Frequency of an isolate (%) = (Number of infected seed (by isolate) /Total seeds)*100%.

Number of infected seed (by isolate) = Percentage of seed isolated from the medium with the highest frequency for the isolate in question* number of ungerminated seeds on the medium.

Total seed = total germinated seed + total ungerminated seed = initial number of seed in seed bag.

Statistical analysis. Balanced ANOVA and General Linear Model Analysis were employed in analyzing frequency of putative, soil-borne pathogenic fungi from treated and undisturbed seedbeds in natural stands, stand treatment and pathogenicity test with MINITAB for

Windows and Microsoft EXCEL. Simple linear regression analysis was used to determine the relationship between the frequency of individual putative pathogenic fungi and seed viability. The degrees of freedom varied by experiment. For fungal frequency analysis from seedbeds in natural stands, 84 degrees of freedom (2 tree species X 7 seedbeds X 6 replications) were used. For the silvicultural systems effect experiment, there were 72 degrees of freedom (2 tree species X 3 types of stands X 6 replications). Multiple regression analysis of frequency of pathogenic fungi on germination was conducted for each tree species, and utilized a total of 66 samples from seedbeds in both natural and disturbed stands, 11 types of treated and undisturbed seedbeds from the three stands (7 seedbeds from natural stand, 2 from the clear-cut stand and 2 from the partially cut stand, with 6 replications for each seedbed type) were included.

3.1.2 *Caloscypha fulgens* and its effects on Engelmann spruce in the ESSF

Caloscypha fulgens was one of the pathogens of conifer seed in the ESSF that we expected to be of major importance in this study. Additional experiments with this fungus included: i) field fruiting body (apothecia) survey; and ii) a test of Engelmann spruce germination on inoculated soil.

Field fruiting body (apothecia) survey. A *C. fulgens* fruiting body survey was conducted from mid- to late June of 1995 and 1996. A line inspection method was used in the survey. The four main survey routes were the B-, D-, F- and the H-transaction line (Figure 1). The distance between each two survey routes was 200 m and the long distance, from the

Sicamous Creek to top of the hill was 1500 m. The surveys covered most of control and partially cut sites.

Effects of *Caloscypha fulgens* on Engelmann spruce --- Inoculation test. During the fruiting body survey in spring of 1995, *Caloscypha* apothecia were common on forest floor seedbeds in natural stands, especially on moss-type seedbeds (MOS) on moist sites. In late June, 1995, three samples of forest floor material, each with a *Caloscypha* fruiting body, and three similar samples collected from a location at least 3 m from the nearest *C. fulgens* fruiting body were collected and stored at 0 to 4 ^{0}C.

In September, 1995, the soil samples were retrieved and thawed. One hundred seeds of Engelmann spruce were sown in each pot. These pots with seeds were put in an incubator at 0 to 5^0 C for 4 months and then moved to room temperature to stimulate seed germination. Ungerminated seeds from both infested soils and uninfested soils were used to isolate *C. fulgens* on water agar medium.

3.1.3 Black mold fungus and its effects on Engelmann spruce and subalpine fir germination

One of the common isolates from the main seed isolation study was an as yet unidentified black mold. This isolate was most common on seedbeds with low germination. Some further research on this fungus was done after frequency estimation, including culturing of the fungus and a pathogenicity test on conifer seeds *in vitro*.

Cultural characteristics of the black mold fungus. Experiments dealing with growth rate on various media and optimum growth temperature for black mold were done during the winter of 1996 and 1997. Knowledge of these attributes is a basic requirement for further study of this fungus.

Optimum growth medium. Two percent water agar, PDA and PSA were the three media selected for this study. Isolates were inoculated into five petri-dishes of each medium. The plates were incubated at 15^0 C for 3 weeks. The diameter of the colony in each plate was measured every 2 days. One-way ANOVA was employed in the data analysis.

Optimum growth temperature. PDA was selected for this experiment since the black mold fungus showed fastest growth on this medium although no significant difference from that on PSA medium. Five temperatures (5, 10, 15, 23, and 30^0C) were chosen to incubate the fungus. 30 plates of the medium were inoculated with black mold fungus and three of each were maintained at one of the five temperatures. Diameter of the colony in each plate was measured along two marked lines every 3 days. There were total 7 measurements within the incubation period of 21 days.

Data analysis. Analysis of variances was employed to examine temperature and time effects on black mold fungus growth *in vitro.*

Pathogenicity test of the black mold fungus on Engelmann spruce and subalpine fir. One of the common isolates from dead seed was an as yet unidentified black mold. This experiment was designed to determine the pathogenicity of the black mold isolate.

Engelmann spruce seed (Seedlot # 29751, see Table 5) that had been stored in a refrigerator at 0^0 C, was soaked in running tap water for 24 hr. Before the soaking, seeds were put in 10% bleach solution for 5 minutes to reduce fungi on the seed surface. Seeds were dried at room temperature for 6 hr. and put in refrigerator at 0 to 5^0 C for 21 days. Seeds were removed to room temperature for 12 hr. before sowing.

Subalpine fir seeds were collected by Bev Athirs at Gavin Lake (near Williams Lake, B.C.) in late August, 1996. These seeds were soaked in running tap water for 2 hr, then soaked in 10% bleach solution for 10 min. and back to tap water for another 46 hr. The seeds were dried for 12 hr, and kept at 0 - 5^0 C for 28 days. Then the seeds were removed to room temperature for 24 hr before sowing.

Twenty plates of PSA medium were inoculated with the black mold. Three, 0.2 to 0.3 cm inoculum plugs were transferred to each plate so that the fungus could quickly grow over the plate from the three sources. Another 20 plates of PSA served as control. The 40 plates were incubated at 15^0 C for 12 days, at which time colonies were 1.5 cm in diameter and ready for seed sowing.

Ten seeds of Engelmann spruce or subalpine fir were placed on each inoculated plate. The same number of seeds placed on non-inoculated plates served as the control. The plates (20 each with Engelmann spruce or subalpine fir seeds) were incubated at 15^0 C for 35 days. Within the period, seed germination on each plate was examined twice a week. Percentage of germination was counted. One-way ANOVA was used to analyze the data.

3.1.4 Survey of Engelmann spruce and subalpine fir seedling diseases in natural and disturbed stands

A survey of Engelmann spruce and subalpine fir seedling diseases was conducted during 1995 and 1996 growing seasons. Routine inspection and permanent plot survey were used in seedling disease survey.

Routine inspection was conducted during other survey work, such as the seedbed type and naturally regenerated seedling survey, *Caloscypha fulgens* fruiting survey, and seedling development survey and was carried on during the two growing seasons. Seedlings were examined for typical diseases during growing season. In late June to early July, special attention was given to brown felt blight disease (*Herpotrichia juniperi* (Duby) Petr.). During July to late of August, attention was focused on needle disease, and other damage. When a typical disease was found, 100 seedlings nearby or seedlings with a 20-m^2 plot (4 m X 5 m) were examined to measure disease incidence.

Labeled, 1 to 5 year-old seedlings on permanent plots for the seedling development study were examined for fungal disease or other damage agents every 2 weeks through the whole growing season. The dead, dying or damaged seedlings were recorded and the pathogen or damage agent was noted.

3.2 Results

3.2.1 Identity of fungal isolates from Engelmann spruce and subalpine fir seed

Fungi from original seed source. Both Engelmann spruce and subalpine fir seeds were tested for any potential putative pathogenic fungi from original seed sources after stratification. Three genera of fungi were commonly isolated from on Engelmann spruce seeds and five from subalpine fir. Species of *Trichoderma* and *Penicillium* were the most common fungi found on both Engelmann spruce and subalpine fir seeds with more than 50% each. Twenty-three percent of both species yielded a *Phoma* species. Another two saprophytic fungi found only on subalpine fir seeds were species of *Rhizopus* and *Memnonilla*. Pathogenic seed-borne fungi, such as *Caloscypha fulgens*, *Sirrococcus* sp., *Fusarium* spp., and *Cylindrocarpon* spp. were not found on seed of either of the two tree species of seed (Table 31).

Table 31. Fungi from stratified Engelmann spruce and subalpine fir seed from Tree Seed Center, BCMOF*

Fungus	Engelmann spruce				Subalpine fir			
	Isolate	%	T (^{0}C)	Medium	Isolate	%	T (^{0}C)	Medium
Memnonilla sp.	-	0.0	23	WA	+	1.6	23	WA
Penicillium spp.	+	59.3	15	WA	+	68.5	15	WA
Phoma sp.	+	22.7	5	PSA	+	23.3	5	PSA
Rhizopus spp.	-	0.0	23	PDA	+	5.0	23	PDA
Trichoderma spp.	+	72.0	23	WA	+	50.0	5	PSA

* The highest germination during fungal assay was 90% for Engelmann spruce on WA medium and 40% for subalpine fir on KMB medium at room temperature.

Soil-borne fungi seed-trapped from forest floor seedbeds in natural stands. The fungi isolated from seeds on forest floor seedbeds belonged to 10 from Engelmann spruce seed and subalpine fir. *Sirococcus* sp. was only found on spruce seeds from mineral soil and *Mucor* sp. occurred only on subalpine fir seed from moss-type seedbeds (MOS) and organic layer of soil (Table 32).

A black mold fungus (not as yet identified) and *Rhizoctonia* spp. were the two most common probably pathogenic fungi isolated from both Engelmann spruce and subalpine fir

Table 32. Fungi from Engelmann spruce and subalpine fir seed over-wintered on seedbeds in natural stands

Fungus	Engelmann spruce				Subalpine fir			
	L and F1 layer		H layer		L and F1 layer		H layer	
	Litter	Moss+ L	Organic	Mineral	Litter	Moss+ L	Organic	Mineral
Black mold fungus	+	+	+	+	+	+	+	+
Botrytis sp.	+	+	+	+	-	+	-	+
Caloscypha fulgens	-	+	-	+	-	+	-	+
Fusarium spp.	-	-	-	+	-	-	-	+
Mucor spp.	-	-	-	-	-	+	+	-
Penicillium spp.	+	+	+	+	+	+	+	+
Phoma sp.	+	+	-	-	+	+	-	-
Rhizoctonia spp.	+	+	+	+	+	+	+	+
Rhizopus spp.	+	+	-	-	+	+	-	-
Sirrococcus sp.	-	-	-	+	-	-	-	-
Trichoderma spp.	+	+	+	+	+	+	+	+

seed. Only *Penicillium* spp. and *Trichoderma* spp. which we do not consider to be pathogenic, and which were common on the seedlot before sowing, were more common. *Caloscypha fulgens* was isolated from seed from moss-type forest floor seedbeds (MOS) and mineral soil (MIN). Other seed or seedling pathogens isolated from seeds over-wintered on site were *Fusarium* spp., *Cylindrocarpon* spp., *Sirrococcus* sp. and *Botrytis* sp. These fungi were only isolated from seeds on mineral soil seedbeds except Botrytis sp. which also found on seeds from forest floor and organic soil

3.2.2 Frequency of putative pathogenic fungi from undisturbed and treated seedbeds in natural and disturbed stands in the ESSF

Most of the isolates listed in Table 32, are considered to be non-pathogenic on seed. *Caloscypha fulgens* and *Rhizoctonia* have been previously reported as seed pathogens (Hayashi and Endo 1975; Huang and Kuhlman 1989; Ito *et al.* 1955; Sutherland 1987). These plus the as yet unidentified black mold isolate were considered the isolates of interest, and are discussed in detail later.

Frequency of putative pathogenic fungi from conifers on seedbeds in natural stands. On natural forest floor seedbeds in the Sicamous Creek Research forests, the black mold isolate and *Rhizoctonia* spp. were the most common soil-borne fungi isolated from seed. *Caloscypha fulgens* was found on seeds from both moss-type seedbeds (MOS) and mineral soil (MIN). Analysis of variance shows that the frequency of *C. fulgens* was not significantly different between species, seedbeds and species-seedbeds interaction, but that the frequency of both the black mold and *Rhizoctonia* was significantly different between species and among seedbeds (Table 33).

Table 33. Analysis of variance for effects of seedbed treatment on frequency of putative pathogenic fungi from conifer seed over-wintered on seedbeds in natural stands

Table 33-1. Analysis of Variance for frequency of *Caloscypha*

Source	df	SS	MS	F	P
Species	1	4.76	4.76	0.22 [ns]	0.643
Seedbeds	6	151.88	25.31	1.15 [ns]	0.341
Species X Seedbeds	6	98.89	16.48	0.75 [ns]	0.611
Error	70	1536.70	21.95		
Total	83	1792.23			

[ns] Not significantly different at 0.05.

Table 33-2. Analysis of Variance for frequency of the black mold fungus

Source	df	SS	MS	F	P
Species	1	2928.0	2928.0	5.85 *	0.018
Seedbeds	6	17635.8	2939.3	5.88 *	< 0.001
Species X Seedbeds	6	2173.3	362.2	0.72 ns	0.632
Error	70	35018.3	500.3		
Total	83	57755.5			

* Significantly different at 0.05.

Table 33-3. Analysis of Variance for frequency of *Rhizoctonia*

Source	df	SS	MS	F	P
Species	1	3279.3	3279.3	10.11*	0.002
Seedbeds	6	8750.0	1458.3	4.50 *	0.001
Species X Seedbeds	6	1001.8	167.0	0.51 ns	0.795
Error	70	22701.4	324.3		
Total	83	35732.5			

The low frequency of *C. fulgens* was surprising. *Caloscypha fulgens* apothecia were found in most of the area in late June of 1995 (see below). On natural forest floor seedbeds, the average frequency of *C. fulgens* was 1.1% from subalpine fir seed and 0.7% from Engelmann spruce seed (Table 34). So it appears that *C. fulgens* does not play a significant role in the loss of viability of seed over-wintering on forest floor. This fungus will be discussed again in Section 3.2.3.

Table 34. Overall frequency of putative pathogenic fungi isolated from Engelmann spruce and subalpine fir seed in natural stands [1].

Species	Total Germination (%)	Percentage of Seed with fungus: *Caloscypha* ns (%)	Black mold (%)	*Rhizoctonia* (%)
Engelmann spruce	33.3 a	0.7	16.7 b	11.8 b
Subalpine fir	22.3 b	1.1	28.5 a	24.3 a
D-Critical Value	7.2	---	9.8	7.9

[1] Average 2100 spruce and 1050 fir seeds per plot with 6 replications on each type of seedbed;

Overall, the average frequency of the black mold and *Rhizoctonia* isolated from Engelmann spruce seed is significantly less than that from subalpine fir (Table 34). This was largely attributable to lower frequencies on altered forest floors; the differences were not significant on MOS and LIT (Table 35).

On undisturbed seedbed, the frequency of the black mold and *Rhizoctonia* from Engelmann spruce and subalpine fir seed varied with seedbed type. The highest frequency of the black mold on both Engelmann spruce and subalpine fir was isolated from moss-type seedbeds (MOS). The lowest was from rotten logs (LOG). For *Rhizoctonia*, the highest frequency was on litter-type seedbeds (LIT) for both tree species. The lowest was from spruce seed on rotten logs (Table 35).

Table 35. Frequency of pathogenic fungi from Engelmann spruce and subalpine fir seed over-wintered on undisturbed and treated seedbeds in natural stands [1,2]

Seedbed Type	Engelmann spruce				Subalpine fir			
	Total	Percentage of seed with fungus			Total	Percentage of seed with fungus		
	Germination	*Caloscypha* *ns*	BM	*Rhizoctonia*	Germination	*Caloscypha* *ns*	BM	*Rhizoctonia*
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
LOG	37.9 b	0.0	0.0 c	1.7 b	23.1 ab	0.0	1.4 d	20.6 b
MOS	14.0 c	0.2	52.5 a	14.2 ab	12.3 b	1.2	49.0 a	24.3 b
LIT	12.2 c	0.0	29.0 ab	31.8 a	11.8 b	0.0	36.2 ab	49.9 a
MOSR	42.7 ab	1.0	13.7 bc	7.4 b	19.1 b	0.0	31.1 abc	25.3 b
LITR	38.4 ab	0.0	10.7 bc	16.0 ab	24.3 ab	0.0	21.7 bcd	16.7 b
MIN	57.3 a	1.4	3.2 c	3.5 b	41.8 a	6.7	8.0 cd	7.9 b
MIX	30.8 bc	2.0	7.6 bc	7.8 b	23.4 ab	0.0	32.1 abc	25.1 b
D-Critical Value	19.1	---	25.8	20.8	19.1	---	25.8	20.8

[1] Average 300 spruce and 150 fir seeds per plot with 6 replications on each type of seedbed.

[2] Percentages on seedbeds with same letter are not significantly different from each other.

[ns] No statistically difference among seedbeds.

Effects of seedbed treatment on frequency of putative pathogens in natural stands

The seedbed treatment significantly reduced the frequency of the black mold and *Rhizoctonia* spp. from Engelmann spruce seed (a=0.05). The best seedbed treatment was removal of the whole forest floor (MIN). Other treatments involving the removal of part of the organic horizons or mixing them with mineral soil, did reduce seed infection but no statistically significantly differences (Table 35). A very low percentage of *C. fulgens* was found on treated seedbeds of MIN, MIX and MOSR. There is no significant difference for *C. fulgens* frequency on Engelmann spruce seeds among those seedbeds (a=0.05).

For subalpine fir, the frequency of the black mold and *Rhizoctonia* on treated seedbeds was higher than that from Engelmann spruce. Frequency of the black mold was only significantly reduced on MIN and was more variable on other treated seedbeds; hence there was less clear distinction between seedbeds. The order of seedbeds was not significantly different than in Engelmann spruce (Table 35). *Caloscypha fulgens* was found on both MOS and MIN seedbeds with low frequency (Table 35).

Effects of silvicultural systems on frequency of putative fungi on conifer seeds

Generally speaking, the stand type had no significant effects (a=0.05) on frequency of putative pathogenic fungi on seeds of Engelmann spruce and subalpine fir. Also there was no significant difference between the two species. The frequency of the black mold and *Rhizoctonia* was significantly different among the seedbeds and the combinations of seedbed and stand (Table 36).

Table 36. Analysis of variance for effects of silvicultural systems effects on frequency of pathogenic fungi on MOS and MIN

Table 36 - 1. Analysis of Variance for frequency of *Caloscypha*

Source	df	SS	MS	F	P
Species	1	0.15	0.15	0.01	0.941
Stand	2	60.94	30.47	1.16	0.320
Seedbeds	1	6.17	6.17	0.23	0.630
Species X stand	2	99.70	49.85	1.90	0.159
Species X Seedbeds	1	40.93	40.93	1.56	0.217
Stand X Seedbeds	2	99.85	49.93	1.90	0.158
Species X Stand X Seedbeds	2	20.50	10.25	0.39	0.678
Error	60	1575.46	26.26		
Total	71	1903.69			

Table 36 -2. Analysis of Variance for frequency of the BM fungus

Source	df	SS	MS	F	P
Species	1	1218.9	1218.9	2.00	0.163
Stand	2	2030.0	1015.0	1.66	0.198
Seedbeds	1	14891.5	14891.5	24.40* *	< 0.001
Species X Stand	2	1899.4	949.7	1.56	0.219
Species X Seedbeds	1	211.4	211.4	0.35	0.558
Stand X Seedbed	2	4342.7	2171.3	3.56 *	0.035
Species X Stand X Seedbed	2	788.8	394.4	0.65	0.528
Error	60	36625.6	610.4		
Total	71	62008.3			

* Significantly different at 0.05.

** Significantly different at 0.01.

Table 36 - 3. Analysis of variance for frequency of *Rhizoctonia*

Source	df	SS	MS	F	P
Species	1	141.0	141.0	0.30	0.586
Stand	2	2631.1	1315.6	2.79	0.069
Seedbed	1	13431.3	13431.3	28.52* *	< 0.001
Species X Stand	2	192.5	96.3	0.20	0.816
Species X Seedbed	1	7.3	7.3	0.02	0.901
Stand X Seedbed	2	4074.3	2037.1	4.33*	0.018
Specie X Stand X seedbed	2	156.7	78.3	0.17	0.847
Error	60	28253.3	470.9		
Total	71	48887.5			

The frequencies of putative pathogens from spruce seeds on mineral soil seedbeds (MIN) in all three silvicultural treatments were significantly lower ($p<0.001$) than those on natural seedbeds (MOS) except in the clear-cut stands, in which black mold was also low on MIN. There were two substantial changes in fungal frequency from Engelmann spruce seed on natural seedbeds (MOS). First, the frequency of black mold in forest floor seedbeds in clear-cut stands was substantially reduced, from 52.5% in natural stands and 31.1% in partially cut to 0.3% in clear-cut stands (Table 37). The second big change was the frequency of *Rhizoctonia* on forest floor seedbeds (MOS) in partially cut stand. Its frequency was significantly increased compared with that on same seedbeds in natural stand and clear-cut stand.

For subalpine fir seed, the silvicultural system treatment did not significantly (a=0.05) affect the frequency of putative pathogenic fungi. On mineral soil exposed by scarification, the frequency of all putative pathogens was significantly less than on MOS. The only significant interaction was on the forest floor seedbeds (MOS) in partially cut stands, where the frequency of *Rhizoctonia* was significantly higher than on the same type of seedbeds in both natural and clear-cut stands (Table 37).

Relationship of percentage frequency of pathogenic fungi with conifer seed germination

In the previous section it was shown that the frequency of pathogenic fungi varied among seedbeds. This section deals with the relationship between seed viability and the frequency of pathogens. Seedbeds with a high frequency of pathogens had lower seed germination rates, which indicates that seed germination decline may be attributed to seed infection. In this section, regression analysis for individual tree species were conducted to test for relationship between frequency of putative pathogenic fungi and seed viability.

Table 37. Frequency of putative pathogenic fungi isolated from Engelmann spruce and sublapine fir seed as percentage of seed sown on seedbeds in natural and disturbed stands [1,2]

Stand	Seedbed	Engelmann spruce				Subalpine fir			
		Total	Percentage of seed with fungus			Total	Percentage of seed with fungus		
		Germination	*Caloscypha* [ns]	BM	*Rhizoctonia*	Germination	*Caloscypha* [ns]	BM	*Rhizoctonia*
		%	%	%	%	%	%	%	%
Partially cut	MOS	2.7 d	4.9	31.1a	55.4 a	7.4 b	0.0	41.4 a	48.9 a
	MIN	45.7 ab	0.8	0.3 b	2.1 c	53.5 a	0.2	8.2 b	5.1 d
1-ha clear-cut	MOS	28.3 bc	0.0	0.3 b	23.2 b	10.7 b	0.0	33.1 a	30.9 b
	MIN	66.3 a	0.0	4.4 b	7.3 c	36.9 a	0.0	12.3 b	10.8 cd
Natural	MOS	13.9 cd	0.2	52.5 a	14.2 b	12.3 b	1.2	49.0 a	24.3 bc
	MIN	57.3 a	1.4	3.2 b	3.5 c	41.8 a	6.7	8.0 b	7.9 d
D - Creiticl value		22.8	---	20.8	15.3	22.8	---	20.8	15.3

[1] Approximately 300 spruce and 15 fir seed per plot with 6 replications from each seedbed type

[2] Percentage on seedbeds with same letter are not significantly different from each other

[ns] No statistically significant difference among seedbeds

There was a significant linear relationship between spruce seed viability and the frequency of Black mold, *Rhizoctonia*, and *Caloscypha* present as independent variables. In simple linear models, both the black mold fungus (BM) or *Rhizoctonia* showed very significant regression relationship ($p<0.001$) (Tables 38-1 and 38-2), while *Caloscypha* was significant at $p=0.023$ (Table 38-3). In a regression model including all three pathogens as independent variables, partial F tests showed that frequency of both BM and *Rhizoctonia* were significantly related to seed viability at $p<0.001$ (Tables 39-1 and 39-2) but that of *Caloscypha* was not.

The relationship between putative pathogens and subalpine fir seed viability was similar to that for Engelmann spruce. The frequency of BM and *Rhizoctonia* had a significant linear relationship with viability of subalpine fir (Tables 40-1 and 40-2) and multiple linear regression models (Tables 41-1 and 41-2). However, the value of coefficient of determination (R^2) in the multiple linear model with independents of BM and *Rhizoctonia* was 41.5% (Table 41-2), less than that of Engelmann spruce (R^2 = 54.3) (Table 39-2).

Particular attention is given to the constants in the two multiple regression models. The constant, 51.9% for Engelmann spruce and 38.3% for subalpine fir, indicates seed viability of the two tree species. When both BM and Rhizoctonia are absent on the forest floor seedbeds, the viability for spruce and fir from the models is 51.9% and 38.3% respectively. Compared with the original seedlot germination percentage (96% for spruce and 77% for fir), the difference, about 40% loss in viability, between the constant and that of the original seed lot is presumably due to factors other than pathogens.

Table 38. Simple linear regression analysis for frequency of the black mold fungus, *Rhizoctonia* and *Caloscypha* versus Engelmann spruce seed viability in the ESSF

Table 38-1. Simple linear regression analysis for frequency of the black mold fungus vs. Engelmann spruce seed germination

Regression equation

Germination (%) = 45.4 - 0.639 BM

Predictor	Coef	Stdev	t-ratio	p
Constant	45.350	3.140	14.44	<0.001
BM	-0.640	0.105	-6.07	<0.001

s = 20.68 R-sq = 36.5% R-sq(adj) = 35.5%

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	1	15744	15744	36.80**	<0.001
Error	64	27377	428		
Total	65	43121			

Table 38-2. Simple linear regression analysis for frequency of *Rhizoctonia* versus Engelmann spruce seed germination

Regression equation

Germination (%) = 45.8 - 0.774 *Rhizoctonia*

Predictor	Coef	Stdev	t-ratio	p
Constant	45.828	3.474	13.19	<0.001
Rhizoctonia	-0.774	0.148	-5.24	<0.001

s = 21.72 R-sq = 30.0% R-sq(adj) = 28.9%

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	1	12941	12941	27.44**	<0.001
Error	64	30180	472		
Total	65	43121			

Table 38-3. Simple linear regression analysis for frequency of *Caloscypha* vs. Engelmann spruce seed germination

Regression equation

Germination (%) = 36.8 - 2.77 *Caloscypha*

Predictor	Coef	Stdev	t-ratio	p
Constant	36.816	3.269	11.26	<0.001
Caloscypha	-2.773	1.195	-2.32	0.023

s = 24.93 R-sq = 7.8% R-sq(adj) = 6.3%

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	1	3349.9	3349.9	5.39*	0.023
Error	64	39770.7	621.4		
Total	65	43120.6			

Table 39. Multiple linear regression analysis for frequency of pathogenic fungi versus Engelmann spruce seed viability in the ESSF

Table 39-1. Multiple linear regression analysis of frequency of the BM, *Rhizoctonia* and *Caloscypha* versus Engelmann spruce seed germination

Regression equation

Germination (%) = 52.8 - 0.477 BM - 0.582 *Rhizoctonia* - 1.60 *Caloscypha*

Predictor	Coef	Stdev	t-ratio	p
Constant	52.764	3.101	17.01	< 0.001
BM	-0.4768	0.097	-4.90	< 0.001
Rhi	-0.5815	0.1266	-4.59	< 0.001
Cal	-1.6032	0.8765	-1.83	0.072

s = 17.83 R-sq = 54.3% R-sq(adj) = 52.1%

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	3	23401.7	7800.6	24.53**	< 0.001
Error	62	19718.9	318.0		
Total	65	43120.6			

SOURCE	df	SEQ SS
BM	1	15743.6
Rhizoctonia	1	6594.1
Caloscypha	1	1064.0

Table 39-2. Multiple linear regression analysis of frequency of the black mold fungus and *Rhizoctonia* versus Engelmann spruce seed germination

Regression equation

Germination (%) = 51.9 - 0.516BM - 0.576 *Rhizoctonia*

Predictor	Coef	Stdev	t-ratio	p
Constant	51.855	3.118	16.63	< 0.001
BM	-0.516	0.097	-5.34	< 0.001
Rhizoctonia	-0.576	0.129	-4.47	< 0.001

s = 18.16 R-sq = 51.8% R-sq(adj) = 50.3%

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	2	22338	11169	33.86	< 0.001
Error	63	20783	330		
Total	65	43121			

SOURCE	df	SEQ SS
BM	1	15744
Rhizoctonia	1	6594

Table 40. Simple linear regression analysis for frequency of the black mould fungus and *Rhizoctonia* versus subalpine fir seed viability in the ESSF

Table 40-1. Simple linear regression analysis for frequency of the Black Mold (BM) vs. subalpine fir seed germination

Regression equation

Germination (%) = 31.0 - 0.284 BM

Predictor	Coef	Stdev	t-ratio	p
Constant	31.009	2.594	11.95	< 0.001
BM	-0.284	0.069	-4.13	< 0.001
s = 15.99	R-sq = 21.1%	R-sq(adj) = 19.8%		

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	1	4369.8	4369.8	17.08	< 0.001
Error	64	16371.1	255.8		
Total	65	20740.9			

Table 40-2. Simple linear regression analysis for frequency of *Rhizoctonia* versus subalpine fir seed germination

Regression Equation

Germination (%) = 31.04 - 0.306 *Rhizoctonia*

Predictor	Coef	Stdev	t-ratio	p
Constant	31.036	2.658	11.68	< 0.001
Rhi	-0.306	0.077	-3.97	< 0.001
s = 16.13	R-sq = 19.7%	R-sq(adj) = 18.5%		

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	1	4092.3	4092.3	15.73	< 0.001
Error	64	16648.7	260.1		
Total	65	20740.9			

Table 41. Multiple linear regression analysis for frequency of pathogenic fungi versus subalpine fir seed viability in the ESSF

Table 41-1. Multiple linear regression analysis for frequency of the black mold fungus, *Rhizoctonia* and *Caloscypha* vs. subalpine fir seed germination

Regression Equation

Germination (%) = 39.2 - 0.301 BM - 0.321 Rhi - 0.658 Cal

Predictor	Coef	Stdev	t-ratio	p
Constant	39.247	2.720	14.43	< 0.001
BM	-0.301	0.059	-5.12	< 0.001
Rhi	-0.321	0.065	-4.92	< 0.001
Cal	-0.658	0.340	-1.94	0.057
s = 13.58	R-sq = 44.9%	R-sq(adj) = 42.2%		

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	3	9305.4	3101.8	16.82	< 0.001
Error	62	11435.5	184.4		
Total	65	20740.9			

SOURCE	df	SEQ SS
BM	1	4369.8
Rhi	1	4243.7
Cal	1	691.9

Table 41-2. Multiple linear regression analysis of frequency of the black mold fungus and *Rhizoctonia* vs. subalpine fir seed germination

Regression Equation

Germination (%) = 38.3 - 0.289 BM - 0.312 *Rhizoctonia*

Predictor	Coef	Stdev	t-ratio	p
Constant	38.268	2.730	14.02	< 0.001
BM	-0.289	0.060	-4.85	< 0.001
Rh	-0.312	0.066	-4.70	< 0.001
s = 13.87	R-sq = 41.5%	R-sq(adj) = 39.7%		

Analysis of Variance

SOURCE	df	SS	MS	F	p
Regression	2	8613.5	4306.8	22.37	< 0.001
Error	63	12127.4	192.5		
Total	65	20740.9			

SOURCE	df	SEQ SS
BM2	1	4369.8
Rhi2	1	4243.7

3.2.3 *Caloscypha fulgens* and its effect on conifer seed germination on seedbeds in the ESSF

***Caloscypha fulgens* apothecium survey.** *Caloscypha fulgens* is one of the major pathogenic fungi to be investigated in this study. The apothecium survey was designed to determine where this fungus was present in this area and under which conditions it occurred. The *C. fulgens* fruiting body (apothecium) survey was conducted from mid June to early July in 1995 and 1996 just after snow melt.

In 1995, snow started melting at the lowest elevation in mid June and was last seen at the top of the hill at D-1650 on July. Apothecia of *C. fulgens* were found in most of the control and partially cut stands.

In natural stands, the yellow-orange color, cup-shaped apothecia (1 to 3 cm diameter) of *C. fulgens* appeared just at edge of the melting snow pack on moss/litter-type (MOS) forest floor seedbeds, usually on flat ground under shade, where snow melts slowly. Cool and wet conditions are likely required for *C. fulgens* apothecium development. No apothecia of *C. fulgens* were found on litter-type seedbeds (LIT), where snow usually melts 1 to 2 weeks earlier and the surface layer of the forest floor dried out quickly. Apothecia lasted on the ground for 2 weeks and disappeared when it got warmer. In early July, 1995, apothecia of *C. fulgens* could still be found on top of hill near a stream with flowing water melted from a deep snow pack.

In disturbed stands, apothecia of *C. fulgens* were found in partially cut stands only where impact on the forest floor was minimal. No apothecia of *C. fulgens* were found on disturbed forest floor seedbeds in partially cut or on any seedbeds in clear-cut sites where snow was melt more than 10 days earlier than in undisturbed stands.

In the spring of 1996, only a few apothecia of *C. fulgens* were found (in B2 on June 27th). The 1996 spring in this area was 10 days late compared with the previous year. When we arrived to the Sicamous Creek site on June 14, 1996, snow still covered most forest ground to an average depth of about 10 cm, the deepest being more than 1 m. When we finished recovering seedbags at the end of June, snow was still present on some open ground at the middle hill in natural stands. In early July, the snow disappeared within a few days and the weather warmed quickly with daytime temperature as high as 10^0 C. The short cool-wet period in the Sicamous Creek Research forest might be the reason that only a few apothecia of *C. fulgens* were found in this area in 1996.

In conclusion, our survey results demonstrate that *C. fulgens* is present on natural and undisturbed forest floor seedbeds in this area.

Effects of *Caloscypha fulgens* on spruce seed germination: Pathogenicity test

Caloscypha fulgens pathogenicity on Engelmann spruce was tested by placing seed on infested and control soils under laboratory conditions from September 1995 to May 1996 (Figure 4). Both the percentage of spruce germination and the frequency of *C. fulgens* were significantly different between infested soil and the control (Tables 42-1 and 42-2). Engelmann spruce germination on infested pots was lower than on controls and *C. fulgens* was isolated from 64.7% of ungerminated seeds or 45.7% of total sown seeds on the infested soil (Table 42-3).

Table 42. Test of *Caloscypha fulgens* pathogenicity to spruce seed germination

Table 42-1. Analysis of variance for Engelmann spruce seed germination

Source	df	SS	MS	F	P
Treatment	1	2000.8	2000.8	40.54**	0.003
Error	4	197.4	49.4		
Total	5	2198.2			

Table 42-2. Analysis of variance for frequency of *Caloscypha fulgens*

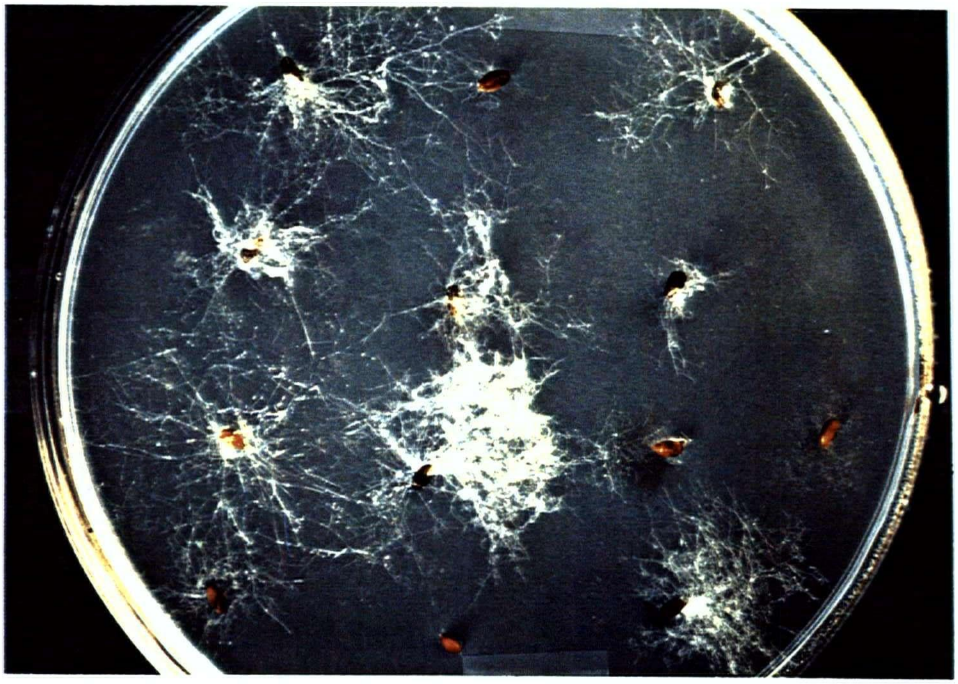
Source	df	SS	MS	F	P
Treatment	1	3136.3	3136.3	43.46*	0.003
Error	4	288.6	72.2		
Total	5	3424.9			

Table 42-3. Percentage of Engelmann spruce germination and infection by *Caloscypha fulgens*

Treatment	N *	Germination (%)	*Caloscypha* (%)
Infested	3	30.15 b	45.73 a
Control	3	66.67 a	0.0 b

* 100 seeds per pot with 3 replications for each treatment

A. Germination of Engelmann spruce on *Caloscypha* -infested soil (right) and non-infested soil (left) under laboratory condition



B. *C. fulgens* isolated from ungerminated spruce seeds on WA

Figure 4. *Caloscypha fulgens* and its effect on Engelmann spruce seed viability

3.2.4 The black mold fungus and its effects on Engelmann spruce and subalpine fir germination and seedling development

A black mold fungus was frequently isolated from Engelmann spruce and subalpine fir seeds over-wintered on forest floor seedbeds in the Sicamous Creek Research Forests in 1996. Analysis of variance and regression showed that there was a significant negative relationship between conifer seed viability and the frequency of isolation of BM, a single seed bag being the sampling unit. This section describes the black mold isolate and the results of pathogenicity tests.

Description of the black mold fungus. The black mold fungus is a non-spore-forming fungus belonging to the Deuteromycotina. The hyphae are dark brown and branched angularly, with side branches septate near the main hyphae, but not constricted basely, and 6 to 11 mm in diameter. No sclerotia were found in cultures on PDA, PSA and WA media. The fungus looks much like *Rhizoctonia* just based on the morphology of the hyphae, however, the colonies are darker, the branches are not constricted and no sclerotia form. Addition work needs to be done to identify this fungus.

Culturing characteristics of the black mold fungus

Optimum medium for black mold fungus. Two rich-nutrient media (PDA and PSA), one poor nutrient medium, (WA) were used in this experiment. After 21 days incubation at 15^0C, diameter of the black mold growth was substantially better on PDA and PSA than on WA (Tables 43-1 and 43-2). A multiple range test showed that average growth of the BM was significantly higher on PDA and PSA than on WA but not significantly different between

PDA and PSA (Table 43-2). The result suggested that this fungus grows better on rich-nutrient media such as PDA and PSA than on nutrient- poor media such as WA.

Table 43. Growth of the black mold fungus in vitro

Table 43-1. Analysis of variance for growth of the BM on three media

Source of Variation	df	SS	MS	F	P-value	F crit
Among Media	2	73.508	36.754	124.262**	< 0.0001	3.682
Error	15	4.437	0.296			
Total	17	77.944				

Table 43-2. Growth of the black mold fungus on three media*

Media	Diameter of colony (cm)	Variance
PDA	5.8 a	0.518
PSA	5.6 a	0.294
WA (2%)	1.4 b	0.076
D - Critical value	0.6	

* Diameter of the BM colony was the average of 6 replications after 21-day incubation at 15^0 C

Optimum growth temperature for black mold fungus. Growth of the black mold fungus on PDA at different temperatures is showed in Figure 5. There was a significant growth difference between temperatures (Table 44). Optimum growth occurred at 15^0 C, and 10^0 C was the second best. Growth at room temperature (average 23^0 C), at which most fungi grow well, was the poorest (Figure 5). A noticeable phenomenon was the growth rate of the black mold fungus at 5^0 C. At this temperature, growth was slow within the first a few days and then speeded up. After 7 to 10 days, its growth was higher than at room temperature and at 30^0 C. The latter had good start within first week, but declined (Figure 5). These results indicate that the black mold fungus is adapted to sustained growth under cool or cold conditions.

Table 44. Analysis of variance for growth of the BM fungus at different temperature

Source	df	SS	MS	F	P
Day	6	127.71	21.286	670.16 **	< 0.001
Temperature	4	38.70	9.675	304.61 **	< 0.001
Day X Temperature	24	14.45	0.602	18.95 **	< 0.001
Error	70	2.22	0.032		
Total	104	183.08			

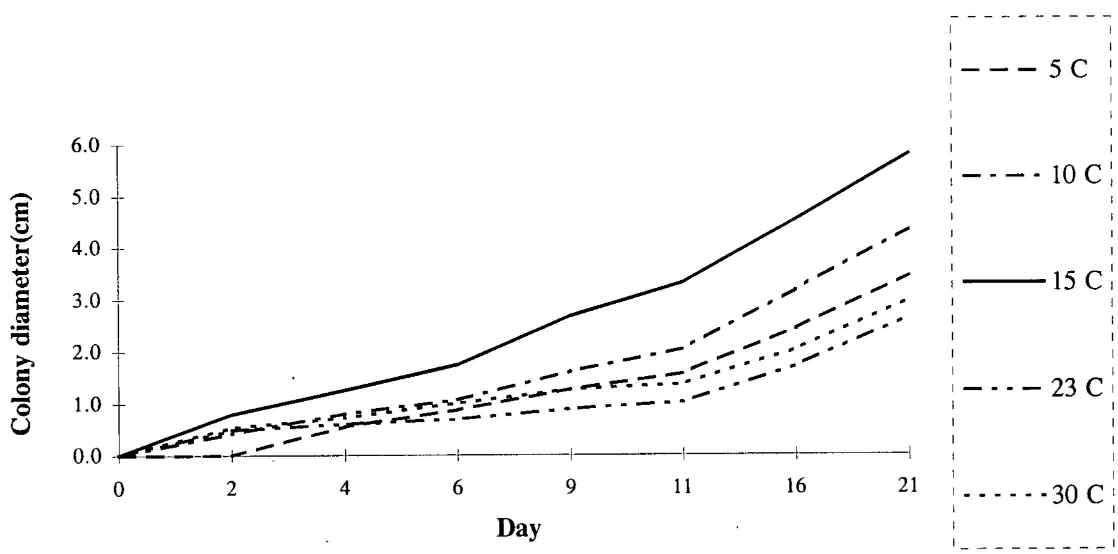


Figure 5. Growth of the BM on PDA at different temperatures

Effect of the black mold fungus on conifer seed and seedlings ------ Pathogenicity test

Engelmann spruce and subalpine fir seeds were placed on PSA inoculated with the black mold fungus (BM) to test for pathogenicity on seed of the two tree species (Figure 6).

At 15^0 C, Engelmann spruce germination on inoculated PSA (41%) was significantly lower than on non-inoculated PSA (57%), F value = 13.6, p=0.0017 (Tables 45-1 and 45-2).

Table 45. Effects of the black mold fungus on Engelmann spruce seed germination *in vitro*

Table 45-1. Engelmann spruce germination on PSA media inoculated with the BM fungus

Treatment	Total Seed	Germinated	Percentage (%)	Variance
Control	100	57	57 a	9
Inoculation	100	41	41 b	9.8

Table 45-2. Analysis of variance for effect of the BM fungus on spruce seed germination

Source of Variation	df	SS	MS	F	P-value	F crit
Between Groups	1	12.8	12.8	13.553*	0.0017	4.4139
Within Groups	18	17	0.94			
Total	19	29.8				

The effect of the black mold fungus on subalpine fir seed germination was not clear due to the low germination rate of the subalpine fir seedlot used. No subalpine fir seed germinated on inoculated PSA medium after 21 days but that was not statistically different from the control (2%). Germination of this seedlot of subalpine fir on wet paper at room temperature was only 14.7% (Table 46).

Table 46. Subalpine fir seed germination on wet paper and PSA medium

Treatment	Total Seed	Germinated	% of Germination
Wet Paper	150	22	14.7%
PSA (without BM)	100	2	2.0%
PSA (with BM)	100	0	0.0%

Effect of black mold fungus on Engelmann spruce seedling development. Observation of germinant development on both inoculated and control media (without inoculation) was continued for another 14 days. Survival of the new seedlings on the inoculated media was significantly less than on control media (Table 47-1). On the inoculated media, new seedlings gradually died due to the root infection by black mold fungus. After 2 weeks, only 6 out of 41 seedlings were still alive. On the control media, 51 seedlings out of 57 new seedlings survived two weeks (Table 47-1). Analysis of variance showed no significant difference between numbers of germinants and surviving seedlings on control media (Table 47-2), but the number of surviving seedlings after 14 days on media inoculated with black mold fungus was significantly lower than the number of germinants (Table 47-3).

Table 47 Effects of the BM fungus on Engelmann spruce seedling development *in vitro*

Table 47 - 1. Survival of Engelmann spruce seedlings on inoculated and control PSA media

Treatment	Number of Germinants	Number of Survived seedling	Percentage of Survival
Control	57	51	89.5%
Inoculated	41	6	14.6%

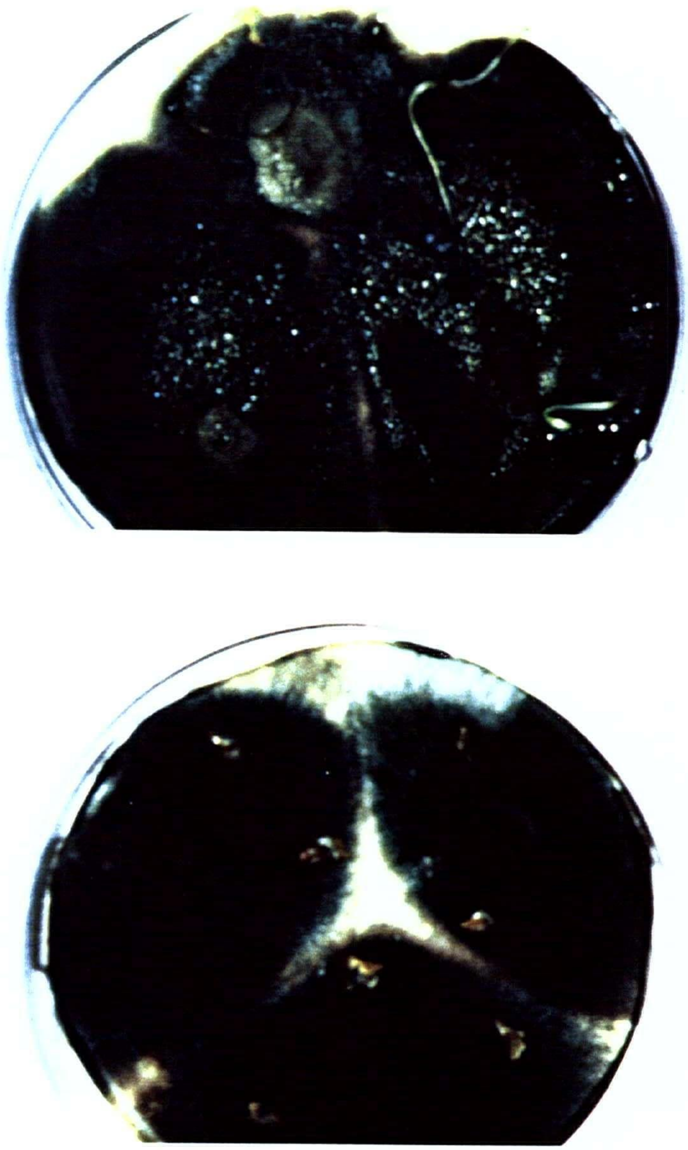


Figure 6. Engelmann spruce (top) and subalpine fir (bottom) seed germination on PSA inoculated with the black mold fungus

Table 47 - 2. Analysis of variance between number of Engelmann spruce germinants and survived seedlings on control PSA media

Source	df	SS	MS	F	P-value	F crit
Between Groups	1	2.45	2.45	1.96 ns	0.179	3.007
Error	18	22.5	1.25			
Total	19	24.95				

Table 47 - 3. Analysis of variance between number of spruce germinants and survived seedlings on PSA media inoculated with the black mold fungus

Source	df	SS	MS	F	P-value	F crit
Between Groups	1	101.25	101.25	83.986 ***	< 0.0001	3.007
Within Groups	18	21.7	1.206			
Total	19	122.95				

3.2.5 Survey of Engelmann spruce and subalpine fir seedling diseases in stands

Survey of brown felt blight disease (*Heroptrichia juniperi*) on Engelmann spruce and subalpine fir seedling/saplings was carried on during the spring of 1995 and 1996. No brown felt blight was found on either Engelmann spruce or subalpine fir seedlings and young trees (5 to 15 years old) in natural stands.

Needle rust of fir (*Pucciniastrum epilobii* B. Otth.) was found on 4 to 15 years old seedlings and young trees of subalpine fir in the Sicamous Creek Research forests. Percentage of infected young trees by this fungus was 78% on the road side near the camp and 6.8% of seedlings on rotten logs in the natural stands.

About 2% of seedlings on rotten logs were damaged by an unknown needle insect in natural stands.

3.3 Discussion

A black mold fungus, *Rhizoctonia* and *Caloscypha fulgens* are the three major pathogenic fungi found in natural forest floor seedbeds in Sicamous Creek Research forests, in the ESSFwc2 subzone, in the Southern Interior of British Columbia. The black mold and *Rhizoctonia* reside mainly on L + H + F layer of forest floor seedbeds and had a highly significant negative regression relationship with viability of Engelmann spruce and subalpine fir seed over-wintered on forest floor seedbeds in natural and disturbed stands. *Caloscypha fulgens* resides on undisturbed forest floor seedbeds in natural and partially cut stands. Although this fungus was not one of the pathogens which severely reduced viability of Engelmann spruce and subalpine fir on forest floor during 95/96 winter, *C. fulgens* was commonly found as apothecia on forest floor seedbeds in June, 1995, and could be a potential pathogen damaging Engelmann spruce seed under suitable environmental conditions.

Black mold fungus. The black mold fungus is an as yet unidentified, sterile fungus which occurred on forest floor seedbeds with high frequency. It is a low-temperature, Racodium-like fungus (Zhong 1995), which can grow well at 5^0 C although its optimum growth temperature is 15^0C and could infect conifer seeds before snow melt. In natural forest stands, black mold fungus is common in moss-type (MOS) and litter-type (LIT) seedbeds. It was isolated from ungerminated Engelmann spruce and subalpine fir seeds over-wintered on natural and disturbed forest floor seedbeds at Sicamous Creek Research forests with high frequency. Pathogenicity test showed this fungus significantly reduced Engelmann spruce seed germination. Infected Engelmann spruce seed was covered with a black sheet of hyphae. The highest percentage of infected Engelmann spruce in one seed-bag from a natural moss-type forest floor was 100%. However, in a laboratory pathogenicity test, the effect of black mold fungus on Engelmann spruce seed germination was not very pronounced (16% less than control). The frequency of diseased seed infected and killed by this fungus from seedbags over-wintered on forest floor seedbeds was much higher. The difference may be the result of several reasons. Firstly, black mold fungus might infect a dormant conifer seed more effectively than germinating seeds in. In the lab experiment, i) stratified Engelmann spruce seed was used, and ii) the temperature was maintained at 15^0 C. Although this temperature was the optimum temperature for black mold fungus growth in culture, it is also good for Engelmann spruce seed germination. Furthermore, germinating seed may be more resistant to infection. Secondly, black mold might need more time to infect conifer seed under cold conditions. Under natural conditions, conifer seed that falls on forest floor seedbeds in the fall will stay under snow more than 7 months until next June to start germination. In our experiment, seed was exposed to the black mold for 7 days at 5^0 C and another 10 to 14 days at 15^0 C. It might not have been long enough for the low-temperature fungus infection.

The effect of the black mold on subalpine fir seed viability was not clear due to the low germination capacity of the seedlot used. The percentage of the black mold isolated from subalpine fir seed was significantly higher than from Engelmann spruce seed. Total germination of subalpine fir seed over-wintered on site was also lower than that of Engelmann spruce seed. The low germination rate on natural forest floor seedbeds (MOS and LIT) and the high frequency of black mold isolated from the ungerminated subalpine fir seeds indicate that there is an effect of the black mold on subalpine fir seed viability. A significant regression relationship of the black mold fungus with subalpine fir seed germination also proved this effect. However, further pathogenicity tests, using conditions that mimic those conditions on the forest floor under snow should be conducted.

Further study of the effect of the black mold fungus on germinants before emerging also needs to be undertaken. Black mold fungus damaged Engelmann spruce germinants *in vitro*. They caused 85% of Engelmann spruce germinants or young seedlings to die within 2 weeks. It was noticed that a large portion of germinants failed to emerge on forest floor seedbeds. Perhaps this was caused by black mold, although this study was not able to confirm this.

***Rhizoctonia* spp.** *Rhizoctonia* species (isolates of this genus were not identified to species) comprise another group of soil-borne fungi found with high frequency on all forest floor seedbeds in natural stands, as well as on seedbeds in partially cut and clear-cut stands at Sicamous Creek Research forest. *Rhizoctonia* spp. mainly occurred in surface and organic layers of soils on litter-type and moss-type forest floor seedbeds (LIT and MOS), but they also occurred on mineral soil seedbeds in natural and disturbed stands. A significant negative regression relationship of *Rhizoctonia* frequency with Engelmann spruce and subalpine fir seed germination shows that this fungus apparently reduces seed viability on natural forest floor seedbeds. However, no further study of this fungus was undertaken due to time limitation.

Isolates of *Rhizoctonia* were recognized as sterile (non-spore-forming) fungi with constrictions near branch junctions on hyphae (Watanabe 1994). *Rhizoctonia* is characterized by: a) branching near the distal septum of cells in young vegetative hyphae; b) the absence of clamp connections and rhizomorphs; and c) sclerotia differentiated into rind and medulla (Sneh *et al.* 1991). One of the best known pathogenic fungi from this genus is *Rhizoctonia solani* which causes a root rot disease of coniferous tree seedlings in bareroot nurseries (Chinese Forestry Academy 1979; Huang and Kuhlman 1989; Ito *et al.* 1955). It is one of a group of soil-borne fungi which cause conifer seedling damping-off disease all over the world. This is the first time that this fungus has been reported to cause loss of conifer seed viability on forest floor seedbeds in the ESSF. Further study of this fungus, such as identification of species, pathogenicity and behavior of this fungus on site are needed.

Caloscypha fulgens. *Caloscypha fulgens* is one of the important seed-borne pathogenic fungi in British Columbia and North America (Sutherland *et al.* 1978; Wicklow-Howard and Skujins 1980). Apothecia of this fungus were commonly found on undisturbed forest floor seedbeds in this area in early spring of 1995. *Caloscypha fulgens* was trapped with Engelmann spruce seed from samples of moss-type, litter-type and mineral soil seedbeds under laboratory condition. More than 50% of Engelmann spruce seed on *Caloscypha*-infested soil lost germinability compared with the seed germination on uninfested soil. These results under laboratory conditions suggest that *C. fulgens* is one of pathogenic fungi present in natural forest floor seedbeds and able to kill Engelmann spruce seed during winter on site. It may be one of the agents which cause the low germination on natural forest floor seedbeds in the ESSF.

Caloscypha fulgens is mainly present in/on moss+litter type (MOS) seedbeds in natural stands. It also occurred on most treated or disturbed seedbeds in natural and disturbed stands except on the seedbeds on which surface layer of litter was removed (LITR). A surprising result was that *C. fulgens* could stay in mineral soil. The highest percentage of subalpine fir seed infected by *C. fulgens* on mineral soil seedbeds in natural stand was 6.7% during the 1995-96 winter (Table 31). In partially cut stands, more Engelmann spruce seed was infected by this pathogen on forest floor seedbeds (Table 33). Generally, there was no significant difference in frequency of *C. fulgens* on various types of forest floor seedbeds in this area.

However, this fungus is not always present on the forest floor seedbeds in the same area with the same high frequency and its damage to conifer seed might vary from year to year. This is shown not only by the fact that few apothecia of the fungus were found in 1996, but also by the low frequency of this fungus trapped from forest floor seedbeds in natural and disturbed seedbeds in spring of 1996. The implied comparison between specially selected soil samples with *C. fulgens* apothecia tested in lab and general plot samples the next year is hardly fair. Infection by *C. fulgens* was correlated with moisture, rainfall, temperature and all combinations of these parameters (Sutherland 1981). Whether *C. fulgens* field behavior is affected by same parameters in high elevation conifer forests is not known.

Seedbed treatment can effectively reduce infection by the black mold and *Rhizoctonia* on forest floor seedbeds in natural and disturbed stands in the ESSFwe2 subzone. The best seedbed treatment for protecting Engelmann spruce and subalpine fir seeds from infection by soil-borne pathogens, such as the black mold and *Rhizoctonia* in natural stands is to remove all surface organic layers, exposing the mineral soils. In disturbed stands, cutting pattern does not have a significant effect in reducing infection by soil-borne fungi on forest floor when seedbed treatment is not undertaken. Moreover, frequency of pathogenic fungi was increased in partially cut stand. To improve natural regeneration in logged stands, site preparation, such as scalping, is required to decrease seed viability loss from soil-borne pathogenic fungi. However, since cutting treatments were very recent, things may still be changing.

Chapter IV. Conclusions

There are three major forest floor seedbed types in natural stands in the Sicamous Creek Research forest, the ESSFwc2 subzone. They are rotten logs (decaying wood) covered with moss (LOG), moss-type seedbeds (MOS), and litter-type seedbeds (LIT), covering 14.9, 72.5 and 4.2% of the surface area, respectively. A further 8.3% of the area is considered unsuitable for seedling establishment. Natural regeneration of Engelmann spruce and subalpine fir is largely limited to rotten log seedbeds (LOG). Natural regeneration on moss-type seedbeds (MOS) and 'pure' litter-type seedbeds (LIT) is very poor. There were no Engelmann spruce seedlings naturally regenerated on these seedbeds and only 0.16 subalpine fir seedlings per square meter.

The poor natural regeneration on forest floor seedbeds in natural stands mainly results from the poor seed germination and germinant pre-emergence loss. Average germination of Engelmann spruce was only 12.2% on litter-type seedbeds (LIT) and 14% on moss-type seedbeds (MOS), which was only one third of the germination rate on rotten logs with mosses (LOG). Average germination of subalpine fir was even worse, only 11.2% on litter-type seedbeds and 12.3% on moss-type seedbeds. Neither Engelmann spruce nor subalpine fir seedlings grow out soil of on litter-type seedbeds and only 6% of Engelmann spruce and 0.2% of subalpine fir seedlings emerged from soils on moss-type seedbeds.

The poor seed germination of Engelmann spruce and subalpine fir on natural forest floor seedbeds is mainly caused by some pathogenic or putative pathogenic fungi. Black mold (an unidentified sterile fungus) and *Rhizoctonia* spp. were found most responsible for the low seed germination on forest floor seedbeds in natural stands.

The black mold fungus is the most important soil-borne pathogenic fungus on forest floor seedbeds in the ESSFwc2 subzone. It is a low-temperature fungus, which grows well at 5 to 15^0 C, and can infect and kill both Engelmann spruce and subalpine fir seed on forest floor seedbeds during the cold winter season. On moss-type seedbeds (MOS), an average 52.5% of Engelmann spruce seed and 49% of subalpine fir seed were infected by the black mold fungus. On litter-type seedbeds (LIT), 29% of Engelmann spruce seed and 36.3% of subalpine fir seed were infected by this fungus.

Rhizoctonia spp is the second most important fungus responsible for the poor seed germination of both Engelmann spruce and subalpine fir on forest floor seedbeds, especially on litter-type seedbeds in natural stands. *Rhizoctonia* had higher infection on both Engelmann spruce and subalpine fir seed on litter-type seedbeds than on moss-type seedbeds. An average of 50% of subalpine fir seed and 31% of Engelmann spruce seed was infected by *Rhizoctonia* on litter-type seedbeds, while only 24% of subalpine fir and 14.2% of spruce seed were infected on moss-type seedbeds.

Caloscypha fulgens is another pathogenic fungus found on forest floor seedbeds in this area. This fungus was isolated from both moss-type and litter-type seedbeds, and it was also found on both Engelmann spruce and subalpine fir seeds over-wintered on organic soil and mineral soil seedbeds. More than 50% of Engelmann spruce seed lost their viability on Caloscypha-infested soil under laboratory conditions. But the frequency of *C. fulgens* on forest floor seedbeds in the ESSFwc2 was not stable and may vary in different year. For example, in spring of 1995, appothecia of *C. fulgens* were very common on undisturbed forest floor seedbeds and very high frequency of this fungus was trapped by Engelmann spruce seed on selected samples of moss-type seedbeds. In 1996, there were very few appothecia of this fungus found on forest floor seedbeds and very low frequency of *C. fulgens* was found on both Engelmann spruce and subalpine fir seeds over-wintered on these seedbeds. Although low percentage of *C. fulgens* was found on conifer seed over-wintered on forest floor seedbeds, special attention should be given to this pathogen since it may cause severe damage of conifer seed on natural forest floor seedbeds in a suitable year.

Seedbed treatment can effectively reduce the frequency of pathogenic fungi on forest floor seedbeds and improve germination of both Engelmann spruce and subalpine fir. Removing soil layers above mineral soil (MIN) is the most effective way of reducing frequency of pathogenic fungi since most of these pathogens are living in surface and organic layers of soils. Frequency of black mold fungus infection on mineral soil was only 3.2% for Engelmann spruce and 8% for subalpine fir. Frequency of *Rhizoctonia* on mineral soil was also significantly reduced compared with that on litter-type seedbeds, (3.5% for Engelmann spruce and 7.9% for subalpine fir).

Removing the surface litter layer of forest floor seedbeds can also reduce frequency of pathogenic fungi on Engelmann spruce seed and significantly improve spruce seed germination. But this treatment does not significantly reduce the frequency of pathogenic fungi on subalpine fir, nor does it improve subalpine fir seed germination.

Harvesting, whether by clearcutting or partial cutting, has no significant effect on the frequency of pathogens and does not improve conifer seed germination on forest floor seedbeds when site preparation is not conducted. When the forest floor was scalped, frequency of infection by pathogenic fungi was significantly reduced and both Engelmann spruce and subalpine fir seed germination rates were significantly improved.

LITERATURE CITED

Alexander, R.R. 1974. Silviculture of subalpine forest in the central and southern Rocky Mountains: the status of our knowledge. U.S. Dep. Agric. For. Ser., Rocky Mtn. For. Range Exp Sta., Res. Pap. RM-121.

Alexander, R.R. 1984. Natural regeneration of Engelmann spruce after clearcutting in the Central Rocky Mountains. U.S. Dep. Agric. For. Ser., Rocky Mtn. For. Range Exp. Sta., Res. Note RM-426.

Alexander, R.R. 1987. Ecology, silviculture and management of the Engelmann spruce. U.S. Dep. Agric. For. Ser. Agri. Handbook No. 659.

Allen, E., D. Morrison, and G. Wallis. 1996. Common tree diseases of British Columbia. Natural Resources Canada, Canadian Forest Service, Victoria, British Columbia.

Arlidge, J.W.C. 1967. The durability of scarified seedbeds for spruce regeneration. British Columbia Dep. Lands, Forest and Water Resources, For. Serv., Victoria, B.C. Res. Note 42.

Barnett, H.L. and B.B. Hunter. 1987. Illustrated genera of Impergect fungi (Fourth edition). MacMillan Publishing Company, New York.

Barron, G.L. 1968. The Genera of Hyphomycetes from Soil. The Williams & Wilkins Company, Baltimore.

Bassman, J.H. 1989. Influence of two site preparation treatments on ecophysiology of planted *Picea engelmannii* X *P. glauca* seedlings. Can. J. For. Res. 19:1359-1370.

Beaudry, L.J. and L.D. McCulloch. 1989. Forest regeneration in ICHg, Price Rupert Forest Region: a problem analysis. For. Can. and B.C. Min. For., Victoria, B.C. FRDA Rep. 090.

Blaisdell, E.L. 1993. Statistics in Practice. Saunders College Publishing, Harcourt Brace College Publishers, Montrial.

Boyd, R.J. and G.H. Deitschman. 1969. Site preparation aids natural regeneration in western larch - Engelmann spruce strip clearcuttings. U.S. Dep. Agric., For. Ser. Intermtn. For. Range Exp. Sta. Res. Pap. INT-64.

British Columbia Ministry of Forests. 1990. Summary of backlog not satisfactory restocked forest land. Victoria, B.C.

Butt, G. 1988. Forest regeneration in the ESSFa subzone: a problem analysis. For. Can. and B.C. Min. For., Victoria, B.C. FRDA Project 3.39. Unpublished Report.

Butt, G. 1990. Ingress of Engelmann spruce and subalpine fir in Southeastern British Columbia. For. Can. and B.C. Min. For., Victoria, B.C. FRDA Project 3.61 Unpublished Report.

Butt, G. and A. Vyse. 1992. Regeneration of Engelmann spruce and subalpine fir in southern British Columbia following clear-cutting - Project 3.61. For. Can. and B.C. Min. For., Victoria, B.C. FRDA Memo No. 210.

Carmichael, J.W., W.B. Kendrick, I.L. Conners and L. Sigler. 1980. Genera of Hyphomycetes. The University of Alberta Press. Edmonton, Alberta, Canada.

Cheng, D.S. 1989. Studies on *Racodium therryanum* caused in-soil seed rot with special reference to its impact on natural regeneration of Yezo spruce. Research Bulletin of the College Experiment Forests. Hokkaido University. 46: 529-575.

Cheng, D.S. and T. Igarashi. 1990. Comparison of susceptibility to *Racodium therryanum* infection of seeds and seedlings of four coniferous species. Research Bulletin of the College Experiment Forests, Hokkaido University. 47:125-136.

Chinese Forestry Academy. 1979. Forest diseases in China. Forestry Press, Beijing, China.

Clark, J.D., L.W.W. Lehrle, and J.H.G. Smith. 1954. Scarification in Engelmann spruce alpine fir forests. B.C. For. Serv., Victoria, B.C. Res. Note 25.

Coate, K.D., S. Haeussle, S. Lindeburgh, R. Pojar and A.J. Stock. 1994. Ecology and Silviculture of Interior Spruce in British Columbia. For. Can. and B.C. Min. For., Victoria, B.C. FRDA Report 220.

Cochran, W.G. 1977. Sampling Techniques (Third Edition). John Wiley & Sons, New York.

Coupe, R., A.C. Stewart and B.M. Wilkeem. 1991. The Engelmann spruce and subalpine fir zone. In Meiginger, D. and J. Pojar (editors). Ecosystems of British Columbia. B.C. Ministry of Forests. Special Report Series No. 6.

Daniel, T.W. and G. Glatzel. 1966. Duff, a lethal seedbed for over-wintering Engelmann spruce seeds. Proc. Sixth World Forestry Congr., Madrid. 2, 1420-1424.

Daniel, T.W. and J. Schmidt. 1972. Lethal and nonlethal effects of the organic horizons of forested soils on the germination of seeds from several associated conifer species of the rocky mountains. Can. J. For. Res. 2: 179-184.

Day, R.J. 1964. The micro environments occupied by spruce and fir regeneration in the Rocky Mountains. Can. Dep. For., For. Res. Br. Publ. No. 1037.

Dennis, J., J. W. Zhong and J.R. Sutherland. 1993. Forestry Canada tree seed bioassay - 1992. Silviculture of British Columbia. Pages 6-8.

Diamadis, S. and W.D. Minter. 1983. Contribution to the microflora of Greek fir. Dasiki Erevna (Greece) V. IV(2): 131-136 (English Abstract).

Dobbs, R.C. 1972. Regeneration of white and Engelmann spruce: a literature review with special reference to the British Columbia Interior. Environ. Can., Can. For. Serv., Victoria, B.C. BC-X-69.

Domsch, K.H., W. Gams and T. Anderson. 1980. Compendium of soil fungi. Volume I. Academic Press, New York.

Dyer, E.D.A. and D.W. Taylor. 1971. Spruce beetle brood production in logging slash and wind-thrown trees in British Columbia. Environ. Can., Can. For. Serv., Victoria, B.C. BC-X-62.

Egger, K.N. and J. W. Paden. 1986. Pathogenicity of postfire ascomycetes (Pezizales) on seeds and germinants of lodgepole pine. Can. J. Bot. 64:2368-2371.

Epners, Z. 1964. A new psychrophilic fungus causing germination failure of conifer seeds. Can. J. Botany 42: 1589-1604.

Farnden, C. 1994. Forest regeneration in the ESSF zone of north-central British Columbia. Environ. Can., Can. For. Serv., Victoria, B.C. Information Report BC-X-351.

Geier-Hayes, K. 1987. Occurrence of conifer seedlings and their microenvironments on disturbed sites in Central Idaho. U.S. Dep. Agric. For. Serv., Intermtn. For. Range Exp. Sta. Res. Pap. INT-383.

Gilmour, J.G. and J. Konishi. 1965. Scarification in the spruce-alpine fir type forest of the Price George Forest District. B.C. For. Serv., Victoria, B.C., For. Manage. Note 4.

Glew, D.R. 1963. The results of stand treatment in the white spruce - alpine fir type of the northern interior of British Columbia. Dep. Lands, Forest, and Water Resources, B.C. For. Serv., Victoria, B.C., For. Mange. Note 1.

Griffith, B.G. 1931. The natural regeneration of spruce in Central British Columbia. For. Chron. 7:204-219.

Harrey, R.D., Jr. 1980. Mortality from *Caloscypha fulgens* and other fungi on spruce seed in Oregon and Washington. Plant Disease 64:223-224.

Harvey, A.E., M.F. Jurgensen, M.J. Larsen, and R.T. Graham. 1987. Decaying organic materials and soil quality in the inland Northwest: a management opportunity. U.S. Dep. Agric. For. Serv., Intermtn. For. Range Exp. Sta., Gen. Tech. Rep. INT- 225.

Hayashi, K. and K. Endo. 1975. Effect of soil fungi and drought on the germination of Todo-fir seed. Bulletins of the Government Forest Experiment Station, Meguro. No. 274.

Hiratsuka, Y. 1987. Forest Tree Diseases of the Prairie Provinces. Government of Canada, Canadian Forestry Service, Information Report NOR-X-286. 141 p.

Hope, G. 1997. Effects of silvicultural systems on soil productivity. In Hollstedt, C. and A. Vyse (editors). 1997. Sicamous Creek Silvicultural Systems Project: Workshop Proceedings. April 24-25, 1996. Kamloops, British Columbia, Canada. Res. Br. B.C. Min. For., Victoria, B.C. Work Pap. 24/1997. PP 79-85.

Huang, J. W. and E. G. Kuhlman. 1989. Recovery and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia*-like fungi in forest nurseries. Plant Dis. 73:968-972.

Igarashi, T. and D.S. Cheng. 1988. Fungal damage caused by *Racodium therryanum* to regeneration of Japanese larch by natural seedling. Research Bulletin of the College Experiment Forests, Hokkaido University 45(1):213-219.

Ito, K., S. Konatani and H. Kondo. 1955. Web-blight fungus of Japanese larch seedlings. Bull. Gov. Forest Exp. Sta. 79:43-63.

Johnson, E.A. and G.I. Fryer. 1996. Why Engelmann spruce does not have a persistent seed bank. Can. J. For. Res. 26:872-878.

Jull, M., C. Delong, A. Eastham, R.M. Sagar, S. Stevenson and R.L. Delong. 1996. Testing Silvicultural Systems of the ESSF: early results of the Lucille Mountain Project. Forest Research Note. PG-01. Unpublished Paper.

Knapp, A.K. and W.K. Smith. 1982. Factors influencing understory seedling establishment of Engelmann spruce and subalpine fir in southeast Wyoming. Can. J. Bot. 60: 2753-2761.

Konish, J. 1985. Provenance and seed quality. In Interior spruce seedling performance: state of the art. Northern Silv. Comm. Proc., Prince George, B.C., Feberary 5-6, 1985.

Kowalski, S. 1980. *Cylindrocarpon destructans* (*Nectria radicicola*) as a cause of mortality in naturally regeneration Abies alba in some mountain forests in Poland. Acta-Agratia-et-Silvestria, Silvestris 19:57-73 (English Abstract).

Kuhnke, D.H. 1989. Silviculture statistics for Canada: an 11-year summary. Environ. Can., Can. For. Ser., Edmonton, Alta. Inf. Rep. NOR-X-301.

Larios, J. M. *et al.* 1988. Study of the fungi which grows in the reflect vegetation *of Abies pinesapo* Boiss in Spain. Acta Botania Malacitana 13:91-110 (English Abstract).

Lees, J.C. 1972. Site factors contributing to the spruce regeneration problem in Alberta's mixed wood. In white spruce: the ecology of a northern resource. R.G. McMinn (Editor). Can. For. Ser., Edmonton, Albta, Inf. Pep. NOR-X-40, PP 8-14.

Lloyd, D. and A. Inselberg. 1997. Ecosystem mapping for the Sicamous Creek Silvicultural Systems research site. In Hollstedt, C. and A. Vyse (editors). 1997. Sicamous Creek Silvicultural Systems Project: Workshop Proceedings. April 24-25, 1996. Kamloops, British Columbia, Canada. Res. Br., B.C. Min. For., Victoria, B.C. Pap. 24/1997. Pages 79-85.

Lindman, H.L. 1992. Analysis of variance in Experimental Design. Springer-Verlag, New York.

McCaughey, W.W. and W.C. Schmidt. 1987. Seed dispersal of Engelmann spruce in the intermountain west. Northwest Sci. 61(1):1-6.

Mittal, R.K. and B.S.P. Wang. 1987. Fungi associated with seeds of eastern white pine and white spruce during processing and seed extraction. Can. J. For. Res. 17: 1026-1034.

Mittal, R.K. and B.S.P. Wang. 1993. Effects of some seed-borne fungi on *Picea glauca* and *Pinus strobus* seeds. Eur. J. Forest Path. 23: 138-146.

Noble, D.L. and F. Ronco, Jr. 1978. Seedfall and establishment of Engelmann spruce and subalpine fir in clearcut openings in Colorado. U.S. Dep. Agric. For. Ser., Rocky Mountains. For. Range Exp. Sta. Res. Pap. RM-200.

Noble, D.L. and R.R. Alexander. 1977. Environmental factors affecting natural regeneration of Engelmann spruce in the Central Rocky Mountains. For. Sci. 23(4):420-429.

Noble, D.L. and W.D. Shepherd. 1973. Grey-headed juncos is important in first season mortality of Engelmann spruce. J. For. 71(12):763-765.

Paden, J.W., J.R. Sutherland and T.A.D. Woods. 1978. *Caloscypha fulgens* (Ascomycotina, Pezizales): the *Geniculodendron pyriforme* (Deuteromycotina, Hyphomycetes). Can. J. Botany 56:2375-2379.

Parish, R. 1997. Age and size structure of the forest at Sicamous Creek. In Hollstedt, D. and A. Vyse (editors). 1997. Sicamous Creek Silvicultural Systems Project: Workshop Proceedings. April 24-25, 1996. Kamloops, British Columbia, Canada. Res. Br., B.C. Min. For., Victoria, B.C. Work Pap. 24/1997. PP 16-31.

Philips, D.H. and D.A. Burdekin. 1992. Diseases of Forest and Ornamental trees. Second Edition. The MacMillan Press Ltd., London and Basingstoke.

Place, I.C.M. 1955. The influence of seedbed conditions on the regeneration of spruce and subalpine fir. Can. Dep. North. Affair and Nat. Resources, For. Bri., Bull. 117.

Roe, A.L., R.R. Alexander and M.D. Andrews. 1970. Engelmann spruce regeneration practices in the Rocky Mountains. U.S. Dep. of Agric. For. Ser. Washington, DC, Prod. Res. Pap. 115.

Roe, A.L. and W.C. Schmidt. 1964. Factors affecting natural regeneration of spruce in the intermountain region. U.S. Dep. of Agric. For. Ser. Intermtn. For. Range Exp. Sta. Ogden, UT. 68 p.

Ronco, F. 1970. Chlorosis of planted Engelmann spruce seedlings unrelated to nitrogen content. Can. J. Bot. 48:851-853.

Ronco, F. 1967. Lessons from artificial regeneration studies in a cutover beetle-killed spruce stand in Western Colorado. U.S. Dep. of Agric. For. Ser. Rocky Mountain For. Range Exp. Sta. Res. Note RM-90.

Rowe, J.S. 1955. Factors influencing white spruce reproduction in Manitoba and Saskatchewan. Canada Dep. North. Affairs and Nat. Resources, For. Res. Div., Tech. Note 3.

Salt, G.A. 1974. Etiology and morphology of Geniculodendron pyriforme gen. et sp. nov., a pathogen of conifer seeds. Trans. Br. Mycol. Soc. 63:339-351.

Sato, K. 1964. Pre-emergence damping-off on coniferous seedlings caused by the pathogenic fungi of snow blight at low temperature (I). J. Jpn. For. soc. 46:171-177.

Sato, K., T. Shoji and N. Ota. 1960. Studies on the snow molding of coniferous seedlings-II Dark snow blight caused by *Rhacodium therrryanum* Thurem. Bull. Gov. For. Exp. Sta. No.124:21-112.

Scagel, A. 1987. A problem analysis of backlog NSR in the Northern ESSF zone. For. Can. and B.C. Min. For., Victoria, B.C. FRDA Project 1.17. B.C. Min. For., Res. Sect. Williams Lake, B.C. Unpublished Report.

Schonhar, S. 1987. Studies on the occurrence of fungal parasites on fine roots of 70-90 years-old spruce (Picea abies Karst.). Mitteilungen des Vereins fur Forstliche Standrtskunde und Forst pflanzenzuchtung, 33:77-80 (English Abstract).

Singleton, L.L., J.D. Mihail and C.M. Rush. 1992. Methods for research on soil-borne phytopathogenic Fungi. APS Press, The American Phytopathological Society. St. Paul, Minnesota. 265 P.

Smith, J.H.G. 1955. Some factors affecting reproduction of Engelmann spruce and alpine fir. Dep. Lands and For., B.C. For. Serv., Victoria, B.C.

Sneh, B., L. Burpee and A. Ogoshi. 1991. Identification of *Rhizoctonia* species. SPS Press, The American Phytopathological society. St. Paul, Minnesota, USA.

Sullivan, T.P., J.R. Sutherland, T.A.D. Woods and D.S. Sullivan. 1984. Dissemination of the conifer seed fungus *Caloscypha fulgens* by small mammals. Can. J. For. Res. 14:134-137.

Sutherland, J.R. 1979. The pathogenic fungus *Caloscypha fulgens* in stored conifer seeds in B.C. and relation of its incidence to ground and squirrel cached collected cones. Can. J. For. Res. 9:129-132.

Sutherland, J.R. 1981. Time, temperature, and moisture effects on incidence of seed infected by *Caloscypha fulgens* in Sitka spruce cones. Can. J. For. Res. 11:727-730.

Sutherland, J.R. and R.S. Hunt. 1991. Diseases in Reforestation. In Lavender, D. P., R. Parish, C.M. Johnson, G. Mountgomery, A.Vyse, R.A. Willis, and D. Winson (editors). Regenerating British Columbia's Forests. University of British Columbia Press, Vancouver, B.C. pp 266-278.

Sutherland, J.R., T. Miller and R.S. Quinard. 1987. Cone and Seed Diseases of North American Conifers. North American Forestry Commission Publication No.1, 77p.

Sutherland, J.R., Shrimpton, G.M. and Sturrock, R.N. 1989, Diseases and insects in British Columbia forest seedling nurseries. FRDA Report No.065.

Sutherland, J.R. and T.A.D. Woods. 1978. The fungus *Geniculodendron pyriforme* in stored Sitka spruce seeds: Effects of seed extraction and cone collection methods on disease incidence. Phytopathology 68:747-750.

Thompson, S.K. 1992. Sampling. A Wiley-Interscience Publication, John Wiley & Sons, Inc. Toronto. PP. 126-138.

Urosevic, B. 1961. The influence of saprophytic and semi-parasitic fungi on the germination of Norway spruce and Scots pine seeds. Proc. Int. Seed Test. Assoc. Vol. 26: 537-556.

Vyse, A. 1997. The Sicamous Creek Silvicultural Systems Project: how the project came to be and what it aims to accomplish. In Hollstedt, D. and A. Vyse (editors). 1997. Sicamous Creek Silvicultural Systems Project: Workshop Proceedings. April 24-25, 1996. Kamloops, British Columbia, Canada. Res. Br., B.C. Min. For., Victoria, B.C. 1997. Work Pap. 24/1997. PP 4-14.

Wang, J. R. 1997. Temperature under snow in the ESSF, Prince George area. Personal communication.

Watanabe, T. 1994. Pictorial Atlas of Soil and Seed Fungi. C.R.R. Press, Inc. Boca Raton, Florida.

Weetman, G. and A. Vyse. 1990. Natural Regeneration. In Lavender, D.P., R.Parish, C.M. Johnson, G. Mountgomery, A.Vyse, R.A. Willis, and D. Winson (editors). Regenerating British Columbia's Forests. University of British Columbia Press, Vancouver, B.C.Wicklow-Howard, M.C. and J. Skujins. 1980. Infection of Engelmann spruce seed by *Geniculodendron pyriforme* in Western North America. Mycologia 72: 406-410.

Widden, P. and D. Parkinson. 1973. Fungi from Canadian coniferous forest soils. Can. J. Botany 51: 2275-2290.

Woodard, P.M. and G. Cummins. 1987. Engelmann spruce, lodgepole pine and alpine fir seed germination success on ashbed conditions. Northwest Sci. 61(4):233-238.

Woods, T.A.D., S. H. Farris and J.R. Sutherland. 1982. Penetration of Sitka spruce seeds by the pathogenic fungus *Caloscypha fulgens*. Can. J. Botany 60:544-548.

Young, J. and C.G. Young. 1992. Seeds of Woody Plants in North America, Revised and Enlarged Edition. Dioscorides Press, Portland, Oregon.

Zhong, J. W. 1995. Pathology of seeds and seedlings in Engelmann spruce dominated stands, Interior of British Columbia. Thesis proposal for Master of Forestry.