

Fungal Diseases in Forest Nurseries in Finland

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Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*) are the major tree species grown in Finnish forest nurseries where 99% of the seedlings are grown in containers first in plastic-covered greenhouses and later outdoors. The main diseases on conifer seedlings are Scleroderris canker (*Gremmeniella abietina*), Sirococcus blight and cankers (*Sirococcus conigenum*), snow blights (*Herpotrichia juniperi* and *Phacidium infestans*) and needle casts (*Lophodermium seditiosum* and *Meria laricis*). Also grey mould (*Botrytis cinerea*) and birch rust (*Melampsorium betulinum*) are among the diseases to be controlled with fungicides. During last years Scleroderris canker has been a problem on Norway spruce, which has been since 2000 the most common species produced in Finnish nurseries. Root die-back (uninucleate *Rhizoctonia* sp.) on container-grown spruce and pine was a problem in the 1990s. Today the disease has become less common in modern nurseries due to improvements in hygiene and cultivation practice. Since 1991 stem lesions and top dying caused by *Phytophthora cactorum* has been a problem on birch. The ongoing climate change has already had effect on rusts and powdery mildews as well as other fungi infecting leaves. All diseases, which gain high precipitation and warm and long autumns. For same reasons winter stored seedlings need sprayings against grey mold. Fungal infections are also possible during short-day (SD) treatment, that is necessary for summer and autumn plantings and a beneficial step prior freezing temperatures outside or in freezer storage. Growers are encouraged to use cultural and integrated pest management techniques such as better nursery hygiene, including removing plant debris in nursery growing areas and hot water washing of containers plus removal of diseased, spore-producing seedlings and trees around the nursery.

Keywords damping-off, grey mold, root dieback, needle casts, snowblights, scleroderris canker, *Sirococcus*, pine twisting rust, stem lesions and top dying, leaf lesions, *Venturia*, powdery mildews

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1 Introduction

1.1 Tree Species

The Finnish tree flora is dominated by Scots pine (*Pinus sylvestris* L.) (50%), Norway spruce (*Picea abies* (L.) Karst.) (30%) and several (mostly *Betula* spp.) broad-leaved species (20%). Prior to the 1960's, reforestation in Finland relied mainly on natural regeneration. Today, natural methods are used in 14% (21 000 ha) of the annual reforested area (150 000 ha), of which 64% is planted with seedlings (96 000 ha) or 22% is seeded (33 000 ha mainly with Scots pine). The area that is reforested with planted seedlings has almost tripled since 1960 (Finnish Statistical Yearbook... 2009).

In 2007, 168 million seedlings were delivered from nurseries to reforestation sites. The majority of seedlings were Norway spruce (66%), followed by Scots pine (31%), silver birch (*Betula pendula* Roth) (2%), downy birch (*B. pubescens* Ehrh.) (0.02%) and other tree species (0.98%), the most important of which were Siberian larch (*Larix sibirica* Ledeb.), lodgepole pine (*Pinus contorta* Douglas ex Loudon), Carelian curly birch (*B. pendula* var. *carelica* (Merklin) Hämet-Ahti) and black spruce (*Picea mariana* (Miller) Britton, Strens & Poggenb.). Most seedlings are produced by 13 large nurseries (production area >10 000 m²), by 8 smaller nurseries (production area >5000 m²) and 48 local, mainly family-owned nurseries (production area <5000 m²) (pers. comm. Kari Leinonen, Evira).

Older nurseries are located in former agricultural land, but most of nurseries built in 1960–80's were established on forest land with sandy soil where bareroot nursery was easy to start. Thus many nurseries are surrounded by Scots pine or Norway spruce forests.

1.2 Nursery Technology and Culture Practices

Beginning in the early 1980's, the proportion of seedlings produced in containers has increased from 29% to 99% and effectively replaced bare-root methods (Yearbook of forest ... 1980, Finnish

Statistical Yearbook ... 2008). Modern container trays are composed of hard plastic cells with air slits at regular intervals on the sides to stimulate natural air pruning of roots around the periphery of the root plug. In addition ventilation holes between the cells are designed to promote air circulation between seedlings for controlling the grey mold fungus, *Botrytis cinerea* Pers. ex Nocca & Balb. (Peterson and Sutherland 1989). Most seedlings are cultured in densities of 400–900 m⁻² (conifers) or 150–400 m⁻² (hardwoods), depending on seedling size and age. Container cells are filled with medium-texture, low-humified *Sphagnum* peat that is often supplemented with dolomite lime and a base fertilizer. Seeds are sown with automatic seeding equipment and seeds are covered with a thin mulch of vermiculate, grit or sawdust. The pH of growth medium used varies between 4.5–5.1 (Rikala and Heiskanen 1995). Fertilizers are provided as fertigation to the growing seedlings according to the electrical conductivity (target 0.8–1.5 mS cm⁻¹ depending on the stage of development of seedlings) of press-water extracts from the growth medium (Juntunen and Rikala 2001) and they are watered with mobile irrigation booms or fixed overhead sprinklers (Tervo 1999, Poteri 2003). Need of irrigation is weighting container trays weekly and trying to keep peat moisture between 40–50% (V/V).

The first crop of one-year-old Norway spruce seedlings is started in heated greenhouses under photoperiodic lighting in March or April. These seedlings are transferred outdoors in the beginning of June or July, respectively. The second crop aiming to two-year-old seedlings is kept inside until the beginning of October and then moved outdoors where they remain for winter and the second growing season. Scots pine seedlings are sown in May and the seedlings are grown in the greenhouse until June–July when they are moved to a hardening compound. Birch seedlings are grown for one-year-old, first in greenhouse and later outdoors since late June.

The plastic films and structures of greenhouses cut 10–40% of light depending on the thickness and age of plastic. Some nurseries use also white-wash to decrease evaporation and temperature during hot days. No shelters are used in outdoor compounds. The temperature and relative humidity in greenhouses are recommended to keep

about 22/15°C and 50/70% (day/night) respectively. Temperature may, however, rise to 30–35°C during sunny days and humidity to 90% in cool, cloudy periods.

The seedlings to be planted in summer or autumn are normally short-day (SD) treated to speed the hardening process so that seedlings stand better autumn frosts (Luoranan et al. 2007, Kohmann and Johnsen 2007). Seedlings stored in freezer storage or outside might also benefit from SD-treatment to tolerate freezing temperatures. The 2–3 week treatment consists of placing the seedlings behind black curtains (in a greenhouse or in an open compound) to shorten the photoperiod during the growing season to 12–14 from 16–20 hours depending on the origin of seeds. The starts of the treatments varied from the late June to the beginning of August depending on the planting time.

Approximately 40–50% of the seedlings are removed from the containers in the autumn and packed in cardboard boxes. Pallets of stacked boxes are wrapped often with a thin plastic sheet to protect seedlings from drying and they are stored at –1 to –4°C for 6–7 months. The rest of seedlings are left to overwinter under snowcover which may be thin or even lack on some winters in southern part of the country. By storing seedlings at freezer storages, risks related to outdoor storage are avoided and greater flexibility in seedling delivery in spring time benefits both nursery and foresters.

The Forest Decree (1200/1996) states that regeneration should be carried out using an adequate amount of material that is suitable for the reforestation site with respect to its origins and properties. The quality of nursery seedlings in Finland is supervised by EVIRA (the Finnish Food Safety Authority) in accordance with an EU directive (1999/105/EY), the Act on Trade in Forest Reproductive Material (241/2002) and the related Decree of the Ministry of Agriculture and Forestry on the marketing of forest reproductive material (1055/2002). According to the legislation, seedlings shall be healthy, viable and otherwise suitable for forest reproduction at the time of sale. A seedling is not considered to meet the requirements if it is harboring disease, insects, abiotic damage, or if the roots or shoots have defects that make it unsuitable for planting.

Two EVIRA inspectors carry out supervisory surveys in the autumn and spring to help prevent the planting of diseased or otherwise low quality seedlings and to advise nursery managers how to deal with poor quality stock.

In this article, we review the current knowledge concerning common and emerging diseases of tree species used in reforestation. We also describe which kind of effects the changes in the technology and practice used in tree nurseries have had on certain diseases. This review is provided in support of recent literature and manuals concerning the identification and management of tree nursery diseases in Canada (Sutherland et al. 1989), USA (Peterson and Smith 1975, Landis 1989, Hamm et al. 1990) and Fennoscandia (Poteri 2008).

2 Diseases

2.1 Environmental Conditions and Disease

As in most cases of poor health, environmental factors play an important role in the probability of infection and transmission of the pathogen. The most important effect of climate change will be the increase of environmental extremes, which in turn have effects on interactions between abiotic and biotic diseases (Boland et al. 2004, Desprez-Loustau et al. 2007). However, most diseases known to cause problems in forestry species today are fungal and gain access to the host plant during periods of physiological stress or through localized sites of damaged or diseased tissue.

In container production, the use of greenhouses with temperature regulation, selected seed, pathogen-free growth medium, irrigation and fertilization results in good seedling growth but may also provide ideal conditions for many biotic diseases. Modern nurseries must optimize culture conditions to maximize seedling production while minimizing the risk of a disease outbreak. In addition to culture conditions, abiotic stress caused by environmental conditions, e.g., frost, or injury can also expose rapidly growing seedlings to fungal attack.

While the short-day treatment is necessary for

summer and autumn plantings and a beneficial step prior freezing temperatures, reduced photosynthesis and increased respiration and humid conditions within the blackout curtains can weaken seedlings and encourage fungal infections. Risks associated with high humidity and depletion of carbohydrates are also high when seedlings are packed into closed boxes for storing and transportation.

The following sections treat each disease in turn and describe the pathogen(s) involved, their biology, the tree species affected, symptoms and their management. Although, a number of fungicides have been authorized for the control of diseases, we do not list them here since the use of products available today might be restricted in near future.

2.2 Failure to Germinate and Damping-off

Damping-off is a fungal disease of young seedlings that causes mortality during the first few weeks after germination. Pre-emergence damping-off is difficult to diagnose because the affected seeds do not germinate. The soil- or seed-borne fungi associated with the disease are not host-specific and they cause rapid decay and mortality of germinating seeds (Lilja 1979, Sutherland et al. 2002) and developing seedlings (Mikola 1952, Vaartaja 1952, Hanioja 1969). The classic symptom of post-emergence damping-off is decay of the hypocotyl at the ground level, but the point where infection starts is non-specific. In many cases there is no clear separation between post-emergence damping-off and root rot on young seedlings. Indeed, many pathogens that infect the succulent tissues of young seedlings can cause both damping-off and root rot (Sutherland and Davis 1991).

Most pathogens that cause damping-off are fungal and mainly reproduce via the asexual stage, e.g., *Alternaria*, *Cylindrocarpon*, *Cylindrocladium*, *Fusarium*, *Trichothecium* and many species of Mucorales (Mittal and Wang 1993, Sutherland et al. 2002). *Pythium* and *Phytophthora* spp. (Chromista) infect plants with swimming asexual zoospores but are carried by organic material in soil or seeds and survive as oospores or chlamydospores (Vaartaja and Gram 1956, Perrin

and Sampagni 1986, Lilja 1994). *Rhizoctonia* species are also important (Vaartaja and Gram 1956, Perrin and Sampagni 1986).

Rust fungi (*Chrysomyxa pirolata* Wint. and *Thekopsora areolata* (Fr.) Magn.) cause cone rusts that destroy seed production totally or interfere with seed germination. Similarly, the ascomycetes *Ciboria betulae* (Wor. ex Nav.) W.L. White and *C. alni* (O. Rostr.) N.F. Buchw. cause seed problems in birch and alder (*Alnus* spp.), respectively. While the majority of pathogens associated with forest tree seeds are fungi, seed-borne viruses and numerous species of bacteria have also been reported (Mittal et al. 1990).

In nursery containers, *Pythium* and *Fusarium* spp. are the most common causes of damping-off (Sutherland and Davis 1991, James et al. 1991). *Fusarium oxysporum* subsp. *pini* (Hartig) Snyder & Hansen and *F. avenaceum* (Corda, Fr.) Sacc. were isolated from the seeds of Scots pine and found to cause post-emergence damping-off in pathogenicity tests (Lilja et al. 1995). Recently, cone pathogen *Sirococcus conigenus* (D.C.) P.F. Cannon & Minter has also caused seed borne damping-off in container-grown Norway spruce (Lilja et al. 2005). Another cone pathogen, which was shown to lower the quality of Norway spruce seeds was *Chrysomyxa pirolata* (Tillman-Sutela et al. 2004). Conidia of *Thysanophora penicilloides* (Roum.) Kend. were also found on the outermost layer of the seeds incapable to germinate (Tillman-Sutela et al. 2004).

Control and prevention of damping-off and root rot fungi requires an effective program that integrates good hygiene, cultivation practice and chemical control. A major source of fungi that cause damping-off is contaminated soil on cones and nursery containers (e.g., Kohmann and Børja 2002, Sutherland et al. 2002). Thus, equipment, tools and containers used during seed collection, storage and cultivation should be kept clean and pathogen free (Landis 1989). The risk of damping-off can also be reduced by using high quality seed and sowing when temperature is high enough to promote rapid and even germination (Vaartaja 1952, Gibson 1956, Perrin and Sampagni 1986, Lilja et al. 1995). Care should also be taken to maintain growth medium with suitable wetness and pH, as soggy and alkaline conditions favour damping-off and root rot (Landis 1989, Suther-

land and Davis 1991, Heiskanen 1993, 1995). Fresh *Sphagnum* peat is a pathogen-free growth medium that includes antagonistic microbes and has an acidic pH to reduce losses (Tahvonen 1982).

2.3 Grey Mold

Early symptoms of grey mold on Norway spruce include discoloured spots on needles (Petäistö et al. 2004). Advanced infections are easily identified by the presence of greyish, cotton-like mycelia and spores borne on tree-like conidiophores. The pathogen *Botryotinia fuckeliana* (de Bary) Whetzel is a common ascomycete saprophyte in nurseries with an asexual stage, *Botrytis cinerea* (Lilja 1986, Gregory and Redfern 1987, Peterson et al. 1988, Sutherland and Davis 1991). *B. cinerea* infection often appears after abiotic damage, such as that caused by frost, fertilizers or herbicides (Sutherland and Davis 1991) although low light intensity coupled with environment stress (e.g., 30–40°C or prolonged drought) might also be important predisposing factors (Zhang and Sutton 1994, Zhang et al. 1995). *B. cinerea* can also infect plants in combination with other fungi, e.g., coupled with *Phomopsis* sp., or to colonize necroses caused by other diseases such as pine twisting rust (Domanski and Kowalski 1988, Hansen and Hamm 1988).

Venn (1981) isolated *B. cinerea* from mouldy needles of barerooted Norway spruce seedlings in cold storage. Infections were common in the lower, shaded branches and were probably initiated in seedling beds (Venn 1979). Petäistö (2006) also found *B. cinerea* to be an important fungal pathogen during freezer storage, where an infection spread readily from diseased seedlings to healthy ones inside cardboard boxes during thawing.

Grey mold spores are spread by man, flying insects (e.g., *Bradysia* spp.: James et al. 1995), wind and greenhouse ventilation (Sutherland and Davis 1991). Spores germinate between 0 and 25°C and the rate is temperature dependent, being optimal 7–20°C (Petäistö 2006). Russell (1990) found that infection can take place after just three hours at 15–20°C and 98% relative humidity (RH) if there is free water on plant surfaces. Although

it was believed that grey mold infection began on the lowest, weakened or dead parts of seedlings before invading healthy tissue (Sutherland and Davis 1991), infection of Norway spruce was found to be more serious on the upper parts of seedlings at 6°C and 80–90% RH (Petäistö 2006).

It has been shown that grey mold fails to develop in black spruce seedlings with completely green foliage (Zhang 1992, Zhang and Sutton 1994) and Norway spruce seedlings were found to be tolerant at the end of growing season (Petäistö et al. 2004).

Mittal et al. (1987) recommend keeping the microclimate within the canopy as dry and well aerated as possible in order to reduce the incidence of grey mold. This is achieved by regulating the seedling density, irrigation and ventilation of nursery greenhouses. Irrigation in the morning ensures rapid drying of foliage during day time and it has also been recommended to brush seedling tops with plastic pipe or a wooden dowel after irrigation to dislodge water droplets and encourage drying (James et al. 1995). There are situations, however, when fungicide treatments against grey mold are needed, e.g. packing of seedlings into airtight boxes after rainy periods in autumn.

2.4 Root Dieback

In Scandinavia, root rot of Norway spruce and Scots pine seedlings was first recorded in Norway and termed 'root dieback' (Galaaen and Venn, 1979). Typical symptoms for the disease are stunted growth of shoots and roots and the patchy occurrence of diseased plants. Partial or total death of rootsystem has been evident for part of the seedlings (Venn et al. 1986, Lilja 1994, Børja et al. 1995, Hietala 1995, 1997).

The fungal root flora of seedlings suffering from root dieback includes, at least, species of *Pythium* and *Cylindrocarpon* and uninucleate *Rhizoctonia* (Galaaen and Venn 1979, Unestam et al. 1989, Lilja et al. 1992, Lilja 1994). In Norway, inoculations with a *Pythium dimorphum* J. W. Hendrix & W. A. Campbell caused some seedlings to suddenly die after two weeks, but those that survived grew well (Venn et al. 1986,

Børja et al. 1995). This species readily infects the roots of 10- to 12-day-old Norway spruce seedlings and causes tissues above the root-hair zone to become dark brown and the hypocotyl necrotic while the root tips remain light coloured and otherwise healthy (Børja et al. 1995). In addition to the cellular and tissue changes noted by Børja et al. (1995), Sharma et al. (1993) found more than 30 different PR-proteins in infected roots, emphasizing a host response to *P. dimorphum*. In Sweden, *Cylindrocarpon destructans* (Zins.) Scholt and *Pythium* spp. were the most common fungi isolated from diseased roots (Unestam et al. 1989, Beyer-Ericson et al. 1991). However, none of the *Cylindrocarpon* species isolated from Finnish material were pathogenic (Lilja et al. 1992). In inoculation experiments, both uninucleate *Rhizoctonia* sp. and *Pythium* spp. caused damping off like disease on seedlings younger than 5–6 weeks, having a sparse root system. In older seedlings only *Rhizoctonia* sp. spreads throughout the root system, resulting in stunted growth of shoot and roots. The diseased seedlings are, however, alive and green, and have a normal apical bud at the end of growing season (Hietala 1995, 1997, Lilja et al. 1996a, Lilja and Rikala 2000).

It has been hypothesised that root dieback is a disease of successive infections or two separate diseases. In the first case the infection with uninucleate *Rhizoctonia* sp. results in a high moisture content in the growth medium, because the infected roots cannot fully utilize the water supplied during irrigation. Wet conditions favour Pythiaceae fungi and promote secondary attack by *Pythium* spp. or other saprophytic species (Lilja 1994, Unestam et al. 1989). The second case is supported by the fact that the infection patterns of *P. dimorphum* and uninucleate *Rhizoctonia* sp. are different. Whereas, as described before, *P. dimorphum* does not infect root tips (Børja et al. 1995), infection by uninucleate *Rhizoctonia* sp. starts from the root tips, which become pigmented and can not divide and grow (Hietala 1995, 1997). The infection could be observed on the surface of lateral root tips, but there were also hyphae inside cortical cells in the main root (Hietala 1995, 1997).

In contrast with the other pathogenic species of *Rhizoctonia* that exhibit bi- or multinucleate cells, cells taken from hyphae that were actively grow-

ing in seedlings suffering from root dieback were uninucleate (Hietala et al. 1994). Furthermore, the *in vitro* form of the fruiting body and DNA comparisons suggest that the uninucleate *Rhizoctonia* might actually be *Ceratobasidium bicorne* Erikss. & Ryv. (Hietala et al. 1994, 2001). All known anamorphs of other *Ceratobasidium* spp. have binucleate cells (Sneh et al. 1991), and in a RAPD analysis uninucleate *Rhizoctonia* sp. isolates from Norway and Finland had banding patterns that were different from *Ceratobasidium* testers with binucleate anamorphs or other binucleate isolates of *Rhizoctonia* (Lilja et al. 1996a).

Seedlings occasionally suffer root dieback without conspicuous symptoms, especially when infected with uninucleate *Rhizoctonia* but without any secondary infection. Seedlings in this condition are shorter than disease-free stock but otherwise appear healthy. Infected seedlings should always be culled since they either suffer increased mortality or reduced growth rates after outplanting (Lilja and Rikala 2000). Today it is possible to detect the disease in planting material using a DNA based test. One benefit of this test is its higher sensitivity compared to the traditional isolation (Hantula et al. 2002).

Root dieback has become less common in modern nurseries due to improvements in hygiene (Iivonen et al. 1996, Kohmann and Børja 2002) and cultivation practice as well as targeted treatments, i.e., immersion in 80°C water baths for 1 minute kills *Rhizoctonia* sclerotia (Iivonen et al. 1996). One of the most important factors in modern cultivation practice is that containers are supported on racks during the growing season which improves ventilation and drainage and thus prevents waterlogging of roots, which has shown to increase the incidence of root dieback (Venn et al. 1986, Unestam et al. 1989, Lilja et al. 1998).

2.5 Needlecasts

In Fennoscandia, needlecast affects mainly Scots pine and Siberian larch. Initial symptoms of the disease may be visible on pines in September–October as small yellow to brown spots on needles (Diwani and Millar 1981, 1987). Infected needles turn brown and fall from the tree the following spring (Martinsson 1975, Kurkela 1979).

Lophodermium is a genus of ascomycete fungi that contains both needlecast pathogens and host-specific endophytes of conifers (Sieber 1988, Müller and Hallaksela 1998). Although Minter et al. (1978) recognised at least four species of *Lophodermium* which can infect pine needles, only *L. seditiosum* Minter, Staley & Millar is considered to be pathogenic (Diwani and Millar 1987, Kurkela 1979). Needlecast in larches is caused by the ascomycete *Meria laricis* Vuillemin.

Ascocarps of *L. seditiosum* mature on fallen needles (Diwani and Millar 1990) and begin to release ascospores in late summer and peak between September and October in Europe (Hanso 1968, Uscuplic 1981) and North America (Nicholls and Skilling 1974). High precipitation during late summer and autumn creates favourable conditions for infection (Kurkela 1979, Diwani and Millar 1981). Due to the less severe climate, southern populations of *L. seditiosum* are more stable and can supply infective propagules to northern latitudes (Hanso 1968, Kurkela 1979). Climatic factors such as lower temperatures during autumn and early snow cover may explain the lower risk of needlecast in the northern part of Finland (Kurkela 1979).

L. seditiosum preferentially infects green primary and secondary first-year needles (Lazarev 1981) via ascospores (Minter 1981a, b, Lazarev 1981). Germinating ascospores form germ tubes ending in melanized, appressorium-like structures (Diwani and Millar 1981) that penetrate the cuticle and epidermis (Staley 1975). The yellow margin of the lesion is caused by starch-free cells in the mesophyll (Diwani and Millar 1981). Furthermore, the amount of chlorophyll and carotenoid pigments in infected needles is 1.2–3.8 and 1.3–2.4 times lower than in healthy ones, respectively (Savkina 1989).

Seedlings infected with *L. seditiosum* may be undetected as they can appear healthy when planted in the early spring. Typically, infected seedlings do not survive planting stress (Lilja 1986) and so there is a need to identify latent infections in material stored below 0° C prior to planting. Stenström and Ihrmark (2005) have developed species-specific PCR primers which detect also latent infections of *L. seditiosum*.

In Finland, needlecast caused by *M. laricis* has mainly been a problem in Siberian larch during

their second growing season (Poteri 2008). Infection occurs during the early part of the growth period while succulent foliage is present on second year seedlings and during periods of wet weather. Infected needles turn brown quite suddenly, wither and soon fall from the tree. The pathogen overwinters in fallen needles and remains infective to local seedlings for at least two years. Seedlings can escape infection if dry conditions prevail during the needle growth period.

Lophodermium needlecast was among the first diseases to be controlled with fungicides in Finnish nurseries (Jamalainen 1956a). Although severe epidemics only occur under particular conditions (Kurkela 1979), the potential economic loss is so great that annual application of fungicides is standard in southern and central Finland (Lilja 1986, Poteri 2008). Larch needlecast can be reduced by producing only 1-year-old seedlings.

2.6 Snow Blights

Seedlings suffering from snow blights are usually detected following snow-melt in the spring. Withered needles below the snowline are matted together with grayish or black brown mycelium and infected seedlings occur in patches (Peace 1962). Snow blights of conifers are mainly caused by two ascomycete fungi: *Phacidium infestans* P. Karst. and *Herpotrichia juniperi* (Duby) Petrak. The later, the black snow mold, is connected with Norway spruce in the nurseries but in the forest it is most common on *Juniperus* and could be found on pines, too. Whereas *P. infestans* can infect conifers generally (Björkman 1948, Kujala 1950, Roll-Hansen 1987), although it has been considered to be an important pathogen of *Pinus* in northern parts of Europe and Asia where snow cover is deep and prolonged. Recent observations in Finnish nurseries and inoculation trials suggest that *P. infestans* also infects container seedlings of Norway spruce (Petäistö and Hantula 2009).

P. infestans infects needles in the autumn via ascospores and continues until the snow covers the apothecia or they become exhausted (Kurkela 1996). Mature spores continue to be released at temperatures close to 0° C (Kurkela 1995a). Although heavy rain decreases spore liberation,

free water is required to initiate ascospore release and a maximum dispersal is reached within 4–6 h of suitable rain. *P. infestans* can also spread from branch to branch under the snow by mycelial growth. The fungus continues to grow at a few degrees below freezing point (Björkmann 1948). In the laboratory, although growth took place as low as -5°C (Björkman 1948). According to Vuorinen and Kurkela (1993) CO_2 concentration and activity of *P. infestans* under snow reached the maxima at higher temperatures (-3° to 0°C) in late winter when the mycelial colonies achieved their greatest extension.

Snow blights, usually occur in northern latitudes and at high altitudes in the south where there is sufficient snow cover during winter (Jamalainen 1956b, 1961). However, according to a recent study *P. infestans* was able to infect seedlings both outdoor and indoor in a freezer storage (Petäistö and Hantula 2009). The freezer storage result proves that the disease can develop without snow cover. In Estonia *H. juniperi* infections have been recorded up to a height of three meters in Norway spruce in dense forest stands during moist winter conditions where only a few inches of snow covered the ground (Hanso and Törva 1975).

Effective control of snow blights in nurseries requires spraying with fungicides. Seedlings overwintering outdoors should be sprayed as late as possible in the autumn before the formation of permanent snow cover. As the onset of winter can be unpredictable, treatment may have to be repeated several times.

2.7 Scleroderris Canker

Recently, a new type of disease similar to Scleroderris canker was found on nursery grown Norway spruce seedlings in Norway and Finland (Børja et al. 2006, Petäistö 2008). Symptoms were similar to those observed on Scots pine: infected needles browned from the base and eventually dropped from the seedling. Necrotic lesions were also present on Norway spruce shoots (Børja et al. 2006). In inoculations the browning of needles first occurred in the mid section of the shoot whereas on pine at the top of the shoot (Petäistö 2008). Typical for Scots pine seedlings is also drooping of needles (Poteri 2008).

The ascomycete *Gremmeniella abietina* (Lagerb.) Morelet var. *abietina* (Petrini et al. 1989) is the causal agent of Scleroderris canker in conifers of Fennoscandia. Although the sexual stage of *G. abietina* was described from Norway spruce growing naturally (Lagerberg 1913), Scleroderris canker has periodically destroyed a lot of Scots pine seedlings in nurseries.

On the basis of immunological, biochemical and DNA profiles, *G. abietina* has been divided into North American, Asian and European races (Dorworth and Krywienczyk 1975, Lecours et al. 1994, Hamelin et al. 1993, Müller and Uotila 1997). Within the European race, three separate biotypes have been recognized: one affecting large trees (LTT), one affecting small trees (STT) and an alpine type (Hellgren and Högborg 1995, Hamelin et al. 1996, Petäistö et al. 1996). Based on new information on the epidemiology, morphology and genetics Laflamme has suggested in his review on the genus that the different races and biotypes represent different species and should be redescribed (Laflamme 2002).

While LTT and STT both infect Scots pine seedlings in nurseries (Uotila 1983, Børja et al. 2006), inoculation experiments using conidia suspensions or live mycelia suggested that LTT was a more potent pathogen than STT (Uotila and Terho 1994, Terho and Uotila 1999). Furthermore, LTT has been implicated as the pathogen responsible for epidemics that occur commonly in forests and nurseries after cool and wet growing seasons preceded by mild winters (Kurkela 1981, Sairanen 1990, Børja et al. 2006).

G. abietina has a life cycle of 2–3 years, with asexual conidia being formed on spruce 2 years and on pine 1 year after infection (Petäistö 2008). Ascocarps will form 3 years after infection or later. Infection by *G. abietina* proceeds several weeks after spore germination when it penetrates the periderm and subsequently invades shoot tissues by the end of the growing season (Lang and Schütt 1974, Siepmann 1976, Patton et al. 1984). During their first growing season Scots pine seedlings are most susceptible to infection when buds form (Petäistö 1999). Second-year seedlings, however, have a higher infection risk during the active shoot growth (Petäistö and Laine 1999). Similarly on spruce the susceptible period begins on the first year seedlings when buds form

in the second half of the growing season and on the second-year seedlings during the active shoot growth in the first half of the growing season (Petäistö 2008). During humid conditions a peak in conidiospore release have been found in June–July, although conidiospores might be present in the air through the whole summer (Petäistö and Heinonen 2003).

Winter storage temperatures influence infection rate in Scots pine seedlings; those overwintering at -7°C and -3°C were more diseased than those kept at 0°C (Petäistö and Laine 1999). Seedlings stored at lower temperatures broke bud later than those kept warmer, suggesting that a physiological factor related to growth initiation may increase vulnerability (Petäistö and Laine 1999). In addition factors that retard shoot maturation increase host-plant susceptibility to *G. abietina* infection (Donaubauer 1972, Uotila 1988). For example, increased nitrogen levels (Ylimartimo 1991, Barklund 1993), incomplete lignification due to rapid growth, low temperatures and light intensity during the summer (Petäistö and Repo 1988) and certain amino acids that stimulate *G. abietina* (Gezelius and Näsholm 1993).

Infected seedlings usually appear green and healthy immediately after the snow has melted. Development of the symptoms depends on the weather conditions and may take a number of weeks before damage becomes clearly visible. This can cause problems in the grading of seedlings for outplanting. A PCR-based *Gremmeniella* detection system have been developed in Canada (Hamelin et al. 2000).

2.8 *Sirococcus* Blight and Cankers

Needles of infected conifer seedlings turn brown at the base and eventually detach. The fungus grows into elongated shoots, where cankers form and subsequently cause restrictions that cause shoot tips to curl over and form a crook. In Finland, cankers were formed on the lower part of current-year stems of 2-year-old Norway spruce seedlings, without a crook formation, in July in areas where needles had fallen off (Lilja et al. 2005).

Sirococcus conigenus is a mitosporic fungus affecting cones and shoots of several species of

conifers in temperate and boreal forests of Europe and North America (Sutton 1980, Sutherland et al. 1981, Sutherland 1987, Halmschlager et al. 2000, Tian Fu and Uotila 2002, Smith et al. 2003). This asexually-reproducing fungus infects seeds and the current-year shoots with conidia borne in small, black pycnidia on cone scales, needles or shoots when the humidity is high. Part of the new infections in the nursery originate from contaminated seeds. After seed germination spores formed in pycnidia on diseased seedlings spread the disease to new seedlings (Sutherland et al. 1981, Sutherland 1987). Low light intensity alone or in conjunction with low temperature before inoculation predisposed seedlings to *Sirococcus* infection (Wall and Magasi 1976). In Austria, imbalanced tree nutrition was associated with an increased incidence of *Sirococcus* shoot blight on Norway spruce; analysis of needles from healthy and diseased trees revealed significant differences in the foliar contents of Mg, Ca, P and Mn (Anglberger et al. 2002, 2003).

Based on culture characteristics, conidium morphology and DNA sequences, Smith et al. (2003) divided *S. conigenus* into two groups: “P group” found in *Larix*, *Picea*, *Pinus* and *Cedrus deodara* (D. Don) G. Don, and “T group” found in hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and *Cedrus atlantica* (Endl.) G. Manetti & Carr. A third group consisting of isolates found in *Picea* of Canada and Switzerland was subsequently confirmed with rDNA ITS data and named “S group” (Konrad et al. 2007). Within this classification, Konrad et al. (2007) also found distinct haplotypes in P group, some of which corresponded to geographical boundaries or to particular host species, e.g., haplotype group III was restricted to Aleppo pine (*P. halepensis* Miller) in Spain and haplotypes of group IV were from different host species in Canada. Rossman et al. (2008) surveyed morphological and nucleotide sequence data in the groups and, based on their findings, redescribed *S. conigenus* [corresponding to P group in Smith et al. (2003)] and formally named the S and T groups as *S. piceicola* Rossman, Caslebury, D.F. Farr & Stanosz and *S. tsugae* Rossman, Caslebury, D.F. Farr & Stanosz, respectively.

The postgermination spread of the pathogen occurs from disease loci originating from seed-born inoculum (Sutherland et al. 1981, Suth-

erland 1987). Thus the mishandling of cones or seeds is one source of this pathogen. The contamination of seeds often occurs via soil during cone collection or storage (Sutherland et al. 2002). The inclusion of old, infested cones in current-year's collections, can also be a source of *S. conigenus* (Sutherland et al. 2002). High hygiene during collection and storage and culling of damaged seeds will decrease the risk of seedborne inoculums. Sometimes the disease can be traced to infected cones on nearby forest trees. In such cases it may be worthwhile to remove such trees (Hamm et al. 1990).

2.9 Pine Twisting Rust

Infected shoots may become girdled and when the lesion is confined to one side of the shoot, normal growth on the opposite side results in bending or twisting of the shoot.

Pine twisting rust is caused by *Melampsora pinitorqua* (Braun) Rostr. The disease occurs sporadically in nurseries (Kurkela and Lilja 1984) and is a serious threat to pine sapling stands (Kujala 1950, Jalkanen and Kurkela 1984).

Gäumann (1959), Rostrup (1884), Hartig (1885) and Klebahn (1903) have described the life cycle of *M. pinitorqua*. Its alternate host is aspen (*Populus tremula* L.) and the rust overwinters as the telial stage on fallen aspen leaves. The microclimate during the winter may influence the condition of the teliospores; teliospores on leaves collected in the autumn did not germinate until early the following May and only if they were stored outdoors (von Weissenberg 1980). In nature, teliospores germinate in the spring following rain and produce basidiospores to infect the current years' leading shoots of Scots pine. Kurkela (1973a, b) studied the dispersal of basidiospores during June in three consecutive years and found basidiospores were formed and released from +0 to 27°C (peaking at 15–20°C) and that the maximum number of spores and densities varied greatly between years. Rain has a critical influence on the formation and liberation of basidiospores as well as on their infection rate (Klingström 1963, Kurkela 1973a). Spermogonia produced yellow-orange aecidia within 10–14 days of infection (Kurkela 1973a). Aecidiospores

are wind-dispersed and infect aspen where the uredial and telial stages are produced on the undersides of leaves. Aeciospores and urediniospores are dispersed mainly during dry weather and according to a diurnal rhythm (Taris 1966, 1968, Kurkela 1973a), although sudden rains can briefly increase the spore density of aeciospores (Kurkela 1973a). Finally, several cycles of urediniospore production and infection on aspen enable the fungus to spread rapidly during late summer and early autumn (Kurkela 1973a).

Resistance of pines to pine twisting rust is partly dependent on genetic factors (Klingström 1969, von Weissenberg 1973, Martinsson 1980). Inhibitory compounds such as resin acids were extracted from the annual shoots of Scots pine and loblolly pine (*P. taeda* L.) and found to affect the germination of basidiospores (Klingström 1963, von Weissenberg 1973). Trees with a high growth rate were found to be more susceptible to the disease than those growing more slowly (Klingström 1969, Jalkanen and Kurkela 1984). Disease incidence in nurseries is greatest near aspen. Elimination of aspen usually affords adequate disease control.

2.10 Birch Rust

Yellow urediniospores appear in late summer as pustules on the undersides of leaves. The fungus usually has its uredinal and telial stages on the leaves of *Betula* spp., although they sometimes also occur on the leaves of some *Alnus* spp. (Roll-Hansen and Roll-Hansen 1981). The aecial stage has been reported to develop on the needles of *Larix* sp. (Klebahn 1904). Birch rust is caused by *Melampsorium betulinum* (Fr.) Kleb. in Basidiomycetes. However, inoculation trials with basidiospores of *M. betulinum* in Finland failed to infect *Larix* sp. (Liro 1906, pers. comm. Marja Poteri, Finnish Forest Research Institute).

Poteri (1992) suggested that *M. betulinum* has two *formae speciales*. When urediniospores collected from silver birch (*B. pendula*) and downy birch (*B. pubescens*) were used in cross-inoculation trials, downy birch showed partial resistance to silver birch rust in the form of necrotic lesions at infection sites and reduced production of new urediniospores. In contrast, downy birch

rust was equally virulent in both birch species and no hypersensitivity reactions were found even though several different clones of silver birch were tested (Poteri 1992, Poteri and Ryyänänen 1994). Scanning electron microscopy of appressoria formation, location and penetration of stomata failed to recognize any of these factors as the basis for resistance (Poteri and Ryyänänen 1994). Silver birch clones were found to have different resistance to silver birch rust, and clones grown at low nitrogen levels were more resistant than those grown at higher levels (Poteri and Rousi 1996). Seedlings grown at high density and with a high nitrogen supply create wetter conditions on leaf surfaces and favour urediniospore germination (Sharp et al. 1958).

M. betulinum is capable of overwintering in buds or fallen leaves as uredinial mycelium (Liro 1906) or as urediniospores (Dooley 1984). Thus, outbreaks can be minimized by routine examination of seedlings and strict hygiene in the nursery. The epidemic phase of *M. betulinum* occurs as the uredinal stage in late summer. It is necessary to control birch rust in nurseries because of growth reduction and high mortality after out-planting (Lilja 1973).

2.11 Stem Lesions and Top Dying of Birch

Necrotic stem lesions and top dying on silver birch seedlings have been in Finland a severe problem with container-grown silver birch seedlings (Lilja et al. 1996b, Juntunen 2000). In 1991, *Phytophthora cactorum* (Lebert et Cohn) Schröter was first time isolated from these lesions and inoculations of birch stems with this Oomycetous Chromista in class Pseudofungi resulted in necrotic lesions identical to those on birch seedlings in nurseries (Lilja et al. 1996b, Hantula et al. 1997, 2000). How this non-native species was introduced to Finland stays unclear (Hantula et al. 2000).

Symptoms caused by *P. cactorum* vary according to seedling development. Following heavy rains in late June, the first lesions often appear and seedlings can suffer crown dieback. Seedlings infected at an early or succulent stage often die. When the bark is more suberized, lesions are more commonly born at the base of stems where

moisture collects and a wetter microhabitat can persist. Older infected seedlings either die or snap depending on the place of the lesion. Snapped seedlings can develop a new leader or produce a new shoot from the base because the pathogen does not infect roots (Lilja et al. 1996b, 2007).

Strict nursery hygiene has proven effective in the control of stem lesion as pathogen can overwinter in organic material (Landis 1989). It is also important to keep the microclimate within birch seedling stands as dry and well ventilated as possible. *P. cactorum* is capable of infecting seedlings through intact bark, although disease severity was in general higher in seedlings in which inoculations were made on leaf scars (Lilja et al. 1996b, 2007). Thus wounds, even small ones, increase the risk of stem lesions.

2.12 Leaf Lesions of Birch

Regular, round, pale green lesions up to 1 cm in diameter are visible on both leaf surfaces of many birch species, including silver and downy birch (Mix 1949). Lesions are initially a different shade of green from the healthy leaf surface and they later turn brown. Common fungi sporulating on birch leaf lesions in Finland are 1) *Asteroma* sp., 2) *Gloeosporium* sp. or *Discula* sp. (*Cylindrosporium*), and 3) *Marssonina betulae* (Lib.) Sacc. (Kurkela 1995b). *Phomopsis* and several species of Fungi Imperfecti are also associated with lesions on birch leaves and have occasionally been observed on nursery seedlings of silver birch.

Cylindrosporium conidia germinate readily on agar to produce strands of several anastomosing parallel hyphae. In inoculation trials, this fungus has produced leaf lesions. *Asteroma* conidia are produced on the same kind of lesions as *Cylindrosporium* but are smaller (Kurkela 1995b, Paavolainen et al. 2000) and have not been cultured on agar. It is possible that *Asteroma* is the microconidial form of *Cylindrosporium*, which was shown to be the anamorph of an ascomycete, *Pyrenopeziza betulicola* Fuckel (Paavolainen et al. 2000). *M. betulae* is easily distinguished from *Cylindrosporium* on the basis of conidial morphology and it may exhibit a teleomorph similar to *Drepanopeziza*. All three fungi occur

in nurseries and in open forest areas. *Asteroma* and *Cylindrosporium* have been found only on leaf lesions but *M. betulae* may also infect growing shoots.

Phomopsis grows along petioles into the growing shoot. The same type of *Phomopsis* has been found in Ontario on the dying tops of yellow birch (Horner 1956). Its perfect stage was designated as *Diaporthe eres* f. sp. *betulae* Nits. (Arnold 1966). Arnold (1970) believed that the fungus could be a potentially serious pathogen for seedlings subjected to low light intensity and high humidity in their natural habitat.

Most of the Fungi Imperfecti associated with lesions on birch leaves are ascomycetes that overwinter on leaf litter. The first lesions, which produce conidia, usually appear in the beginning of July at the earliest. According to Redlin (1995), *Gloeosporium betularum* (Ellis & G. Martin) von Arx in the US is conspecific with a teleomorphic fungus belonging to the family Gnomoniaceae. *Gnomonia intermedia* Rehm occurs in Central Europe (Monod 1983) and also in Finland on birch leaf litter, but its hyphal cultures are different from the conidial isolations obtained from leaf lesions. The fungus is common in nurseries and on young birches in the forest, but it is apparently harmless.

2.13 Venturia Leaf and Shoot Blights

Leaves and shoots of aspen and hybrid aspen *Populus tremula* x *P. tremuloides* Michx are subjected to leaf and shoot blight disease caused by *Venturia* species. The typical symptoms of black shoot blight are crooked, blackish shoots ("shepherd's crooks") and blackened leaves. Usually *Venturia* diseases are not killing the plants. However, death of shoots will stop the height growth and leads to rejection of the plants from the nurseries. Severe defoliation after outplanting will lead to growth losses, and increased mortality by weed competition and secondary pests and pathogens.

The taxonomy of *Venturia* species has not been well documented. At least two species occur on aspen and hybrid aspen, *V. tremulae* Aderhold (anamorph *Pollaccia radiosia* (Lib.) Bald. & Cif.) and *V. macularis* (Fr.:Fr.) E. Müller & Arx, which

has been considered as saprophyte or non-aggressive pathogen (Morelet 1985). On *Populus* section *Tacamahaca*, the disease is caused by *V. populina* (Vuill.) Farb. (anamorph *Pollaccia elegans* Serv.) and few years ago described species *V. inopina* G. Newc. (Newcombe 2003). In northern Europe, a majority of the shoot blight symptoms on hybrid aspen and aspen are caused by *V. tremulae* (Kasanen et al. 2004).

The pseudothecia of the fungi are ball shaped of 0.2 mm diameter. The pathogen overwinters in the sexual stage and the ascocarps are embedded in the host bark or leaf tissue, which the pathogen had killed. As the ascocarp further develops, the plant surface breaks and ascospores are released from ostiole. Sporulation occurs in rainy weather throughout summer (Kasanen and Kurkela, unpublished data). The primary infections by ascospores are followed by asexual conidial production.

The *Venturia* species have high migrational potential and usually population subdivision is seen only due to other factors than geographical distance (Tenzer et al. 1999, Kasanen et al. 2004). Therefore, the only feasible management strategy is to use mixtures of resistant or moderately susceptible clones.

2.14 Powdery Mildews

Powdery mildews appear as a dusty white to gray coating over leaf surfaces or other plant parts. The diseases are caused by a group of ascomycetous fungi (Erysiphaceae) growing superficially on the leaves and only the haustoria penetrate into cells. Some of them appear as dense white mycelium on the leaf upper surface like *Microsphaera* species on oak or *Uncinula* on maple and willow, and some form sparse and loose hyphal net, almost invisible without magnification. Conidia are formed sometimes very abundantly like a powdery mass on the infected leaves. Teleomorph of Erysiphaceae fungi is called cleistothecia which develop among the mycelium and can be seen by naked eye as black dots when ripe. Cleistothecia are like perithecia but without special opening for liberating spores. The most Erysiphaceae fungi have specially formed cleistothelial hairs which are used to identify different genera. Powdery

mildew infection may cause distortions in young growing leaves.

Powdery mildews are large group of fungi having specialized pathogens for most of plant species except conifers (Blumer 1933). Maple (*Acer platanoides* L.) has common powdery mildew disease caused by *Uncinula tulasnei* Fuckel. Willows are hosts of *Uncinula salicis* (D.C.) Winter. At least two *Microsphaera* species, *M. alphitoides* Griff. & Maubl. and *M. hypophylla* Nevodovskij are able to infect oak (*Quercus robur* L.).

U. tulasnei may infect massively maple sprouts in late summer. Small white circular mycelial spot appear on the upper side of the leaves and often on the bark of growing shoots too. The spots grow together and may finally cover the whole leaf surface. In such cases the fungus retards the growth.

Microsphaera fungi infect the both sides of oak leaves. White mycelial matt grows usually from the middle of the leaf and may cover gradually the whole leaf surface. By *M. alphitoides* Black cleistothecia appear in the oldest part of mycelium, mainly on the upper side of the mature leaves. *M. hypophylla* infects growing shoots already in the early summer producing cleistothecia on the lower side of the leaf (Roll-Hansen 1961).

The conidia of powdery mildews are spread by wind from host to host. Mature cleistothecia may also crack open during the summer and liberate asci and ascospores for new infection. There are two ways of overwintering of these fungi: 1) ascospores, remained in cleistothecia in plant debris in winter, liberate in the spring and are then carried by wind to the host leaves, 2) conidia or mycelium may overwinter between bud scales and then grow out to cause new infection in the early summer. The spreading of powdery mildews does not depend on rains or dew. Conidia may germinate in humidity from 40–100% and in the temperatures around 20°C. Diffuse light apparently favors the infection. Some important facts to avoid powdery mildew infection: do not grow plants in dense patches, arrange good aeration, grow the plants in direct sunlight, avoid excess fertilization with nitrogen, use proper fungicides if necessary.

3 General Conclusions

Factors such as high seedling densities, irrigation, fertilization, and herbicides, that make nursery production of conifers successful may also encourage their pathogenic fungi. Strict hygiene, use of good quality seed, pest-free growth medium, clean containers and growing-area surfaces, together with cultural practices such as proper irrigation, ventilation, fertilization, seedling densities and removal of weeds all discourage or prevent the entry and survival of pathogens causing damping-off, grey mold, root dieback and stem lesions. There are also diseases such as Scleroderris canker, birch rust, snow blights and Lophodermium needlecast which require annual application of fungicides. For environmental reasons, the use of fungicides should be minimized and carefully-controlled sprayings must be carried out at the correct time. A knowledge of pathogen life cycles and the conditions which predispose seedlings to disease guarantees effective and economical management. In Finland, rainfall in summer and autumn can be so high that the use of folding or retractable roofs might improve the quality of seedlings (Bartok 2005). Because diseased seedlings may appear green and healthy immediately after winter, their detection in the short time between lifting and outplanting in the springtime remains a problem. Symptoms are dependent on weather conditions and it may be several weeks before diseases such as Scleroderris canker and Lophodermium needlecast become clearly visible. Modern tools, based on molecular biology, can help to identify diseases in seedlings although their current cost may prohibit their routine application. Only healthy seedlings survive after outplanting and can guarantee the future of nurseries and artificial reforestation techniques.

Changes and adaptations in forest nursery technology have occurring in last ten years, but these may need to occur at an accelerated rate because of rapid changes in climate. In future abiotic diseases associated with environmental extremes are expected to increase (Boland et al. 2004, Desprez-Loustau et al. 2007). Also diseases as rust, powdery mildews as well as other fungi infecting leaves gain higher precipita-

tion (Desprez-Loustau et al. 2007). The climate change might also improve the establishment of introduced species into new geographical areas. With time, we expect more non-native pathogens to be introduced in Finnish nurseries.

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