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THE EFFECT OF CROP QUALITY AND PRE-TREATMENT ON GERMINATION IN SCOTS PINE AND NORWAY SPRUCE SEEDS

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DEPARTMENT OF BIOLOGY,
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THE EFFECT OF CROP QUALITY AND PRE-TREATMENT ON GERMINATION IN SCOTS PINE AND NORWAY SPRUCE SEEDS

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Abstract

Weather conditions during the growing season are determining the size and quality of the Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seed crop in northern areas. Pathogens, fungi, and insects also have an effect on seed crops. The varying quality of seeds from forest stands and seed orchards does not full fill the germination requirements of tree nurseries. Multi-phase pre-treatment are therefore used in forest tree seed centres to improve seed lots quality.

The main objectives of this study were to analyse long-term variation in the size and quality of Scots pine seed crops in Northern Finland. Determine the impact of fungal injuries on the structures of Norway spruce seeds. To detect changes in the germination capacity and rate of Norway spruce seeds during pre-treatment phases and to determine the impacts of short-term and long-term storage on the germination of treated seeds.

The study found that in most years, regeneration of Scots pine in Northern Finland is limited by quantity as well as quality the seed crop. The long-term average of the Scots pine seed crop was 77seeds/m^2 and the long-term average expected germination percentage was 61%. Aeciospores of the inlad spruce cone rust *Chrysomyxa pirolata* (Körnicke) Wint. were found to form inside Norway spruce seeds, destroying the nucellar layers and reducing germination of seeds. In general, the germination capacity and rate of Norway spruce seeds increased during pre-treatment phases. The germination capacity of seeds increased about 30% and the rate by more than 40% during pre-treatment. During long-term storage the germination capacity and rate of pre-treated Scots pine seeds were preserved better in frozen storage than in cool storage. It was found that pre-treated Scots pine forest stand seeds can be stored for several years in frozen conditions. The germination capacity and rate of pre-treated orchard seeds were effected significantly more than those from forest stands. It is therefore recommended that Scots pine seeds from orchards be stored without pre-treatment. The germination capacity and rate of treated Norway spruce seeds from orchards was not significantly different after one year of storage.

Keywords: expected germination percentage, fungal injuries, germination capacity, germination rate, IDS treatment, pre-treatment, seed crop, storage

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The idea for study one was planned together with Mikko Hyppönen, Ville Hallikainen, Marja-Liisa Sutinen, and Tatu Hokkanen. The cone collection for seed crop quality analyses were made by the staff of Metsähallitus and x-ray analyses were carried out by employees of Forelia Co. The author saved seed quality information in a data base. The seed crop size was determined by Tatu

Hokkanen. The author planned the study, made statistical analyses with help of Juha Hyvönen, edited the pictures and wrote the original manuscript and its revised version.

The study idea of the second paper was planned together by the authors. The author and Enni Pesonen collected seed samples for the study. The author prepared microscopy samples for FESEM and stained seed samples with Annneli Kauppi, cut microscopy sections by microtome with Leena Seitamaki and Paula Kylmänen, and used FESEM photography with Anneli Kauppi and Eila-Tillman-Sutela. The author studied seed samples with fluorescence microscopy, measured the dimensions of aciospores, and edited picture pages of the third manuscript and took part in the writing of it. The manuscript and its revised version were mainly written by Eila Tillman-Sutela.

The study idea for the third work was planned by Eila Tillman-Sutela, who also provided the basic guidelines for implementation of the study. The author took part in the IDS treatment of seeds along with the staff from Forelia Co., made germination tests, studied seed samples by x-ray radiography, saved germination test results in a data base, carried out statistical analyses, took part in FESEM photography with Annneli Kauppi and Eila Tillman-Sutela, edited the pictures, and wrote an English manuscript of the second paper. The manuscript revised version was written by all three authors.

The fourth study was planned by Eila Tillman-Sutela and the author. The author made germination tests, studied seed samples by x-ray radiography, saved germination tests in a data base, carried out statistical analyses, and wrote the original and revised manuscript.

Oulu, January 2009 Anu Hilli

List of original papers

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II Tillman-Sutela E, Kauppi A, Hilli A & Kaitera J (2004) Fungal injury to seed tissues of Norway spruce, *Picea abies* (L.) Karst. Trees 18: 151–156.

III Tillman-Sutela E, Hilli A & Kauppi A (2003) Germination changes of *Picea abies* seeds at water-based pretreatments. Seed Technology 25: 168–182.

IV Hilli A, Tillman-Sutela E & Kauppi A (2003) Germination of pretreated Scots pine seeds after long-term storage. Canadian Journal of Forest Research 33: 47–53.

Contents

Introduction

The size and quality of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seed crops varies greatly between years, stands and individual trees. The variation between years is mainly an effect of temperature conditions during the reproductive cycle, while the variation between stands and individual trees depends on microclimatic conditions, site conditions, tree size and genetic features (Sarvas 1962, Hagner 1965, Koski & Tallqvist 1978, Leikola *et al.* 1982). The number of empty and dead seeds, pathogens, fungi, and insects all reduce the size and quality of seed crops (Heikinheimo 1932, Sarvas 1962, Nikula & Jalkanen 1990). Good seed years occur only a few times a century in northernmost Finland (Renvall 1912, Henttonen *et al.* 1986).

In order to ensure a supply of forest regeneration material for northern Finland, seed orchards of Scots pine and Norway spruce have been established in southern Finland (Sarvas 1970, Nikkanen *et al.* 1999). A high level of background pollination from southern forests reduces the use of seed orchard seedlings in Lapland and in the northern parts of the Province of Oulu because the seedlings are not adapted to the utilization area (Harju & Muona 1989, Nikkanen *et al.* 1999, Pakkanen *et al.* 2000, Parantainen & Pulkkinen 2003). Thus in northern Finland, forest regeneration depends on the size and quality of seed crops collected from natural stands.

The germination capacity and rate of seeds collected from Scots pine and Norway spruce forest stands or from seed orchards do not fulfill the requirements of germination needed in nurseries, where the aim is to sow only one seed in each pot. Thus to improve germination percentage and to reach a homogeneous germination rate, a multi-phased pre-treatment is used in forest tree seed centers (Fig. 1). At present, variation in the germination capacity and rate of Scots pine seed lots can be successfully minimized using the multi-phased pre-treatment (Bergsten 1988, Tillman-Sutela 1995, 1996) but using the same procedure on Norway spruce seeds has rarely raised the germination capacity and rate to a satisfactory level. The proportion of Norway spruce seedlings in the total amount of seedlings produced in nurseries is almost 70% because spruce forests are often regenerated using planting (Finnish statistical yearbook of forestry 2007). There is therefore demand from the forest nurseries for high-quality spruce seeds.

Seeds collected in fall must be stored at least until the following growing season. Since northern Scots pine and Norway spruce stands do not produce abundant cone crops with high germination capacity every year (e.g. Sarvas 1962,

Ryynänen 1982), there is also a need for long-term storage. The germination capacity of non-treated Scots pine and Norway spruce (Huss 1967, Kamra 1967a,b), and *Pinus elliottii* Engelm. and *Pinus echinata* Mill. (Barnett & Vozzo 1985) seed is known to be well-preserved for several years in frozen storage, but research on the storage of pre-treated seeds is scant and has been performed on short-term storage only (Tillman-Sutela 1996).

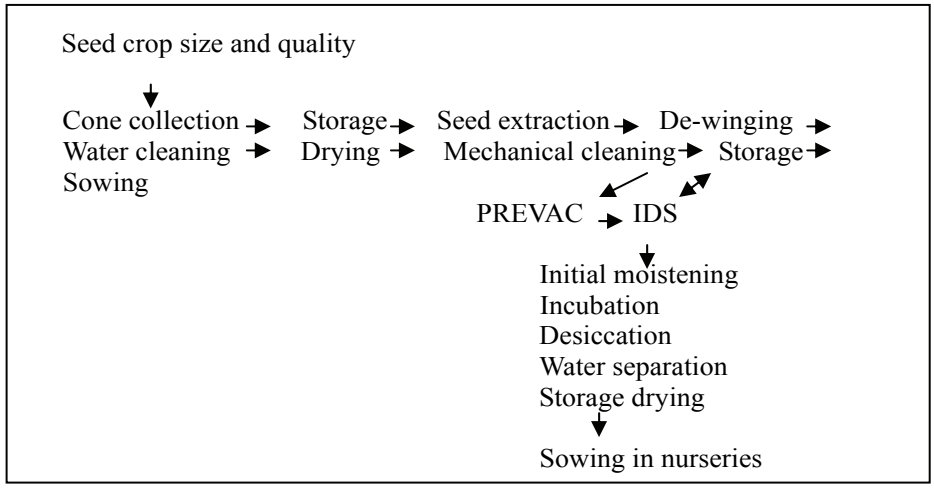

Fig. 1. The multi-phase pre-treatment of forest tree seeds from cone collection to nursery sowing. The seed crop is collected if the size and quality is good. Modified from Tillman-Sutela *et al.* 2003. The picture is reprinted with permission from the Association of official seed analysts and the Society of commercial seed technologists.

1.1 Seed crop size

The size of seed crop is determined by the formation of regenerative buds, flowering, pollination, and seed maturation (Sarvas 1962, Owens *et al.* 2001). Temperature conditions influence the seed crop size of Scots pine and Norway spruce from flower initiation to seed dispersal (Sarvas 1970, Kardell *et al.* 1973 Leikola *et al.* 1982, Parantainen & Pulkkinen 2003). Dry, sufficiently windy weather enhances the pollination and consequently the seed crop of Scots pine and Norway spruce (Sarvas 1962, Nikkanen *et al.* 2002).

Earlier studies have also shown that tree characteristics have an influence on the seed crop. A positive correlation has been found between the number of cones and the diameter of trees at breast height (Hagner 1965, Karlsson 2000), whereas Lähde (1976) has shown that the taller and older the tree, the greater the cone crop. The number of viable seeds per cone (Karlsson 2006) and seed production (Mikola 1987) is increased significantly by fertilization. Karlsson and Örlander (2002) have also reported that mineral nutrients are major factors limiting seed production. Further, genetic factors have an effect on the size of seed crops. Previous studies have noted variation between forests and clones in both female and male flowering (Sarvas 1962, Koski & Tallqvist 1978, Saarsalmi *et al.* 1994). Furthermore, large differences in the quantity and quality of the seed crops among clones have also been found (Saarsalmi *et al.* 1994, Nikkanen *et al.* 2002).

Most Scots pine crops fall within boundaries of 0–250 seeds/m^2 (Heikinheimo 1937,1948, Sarvas 1962, Hagner 1965, Koski & Tallqvist 1978, Parviainen & Seppänen 1994, Béland *et al.* 2000, Hokkanen 2000, Karlsson & Örlander 2000, Hannerz *et al.* 2002), although larger crops have been reported (Koski & Tallqvist 1978). The crop size of Norway spruce varies generally from 0 to 280 seeds/m^2 (Heikinheimo 1948, Nilsson *et al.* 2002).

1.2 Seed crop quality

The proportion of Scots pine seeds expected germination percentage has been found by previous studies to vary widely – between 0–97% (Ryynänen 1982, Harju *et al.* 1996, Juntunen & Neuvonen 2006) and from 70 to 100% (Sahlén & Bergsten 1994). Temperature requirements are the most important in seed maturation (Simak 1972, Kardell *et al.* 1973, Sarvas 1962, 1970, Henttonen *et al.* 1986, Sahlén & Bergsten 1994). According to Sarvas (1970), 845 degree days (d.d.) are needed for 50% of Scots pine seeds to mature anatomically (an embryo occupying ¾ or more of the embryo cavity), and most of the seeds reach maturity between 900 and 1,100 d.d. (Sarvas 1970, Nygren 1987, Almqvist *et al.* 1998). The temperature requirements for Norway spruce seed maturation are lower than those for pine, a germination capacity of 95% is reached at an annual heat sum of 875 d.d. (Almqvist *et al.* 1998). Further, the maturation of the surface structure of Scots pine seeds have been reported to respond a disturbed photoperiod (Tillman-Sutela *et al.* 1998) and thus the length of the photoperiod has an effect on seed maturation (Sahlén & Bergsten 1994).

The number of dead, empty, and damaged seeds reduces the quality of seed crops. The average proportion of empty seeds in a Scots pine seed crop has been found to vary between 15% and 20% and with a larger variation in Norway spruce – between 45% and 60% (Heikinheimo 1932, Sarvas 1962, Nygren 1986). The percentage of empty Norway spruce seeds has been found to be high, even in seeds collected from seed orchards (Nikkanen *et al.* 2002, Tillman-Sutela *et al.* 2003). The proportion of dead seeds and abnormal germinants also reduces the germination capacity of seed crops harvested by a variable degree (Bergsten 1988, Tillman-Sutela 1995, Hilli *et al.* 2003, Wennström *et al.* 2007).

Pathogens, fungi, and insects also reduce the size and quality of seed crops. The most important insects causing injuries in Scots pine and Norway spruce cones and seeds are *Dioryctria abietella* Den. & Schiff., *Eupithecia abietaria* (Goeze) *E. pini* Retz*., Eupithecia analoga* Diakonoff., *Plemeliella abietina* Seitn., and *Pissodes validirostis* Sahlberg (Nikula & Jalkanen 1990, Uotila & Kankaanhuhta 2003). Cone pathogens especially impair Norway spruce seed crops (Kangas 1940, Rummukainen 1960, Nikula & Jalkanen 1990). *Chrysomyxa pirolata* (Körnicke) Wint. and *Thekopsora areolata* (Fr.) P. Magn. are the only cone pathogens that found to cause economically important losses in Scandinavian seed crops' (Roll-Hansen 1965, 1967). The cone rust *Chrysomyxa pirolata* infect the female flowers of various spruces in spring, and rainy weather at the beginning of summer increases the release of rust spores from the alternate hosts to the hosts where the disease develops (Nelson & Krebill 1982, Crane & Hiratsuka 2000). Seeds of Norway spruce, (Rummukainen 1960) *Picea glauca* (Moench.) Voss. (Summers *et al.* 1986) and *Picea mariana* (Mill.) B.S.P. (Singh & Carew 1990) have all be found to produce fewer seeds with lower germination, if infected by *Chrysomyxa pirolata*. The seed crop can also be lost totally because of fungi infection (Hylander *et al.* 1953, Savile 1950, Ziller 1974, Nelson & Krebill 1982).

1.3 Factors affecting seed germination

Conifer seeds have three main components; the seed coat, the embryo, and the megagametophyte (Kolotello 1997). A hard seed coat can restrict or delay water uptake (Mayer & Poljakoff-Mayber 1963, Bradbeer 1992, Bewley & Black 1994) but the seed coat does not always restrict the imbibition of conifer seeds. For example the imbibition of Scots pine and Norway spruce seeds is regulated by the lipophilic covers surrounding the endosperm, nucellar layers, and nucellar cap

(Tillman-Sutela & Kauppi 1995a,b). Also the seed coat of *Pinus albicaulis* (Engelm.) and *Pinus sibirica* (Du Tour) is permeable (Tillman-Sutela *et al.* 2008) and nucellar and megagametophyte membranes with a hard seed coat restrict the water uptake of *Chamaecyparis nootkatensis* (D. Don) Spach seeds (Ren & Kermode 1999).

In order for seeds to germinate the environmental conditions must be favorable. The requirements for ambient conditions vary between species, but an adequate supply of water, a suitable temperature, and the presence of oxygen are the most important factors for germination (Mayer & Poljakoff-Mayber 1963, Bradbeer 1992, Bewley & Black 1994). Germination begins with water uptake where imbibition rate is determined by the availability of water, the permeability of seed structures regulating the uptake of water, and the conditions during hydration. During the second phase, the water uptake of seeds is quite low. However, major metabolic events take place during this phase. Water uptake increases again when the cells of the radicle extend and germination is completed (Mayer & Poljakoff-Mayber 1963, Bradbeer 1992, Bewley & Black 1994).

The germination of conifer seeds is most rapid in temperatures between 20 ºC and 25 ºC (ISTA 1999). The optimum temperature for the germination of Scots pine is between 20 ºC and 25 ºC (Kamra 1967a) and for Norway spruce 21 ºC (Simak & Kamra 1970, Bergsten 1987, Leinonen *et al.* 1993). The seeds of both species can also germinate in lower temperatures (Kamra 1967a, Leinonen *et al.* 1993, Leinonen & Rita 1995, Savonen 2002), conversely the germination of Norway spruce seeds decreases at temperatures constantly above 25 ºC (Simak & Kamra 1970, Leinonen *et al.* 1993).

Seeds can be divided into those that germinate in light or in darkness and those that are affected by the photoperiod (Mayer & Poljakoff-Mayber 1963). The germination capacity of Scots pine seeds is higher in light than in darkness (Sarvas 1950, Nyman 1963, Kamra 1967a). Norway spruce seeds do not require light for germination at optimal temperatures (Vaartaja 1956, Kamra 1967a, Leinonen *et al.* 1993, Leinonen 1998). However, after cold storage, Norway spruce seeds germinate best in light (Leinonen 1997, Leinonen & de Chantal 1998).

Germination processes can also occur in seeds that do not achieve radicle emergence regardless of favorable ambient conditions for germination. These seeds are said to be dormant (Mayer & Poljakoff-Mayber 1963, Bradbeer 1992, Bewley & Black 1994). Dormancy, the inability of the embryo to germinate, can be divided into coat-imposed and embryo dormancy (Bewley & Black 1994).

Embryo dormancy is common in woody species such as *Picea glauca* (Downie & Bewley 1996), *Chamaecyparis nootkatensis* (Ren & Kermode 1999), and *Pinus taeda* L. (Cooke *et al.* 2002). Dormancy can also be induced in mature, non-dormant seeds by environmental conditions unfavorable for germination or by storage conditions (Bewley & Black 1994, Leinonen 1997, 1998). Inhibitors preventing germination have been found in many species in the seed coat and embryo (Bewley & Black 1994). Abscisic acid (ABA) regulates many processes during a plant's life cycle, for example the prevention of precocious germination and the induction of primary dormancy. Other growth regulators, such as gibberellin, cytokinin, and ethylene have various effects on seed dormancy. In many species, dormancy can be broken by moist chilling at low temperatures (Bewley & Black 1994, Kermode 2005).

1.4 Pre-treatment of seeds

When seed lots of varying germination capacity are used for containerized seedling production, multi-seed sowing is required to minimize the amount of empty containers. Germination capacity and rate have to be over 95% in single-seed sowing. To reach a high germination percentage and a homogeneous germination rate, pre-treatment methods e.g. cleaning process' of seeds and moist chilling have been used (e.g. ISTA 1985, Edwards 1986, Gosling & Rigg 1990, Jones & Gosling 1994). The PREVAC technique (pressure-vacuum) is used to remove empty and damaged seeds from a seed lot. Whereas the IDS method (incubation-desiccation-separation) is used to raise the germination capacity of seed lots and to even out differences between seed germination rates (Lestander & Bergsten 1985, Simak *et al.* 1985, Lestander 1988). Germination begins during the IDS treatment of seeds but it is restricted by controlling the temperature and the availability of water. A temperature of 5–10 ºC is used in the incubation phase of IDS treatment of Scots pine and Norway spruce seeds (Bergsten 1988, Tillman-Sutela 1995, 1996). This restricts germination since it is lower than the optimal seed germination temperature (Kamra 1967a,b, Leinonen 1998). Live and dead seeds imbibe water similarly (Bewley & Black 1994) but their water-binding capacities diverge when the seeds are dried in identical conditions (Simak 1980, Bergsten 1987). Thus, the difference in seed density makes it possible to separate live and dead seeds in water by floating during IDS treatment (Simak *et al.* 1985, Bergsten 1987).

1.5 Aims of the study

Although variation in the size and quality of Scots pine seed crop has been studied, long-term variation in crop size and quality for seeds in northern Finland remains unknown. Pre-treatment methods are used in forest tree seed centres to improve seed quality. Methods such as mechanical cleaning and moist chilling are commonly used in forest tree seed centres, and in some places the PREVAC and IDS methods are used. Nevertheless, the effect of the pre-treatment from start to storage of seeds is poorly understood. Fungal injuries reduce germination capacity of seeds and fungal injuries may also have an effect on the success of the multi-phase pre-treatment. Seed germination commences during pre-treatment, but it is controlled by regulating humidity and temperature, which may affect the viability of seeds in storage.

The main objectives of this study can be divided into the following themes:

1. to analyse long-term variation in the size and quality of Scots pine seed crops in northern Finland (I),
2. to determine the impacts of fungal injuries on the structures of Norway spruce seeds (II, III)
3. to detect changes in the germination capacity and rate of Norway spruce seeds during the multi-phased pre-treatment (III),
4. to determine the impacts of short-term and long-term storage on the germination capacity and rate of pre-treated seeds (III, IV).

2 Material and methods

2.1 Material

The sizes of seed crops were studied in four Scots pine stands, located in Rovaniemi (66°21´N, 26°44´E) and Kittilä (68°01´N, 24°09´E). The seed falls were measured between 1960 and 2004 (I). The seed falls were caught in traps; there were 15 traps in the Rovaniemi I stand and 10 in the other stands (Rovaniemi II, Kittilä I and II). The size of the sample plots was 2500 m^2 in each stand. The seed fall traps were emptied at regular intervals between May and September. After collection, all the seed crop samples were air-dried and weighed.

The expected germination percentage of the pine seed crop was analysed from the stands of Kittilä. The cone samples were collected from the pine stands between the years 1986 and 2004, excluding the years 1987 and 1998 because of the low annual heat sum during these years. The cone samples collected between the end of August and mid-September were removed from the database because the expected germination percentage of seeds changes considerably in August and at the beginning of September (Sahlén & Bergsten 1994). The cones were collected from 10 trees per stand, and 10 cones from every tree were randomly taken into a sample. The cones were collected from different parts of the crown. The number and location of stands from which the cone samples were collected varied annually (I).

Seeds from a Norway spruce seed orchard were used for studying fungal injuries on the seed structures (II) and changes in the germination capacity and rate of seeds during the multi-phased pre-treatment (III). The Norway spruce cones (1200 l) were collected in February 2001 from seed orchard Sv176 (Metsä-Ihala), which is located in central Finland (62°12´N, 24°23´E). The seed orchard is founded using grafts collected from plus trees in northern Finland (66°–69° N, Nikkanen *et al.*1999).

Scots pine seeds from three open-pollinated stands and one seed orchard were used as material in the seed storage study (IV). Both pre-treated and non-treated seeds were studied. The pine seeds were collected in three open-pollinated stands located in Inari (68°50´N), Rovaniemi (66°50´N), and Lieksa (63°15´N) and in a seed orchard Sv141 (Parkkola, 62°12´N, 25°21´E). The seed orchard was founded using grafts collected from plus trees in northern Finland (67°–68° N, Nikkanen

*et al.*1999). The seeds had been cleaned using the PREVAC method to remove empty and damaged seeds (Lestander & Bergsten 1985).

2.2 Methods

2.2.1 Extraction of seeds

The Scots pine seeds were extracted from cones at a temperature of 40 ºC (I). The cones opened approximately after two days in the autumn-collected samples and after one day in the winter-collected samples. A 40-litre sample of Norway spruce cones was separated immediately after cone collection (III). From this sample, 20 cones were opened by hand to get seeds for a primary germination test (III). The rest of the 40-litre cone sample was allowed to open in airflow (20–22 °C). The rest of collected cones (1160 l) were divided into two parts for mechanical seed extraction. One part was treated using the ordinary method, i.e. sprinkling water on the cones at a temperature of 38 °C (II,III). The other part was treated at the same temperature, but the amount of water was restricted (dry-extracted seeds, III).

2.2.2 Pre-treatment and storage of seeds

After extraction the seeds were cleaned and dried (II–IV). The dried Norway spruce seeds were stored for three months at −3 °C before IDS treatment. The stored spruce seeds were incubated for 24 h at 5 °C before the IDS treatment (III). The IDS procedure (Bergsten, 1987) was run for Scots pine seeds in 1991 and 1992 (IV). In the IDS treatment, the pine orchard seeds were incubated for 8 days at 10 ºC or for 30 days at 5 °C. The forest stand seeds were incubated for 30 days at 10 ºC or for 50 days at 5 °C (Tillman-Sutela 1995). The incubated seeds were desiccated in airflow at 20 °C and separated in a water flume into 11 fractions. The treated seeds were dried to 6% (fresh mass) storage moisture content (III, IV). The Norway spruce seeds were stored for one year at a temperature of −18 °C (III). The Scots pine seeds from the three open-pollinated stands were stored for nine years and the orchard seeds were stored for ten years at temperatures of 2 °C (cool storage) and −18 °C (frozen storage) (IV).

2.2.3 Germination tests

The germination percentage and rate of the Norway spruce seeds were tested prior to pre-treatment, immediately after every phase of treatment, and after storage of one year (III). The Norway spruce seeds were germinated at 21 °C, thus differing from International Seed Testing Association (ISTA) rules (1999). After long-term storage the germination percentage and rate of Scots pine seeds were studied in the best and the poorest seed fractions (IV). The Scots pine germination tests were made at 20 °C and constant light (ISTA, 1999). The germination tests were made according to the ISTA rules, using four replications of 100 seeds (ISTA 1985, 1999). The replications were randomly allocated for the germination tests. Seeds with a radicle of at least the length of the seed coat were regarded as germinated, which differs from the ISTA rules (1985, 1999). The germination percentage indicated the percentage of seeds in the replicate that completed germination during a 14-day test. The germination rate of Norway spruce seeds (III) was determined as the proportion of germination that occurred within 7 days from the beginning of the test (Tillman-Sutela 1995). The germination rate of Scots pine seeds indicated the percentage of germination that occurred within 7 days of the test (IV). The germinated seeds were counted daily from the 4^{th} until the 10^{th} day. On the 14^{th} day, the abnormally germinated seeds and non-germinated seeds were also counted.

2.2.4 X-ray radiography and moisture content analyses

The expected germination percentage of the Scots pine seeds was determined by x-ray radiography on the basis of 300 seeds (I). The percentage was calculated from all the seeds and from filled seeds only. The calculation was based on seed maturation analyses on the proportion between embryos and embryo cavities (Simak 1980). Also the amount of empty seeds was analysed with an x-ray radiograph (I). Radiography was also used to follow Norway spruce seed coat opening at the different pre-treatment phases. Samples of 100 seeds were used (III). After the germination tests, radiography was used to classify the non-germinated seeds as live but non-germinated, empty, or dead (III, IV) (Simak *et al.* 1989). Changes in the Norway spruce seed moisture content at the different pre-treatment phases were analysed from 2-gram samples using an infrared dryer (Mettler LP 16-M, III).

2.2.5 Microscopy

The structures of the Norway spruce seeds were examined during the pre-treatment phases using field emission scanning electron microscopy FESEM, JSM 6400F (II-III). The FESEM was used to examine the opening of the seed coat and to determine the location of fungal injuries in the seeds. The fungal injuries were also determined by fluorescence microscopy. Fifty seeds from each pre-treatment phase were fixed in FAA (ethanol, glacial acetic acid and formalin 85:5:10), in which the seeds were also stored. The fixed seeds (100) were dehydrated in an alcohol gradient, critical-point-dried, attached to SEM mounts and sputter-coated with gold and palladium (II-III). Also fluorescence microscopy samples (70) were fixed in FAA, infiltrated, and mounted into Reichert-Jung-Historesin. Longitudinal 4-µm sections were cut on microtome. The unstained sections were studied using fluorescence microscopy with UV excitation. Some sections were stained with Pianeze staining for the differentiation of fungus and host tissues II (Simmons & Shoemaker 1952).

2.2.6 Statistical analyses

General linear models were used to analyse the size and quality of a seed crop. The effects of the annual mean temperature in July, the annual heat sum, the altitude, the location of the stand, and the stand characteristics were used as possible explanatory variables when analysing the size of the Scots pine seed crop. Seed crop measurements of single stand in different years were used as independent observational data in the analyses. Logarithmic transformation (ln) of the dependent variable (seed crop size) was used to stabilize the variance. When analysing the quality of the Sots pine seed crop (the expected germination percentage determined by x-ray radiography), the explanatory variables were the annual heat sum (ripening year), the mean temperature in July, the number of empty seeds, and the altitude. Arc sin square root transformation of the dependent variable (quality of seeds) was used to stabilise the variance (I).

Four replications of 100 Norway spruce seeds were randomly taken from different phase of pre-treatment to estimate the changes of germination capacity and rate through the experiment (III). Variance analyse with multiple comparisons was used to analyse the effect of the treatments. The means of germination capacity and rate during the pre-treatment phases were compared using Tukey's multiple comparison tests to find out the effect of the every phase on seed

germination (III). Further, the effect of the extraction methods on seed germination capacity and rate was studied in each phase using a paired T-test. The means of germination capacity and rate of Norway spruce seed fractions before storage and after frozen storage of one year were compared using the T-test (III).

Variance analyse with multiple comparisons was used to analyse differences between groups (IV). When the ANOVA revealed a significant difference between groups, a multiple comparison was performed using Tukey's test. The means of germination capacity and rate in pre-treated and non-treated stored seeds and control seeds were compared using Tukey's multiple comparison tests to find out the effect of storage temperature (2 °C and −18 °C) on Scots pine seed germination capacity and rate (IV). Further, the means of germination capacity and rate in pre-treated (incubated at 5 °C or 10 °C) and non-treated seeds after long-term storage were compared using Tukey's test to find out the effect of pre-treatment on storage endurance of seeds. The means of germination capacity and rate in pre-treated Scots pine seed lots after long-term storage were compared using a T-test to find out the impact of two incubation temperatures on the storage viability of seeds. The germination capacity and rate of the seed lots prior to storage were used as controls (IV).

In all statistical analyses the normality of the distribution was checked from the residuals. The significance level used in the comparisons was 5%.

3 Results

3.1 Long-term variation of pine seed crop size and quality in northern Finland

During the period of 1960–2004 the long-term average of the Scots pine seed crop in northern Finland was 77 seeds/m^2, varying between 1 and 359 seeds/m^2 (I, Table 3). The long-term average seed crop in the stands of Rovaniemi was 94 and 102 seeds/m^2 and in the Kittilä stands 59 and 58 seeds/m^2 (I, Table 3). The mean annual seed crop exceeded 200 seeds/m^2 five times in the stands of Rovaniemi and only twice in the stands of Kittilä. The mean annual seed crop in the stands was under 10 seeds/m^2 five times during the study period 1960–2004. No proper model was found for determining the size of the seed crop. The correlation coefficients between the seed crop (log-transformed) and the parameters of temperature and stand characteristics, and the altitude were small (I, Table IV).

In the Scots pine stands of Kittilä the long-term average expected germination percentage was 51% in all seeds and 61% in filled seeds (I, Table 2). The average expected germination percentage varied from 5 to 71% in all seeds and from 7 to 81% in filled seeds. The expected germination percentage exceeded 50% seven times during the years 1986–2004. The expected germination percentage also varied greatly between stands within the same year (I, Table 2). The long-term average of empty seeds was 17% and the annual average of empty seeds varied greatly among stands (I, Table 2). A combination of more than 100 seed/m^2 and an expected germination percentage over 50% was observed once during the years 1986–2004.

An annual heat sum of 800 d.d. was needed to achieve an expected germination percentage of 50% (I, Fig. 3). The annual heat sum of the seed-ripening year accounted for 40% of the variation in the annual expected germination percentage of the seed crop (I). When the proportion between empty seeds (%), altitude, and the mean temperature of July were added to the model, the coefficient of determination was 52%.

3.2 Effect of fungal injuries to seed tissues

The fungal injuries found in the tissues of Norway spruce seeds were caused by *Chrysomyxa pirolata* and *Thysanophora penicillioides* (Roum.) Kendrick (II, II

1A–H). The nucellar cap usually seemed to be entire and unbroken and it enveloped the megagametophyte and the embryo (II, III). The nucellar layers inside the seed coat were infected by fungi, and detached cells extruded from the edge of the opened seed coat (II, Fig. IB and III, Fig. 2C). These cell walls of the middle lamellae were disintegrated (II, Fig. IB). Protein and lipid bodies in the megagametophyte were rare or absent (II, III,Fig. 2D–E) and the middle lamellae of the cell walls had a gelatinous appearance (II, Fig. 1H and III, Fig. 2D). The outermost layer of the seed coat, the sarcotesta, was infected in most of the examined seeds by the conidia and conidiophores of *Thysanophora penicillioides* and the wax cover typical of mature conifer seeds was missing (II, Fig. 2A–D).

The surface structure of seeds was normal (II), but the hydrated seeds opened quickly (III). The opening of the seed coat took place already at the extraction phase of the pre-treatment (III, Fig. 2A). Both in the water-extracted and in the dry-extracted seed lots 70% of the seeds had opened their seed coat during the various treatment phases. The proportion of opened seeds was 30% – even in the seed lot extracted in airflow at room temperature (III). The seed coat had usually opened along the ridge extending from the micropyle almost to the edge of the nucellar cap (II, 1A and III, Fig. 2A).

3.3 Multi-phased pre-treatment and germination

The pre-treatment of Norway spruce seeds comprised 11 phases from cone collection to seed storage (III). After the extraction phase the germination percentage was in dry-extracted seed lots 37% and in water-extracted seed lots 44%. The germination rate was about 55% in both seed lots (Fig. 2–3). The differences in the means of germination capacity and rate between the water- and dry-extracted seed lots were mainly insignificant (III, Table 2). The opening of Norway spruce cones was the fastest in warm airflow.

After seed extraction the germination capacity and rate of spruce seeds rose during the water-cleaning phases and declined during the drying phases (Fig. 2–3). Mechanical cleaning alone decreased the germination rate of the water-extracted seeds by 26%-units compared to the germination rate of drying phase. The mean difference was statistically significant (III, Table 1). The germination capacity and rate of seed lots increased by 10–35%-unit during the three months of storage (−3 °C) preceding the IDS treatment, but the mean differences were statistically significant only in terms of the germination rate (III, Table 1). The moisture content of the seeds rose during the cleaning phases, reaching 47% in

dry-extracted seeds and 59% in water-extracted seeds after water cleaning (III, Table 2).

The germination capacity of the seeds was lower after the initial moistening in the IDS treatment as compared to storage-dry seeds (Fig. 2–3). The next phase, incubation, improved the germination capacity of the moistened seeds by only 2–3%-unit, whereas the germination rate increased by 9–12%-unit. The seed moisture content increased modestly in comparison to the initial moistening (III, Table 1); it remained the same after 24 hours and after 4 hours of incubation. After the IDS treatment in the best fractions the germination capacity of seeds was over 70% while the germination rate exceeded 90%. The germination capacity of the seeds in best fractions rose about 30%-unit from the extraction and cleaning phase, while the germination rate rose more than 40%-unit (III).

After the extraction phase the amount of empty seeds was 33% in the dry-extracted and 22% in the water-extracted lot. A great number of empty and dead seeds were sorted out during the mechanical cleaning phase and the rest were separated into the cast-off fraction (Fr11) during the IDS treatment. In the best fractions only 1–3% of the seeds were dead and no empty seeds were observed after the IDS treatment. The proportion of opened seeds in the poorest fraction was 45% in the dry-extracted and 51% in the water-extracted seed lots (III).

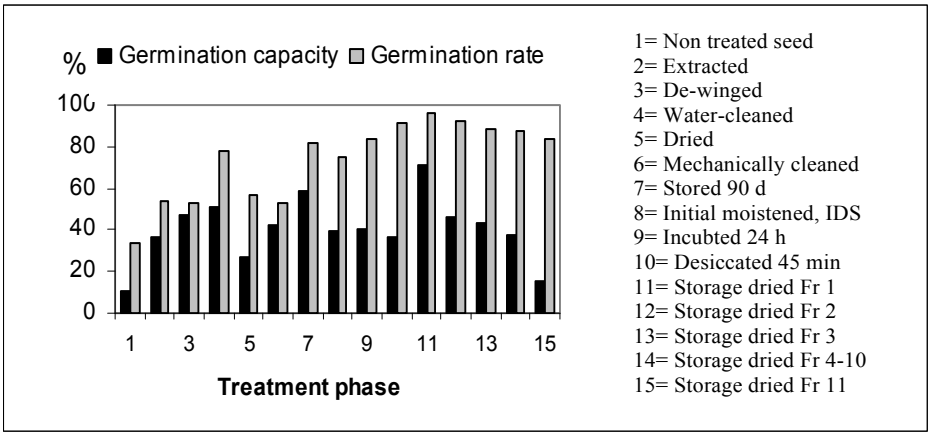

Fig. 2. The germination capacity and rate (%) of dry-extracted seeds after pre-treatment phases. The picture is reprinted with permission from the Association of official seed analysts and the Society of commercial seed technologists.

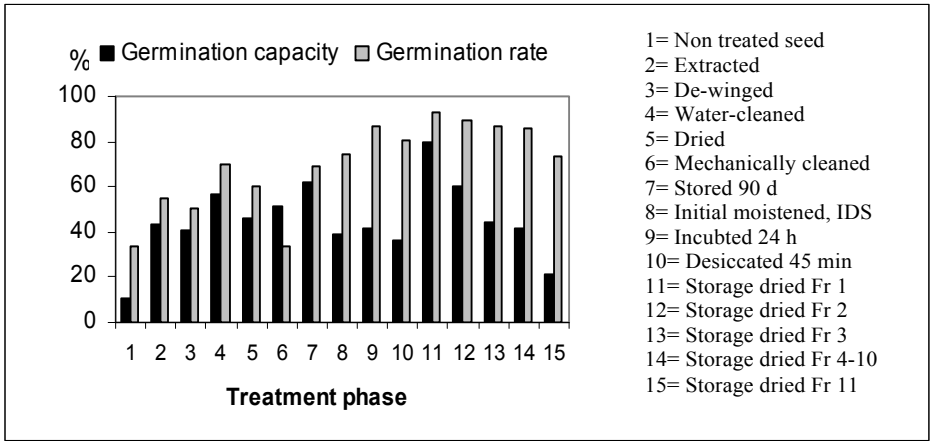

Fig. 3. The germination capacity and rate (%) of water-extracted seeds after pre-treatment phases. The picture is reprinted with permission from the Association of official seed analysts and the Society of commercial seed technologists.

3.4 Germination of pre-treated spruce seeds after short-term storage

After one year of storage at −18 °C the germination capacity of dry-extracted and water-extracted Norway spruce seeds was 78% and 85%, respectively (III, Fig. 3–4). The germination rate of the dry-extracted seeds in the best fraction (Fr1) was 89% and water-extracted seeds 81% after storage of one year (III, Fig. 3–4). The germination rate of the dry-extracted and water-extracted seed fractions decreased on the average by 10%-unit compared to the germination rate prior storage and these mean differences were mainly significant.

3.5 Germination of pre-treated pine seeds after long-term storage

The germination capacity and rate of all pine seed lots studied, either pre-treated or not, impaired at both storage temperatures (Fig. 4–5). The changes at two storage temperatures were parallel, even if the germination capacity and rate maintained better in frozen than in cool store. During long-term storage, the germination rate decreased in most cases more than the germination capacity. Declination of the germination capacity and rate was more obvious in pre-treated seed orchard than in forest stand seeds (Fig. 4). The germination rate of the

orchard seeds decreased by 21–52%-unit and the mean differences were statistically significant at both storage temperatures (IV, Table 2). Differences in the germination capacity and rate of treated and non-treated seeds were insignificant after long-term frozen storage. After cool storage the means of germination capacity and rate were significantly higher in the non-treated seeds than in the treated ones (p-values 0.003–0.034). The germination capacity and rate of the seeds in fraction 10 decreased by 1–64%-unit, depending on the seed lot (IV, Table 2).

Forest stand seeds incubated at 10 °C had a higher germination capacity and rate than those incubated at 5 °C (Fig. 4). The differences were also statistically significant (IV, Table 2). After long-term storage the germination capacity of orchard seeds (Parkkola) remained highest in seeds incubated at 5 °C. On the other hand, the germination rate of the orchard seeds remained highest in seeds incubated at 10 °C (Fig.4, IV, Table 2).

After long-term storage the proportion of abnormal germinants and dead seeds was frequently smaller in frozen-stored than in cool-stored seed lots and the number of non-germinated fresh seeds was small in all seed lots.

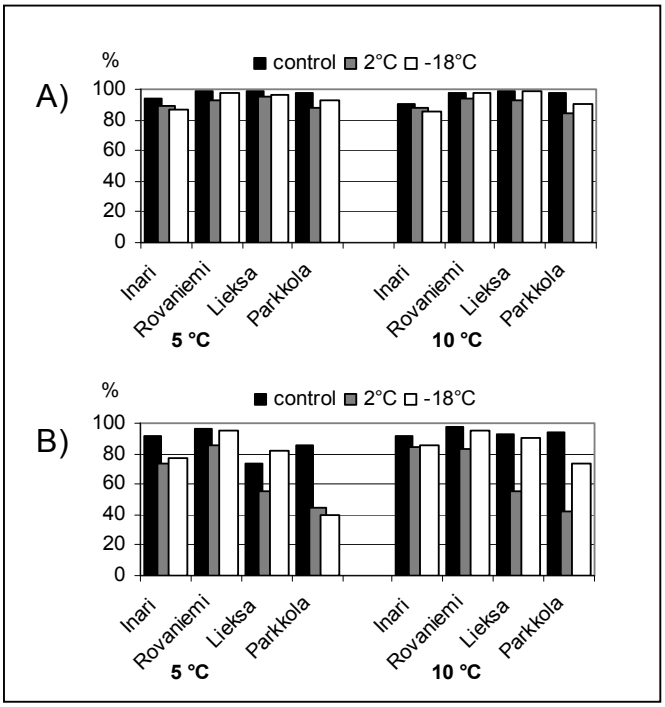

Fig 4. The germination capacity (A) and rate (B) of pre-treated best fraction seeds (incubated 5 °C or 10 °C) after long-term storage. Control = the germination capacity and rate of seeds prior to storage. The picture is reprinted with permission from the NRC Research Press.

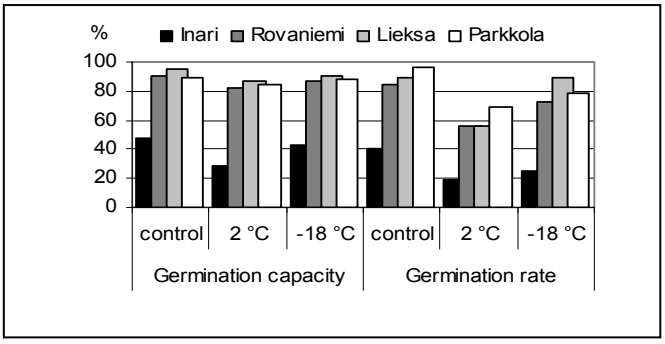

Fig 5. The germination capacity and rate of non-treated seeds after long-term storage. Control = the germination capacity and rate of seeds prior to storage. The picture is reprinted with permission from the NRC Research Press.

4 Discussion

4.1 Long-term variation of pine seed crop size and quality

The crop size of Scots pine seeds varies considerably between years (I). Further, the long-term average annual seed crop (I) was higher compared to previous seed crop measurements in forest stands in Rovaniemi and Kittilä (Koski & Tallqvist 1978) and the crop never failed completely despite being measured over a long time (I). The difference between these results and previous findings (Heikinheimo 1937, Koski & Tallqvist, 1978) may be explained by different sampling methods. Previous studies have shown that there is great variation in the annual Scots pine seed crop between and within stands (Koski & Tallqvist 1978, Karlsson & Örlander 2000, Juntunen & Neuvonen 2006). Therefore, it is important to use a sufficient number of traps to ensure reliable estimates. The result of this study also verify the great annual variation in pine seed crops found previously (Heikinheimo 1937,1948, Hagner 1965, Béland *et al.* 2000, Karlsson & Örlander 2000, Hokkanen 2000, Hannerz *et al.* 2002)

The results of this study show that the pine seed crop in somewhat dependent on temperature conditions during the reproductive cycle (I), this differs from earlier studies, where temperature conditions before the flowering year were found to be more important (Leikola *et al.* 1982). Further, the mean diameter of trees at breast height and height of the trees showed a positive correlation with the seed crop (I). This result was in accordance with some earlier findings (Karlsson 2006, Koski & Tallqvist 1978), however others (Karlsson 2000) have found no significant correlation between tree height and cone production. The length and vitality of the green crown may also have an effect on the size of the seed crop. The correlation between the seed crop and the age of the stand was negative (I), while in previous studies positive correlations have been found (Lähde 1976). This is partly caused by the fact that the oldest forest lies at the highest altitude, and therefore in the hardest climatic conditions.

Scots pine seed ripening is a problem in regions where the annual heat sum is low (I). The results of this study show that 800 d.d. are needed for 50% of seeds to mature anatomically. The same critical annual heat sum has been reported by Harju *et al.* (1996) although Almqvist *et al.* (1998) and Juntunen & Neuvonen (2006) have reported that a germination capacity of 50% is attained in pine seeds at 725–750 d.d. However, Henttonen *et al.* (1986) and Sarvas (1970) have found

that a germination level of 50% calls for an annual heat sum greater than 800 d.d. Different methods used in determining seed maturity can explain the difference between the results. Seeds from different localities may also respond differently to the annual heat sum accumulation. This idea is supported by Nygren and Pulkkinen (1994), who have found that the average annual heat sum required for reaching 90% of the seeds' dry mass is 755 d.d. for northern clone seeds and 954 d.d. for southern clone seeds. The results presented here also show that the annual heat sum of the seed-ripening year accounts for 40% of the variation in the annual expected germination percentage of the seed crop (I). Lähde (1976) has reported that the annual heat sum accounts for only 5% of the variation in the pine seed germination capacity, but as much as 33% of the variation in the seed germination rate.

The number of empty Scots pine seeds varied greatly between stands, and the long-term average of empty seeds was 17% (I). One reason for the large differences in the annual average of empty seeds could be the effect of climatic conditions during flowering. The proportion of full pine seeds depends on the success of pollination: the poorer the pollination, the greater the percentage of empty seeds (Sarvas 1962). During the flowering period of pine in northern Finland, rainfall combined with the occurrence of few warm days may reduce the effectiveness of pollination and therefore increase the proportion of empty seeds.

Quantitatively and qualitatively, good Scots pine seed years did not coincide in this study (I). The size and quality of seed crops were studied in different stands which may affect the result. First, the forest studied were of different density. Previous studies have shown that forest density has an effect on seed crop size (Heikinheimo 1948, Béland *et al.* 2000). Second, differences in the microclimatic conditions between forests may influence the seed crop size and quality. Sarvas (1962) has shown that air temperature, relative humidity, and precipitation have an effect on the flowering of pine and thus on the seed crop size. Furthermore, the importance of temperature requirements for anatomical seed maturation has been shown in many previous studies (e.g. Sarvas 1970, Kardell *et. al*. 1973, Henttonen *et al.* 1986, Almqvist *et al.* 1998).

4.2 Fungal injuries and germination

Aeciospores of *Chrysomyxa pirolata* were found to formed inside the Norway spruce seeds (II). The uredinia and telia of *Chrysomyxa pirolata* overwinter on the alternate host (*Pyrola* spp.), germinate, and produce basidiospores that infect

spruce cones at pollination time (Jørstad 1925, Nelson & Krebill 1982, Crane & Hiratsuka 2000). The basidiospores can be disseminated to spruce cones when the micropyle is open. In earlier studies, aeciospores of *Chrysomyxa pirolata* have been detected in *Picea mariana* bract scales and seed wings (Singh & Carew 1990) and in *Picea pungens* Engelm. cone scales (Nelson & Krebill 1982). *Chrysomyxa pirolata* has also been found to cause distortions in spruce cone scales as well as malformed and prematurely opened cones of *Picea pungens* and *Picea mariana* (Nelson & Krebill 1982, Singh & Carew 1990). In this study, injuries caused by *Chrysomyxa pirolata* were mainly found in nucellar layers and the megagametophyte (II). Enzymes produced by the rust probably caused the deterioration of middle lamellae and cell walls of nucellar layers of Norway spruce seeds.

In the fungus-infected Norway spruce seed lot, the germination capacity and rate were low before pre-treatment (III). The lipid and protein bodies of the megagametophyte, typical for Scots pine and Norway spruce seeds (Simola 1974, Tillman-Sutela & Kauppi 1995a,b) were rare or missing and the nucellar layers had deteriorated (II-III). The breakdown and chemical changes of reserve materials occur soon after seed moistening (Mayer & Poljakoff-Mayber 1963). This metabolism could be disturbed by fungal injuries in the Norway spruce megagametophyte and result in a low germination capacity and rate. Previous studies have also shown that the protein body contains structures that store minerals for germination (Pittermann *et al.* 1996). Furthermore, germination tests in previous studies have shown that the first sign of disturbed metabolism is the reduced germination rate of seeds (Marquez-Millano *et al.* 1991). Thus the result of this study confirmed earlier results about reduced germination of various spruce seeds infected by *Chrysomyxa pirolata* (Hylander *et al.* 1953, Rummukainen 1960, Nelson & Krebill 1970, 1982, Sutherland 1981, Singh & Carew 1990).

4.3 Multi-phased pre-treatment and germination

Norway spruce cones opened faster in warm airflow at 20–22 °C than in mechanical extraction (38 °C). Thus the extraction results correspond to the natural opening process of cones: northern Scots pine and Norway spruce forests shed their seeds in spring (Heikinheimo 1932, 1937, Hannerz *et al.* 2002) when the relative humidity of air is low and the cone scales open due to their reduced moisture content (Harlow *et al.* 1964). The results were also in accordance with

previous findings about the advantages of using temperatures from 20 to 30 °C in the extraction of Norway spruce cones (Kangas 1942, Skre 1988). Further, it has been demonstrated that a high extraction temperature also reduces the viability of other conifer seeds (Barnet & McLemore 1970, Jones *et al.* 1997).

In this study, the Norway spruce seed coat opened already during the extraction phase and seed moistening was rapid (III). Several factors may contribute to seed coat opening and the fast moistening of seeds during incubation. First, fungal damage deteriorated the structures of nucellar layers (II, III) that restrict the passage of water into the Norway spruce seed (Tillman-Sutela & Kauppi 1995a) and thus promoted the entrance of water into the seeds and the opening of the seed coat in the micropyle. In spruce seeds, the cells of the endotesta curve outwards at the micropylar canal. When the seeds are moistened the cells expand and the seed coat opens easily at the micropyle (Tillman-Sutela & Kauppi 1995a). Second, the rapid seed moistening could be caused by the hyphomycete of secondary fungi. The hyphomycete of *Thysanophora penicillioides* appeared on the outermost layer of the seed coat, the sarcotesta, where the normal wax coat was missing (II). The layers of the seed coat affect the movement of water into the spruce seed. The sarcotesta is covered by a wax layer and the cells of the sclerotesta are full of wax lamellae (Tillman-Sutela & Kauppi 1995a). The rust fungus infection may have disturbed the development of the wax and thus weakened the protective function of the seed coat structures, promoting opening of the seed coat. Third, for the rapid seed moistening could be the megagametophyte, its structure differed from that of normal nutrient tissue of Norways spruce seeds (Tillman-Sutela & Kauppi 1995a).

The germination capacity and rate of Norway spruce seeds rose during the pre-treatment phases in which water was used, but were impaired during the drying phases (III). The germination process in seeds accelerates when the seed moisture content reaches about 30% (Bewley & Black 1994). The moisture content of the studied seeds after the water-cleaning phase was 47–59%, which enabled embryo cell elongation (III). Drying results in a loss of viability when cell elongation and division have started (Mayer & Poljakoff-Mayber 1963, Bradbeer 1992, Bewley & Black 1994). A decrease in the germination capacity and rate during the drying and mechanical cleaning phases (III) supported this interpretation. The temperature used (30 °C) could be another explanation for low germination after the drying phase. Several studies have shown that the germination capacity and rate of Norway spruce seeds decrease when the temperature exceeds 25 °C (Simak & Kamra 1970, Bergsten 1987, Leinonen *et*

al. 1993). Moisture control is also important during pre-treatment because the combination of high moisture content and low temperature (3–7 °C) can lead to premature germination during pre-treatment (Edwards 1986, Gosling & Rigg 1990, Jones & Gosling 1994).

The germination capacity and rate of cleaned seeds increased during storage prior to IDS treatment (III). One explanation for the increased germination capacity and rate may be that no structurally immature seeds were detected in the microscopic studies of the seed lots (III). Alternatively, the improved post-storage germination capacity and rate can be the result of metabolism at the end of the seed-ripening process (Bewley & Black 1994). This result is also supported by the finding that the seeds of *Pinus albicaulis* and *Pinus sibirica* reach maturity after seed dispersal in a soil seed bank (Tillman-Sutela *et al.* 2008). Further, the germination capacity and rate of non-treated Norway spruce and Scots pine seeds improve during dry-cold storage, which could be the sign of a decreased dormancy level (Sarvas 1974, Nygren 1986, Jones & Gosling 1994, Leinonen & Rita 1995).

The germination capacity and rate of Norway spruce seeds were lower after incubation than after storage for three months (III). These results support earlier findings, according to which moist chilling accelerates the germination rate, but does not always increase the germination capacity of *Picea abies* and *Picea sitchensis* seeds (Jensen *et al.* 1967, Jones & Gosling 1994, Leinonen & Rita 1995). Previous studies have also shown a reduced germination rate in winter-collected and dry-stored Norway spruce seeds after moist chilling (Leinonen 1997). In addition, Tillman-Sutela (1996) has found that when Scots pine seeds with a high germination rate are incubated for a several days at 5 ºC, their germination rate is reduced due to the gradual deterioration of the seeds' nutrient tissue. Mechanical weakening of the megagametophyte after moist chilling has also been noticed in the seeds of *Chamaecyparis nootkatensis* (Ren & Kermode 1999), and *Picea glauca* (Downie & Bewley 1996). Because of the fungal injuries in the studied seeds it is not possible to say whether structural changes occurred in the nutrient tissues after the short incubation time.

The minimum level of germination (95%) for seeds used in single-seed sowing in nurseries was not reached with pre-treatment in the fungus-infected Norway spruce seed lot (III). Previous studies have shown that seeds start to germinate during incubation, and thus it is difficult to achieve a sufficient moisture difference between viable and dead seeds by the IDS method (Bergsten 1987, Tillman-Sutela & Kauppi 1995a). According results from this study, the

empty and dead seeds were sorted out well during the pre-treatment (III), even though a moisture content of 30% was not achieved in the initial moistening or during the incubation phase. The seed moisture content may have remained low because the large nucellar cap was entire (II) and thus restricted the water uptake (Tillman-Sutela & Kauppi 1995a) or because the incubation phase was short. Seeds with the best germination capacity and rate were separated into the first fraction, and the differences between fractions were notable (III). In addition, the germination capacity increased by approximately 30% and the rate by more than 40% during the multi-phased pre-treatment (III). The proportion of opened, fungus-infected seeds varied between 12 and 18% in the best fraction, but the most severely damaged seeds were separated into the cast-off fraction during the pre-treatment (III). Not all fungus-infected seeds were separated into the cast-off fraction. This may be partly explained by the similarity of water-binding capacities of healthy seeds and those with slight fungal injury. Thus, the germination capacity and rate of IDS-treated Norway spruce seeds with fungal damage were left notably lower compared to Scots pine seed lots with a low initial germination capacity and rate (Bergsten 1988, Tillman-Sutela 1995, 1996).

4.4 Storage and germination of pre-treated seeds

Long-term storage of seeds is needed in northern areas where seed lots rarely reach high germination levels (I, Sarvas 1962, Ryynänen 1982). During long-term storage the germination capacity and rate of pre-treated Scots pine seeds were preserved better in frozen storage (−18 °C) than in cool storage (2 °C) (IV). Also the germination capacity and rate of pre-treated Norway spruce seeds remain high after one year in frozen storage (III). The results of this study concur with previous findings on storage temperature, according to which, frozen storage temperatures are the most suitable for the long-term storage of non-treated various pine and Norway spruce seeds (Barton 1961, Huss 1967, Kamra 1967a,b, Barnett & McLemore 1970, Barnett and Vozzo 1985). The results also confirm previous observations on the short-term storage temperature of pre-treated Scots pine seeds (Tillman-Sutela 1995, 1996). Seeds use the reserved nutrients of their cells during storage. Thus increasing the storage temperature intensifies the biochemical reactions (Mayer & Poljakoff-Mayber 1963, Simola 1974, Ryynänen 1980, Bewley & Black 1994) and speeds up the reduction of seed viability. The results of this study also supported earlier findings, that moist chilled seeds (*Pinus*

taeda) can be stored for several years without losing viability, provided that the moisture content is low (McLemore & Barnett 1968).

In the Scots pine northernmost seed lot, the germination capacity and rate were the highest in cool-stored seeds (IV). The low temperature sum of the northern environment restricts the maturation of conifer seeds (I, Sarvas 1962, Owens & Molder 1977, Henttonen *et al.* 1986, Juntunen & Neuvonen 2006). Hence, northern Scots pine seeds often have living cells in the nucellar layers at cone collection (Tillman-Sutela & Kauppi 1995). It is therefore possible that the metabolism of these seeds is active enough at cool storage temperatures to contribute to the ripening of nucellar layers and thus has an influence on germination. The result is supported by Bradbeer & Colman (1967), who reported on active metabolism in many seed species in low temperatures.

After long-term storage the germination capacity and rate of Scots pine orchard seeds were notably lower than those of forest stand seeds (IV). During storage of one year the germination rate of Norway spruce orchard seeds decreased more than their germination capacity (III). Several factors may affect the lower germination capacity and rate of orchard seeds after storage. First, studies of several conifer species have shown a high genetic control over germination capacity and rate and related differences between and within species (Weber & Sorensen 1990, Weber & Sorensen 1992, El-Kassaby *et al.* 2002), which can also be observed after long-term storage. Second, the seeds of different species from different provinces age at different rates. The ageing rate reflects genetic differences which probably are also evident in germination after long-term storage (El-Kassaby & Edwards 1998, El-Kassaby *et al.* 2002). The small differences in flowering phenology between seed orchards with northern clones and surrounding forests (Nikkanen *et al.* 2002, Parantainen & Pulkkinen 2003) leads to cross pollination (Harju & Muona 1989, Pakkanen *et al.* 1991, Pakkanen *et al.* 2000), which increases the genetic variation of seeds.

Embryo growth is closely connected with the annual heat sum (Owens *et al.* 1993, Owens *et al.* 2001); the collection of cones in seed orchards is usually started with reference to the annual heat sum (Sarvas 1970). The Scots pine orchard seeds of this study were collected early in autumn, while the forest stand seeds were collected in winter (IV). Studies have shown that, the maturation of Scots pine surface structures continues until as late as the end of October (Tillman-Sutela *et al.* 1998). Thus, it is possible that the surface structures of Scots pine orchard seeds were not mature at the cone collection time and therefore could not protect the seeds against the stress caused by pre-treatment

and storage. Earlier studies have also shown that the time of cone collection has an impact on seed storage viability (Huss 1951, Barnett & McLemore 1970) because the seeds must reach a sufficient maturity level to survive in storage. Nygren (1987) has shown that the germination percentage of autumn-collected Scots pine seeds is greatly affected by the cone collection time: the germination of seeds collected in November and December is higher than that of seeds collected in September and October. Further, Barnett and McLemore (1970) have shown that after storage of 10 years the germination of *Pinus palustris* Mill. seeds collected in October was higher than that of seeds collected in September.

The growing conditions of the seed orchard differed from the original surroundings of the mother trees. According to previous findings, the maturation of Scots pine seed surface structures is connected to the photoperiod (Tillman-Sutela *et al.* 1998). Thus, the different length of photoperiod of the seed orchard compared to the more northern female reproductive environment may change the maturation schedule of seeds surface structures produced in the seed orchards. This idea is supported by other research, which have shown that aftereffects of the female reproductive environment influence bud phenology, frost hardiness, and the growth of Scots pine and Norway spruce seedlings (eg. Mikola 1982, Lindgren & Wei 1994, Partanen & Beuker 1999, Partanen *et al.* 1998, Hannerz & Westin 2000, Johnsen & Skrøppa 2000, Skrøppa & Johnsen 2000, Skrøppa *et al.* 2007).

The hyphomycete of *Thysanophora penicillioides* occurred on Norway spruce seeds whose wax cover was missing (II, III). Fungal injuries on the seed coat may decrease storage durability because the fenolic components and tannins of the seed coat protect the seed from oxidation and degradation (Zucker 1983).

5 Conclusions

The results presented here confirm that the seed crop size and quality of Scots pine and Norway spruce vary, which makes it important to pre-treat seed lots in forest tree seed centers. The results support earlier findings indicating that temperature conditions during the growing seasons of the reproductive cycle are crucial factors determining the quality of Scots pine seed crop. The results do not support the assumption that a large seed crop entails high quality. In this study the size and quality of Scots pine seed crops were not studied in the same stands although it is known that seed crop quality varies between stands during one year. It is therefore important to study whether quantitatively and qualitatively good crops occur simultaneously in the same stands. The results also confirm the importance of seed crop prediction combined with quality information so that forest owners and foresters can select the best regeneration method for a cut area in any specific year.

According to this study, aeciospores of *Chrysomyxa pirolata* form inside Norway spruce seeds, destroying mainly the nucellar layers. This differs from previous findings that the cone rust infects only bract and cone scales and seed wings. Consequently, it is important to find out how commonly aeciospores of *Chrysomyxa pirolata* appear in the seeds of Norway spruce. This study also confirmed that fungicide application against rust fungi in spruce seed orchards is most effective during pollination.

This study showed that the use of water must be restricted in the extraction and cleaning phases of Norway spruce seeds because the seed coat of a moistened seed opens easily, especially if infected by fungi. The incubation time must be short as well. The temperature used in this study during the drying phase of mechanical cleaning (30 ºC) must be reduced because the high temperature caused a clear decrease in the germination capacity and rate of Norway spruce seeds. Also lowering the temperature used in the seed extraction phase (38 ºC) is recommended. Since fungal injuries destroyed seed structures and lowered the germination of Norway spruce seeds in this study, it is important to study the effects of the different phases of pre-treatment on germination, and this should be carried out using seeds of good quality.

During the years of study (1986–2004), was the expected germination percentage of Scots pine seeds found to be more than 50% in seven years. The combination of more than 100 seeds/m^2 and an expected germination percentage of greater than 50% was observed in one year only. It is clear from these results

that long-term seed storage is necessary to seedling production. This result proved that pre-treated Scots pine forest stand seeds can be stored for several years in frozen conditions. The germination capacity and especially the germination rate of pre-treated orchard seeds decreased notably more than those of forest stand seeds. Thus, Scots pine orchard seeds should be stored non-treated. Otherwise the germination capacity and rate of pre-treated Norway spruce orchard seeds remained nearly unchanged during storage of one year at −18 °C. According to these results, long-term storage of treated Norway spruce seeds is not recommended because seeds of different species and from different provinces age at different rates. Furthermore, the seed coat of Norway spruce seeds often opens during treatments, leading to seed deterioration during long-term storage. In addition, a missing wax cover on the outermost layer of the seed coat can also expose seeds to fungal infections and thus reduce storage durability. More information is needed about the storage endurance of pre-treated seed orchard seeds.

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Original papers

I Hilli A, Hokkanen T, Hyvönen J & Sutinen ML (2008) Long-term variation in Scots pine seed crop size and quality in northern Finland. Scandinavian Journal of Forest Research 23: 395–403.

II Tillman-Sutela E, Kauppi A, Hilli A & Kaitera J (2004) Fungal injury to seed tissues of Norway spruce, *Picea abies* (L.) Karst. Trees 18: 151–156.

III Tillman-Sutela E, Hilli A & Kauppi A (2003) Germination changes of *Picea abies* seeds at water-based pretreatments. Seed Technology 25: 168–182.

IV Hilli A, Tillman-Sutela E & Kauppi A (2003) Germination of pretreated Scots pine seeds after long-term storage. Canadian Journal of Forest Research 33: 47–53.

Original papers are not included in the electronic version of the dissertation.

503. Rinta-aho, Marko (2008) On monomial exponential sums in certain index 2 cases and their connections to coding theory

504. Sallinen, Pirkko (2008) Myocardial infarction. Aspects relating to endogenous and exogenous melatonin and cardiac contractility

505. Xin, Weidong (2008) Continuum electrostatics of biomolecular systems

506. Pyhäjärvi, Tanja (2008) Roles of demography and natural selection in molecular evolution of trees, focus on *Pinus sylvestris*

507. Kinnunen, Aulis (2008) A Palaeoproterozoic high-sulphidation epithermal gold deposit at Orivesi, southern Finland

508. Alahuhta, Markus (2008) Protein crystallography of triosephosphate isomerases: functional and protein engineering studies

509. Reif, Vitali (2008) Birds of prey and grouse in Finland. Do avian predators limit or regulate their prey numbers?

510. Saravesi, Karita (2008) Mycorrhizal responses to defoliation of woody hosts

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512. Korsu, Kai (2008) Ecology and impacts of nonnative salmonids with special reference to brook trout (*Salvelinus fontinalis* Mitchill) in North Europe

513. Laitinen, Jarmo (2008) Vegetational and landscape level responses to water level fluctuations in Finnish, mid-boreal aapa mire – aro wetland environments

514. Viljakainen, Lumi (2008) Evolutionary genetics of immunity and infection in social insects

515. Hurme, Eija (2008) Ecological knowledge towards sustainable forest management. Habitat requirements of the Siberian flying squirrel in Finland

516. Kaukonen, Risto (2008) Sulfide-poor platinum-group element deposits. A mineralogical approach with case studies and examples from the literature

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