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Seed quality attributes in seedling production of Norway spruce (*Picea abies* (L.) Karst.)

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Academic dissertation

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ABSTRACT

The artificial regeneration of Norway spruce (*Picea abies* (L.) Karst.) in the Nordic countries relies on planting containerized seedlings originating from seeds collected from either seed orchards or forest stands. The aim of this thesis is to investigate the effect of seed quality attributes on seedling production and to study whether it is possible to enhance germinability and seedling health through seed soaking treatments. The variation in seed quality among individual trees and clones and the components of seed weight variance are also studied.

Commercial seed lots were screened for microbes and the effect of soaking treatment on microbial abundance was analyzed. The three seed lots contained some pathogenic fungi, but most fungi found were saprophytic. The seed lots differed in their likelihood to suffer from damping-off when germinated in water agar medium but not in peat. With current production methods seed-borne fungi do not have a great impact for seedling health, but altering for example the growing media may increase their importance.

Seedling quality declined with increasing emergence time in an experiment on 1-year-old seedlings in the nursery, thus emphasizing the importance of fast germination. Seed soak-sorting hastened the emergence and increased seedling size more in 1.5-year-old containerized seedlings compared to the 1-year-old seedling crop in addition of decreasing the proportion of cull seedlings.

The proportion of full seeds varied between individual trees and clones in cones collected from a forest stand and from a seed orchard. The average seed weights differed between trees or clones, but intracone variation was the greatest source of seed weight variance. This indicates that weight-based seed sorting may have a smaller impact on the genetic diversity of seed lots than previously thought.

Seed and seedling producers as well as society have different preferences for seed quality attributes and different tools for quality management.

Key words: Cone pests, forest regeneration, genetic diversity, seed physiology, seed production

TIIVISTELMÄ

Kuusi (*Picea abies* (L.) Karst.) on metsänviljelyn pääpuulaji Pohjoismaissa. Suomessa tuotetaan vuosittain noin 100 miljoonaa kuusen tainta. Kuusen siementuotanto on sidoksissa lajin lisääntymisbiologiaan: hyvät siemenvuodet toistuvat harvoin ja merkittävä osa siemenistä on tavallisesti tyhjiä tai hyönteisten vaurioittamia. Tässä väitöskirjassa tarkastellaan siementen laadun eri osa-alueiden vaikutusta paakkutaimituotantoon sekä mahdollisuuksia parantaa siementen ja taimien laatua siementen esikäsittelyillä. Lisäksi tarkastellaan siementen laadun ja painon vaihtelua metsiköstä ja siemenviljelykseltä kerätyssä aineistossa ja vaihtelun merkitystä siemensaantoon.

Väitöskirjan ensimmäisessä osajulkaisussa eristettiin kaupallisista siemeneristä niissä esiintyvät sienet ja tutkittiin siementen liotuskäsittelyiden vaikutusta näiden runsauteen. Tutkituissa kolmessa siemenerässä esiintyi patogeenisiä sienilajeja, mutta suurin osa lajistosta oli saprofyyttistä. Taimipolteoireiden esiintyvyyttä tutkittiin agar-alustalla ja turpeessa tehdyissä orastumiskokeissa. Taimipoltteen yleisyys vaihteli siemenerittäin agar-alustalla, muttei turpeessa, mikä korostaa kasvatusolosuhteiden merkitystä siementen mukana leviävien sienitautien ehkäisemisessä.

Siementen liotuskäsittelyt aikaistivat itämistä laboratoriossa sekä orastumista taimitarhakokeissa. Liotuskäsittelyn avulla voitiin lajitella siemeneristä pois tyhjät ja hyönteisten vaurioittamat siemenet. Työn toisessa osajulkaisussa tarkasteltiin yksivuotiaaksi kasvatetuilla taimilla orastumisajankohdan ja taimien laadun yhteyttä. Mitä myöhemmin taimi orastui, sitä todennäköisemmin siitä kehittyi huonolaatuinen, ns. raakkitaimi. Siementen liotuskäsittelystä oli hyötyä 1,5-vuotiaaksi kasvatettujen taimien kehitykselle. Tulosten mukaan raakkitaimien osuus pieneni ja taimien koko kasvoi liotuskäsittelyn seurauksena. Tulosten perusteella esikäsittelyn vaikutus riippuu itämisen ja taimien alkukehityksen aikaisista olosuhteista.

Väitöskirjan neljännessä osajulkaisussa havaittiin täysien siementen osuuksien vaihtelevan yksittäisten puiden, tai siemenviljelyksillä kloonien, välillä. Metsikköaineistossa myös hyönteistuhojen osuus vaihteli puittain. Siementen keskipainot vaihtelivat niin ikään puiden tai kloonien välillä, mutta varianssikomponenttianalyysin mukaan kävyn sisäinen siementen painon varianssi on käpyjen ja puiden tai kloonien välistä painovaihtelua suurempaa. Siementen painolajittelu vaikuttaa näin ollen vähemmän siemenerän geneettiseen kokoonpanoon kuin aiemmin on ajateltu.

Siementen laatu ei ole siementuottajien, taimitarhatuotannon ja yhteiskunnan näkökulmista yhtenäinen käsite vaan laadun eri osa-alueet ovat niille eri tavoin merkityksellisiä, ja niiden keinot laadun ohjailuun ovat erilaisia.

Avainsanat: Käpytuholaiset, metsänuudistaminen, metsänviljelyaineisto, siementen fysiologia, siementuotanto.

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Doctors Arja Lilja, Tiina Ylioja and Anna Poimala (neé Rytkönen) all contributed to the articles of this thesis. I thank Arja especially for her candid feedback in our various projects during these years, which has improved among other things my writing. Tiina introduced me to the world of cone and seed insects and has showed continuous collegial support. Doctors Marja Poteri and Kari Leinonen were a part of the advisory group of this thesis and have kept tabs of its development in addition of other professional help and collaboration.

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"Suomen metsä on hyvin kallis omaisuus. Niin kallis, että tämä maa metsätönnä olisi kelpaamaton ihmisten asuttavaksi." – Z. Topelius

LIST OF ORIGINAL ARTICLES

This dissertation is based on the following four articles, which are referred to by their Roman numerals in the text. The articles are reprinted with the kind permission of the publishers.

I Himanen, K., Lilja, A., Rytkönen, A. & Nygren, M. (2013). Soaking effects on seed germination and fungal infection in Norway spruce. Scandinavian Journal of Forest Research 28(1): 1−7. https://doi.org/10.1080/02827581.2012.683037

II Himanen, K. & Nygren, M. (2014). Effects of seed pre-soaking on the emergence and early growth of containerized Norway spruce seedlings. New Forests 45(1): 71−82. https://doi.org/10.1007/s11056-013-9392-6

III Himanen, K. & Nygren, M. (2015). Seed soak-sorting prior to sowing affects the size and quality of 1.5-year-old containerized *Picea abies* seedlings. Silva Fennica 49(3) article id 1056. http://dx.doi.org/10.14214/sf.1056

IV Himanen, K., Helenius, P., Ylioja, T. & Nygren M. (2016). Intracone variation explains most of the variance in *Picea abies* seed weight: implications for seed sorting. Canadian Journal of Forest Research 46: 470−477. https://doi.org/10.1139/cjfr-2015-0379

Statement of author's contribution

I Research idea and experimental design were created by Katri Himanen, Arja Lilja and Markku Nygren. Katri Himanen conducted the soaking treatments, germination experiments and the assessment of visual disease symptoms in these tests. Arja Lilja identified the fungi using morphological features and Anna Poimala (née Rytkönen) conducted the PCR identification. Data analysis was done by Markku Nygren, Katri Himanen and Arja Lilja. The article was written by Katri Himanen, Arja Lilja, Anna Poimala and Markku Nygren.

II and **III** Research idea and experimental design were created and data was collected by Katri Himanen and Markku Nygren. Markku Nygren and Katri Himanen conducted the data analysis and the article was written by Katri Himanen and Markku Nygren.

IV Research idea and experimental design were created by Katri Himanen and Markku Nygren. The data was collected by Katri Himanen and Pekka Helenius. Tiina Ylioja offered information and help in insect identification. Data analysis was done by Markku Nygren and Katri Himanen. The article was written by Katri Himanen. Markku Nygren, Pekka Helenius and Tiina Ylioja commented and improved the text.

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1 INTRODUCTION

1.1 Reproductive biology and ecology of Norway spruce

1.1.1 Phylogeny of Norway spruce

Norway spruce (*Picea abies* (L.) Karst.) is a species of the Pinaceae family. The genus *Picea* includes approximately 35 species. The nearest relatives of Norway spruce are *P. meyeri* Rehder & E. H. Wilson, *P. korayensis* Nakai. and *P. asperata* Mast. (Ran et al. 2006, Lockwood et al. 2013) with distribution ranges located in eastern Eurasia.

Norway spruce is divided into two subspecies *P. abies* ssp. *abies* and *P. abies* ssp. *obovata*. The former subspecies has a wide distribution in Europe ranging latitudinally from the Alps and the Balkan mountain range to Fennoscandia and longitudinally from Norway to the Urals. Recent evidence has suggested that the northern populations of Norway spruce are phylogenetically distant from the southern, alpine populations (Lockwood et al. 2013).

P. abies ssp. *obovata* is in some cases separated into its own species *Picea obovata* Ledeb (Lockwood et al. 2013) distributed in northernmost parts of Fennoscandia and Siberia. However, analysis of nuclear DNA has shown *P. abies* spp. *abies* and *P. abies* spp. *obovata* to be extremely closely related (Krutowskii & Bergmann 1995). There exists are large geographical zone of hybridization between the two subspecies. This thesis deals with *P. abies* ssp. *abies* and describes its reproductive biology.

1.1.2 Flowering and seed development

Norway spruce is a monecious, unisexual and wind-pollinated tree. It begins to flower typically at the age of 40–50 years (Lindgren et al. 1977). The flowering is reduced at the age of 120–180 years (Hagner 1955). Flowering entails the differentiation of primordia into female or male flowers. This is possible when the tree has passed the juvenile stage into maturity (Greenwood 1995, Carlsbecker et al. 2004). By default, buds are vegetative and the formation of flowering buds requires an induction mediated by plant hormones. Bud differentiation occurs in Norway spruce in late June–early July the year before flowering (Selås et al. 2002). Female flowers appear in terminal buds, while male flowers are also located in side-buds (Lindgren et al. 1977).

The flowering in conifers, as in many other plants, is induced by climatic conditions which interact with endogenous processes such as carbon storage and hormone levels (Woodward & Silsbee 1994). For Norway spruce, again as for many species in the Pinaceae family, a cool summer two years before flowering and a warm summer the year before flowering predict abundant flower bud formation (Hagner 1958, Selås et al. 2002, Pukkala et al. 2010). Pukkala et al. (2010) found that in southern Finland, a model taking into account the temperature sum cumulated during a 50 day period after achieving 200 d.d. (+ 5 °C threshold) the year before flowering best predicts good and rather good seed years.

In Norway spruce stands the most important stand parameters influencing the abundance of flowering are the age, height and stem diameter of the tree as well as the size of the tree crown in addition to stand density (Andersson 1965, Nikkanen & Ruotsalainen 2000, Pukkala et al. 2010, Nygren et al. 2017). Flowering is most prolific in dominant or co-dominant trees with the greatest exposure to light and warmth (Sarvas 1968, Lindgren et al. 1977). The southern sides of tree crowns also form more flowers than northern sides

(Andersson 1965). Accordingly, trees that are not shaded by other trees begin flowering earlier than trees in closed stands.

In Pinaceae, female flowers appear typically at an earlier age than male flowers, which follow some years later (Ross & Pharis 1987). Male flowers appear in the lower branches and the female flowers in the top of the crown. This reduces the chance of selfing. As the flowering begins the flowers are found in the branch tips, and the male and female flowering regions are clearly separate. As the tree ages the proportion of flowering buds compared to vegetative buds increases, as well as the flowering in the mid part of the crown. However, with further increasing age and decreasing branch vigour, the zonation of female and male flowers again appears more strongly (Ross & Pharis 1987).

The abundance of flowering has also a genetic component (Nikkanen & Ruotsalainen 2000, Nygren et al. 2017). Koski & Tallqvist (1978) found that 1.2% of trees flowered annually in 19 stands followed for 5–16 years (849 trees in total) and that 3.3% of the trees did not flower once. The distribution of the flowering frequency was skewed left, meaning that trees with low flowering frequency were most common. Nikkanen & Ruotsalainen (2000) also observed that a correlation in the flowering abundance of seed orchard clones in different years was usually positive and significant. Almqvist et al. (2001) also reported that fecundity between clones was under strong genetic control.

The peak in the timing of the flowering of Norway spruce takes place at 140 d.d. (+5 °C threshold) (Sarvas 1968), which is usually reached by the end of May or the first week of June in southern Finnish conditions. According to Eriksson et al. (1973) both female and male flowering occurs around 200 d.d. (+2 °C threshold), with differences between clones. The susceptibility of the female strobili to pollen lasts often only a few days (Sarvas 1968, Nikkanen 2001), depending on weather conditions. Luomajoki (1993) found that anthesis lasted 12–20 days in southern Finland when several stands were followed for 11 years.

Pollination is followed by fertilization within a month (Håkansson 1956) at approximately 400 d.d. (+5 °C threshold) (Sarvas 1965). The development of the seed and cone structures is rapid thereafter, with dry mass saturation achieved approximately a month later (Skre & Gjelsvik 1980). Once the dry mass saturation is reached, the water content of the seeds and the cones is still high, often over 50%. The cones are green and lignification is still in process.

According to Sarvas (1965), embryos reached 60% of their final length at an average of 860 d.d. in measurements conducted in Punkaharju, in southern Finland. Skre & Gjelsvik (1980) report that anatomical maturation – the completion of the development of the anatomical structures – takes place at 875 d.d. (+5 °C threshold). Physiological changes which increase germinability and prepare the seeds for a quiescent period continue after the completion of the anatomical maturation. This period is called physiological maturation (Almqvist et al. 1998) or the desiccation phase (Angelovici et al. 2010) during which the water content and respiration of the seeds and their surrounding structures decrease. In northern Europe conifers, this phase continues into early winter (Skre & Gjelsvik 1980, Almqvist et al. 1998).

The seed dispersal in Norway spruce typically occurs in late winter and spring from February to May. According to Heikinheimo (1937), peak dispersal occurs in April. Cone scales open as the cone dries and the winged seeds fall out. The dispersal is wind-assisted and if the seeds fall on snow, further dispersion is caused by wind as well. In warm and dry autumns seed dispersal has been reported to occur already in October–November (Ruotsalainen 1990).

The main structures of Norway spruce seeds are the embryo, residing in the embryo cavity, the megagametophyte storage tissue and the hard seed coat covering filamentous structures. The embryo as well as the megagametophyte are living tissue. The megagametophyte, which comprises the major part of the seed weight, is haploid maternal tissue in conifers (Reich et al. 1994). There is therefore a genetic relationship between the mother tree and seed weight (Roach and Wulff 1987, Castro 1999).

Norway spruce differs from many tree species, such as Scots pine (*Pinus sylvestris* L.), in the regard that even the unpollinated or unfertilized ovules develop into full sized, but empty seeds and cone structures develop similarly regardless of the proportion of full seeds in a cone (Sarvas 1968). Therefore, the quantity and proportion of full seeds cannot be detected visually from the surface of the cone or seed.

1.1.3 Germination of Norway spruce seed

Germination begins with the uptake of water by the quiescent seed and continues with a rapid increase in metabolic activity (Bewley 1997, Nonogaki et al. 2010). Germination is often considered to be completed as the radicle emerges from the seed (Black et al. 2006), while ISTA (International Seed Testing Association) rules determine the germination of conifer seeds to be complete when the radicle has grown to the length of four times the length of the seed coat (International seed testing… 2005).

The water uptake of a seed contains three phases: the first (I) being a rapid, physical uptake of water by the seed desiccated during maturation, referred to as imbibition (Black et al. 2006). In the second phase (II) the water content plateaus and the third (III) phase of further increase in water content occurs after the completion of the germination sensu stricto, as the embryo elongates. In Pinaceae seeds the water uptake takes place from the chalazal end of the seed as the micropyle side contains hydrophobic membranes (Tillman-Sutela & Kauppi 1995, Terskikh et al. 2005). Respiration increases and mitochondrial repair and multiplication begin during phase I of the water uptake as well as the repair of different membrane and protein structures (Bewley 1997, Nonogaki et al. 2010). Cell division and the mobilization of storage tissues occur after germination in phase III of water uptake.

In addition to water availability, germination is affected by temperature. The optimal temperature for Norway spruce seed germination is 20–22 °C (Bergsten 1987, Leinonen et al. 1992). According to Leinonen et al. (1992) 50% germination was achieved at 14 °C and the germination rate was reduced at temperatures above 23 °C. Oxygen availability also affects germination. Kaila (2007) found that an air oxygen content of 8% reduced the germinability of Norway spruce seeds to one fifth compared to ambient oxygen levels (21%). With air oxygen content of 1% the seeds did not germinate at all and did not recover even after returning to ambient conditions. Norway spruce seeds typically germinate equally well in darkness or light in optimal temperatures (Leinonen et al. 1992). The optimal pH for the germination of this species is around 5–6, with decreasing germinability especially in more alkaline conditions (Rikala & Jozefek 1990).

Seed dormancy is determined as the failure of viable seeds to germinate in favourable environmental conditions (Bewley 1997). Primary dormancy occurs after seed maturation while secondary dormancy is a state induced by certain environmental conditions or their combinations. Norway spruce seeds are considered to be shallowly dormant or their primary dormancy in described as relative dormancy. This means that the seeds typically germinate readily without dormancy breaking treatments such as moist-chilling. However,

moist-chilling may widen the temperature range in which the seeds may germinate or increase germination in the dark (Leinonen 1998a). Savonen (1998) also found a positive germination response in nursery conditions in some seed lots of Norway spruce when seeds were sown in moist peat and stored for 3 weeks at +3 to +5 °C prior to moving the containers and placing them in locations with good germination conditions. Despite these observations, in practical seedling production seed dormancy is not considered to be a problem for Norway spruce.

1.1.4 Insects and rust fungi in Norway spruce cones

Several insect species consume seeds or cone tissue of Norway spruce. The seeds especially contain high amounts of high-energy compounds such as lipids and proteins (Pulliainen & Lajunen 1984, Tillman-Sutela et al. 1995) compared to wood and therefore the reproductive structures are favoured by many insects. In addition to pest insects, many parasitic or predatory species to these are commonly found in cones (Bakke 1955, Brockerhoff & Kenis 1996).

The most common Norway spruce cone insect pest species in Fennoscandia are spruce seed moth (*Cydia strobilella* L.), spruce cone worm (*Dioryctria abietella* Den. & Schiff.), cloaked pug (*Eupithecia abietaria* (Goeze)), spruce cone maggot (*Strobilomyia anthracina* (Cherny)) and cone gall midge (*Kaltenbachiola strobi* (Winn.) (Annila 1981, Annila & Heliövaara 1991, Seifert ym. 2000, Rosenberg 2003, Rosenberg & Weslien 2005, Rosenberg et al. 2012). Spruce seed chalcid (*Megastigmus strobilobius* Ratz.) and spruce seed midge (*Plemeliella abietina* Seitn.) are found inside the seeds. *C. strobilella*, *S. anthracina* and *P. abietina* lay their eggs in flowers while the other species oviposit in the developing cones (Annila 1981, Annila & Heliövaara 1991, Brockerhoff & Kenis 1996, Rosenberg et al. 2012).

The proportion of insect infested cones and seeds is highly variable in different years and at different locations. Rosenberg et al. (2012) found that approximately 38% of cones were infested by *E. abietaria* or *Eupithecia analoga* (Diakonoff), in single year experiment in a seed orchard in central Sweden, while *S. anthracina*, *M. strobilobius* and *P. abietina* were present in very small numbers (<2%). In another study in central Sweden (Rosenberg & Weslien 2005) *C. strobilella* was found in 23% and 85% of cones in a research covering two consecutive years. Annila & Heliövaara (1991) report that *D. abietella* was present in 15–25% of cones collected from a seed orchard in a single year in southern Finland. *S. anthracina* on the other hand was present only in few percent of the cones in the same study. Nikula & Jalkanen (1990) observed that 95% of cones were damaged by some insect or cone rust in a good seed year in Finnish Lapland when 30 stands were monitored. *K. strobi* were present in 82% and *C. strobilella* in 36% of the cones. In a study in northern Norway (Bakke 1955) *D. abietella* and *K. strobi* were found in most cones. Seifert et al. (2000) report that 36% to 100% of Norway spruce cones were infested with some cone or seed insect in Switzerland when cones were harvested from 29 sites. *C. strobilella* was the most prominent species.

Thekopsora areolata (Fr.) Magnus causing cherry-spruce rust and *Chrysomyxa pirolata* Wint. which causes inland spruce cone rust are the most significant fungi which damage Norway spruce cones and seeds during their development (Tillman-Sutela et al. 2004, Kaitera 2013). Both infect pistillate cones in early summer via basidiospores that originate from alternate hosts (Kaitera et al. 2009). *T. areolata* is the more common of the two rusts and can cause major losses in seed orchards and stands (Nikula & Jalkanen 1990, Kaitera

2013). Almqvist and Rosenberg (2016) report that 70% of the cones in studied Swedish seed orchards were damaged by cherry-spruce rust in 2006. In a study in one seed orchard in southern Finland approximately 15% of cones were infested with this fungus (Annila & Heliövaara 1991). Kaitera found damage caused by *T. areolata* in 0–88% and *C. pirolata* in 0–8% of the cones when observing 24 stands and 8 seed orchards in Finland in a single year. Nikula & Jalkanen (1990) found cherry-spruce rust in 18% of the cones and inland spruce cone rust in 2.5% of the cones in 30 stands in Finnish Lapland.

1.1.5 The variation in annual seed production and seed crop quality

Norway spruce is a masting tree species (Silvertown 1980, Selås et al. 2002). In southern and central Finland, it flowers abundantly roughly twice a decade (Koski & Tallqvist 1978). Sarvas (1968) followed the flowering in southern Finland for 8–12 years, during which top yields were measured in most stands only once. The top yields were 4.5-fold compared to average crop. After abundant flowering the maximum seed fall can be up to 2500 seeds/m^2 (Koski & Tallqvist 1978). The maximum number of full seeds according to Sarvas (1968) was 850 seeds/m^2.

The annual variation in the abundance of flowering is generally hypothesized to be due to seed predation avoidance. During intervals between mast years predator populations – especially those of monophagous species – decline, reducing seed losses in the following mast year. For Norway spruce this is supported by findings made by Annila (1981) that several consecutive poor seed years caused a significant decline in the populations of *C. strobilella* and reduced damage in the following good seed years. In addition to this, mast seeding satiates predator populations: when predators are unable to consume all the seeds, some survive. The latter phenomenon in particular has driven flowering synchrony between individual trees within a stand as well as on a larger geographical scale (Silvertown 1980). The seeds of individuals that flower during mast intervals are more likely to be eaten than the seeds of trees reproducing synchronously with others.

In addition to predation avoidance the cost of reproduction limits the tree's ability to produce pollen and seeds in large numbers annually (Almqvist et al. 2001). According to Pukkala (1987) radial growth is reduced 19% in good seed years of Norway spruce in southern and central Finland. The seed crop index explains 27% of radial growth index. In a study performed in southern Norway seed production explained 42% of the annual variation in tree ring width (Selås et al. 2002). In addition to the immediate effect on growth, future vegetative growth is also reduced as strobilus in apical position stops the growth of the shoot after flowering (Lindgren et al. 1977). This leads to a reduction in the number of vegetative buds the following year which again reduces both the amount of photosynthesis as well as the tree's ability to flower in the next few years (Andersson 1965).

Besides the annual variation in flowering, the resulting seed crop and the number of full and healthy seeds therein depend on many factors and is subject to both annual and geographical variation. Sarvas (1968) observed that the difference between the potential number of seeds on the basis of the number of flowers and the actual seed crop is 20 percentage points. This indicates that a large number of flowers are destroyed by different pests or abiotic damages. According to Sarvas (1968) the average portion of empty seeds was 66.5% during 12 years of observations in 8 Norway spruce stands, varying from 22% to 100%. Heikinheimo (1937) reports the average empty seed proportion to be 45.2%. The

proportion of empty seeds is the lowest in years of abundant flowering and the highest during years of poor flowering (Heikinheimo 1937, Sarvas 1968).

The production of empty seeds in trees has also been suggested to be an adaptive trait reducing seed predation because empty seeds function as decoys for predators (Fuentes & Schupp 1998, Perea et al. 2013). Interestingly, Perea et al. (2013) found that in elm (*Ulmus laevis* Pall.) a 50% proportion of empty seeds reduced the post-dispersal seed predation significantly while larger proportions did not reduce the losses further.

As mentioned previously, there is a relationship between the abundance of cone and seed feeding insects and the amount of flowering, and that the population sizes of insects are prone to dramatic annual variation (Annila 1981). Andersson (1965) observed that both the proportion of full seeds as well as seeds with insect damage varied between stands in the same flowering and maturation year. Seifert et al. (2000) report variation between regions in the presence of seed feeding insects in Switzerland when their occurrence was observed in two consecutive years.

Cone rusts also exhibit a great amount of annual variation, causing heavy losses to seed crops in some years (Hannerz et al. 2000). Kaitera (2013) on the other hand reports that the occurrence of *T. areolata* and *C. pirolata* was lower in northern Finland compared to southern locations in a good seed year of 2006 when observing 9 seed orchards and 23 stands. In the same study *T. areolata* was more common in seed orchards compared to stands in northern Finland and for *C. pirolata* this was the case in all of Finland. This indicates that both geographical location as well as the production environment – seed orchard or stand – may affect the severity of pest problems.

The weight of the full seeds is also subject to annual variation as the seed weight is affected by the temperature sum of the year of maturation. Therefore, the seed weight differs between years in the same location (Sorensen and Franklin 1977, Borgman et al. 2014). In general, the lightest seeds originate from the northern latitudes and the heaviest seeds originate from the southern latitudes in the Northern Hemisphere (Khalil 1986). The nutritional status of the mother plant also affects the seed weight (Bazzaz et al. 2000, Karlsson and Orlander 2002), meaning that the same genotype may produce seeds of different weights based on the site fertility and the availability of other resources.

1.2 Norway spruce seed and seedling production as a part of regeneration activities in the Nordic countries

Norway spruce is a widely used tree species in the forest industry in Europe and in the Nordic countries. As the demand for wood from final fellings is continuous, regeneration activities need to be commenced on large areas annually. Artificial regeneration is a way to overcome the variation of this species in seed production and the regeneration potential described in the previous chapters. On the other hand, artificial regeneration, especially the seed procurement activities depend on, and must adapt to, the biological profile of this species.

In Finland a seed crop of 100 kg or more has been collected from Norway spruce seed orchards four times in the 2000s: in 2014, 2012, 2008 and 2006 (Amounts of seed… 2015), demonstrating the frequency of modest or good seed crops. As good seed years occur irregularly, the seeds must be in a correct physiological state to endure the storage. This emphasizes the importance of a proper seed production methodology. For example, the absence of pathogens in seeds is necessary to ensure the storability of seeds over poor crop

years. The irregularity of flowering also sets constraints on breeding of Norway spruce. These matters will be described in the following.

During the last 10 years (2007–2016), 95–120 million containerized Norway spruce seedlings have been produced annually in Finland (Number of domestic… 2017). To produce this number of seedlings, 850–1100 kg of Norway spruce seeds have been used annually. At the moment, no seedlings for forest regeneration are produced from cuttings or via somatic embryogenesis in Finland. In addition to the domestic seedling production, 3.9–25.1 million Norway spruce seedlings have been imported, mainly from Sweden during the same period, though with a strongly decreasing trend (Trade in seeds… 2017). Seed imports have varied between 7–289 kg annually in the same time period (Trade in seed… 2017), with Sweden as the main trade partner.

The number of seedlings produced and imported corresponds approximately to an annual planting area of 58 000–76 000 ha with an assumed planting density of 1800 seedlings / ha. During the period of 2006–2015, the total annual area under forest regeneration in Finland was 121 00–154 000 ha (Finnish statistical yearbook… 2014), which is approximately 0.4% of Finnish land area.

In Sweden, Norway spruce seedling production has varied from 182 million to 225 million in the 2010's, which is 54–59% of the total tree seedling production (Produktion av skogsplantor 2016). During the same period 71–80% of the Norway spruce seedlings were produced in containers. Bare-root seedlings are mainly used in southern Sweden.

In Norway, forest cultivation decreased rapidly at the turn of the century. Since then the number of delivered seedlings has slowly increased, and Norway spruce has remained the main tree species for forest cultivation. In the 2010s, 22–34 million Norway spruce seedlings have been produced annually (Leverte bruksplanter 1994–2016).

Norway spruce seeds are acquired from seed orchards and from forest stands after final felling both in Finland and Sweden, while in Norway the seed orchard seed production is sufficient to cover the total use. The proportion of seed orchard seed used in seedling production has varied between 13–70% in Finland in 2005–2016. In Sweden the proportion has increased from 49% to 77% during the same time period (Produktion av skogsplantor 2016). The proportion of imported seed orchard seed has been 1–25% in Finland and 1–12% in Sweden of the total seed use during these years (Finnish statistical yearbook… 2014, Produktion av skogsplantor 2016).

1.2.1 Tree breeding

Tree breeding began in Finland in the 1940s with the selection of plus-trees. These were selected phenotypically, and cuttings were taken from these to form grafts for seed orchards. In plus-tree selection height, growth, health as well as quality attributes, such as the diameter and angle of branches, were prioritized. The process of the early phases of this selection in Finland is described in Oskarson (1995). Similar efforts were made in Sweden (Hannertz et al. 2000). By the end of last century, 2644 plus trees of Norway spruce had been selected in Finland (Yrjänen & Karvinen 2002).

The first Norway spruce seed orchards were established in Finland in 1960s. Over 300 ha were established by 1972, after which followed a pause for over two decades (Nikkanen et al. 1999, Himanen 2016). For the first-generation seed orchards established before the 2000s, 30–50 clones were typically used in a single seed orchard. Initially clones were planted in rows, but a scattered distribution was applied from the 1980s to reduce the chance of selfing.

Most Norway spruce progeny trials in Finland were established from 1980s onwards, which is considerably later than for Scots pine (Nikkanen 2002). Based on these trials, the best parent trees were chosen and planted into so-called 1.5-generation seed orchards. The first of these was established in 2001 (Metsäpuiden siementarvearviotyöryhmän muistio 2011). None of these have yet reached seed production age.

Until the 1980s, most of the seed orchards in Finland were established on forest soils. After that orchards have been predominantly established on old agricultural land to ease management and harvesting practices. The methodology of tree breeding is described, for example, in Ruotsalainen (2014), and the establishment and maintenance of seed orchards in Finland in detail in Antola et al. (2009) and for Sweden in Almqvist et al. (2007).

1.2.2 Norway spruce seed procurement chain in practice

The best practices for conifer seed production and collection are described for Finnish conditions in Helenius (2010) and for Sweden in Almqvist et al. (2007). The magnitude of flowering is typically monitored in seed orchards from bud cuttings or visually in early summer. The cone development as well as the appearance of cone rust infections and insect damage is followed later in July and August (Almqvist et al 2007).

In Finland the decision of whether to collect or not is made in August or early September typically after the collection of cone samples (Helenius 2010). Seed quality is assessed from the samples with radiography or the quality of the cones and seeds are simply assessed visually. The main reasons for deciding against collection is that there may be too few cones per graft, a too limited number of cone-bearing clones or too severe cone rust or insect damage. No regulations or legislation on the minimum number of clones contributing to the seed lot exist in Finland. Similarly to in seed orchards, cone samples are often taken from stands when planning a cone harvest to collect stand seeds. Occasionally the percentage of full and healthy seeds in the cones is so limited that the collection is not done despite the abundance of cones.

The cones are collected typically between the middle of September and the middle of December in Finland. The maturity of the seeds can be assessed with radiography. When the embryo and megagametophyte have reached their full size, the seeds are considered anatomically mature (Simak 1980, Sahlén 1992). This is typically taken as a sign that the collection can be initiated, though allowing further, physiological maturation to occur – i.e. delaying the collection – can increase germination energy (a measure of the rapidity of germination expressed as the percentage of seeds germinating within a given time) and germination capacity (final germination percentage) (Johansson 1954, Almqvist et al. 1998).

In seed orchards cones are collected manually by contractors. The upper crowns of tall seed orchards are collected using boom lifts. The cones of several grafts and clones are mixed together at this point. A seed orchard may be collected in more than one batch in which case each collection forms its own seed lot, i.e. has its own master certificate.

Some contractors clean the cones from needles and branches on the site in a rotating drum, but often this cleaning is done after cone storage just prior to kilning (Helenius 2010). In forest stands cones are collected manually from the tree crowns of the felled trees from the ground. In November–December the crowns may be on top of snow depending on the weather, but for the most part the crowns rest on soil or ground vegetation.

The cone storage before kilning is done in various ways and the length of the storage varies from a few days to several months. The cones may be moved from the original collection bags or buckets to larger metal or plywood containers with a volume of several cubic meters (Helenius 2010). These are held at the seed orchards until they are full, and they are then transported to storage facilities typically with outdoor temperatures but covered from rain. Instead of these containers, cones may be kept in plastic mesh bags of various volumes out on the collection site for days or weeks before moving them to indoor storage facilities.

Typically, the bags are in contact with the soil or ground vegetation during outdoor storage and they are exposed to rain. In Finland freezing temperatures are possible already in September with increasing frequency and severity towards the end of the year. However, warm spells with temperatures reaching up to 15 °C in September–October and up to 10 °C in November are equally typical. Therefore, the cones may experience a wide range of temperatures within short periods of time during outdoor storage.

Kilning is done in large heating cabinets in which warm air is blown through the cone mass. Temperatures reach typically 35–40 °C, but some operators use temperatures up to 55 °C. The cone scales open as they dry and seeds with the seed wings attached to them fall out from healthy cones with light shuffling. If the cones are severely damaged by insects, extra resin is produced leading to the cone scales sticking together and hence reducing the number of extractable seeds. After extraction, the seeds are de-winged in a rotating drum. The seeds are sprayed with water and then drummed. The moist wings expand and fall off the seeds helped by flexible brushes in the drum.

De-winging is followed by seed sorting. This is done to discard empty, insect-infested, and damaged seeds that would otherwise reduce the germination capacity of a seed lot. These seeds may also function as habitats for harmful microbes (Whittle 1977, Sutherland et al. 2002). Sorting is usually done by sieve, or by using blowers, and gravity tables (Kolotelo et al. 2001, Karrfalt 2008). The former sorts seeds by size and the latter two mostly by weight. Light-weight as well as the largest seeds are often removed to ensure the proper functioning of sowing machines in nurseries.

Prior to the sorting process the seeds are dried in air currents until they reach a storage moisture content of 5–7% (fresh weight basis). Moisture content samples are taken prior to packing to check that the target level has been reached. Tests samples are also taken to measure the germination energy and capacity. The final seed lot is then packaged into plastic containers typically holding 5–50 kg. These are stored at low temperatures, either in freezing temperatures of -15 to -18 °C or at +5 °C. The best seed lots are often sold within a year or two, but for some provenances, or for seed lots with low germinability, the storage time may extend to over a decade. To control moisture content and germinability during the storage, seed lots are typically sampled yearly.

1.2.3 Norway spruce seedling production

In the Nordic countries, forest tree seedling production shifted from bare-root to containerized seedlings during the 1980s and 1990s. At the beginning of the 2000s, 99% of Norway spruce seedlings were produced in containers in Finland (Finnish Statistical Yearbook… 2014). The dominant seedling types for Norway spruce have been 1- and 1.5-year-old seedlings for the past 15 years. The latter are also called 2-year-old seedlings. The most commonly used container types are BCC Plantek PL121, PL81 and PL64, with a cell

volume of 50, 85 and 115 cm^3 respectively. A PL121 container is most commonly used in northern Finland. The latter two are both used for 1- and 1.5-year-old seedlings.

The 1-year-old seedling crop is typically sown in a peat substrate in March–April and the seedlings are grown in a plastic house for either the entire growing period or for the first 1–2 months, after which they are moved outdoors. The seedlings may be used for autumn plantations or they are out-planted the following spring after winter storage. The 1.5-year old seedlings are sown in June and the seedlings spend the first summer in the plastic house to both control their growth and to ensure sufficient warmth for the seedlings, which is important for the development of frost hardiness (Rikala 2000). The seedlings are then stored the following winter and grown for the second summer outdoors.

The most common growing medium is low-humified *Sphagnum* peat, which is limed and base-fertilized by the manufacturer (Rikala 2000). The containers are filled and compressed mechanically. A small dent is pressed with a dibbler into the peat surface where the seed or seeds are sown mechanically. The peat surface is then covered with sand, saw dust or vermiculate which reduces evaporation from the peat and reduces the growth of weeds, especially mosses.

The containers are placed on metal frames or pallets for the duration of the growing period to help with the logistics at the nursery and to keep seedlings elevated from the ground. This helps to ensure the proper development of the root system as the airflow under the containers stops the roots from growing out of the container cells (Rikala 2012). The practice also reduces the risk of fungal infections in the seedlings (Venn et al. 1986).

The containers are irrigated to field capacity after sowing to promote even and rapid germination. In the spring plastic houses may be heated to keep the temperature favourable for germination. As the growing season progresses the plastic houses are ventilated to inhibit temperature peaks, but cooling systems are seldom available. This leads to temperatures rising above 30 °C during sunny and warm days on the container level.

After the initial irrigation lasting for a few days the aim is to keep the growth medium near field capacity until the radicle emergence. Watering is either continued with small, daily doses or if the evaporation is small, irrigation is halted to prevent hypoxia which is harmful for germination and the development of the root system. After the germination and emergence phase, the aim is to reduce the water content of the growth medium to the level of 35–45% (Heiskanen 1995, Heiskanen 1999). At the end of the growing period the target moisture content of the peat is closer to 35% to promote the frost hardiness of the seedlings (Rikala 2012).

Fertilization is mostly added in a soluble form via irrigation water. Nitrogen is usually distributed in an inorganic form, but amino acid based organic products are also available (Riikonen & Rikala 2011, Gruffman et al. 2014). The fertilization need is assessed by measuring the conductivity of the water in the growth medium (Rikala 2012).

Short day treatment is applied routinely for the 1.5-year-old seedlings in July–August to stop the height growth, as well as to hasten frost hardiness (Luoranen et al. 2009a). Short day treatment is especially recommended for seedlings to be planted in August–September (Luoranen et al. 2006, Luoranen et al. 2009b). In Finnish nurseries artificial lighting is occasionally used, mostly in winter and early spring for production of small seedlings for transplanting.

Seedling lots are typically sorted during the autumn to cull poor quality seedlings. Finnish legislation determines that the planting stocks marketed must include only healthy and viable seedlings, and seedlings with damaged leading shoots or root systems as well as bent or pest infested seedlings need to be culled (Decree of the… 2002). The decree allows

a maximum of 5% of the seedlings to have such damage in the marketed lots. One of the more common reasons for culling is the small size of the shoot. Short seedlings are difficult to plant especially in mechanized planting, where seedlings are planted deeper than in manual planting to ensure contact with the humus layer (Luoranen & Viiri 2016). Seedlings planted the following spring are either packed in cardboard boxes and stored in freezers, or unpacked seedlings are stored in open fields under natural or artificial snow cover. The handling of freezer stored Norway spruce seedlings prior planting is presented in detail in Helenius (2005).

1.2.4 Pre-sowing treatments to hasten germination

Conifer seeds can be soaked prior to sowing in nurseries to hasten germination (Himanen et al. 2010, Kolotelo et al. 2001). Soaking can be used as a sole treatment or it can be the first step in stratification or moist-chilling targeted to break seed dormancy in species requiring it (Jones & Gosling 1994, MacLachlan et al. 2017).

In soaking treatments, the seeds are soaked in still water for a period of time (Riffle & Springfield 1968, Löyttyniemi 1969, Barnett et al. 1999). The water may be aerated with pumps (Barnett & McLemore 1967, Himanen et al. 2010), or the seeds can be soaked under running water (e.g. Riffle & Springfield 1968, Himanen et al. 2010). Aerating the water and rinsing ensure a sufficient oxygen supply for the seeds during the soak.

A rinse is also thought to remove germination inhibitors and debris carrying fungi (Kolotelo et al. 2001), but the reports on the effect of rinsing on seed-borne fungi are somewhat contradictory. On Colorado blue spruce (*Picea pungens* Engelm.) and on Black Hills spruce (*Picea glauca* var. *albertiana*) (S. Brown) Sarg.) *Fusarium* contamination decreased after soaking under running water (James 1985, 1987), whereas rinsing has been shown to increase its occurrence on Douglas fir (*Pseudotsuga mentziesii* (Mirb.) Franco) seeds (James 1986). Simple and aerated soaking techniques are used in Finnish nurseries growing Norway spruce, but if and how these treatments affect seed fungi or disease outbreak in germinants is unknown.

1.3 The concept of quality in the seed trade

The quality of products is often defined as their fitness for purpose or fitness for use (i) (Juran 1988a). Juran's (1988b) other definition is two-fold: Quality consists of those product features which meet the need of customers and thereby provide product satisfaction (ii), and quality consists of freedom from deficiencies (iii).

In the seed trade, quality can be seen from the perspective of the seed *producer* or from the perspective of the seed *user* in nursery seedling production. Society can be seen as a party in the seed trade as well, as seed and seedling quality attributes have an impact on forest resources, as will be discussed later in the following chapters.

For both seed producers and nurseries, seed quality is mostly seen as a means for income and the first two definitions (i and ii) above of quality apply. From the financial perspective of the seed producers, the purpose of all actions is to maximize the profit in seed sales. This includes both producing a magnitude of seeds for the market, as well as producing them with as little cost as possible. Seed quality demands are therefore defined by the market and the level of quality needed so that the seeds can be sold for the maximum profit. The optimization task is not to produce seeds of the highest possible technical

quality, but to produce the level of quality that is saleable and achieves the highest profit (i.e. the sale price–production cost -difference). It is not in the seed producer's financial interest to produce excess quality which is not financially compensated by the customer, i.e. nurseries, or other parties. The seedling producer is on the other hand interested in the seed's ability to become a healthy, saleable seedling and in the seedling obtaining genetic attributes which are favourable towards selling the seedlings.

Society, however, emphasizes neither the direct (tax) income from the sale of the seeds or seedlings, but the quality attributes of forest reproductive material that will in the long run produce the highest tax income from the forestry sector, the largest forest product export possibilities or ensure the functioning of the forest ecosystem for any and all purposes. Society's targets are therefore linked to the genetic attributes of the forest reproductive material. Society benefits from the use of forest reproductive material with high breeding value and from the use of suitable provenances ensuring the adaptability and productivity of forests in the changing climate. Because legislation on seeds and forest reproductive material emphasizes both questions of provenance and the absence of low germinability seed or seedlings with physical deficiencies (Decree of the … 2002), the third definition (iii) of quality described above can be considered the definition used by society. In the following chapters the quality attributes that are of interest to nursery production and society are presented more closely.

1.3.1 Germinability

In nursery seedling production the aim is to achieve rapid and uniform germination. When seedlings emerge synchronously, irrigation, fertilization and other growing measures can be optimized for all the seedlings, which simplifies the production. When germination occurs over a protracted period, seedling requirements vary across the crop and growing measures are inevitably ill suited for some of the seedlings, leaving them behind in height, diameter or dry weight development or creating an unbalanced root system (O'Reilly and Doody 2006). Germination energy is thus more important for the quality of the seedling crop than germination capacity.

One factor affecting germination energy is the seed weight. Sorensen and Campbell (1993) reported faster germination in heavy Douglas-fir seeds than in lighter seeds, as did Dunlap and Barnett (1983) in a study of Scots pine. Seed weight also correlates positively with seedling size in conifers (Dunlap and Barnett 1983, Reich et al. 1994), although the effect is typically temporary (Mikola 1980, Sorensen and Campbell 1993).

Germination capacity is, however, important for determining whether to use one or multiple seeds per pot, i.e. each seedling. Sowing multiple seeds increases production costs as the extra seedlings need to be plugged as well as due to the direct extra cost of the seeds. Typically, 95 percent germination capacity is held as the limit for single seed sowing (Edwards & El-Kassaby 1996).

According to Finnish legislation the information on germination energy and capacity need to be determined for all marketed seed lots (Decree of the… 2002). The decree advises to use internationally agreed analysis methods which implies the application of ISTA rules (International seed testing… 2005). As the laboratory germination tests are done in close to optimal conditions, the germinability in nursery conditions may be different and are typically poorer as the germination conditions may be sub-optimal in terms of temperature, light, oxygen availability etc. The ability of seeds to germinate is influenced by the individual environmental conditions as well as their interaction (Ahola & Leinonen

1999, Yang et al. 2010). Additionally, aged or weakened seeds typically germinate well in optimal conditions, but sub-optimal or stressful conditions yield poor germination (Zobel 1990). This "hidden" weakness may be due to un-timely cone collection, sub-optimal storage conditions etc. (Sahlén 1992).

1.3.2 Seed-borne microbes and their control during seed handling and diseases caused by them during seedling production

Another attribute of seed quality which is important in seedling production, as well as during seed storage, is the amount and species composition of microbes present in and on the seeds. A large number of fungal species are found on the surface (Urosĕvic 1961, Sutherland et al. 2002, Talgø et al. 2010) and inside the seed coat (Tillman-Sutela et al. 2004) of conifer tree seeds. There are many stages of the production by which tree seeds acquire fungal contaminants. The timing of the cone collection, cone handling and storage prior to kilning, processes in seed-handling and storage can all influence the fungal diversity found in the seed lot as well the effects that the fungi may have on seeds (e.g. Sutherland & Woods 1978, Mittal & Wang 1987, James et al. 1990, Fraedrich & Miller 1995).

Most fungi associated with seeds are harmless or nuisance pathogens, but a number of species are known to reduce the viability and germinability of conifer seeds or cause diseases in seedlings (Mittal et al. 1990, Sutherland et al. 2002, Salerno & Lori 2007). Species for example in genera *Penicillium*, *Aspergillus* and *Cladosporium* are mostly saprophytic and invade dead or damaged seeds. These kinds of fungi may become problematic during long-term storage, especially under warm and humid conditions (Sutherland et al. 2002) that accelerate the aging process and weaken the seeds (Leinonen 1998b, Nowakowska & Rakowski 2002).

Another typical disease caused by seed-borne fungi is damping-off, in which germinants are infected and die before emergence, or necrosis appears in small seedlings near the ground line (Sutherland et al. 2002). Fungi in genera such as *Fusarium*, *Alternaria* and *Phoma* are known to cause damping-off in numerous plant species (Mittal & Wang 1993, Lilja et al. 1995, Salerno & Lori 2007). Root rot is often caused by similar fungi, but in the last decade it has seldom caused problems in Finnish nurseries due to changes is seedling production practices and nursery hygiene. The emergence of damping-off is affected by growing conditions: cold and humid conditions as well as poor lighting increases the chances of the disease. Low pH of the growth media on the other hand inhibits the disease and therefore *Sphagnum*-peat used in containerized seedling production is a good Integrated Pest Management (IPM) practice.

Disease symptoms caused by seed-borne microbes can, however, also appear later on during the seedling production or even after out-planting. *Sirococcus conigenus* (D.C.) P.F. Cannon & Minter is a seedborne pathogen that can cause deadly infections in container germinants and seedlings of Norway spruce (Lilja et al. 2010). The fungus infects growing shoot tissues as well as cones. The disease develops in germinants growing from infected seeds, and the small seedlings dry up. Pycnidia develop in the infected tissue and they produce spores that spread the fungus further into growing seedlings (Sutherland et al. 1981). In cones the pycnidia form after seed dispersal. Therefore, seed lots become contaminated if old cones are collected among healthy, fresh ones.

T. areolata causes cankers in Norway spruce seedlings which are very similar in appearance to *S. conigenus*. Although *T. areolata* appears regularly in cones, the seedlings are infected by spores originating from trees surrounding the nursery, not via seeds.

1.3.3 Genetic attributes

From the societal perspective, the genetic quality of seeds is of most interest. The use of genetically improved forest reproductive material increases growth volumes which allow an increase in the annual available cutting for the future, the availability of raw materials for the forest industry, provides a higher income for forest owners and increases the carbon sequestration per hectare. The enhanced timber quality also adds value for the forestry sector.

Another genetic aspect which is highly relevant for society is the genetic diversity of forest reproductive material. The maintenance of forest genetic resources is paramount for adaptation to climate change and other environmental changes and for the functioning of the forest ecosystem (Alfaro et al. 2014). Forest genetic resources are material for tree breeding programmes aimed at increasing genetic gain, but they are also a basis for ecosystem services provided by the forest (Luck et al. 2003, Schaberg et al. 2008). The longer the rotation period, the higher level of genetic diversity is required to ensure long-term adaptability of the forest.

The level of genetic diversity in forest reproductive material depends in part on the level of genetic gain in the propagation material (Ivetić et al. 2016). Genetic gain is increased by selection, which logically reduces the genetic diversity (Ruotsalainen 2014). In tree breeding programmes a balance is sought in finding short-term genetic gain and the long-term goal of maintaining genetic diversity for future improvement and selection (Rosvall 1999, Ruotsalainen 2014).

The genetic diversity and variation is maintained in breeding populations and care is taken that these populations include even rare alleles (Ruotsalainen 2014). In the seed orchards, the population sizes are smaller than in the breeding populations and thus their genetic diversity is smaller. Further steps in the forest reproductive material production and forest regeneration have an impact on the genetic diversity of forest trees (Edwards & El-Kassby 1996, Ratnam et al. 2014, Ivetić et al. 2016), and therefore, care must be taken in composing seed material for regeneration. The risk of reduced genetic diversity due to the production chain of seeds and seedlings will impact the forest owner and society even if the genetic diversity of the breeding populations would be high.

1.4 Aims of the study and research questions

The purpose of this dissertation is to elucidate the effects and importance of seed quality for containerized Norway spruce seedling production. The regeneration of Norway spruce in northern Europe relies on planting containerized seedlings and they are almost solely produced via seeds. Therefore, the quality of the seeds as well as the seedlings has a great impact from both the ecological and economic points of view on our silviculture and our future forests.

The aim of the dissertation is to assess the importance of different factors of seed quality on the successful production of healthy seedlings. The profitability of seedling production is dependent of the proportion of saleable seedling and a low cull percentage.

The aim is to both investigate which factors and seed quality attributes have negative effects on seedling production and quality, as well as to study whether it is possible to enhance germinability and seedling health through certain production methods. The importance of seed quality for society is also discussed.

The research questions investigated in this thesis are:

1. How does the soaking of Norway spruce seed affect germination and seedling emergence and their synchrony in laboratory and nursery environments? (Studies I, II & III)

2. Can seed soaking be used for sorting out poor quality seed? (II & III)

3. What is the effect of seed soaking on containerized seedling morphology and the cull percentage in seedling lots? (II & III)

4. Does soaking have an effect on fungi present in seed lots, and does it affect the emergence of symptoms of damping-off? (I)

5. What fungi are found in commercial seed lots? What is their significance for the emergence of disease symptoms? (I)

6. What is the level of reduction in seed yields caused by empty seeds and insects feeding directly on seeds? (IV)

7. Does genotypic, maternal variation in seed quality exist? (IV)

8. How much of the variation in seed weight is explained by intertree/interclone and by intercone and intracone variations? (IV)

9. How does weight-based seed sorting affect the genetic base of Norway spruce seed lots? (IV)

2 MATERIALS AND METHODS

2.1 Experimental settings

The research questions were studied in four experimental settings. The first was executed in a laboratory environment. Three commercial Norway spruce seed lots were screened for microbes and the effect of soaking treatment on their microbial abundance was analyzed. The effect of soaking on germination and the emergence of disease symptoms was observed both in peat and water agar media (Figure 1A).

The second and third experimental settings included testing the effect of seed soaking on the emergence and the growth and development of 1 and 1.5-year-old seedlings in a typical setting for commercial seedling production. The seedlings were grown at a research nursery in the Finnish Forest Research Institute (currently Natural Resources Institute

Finland), in Suonenjoki, Finland (62°39’N, 27°03’E, elevation 142 m a.s.l.) (Figure 1B). In both experiments the usefulness of soaking for seed sorting was studied as well. In the study on 1-year-old seedlings, the emphasis was on the synchrony of the seedling emergence as well as the importance of the emergence speed for the quality of the seedlings.

In the fourth experimental setting, cones were collected from both an open pollinated, natural stand (Figure 1C) and from a first-generation seed orchard (Figure 1D) to determine the variation in seed quality and seed weight among and within individual trees or clones.

Figure 1. Environments for acquiring the study materials. For study I, seeds were incubated on water agar plates in a germination cabinet (A). In studies II and III seedlings were grown in a greenhouse in a commercial seedling production setting (B). In study IV cones were collected from a forest stand (C) and from a seed orchard (D). Photos: Katri Himanen.

2.2 Seed material

For studies I–III commercial seed lots were used as the study material. Seed lots with quality attributes – e.g. purity, germinability – typical for seedling production in Finnish nurseries were chosen for the experiments. The seeds were acquired from the seed companies Siemen Forelia Oy and Siemen Tapio Oy (currently Tapio Silva Oy) (Table 1). All seed orchard seeds in the experiments belonged to the qualified class, i.e. they were from first generation seed orchards.

In study IV the seed material was collected from individual trees and grafts. For the forest stand material, cones of seven randomly chosen individual trees were collected from a mature, naturally formed Norway spruce dominated stand in Kuopio (Puijo Hill), central Finland (62°54'N, 27°39'E), 190–215 m above sea level, on 14 March 2011. The seven trees were located at a range of 470 m. Ten litres of cones were collected from the tree crowns of each tree with a boom lift. The cones were collected from three points, i.e., from different sides and heights, in each crown to get a representative sample. Cones with clear rust infection or visible insect damage were not collected.

The seed orchard material for this study was collected from a seed orchard in Joroinen, in central Finland (Sv403 Suhola 1, 62°15'N, 27°42'E, 90 m a.s.l.) on 14 January 2013. The cones were collected from five clones, one graft each. The orchard was established in 1994 on old agricultural land with 35 different clones. The grafts in the seed orchard were originally targeted for a more southern location in Finland. The plus trees from which the grafts originate are located approximately 2° south (latitude) and from 100 to 200 d.d. in warmer conditions than where the orchard is located. Two litres of cones with the same criteria as the forest stand were collected from each graft.

The germinability of seed lots used in studies I–III was measured before the experiments to ensure their viability. Seeds were germinated for 21 days in a germination cabinet (FLOHR Instruments GC 10/11, Netherlands; 20 °C, 16 h day, RH 98 %, 1000–1500 lux) on Petri dishes (Ø = 85 mm) on two layers of blotter paper (Munktell no. 1701, Sweden) moistened with 5 ml of tap water.

Table 1. Norway spruce seed material used and collected for the studies, its origin and area of utilization confirmed by Evira (Finnish Food Safety Authority).

	Seed lot code, master certificate	Year of maturation	Collection environment	Seed orchard number or number of deployment area	Municipality of the seed orchard or stand and its co-ordinates	Utilization area, d.d.
Study I	M29-06-0038	2006	Seed orchard	Sv 170	Jyväskylä, 62°13'N, 25°24'E	860–1060 d.d.
	M29-08-0018	2008	Stand	Pab 1	Pohja, 60°5'N, 23°31'E	60°30'N-60°46'N,25°54'E-26°23'E
	T03-95-0039	1995	Seed orchard	Sv 111	Kangasniemi, 61°56´N, 26°41´E	1060-1260 d.d.
Study II and III	T03-06-0414	2006	Stand	Pab 2	Suonenjoki, 62°37'N; 27°7'E	62°57′N–63°20′N; 26°57′E–27°57′E
	T03-06-0421	2006	Stand	Pab 2	Maaninka, 63°9'N; 27°18'E	62°57′N–63°20′N; 26°57′E–27°57′E
Study IV	-	2010	Stand	Stand located in Pab 2, collection from 7 trees	Kuopio, 62°54'N; 27°39'E	-
	-	2012	Seed orchard	Sv 403, collection from 5 clones	Joroinen, 62°15'N; 27°42'E	-

2.3 Seed treatments

In study I three replications of the following treatments were applied to all three seed lots: (1) control, (2) soaking for 24 h, and (3) soaking for 24 h, with the water changed every eight hours. The soaking was done at 22 ± 1 °C in deionized water (300 ml) in glass beakers (400 ml) on a rocking table (SM 25, Edmund Bühler 7400, Tübingen, Germany), keeping the seeds in motion during the soak. Each replicate contained 600 seeds sampled randomly from the seed lots by an electronic seed counter (Elmor Unit Counter Model 600, Switzerland). After soaking, the seeds were drained on a sieve and air-dried on blotter paper in Petri dishes until they no longer adhered to each other.

In studies II and III seeds were soaked for 15 h in tap water aerated with aquarium pumps (SERA 301, PA, USA) in plastic tubs in incubator cabinets (Snijders Scientific

B.V. ECD01E, Netherlands) at 15 °C under fluorescent light (irradiance 17 W/m^2, red 4.9 µmol/m^2/s, far-red 1.0 µmol/m^2/s). The aim of the aeration was to keep the seeds moving during the soak and to ensure a sufficient oxygen supply for the seeds. After the soak, floating and sunken seeds (i.e. the surface and bottom fraction) were separated and placed on stainless steel sieves. The seeds were surface dried in a heating cabinet (Binder FED 720, Tuttlingen, Germany) at 24 °C and occasionally stirred with a plastic spoon during the drying and removed from the cabinets once they no longer adhered to each other. After the surface drying in study II, 3 x 0.5 g seed samples were drawn randomly from the soaked fractions and from the un-soaked control seeds for a water content analysis. No sample was drawn from the floating fraction of seed lot B due to the small number of seeds contained within this fraction. The water content was measured on a fresh weight basis using a constant temperature oven method (103 °C, 17 h) (International Seed Testing Association 2007).

2.4 Measurements and analysis

2.4.1 Study I

After soaking, the seeds in study I were analyzed for seed-borne microbes and germinated in two media. One hundred seeds from each replicate were plated on malt extract agar (MA: 12 g/l Difco Malt-extract; 12 g/l Difco agar, MA, USA) at a density of 10 seeds per plate and incubated at 22 °C in the dark. The fungal growth on the seeds was examined under a stereomicroscope (Leica MZ6, Switzerland) and the isolations of the fungi were done concurrently after one, two, and three weeks. Isolations onto MA were done by collecting the sporangiophores, spores or mycelia growing on the seeds. Hyphal tips were also isolated from the agar near the seeds. Pure cultures were first separated into groups based on colony colour, growth rate, and pattern as well as possible conidia and conidiophore morphology. The identification of each group was based on morphology and nucleotide sequence.

DNA from fungal mycelia was isolated via phenol extraction and polyethylene glycol precipitation as described by Vainio et al. (1998). The Internal Transcribed Spacer (ITS) region of ribosomal DNA was amplified from all isolates with primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) according to the temperature cycle given in Gardes and Bruns (1993). Reaction conditions were as suggested by the manufacturer of the Dynazyme II DNA-polymerase, except for the primer concentration at 25 µM. Amplicons were purified with a High Pure PCR purification kit (Roche, Germany) and sequenced using the Therm EXCELTM II DNA sequencing kit-LC (for 66 cm gels) (Epicentre) with the labelled primer pair ITS1F and ITS4. The resulting sequences were visualized using the LI-COR global edition IR2 system (LI-COR Inc., USA) following the manufacturer's instructions. BLAST searches were used to compare the obtained sequences with those deposited in GenBank.

In the second experiment of study I, 72 seeds from each replicate were randomly selected and placed on three sterile water agar (15 g/l Difco Bacto agar 0140-01, MA, USA) well plates with 24 individual departments. After sowing one seed into each department, the plates were placed in a germination cabinet (FLOHR Instruments GC 10/11, Netherlands; 20 °C, 16 h day, RH 98%, 1000–1500 lux). A seed was considered to have germinated when the hypocotyl was at least 1 cm long. Germination and the

development of disease symptoms were observed 7, 10, 14, and 21 days after sowing. A seed or a germinant was registered as suffering from disease symptoms when fungal hyphae were observed, or the germinant showed symptoms of damping-off, or necrosis was apparent in some part of the germinating seed or the germinant, if germinant dried up or other disease symptoms emerged during the test.

In addition to this, 180 seeds were sown in plastic trays (Plantek PL224, 24 cm^3 per cavity, 1400 seedlings/m^2) filled with base-fertilized low-humified Sphagnum peat, one seed per cavity. Eighteen seeds were sown in each of 10 blocks. Prior to sowing, the peat was watered to field capacity and a small dent was pressed into its surface in each cavity, into which the seeds were subsequently sown. The seeds were covered with a thin layer of vermiculite. In each tray, seeds from each replicate were sown randomly among cavities. The trays were placed on shelves in two germination cabinets (Snijders Scientific B.V. ECD01E, Netherlands; 20 °C, 16 h day, 2500–9000 lux), with five trays in each cabinet. The seed germination and emergence of disease symptoms was monitored for 28 days. A seed was considered to have germinated when the seed coat or hypocotyl emerged from below the vermiculite. The trays were watered when required to maintain the tray weight at approximately 5 kg.

2.4.2 Studies II and III

In studies II and III seed samples were drawn randomly using an electronic seed counter (Elmor Unit Counter Model 600, Switzerland) from each soak fraction and the dry control from both seed lots. In study II the drawn samples contained 81 seeds and in study III 64 seeds each. The samples were X-rayed (Faxitron MX-20, IL, USA; Fuji medical X-ray film, AD Mammography, exposure time 18 kV, 10 s) to detect empty, insect infested seeds (larva filled) or otherwise damaged seeds. Insect species were identified according to Simak (1955) and Wiersma (1973). Seeds in which the megagametophyte did not fill the seed coat entirely, or in which the embryo did not completely fill the embryo cavity, were considered viable but anatomically immature.

In study II the radiographed seeds from both soaked fractions as well as control seeds were then sown in plastic containers (Plantek PL81F, 81 cells per tray, cell volume 85 cm3, growing density 546 cell/m2, BCC, Iso-Vimma, Finland) filled with base fertilized and limed, low-humified Sphagnum peat (Kekkilä WF6). Each sample of 81 seeds was sown in a single container, with one seed per cell. Each seed was placed in a shallow depression made in the peat surface and then covered with a thin layer of vermiculite. A total of 2916 seeds were sown. The containers were placed in a plastic house in randomized block design on 22nd of April, with one container from each seed lot (A and B) 9 treatment (bottom fraction, surface fraction and control) combination in each of the six blocks. Seedlings were grown amongst a commercial seedling crop sown on the same date and were allowed to grow until the end of September according to normal Finnish nursery practice for 1-year-old Norway spruce seedlings. Temperature and relative humidity at seedling level was logged on an hourly basis (DS1923-F5 Hygrochron, iButton Maxim, CA, USA). Emergence and the condition (possible death, development of disease symptoms) of the germinants were examined and recorded on nine occasions during the first 32 days after sowing.

At the end of the growing season, all live seedlings were measured for height (from the base of the seedling to the uppermost needle tip) and stem diameter (approximately 1 cm

from the surface of the mulch). The cull proportion was determined on the basis of the number of seedlings not qualified for sale according to standard nursery production criteria (Decree of the… 2002, Rikala 2012). A seedling was considered a cull seedling, if its height was <13 cm, if the shoot had branched into several tops of equal height, if there was some other type of growth disturbance in the plant, or if the shoot was damaged so that the recovery of the plant was unlikely, or if the root plug was not intact.

In addition, 20 seedlings from each container were drawn randomly to determine the dry weight of the shoot and root system and to calculate the shoot/root -ratio. The shoot was cut from the surface of the mulch and the root plug was rinsed under running water until all the peat was washed off. Shoots and roots were placed in paper envelopes and dried in a heating cabinet (Binder FED 720, Tuttlingen, Germany) at 60 °C for 72 h, after which they were placed in a desiccator for a minimum of 30 min prior to weighing.

In study III the experimental procedure was similar to that in study II. The seeds were, however, sown on 14th of June into bigger Plantek PL64F plastic nursery containers (64 cells per tray, cell volume 115 cm^3, growing density 432 cells per m^2; BCC Oy, Säkylä, Finland) customary for 1.5-year-old seedlings. The peat substrate was Novagro M1L (Biolan Oy, Eura, Finland). The emergence and condition (death, development of disease symptoms) of each germinant were examined and recorded on days 14, 21, 28 and 49 after sowing and the height, stem diameter and shoot and root dry masses after the first growing season were determined as in study II. After these measurements (early October 2011), the containers were moved to an outdoor area, fenced to prevent browsing, to overwinter under natural snow cover. In May 2012, the seedling containers were moved to an outdoor growing area. The seedlings used for determining shoot and root dry masses the previous autumn were replaced by supplemental seedlings grown from seeds from seed lot A to keep the growing density equal in both growing seasons. These supplemental seedlings were marked and not included in the final measurements.

At the end of the second growing season, the height and stem diameter of all the original seedlings were measured. In addition, a set of 20 seedlings from each container was drawn randomly from the original seedlings to determine the final root and shoot dry mass. Again, the dead seedlings were recorded. In case a seedling drawn for sampling its mass had died, the next available original seedling was chosen. In some containers this led to a sample size smaller than 20 seedlings, but a minimum of 15 seedlings was sampled from all containers. The final quality of all the original seedlings (saleable or culled) was also assessed as in study II with the exception of the height requirement of >15 cm for a saleable seedling.

2.4.3 Study IV

After collection, the cones were cleaned of debris (twigs, needles etc.) and stored at –15 °C. Five cones from each stand tree and four cones from each orchard graft (i.e. clone) were selected for seed extraction. When the seeds were extracted, the cones were allowed to thaw at room temperature, after which they were placed individually in paper envelopes in a heating cabinet at 38 °C. The cones were kept in the cabinet until the cone scales opened completely, for 24 h at the most. Every seed was extracted; those that did not fall out of the cones were removed with tweezers. The seed wings were removed carefully by hand, and the seeds were placed on an adhesive film in a cardboard frame so that each seed could be monitored.

The seeds were then X-rayed as in studies II and III. The seeds were classified as (1) full (the megagametophyte filled the seed coat and the embryo filled the embryo cavity), (2) anatomically immature (the megagametophyte and (or) the embryo did not entirely fill the seed coat or the embryo cavity), (3) severely anatomically immature (the megagametophyte filled less than 60% of the seed coat and (or) embryo was missing or shorter than 50% of the total length of the seed), (4) development stagnated (the megagametophyte and embryo development had commenced but stopped, creating dried remnants of these tissues), (5) empty (the seed coat was entirely empty or only remnants of membrane tissue were visible), (6) larvae infested, (7) insect damaged, (8) mechanically damaged, or with (9) unidentifiable damage (Figure 2). The insect species inside the seed were identified according to Simak (1955) and Wiersma (1973).

The seeds, still attached to the frames, were placed in cabinets (Votsch VCL 0003, Votsch Industrietechnik GmbH, Balingen, Germany) at 20 °C and a relative humidity of 35% for at least 48 h prior to weighing each seed individually (Mettler-Toledo MT5 microscale, Mettler-Toledo, Greifensee, Switzerland). The information on the seed quality and seed weight of each individual seed was combined for data analysis as well as to create scenarios of seed loss during seed sorting.

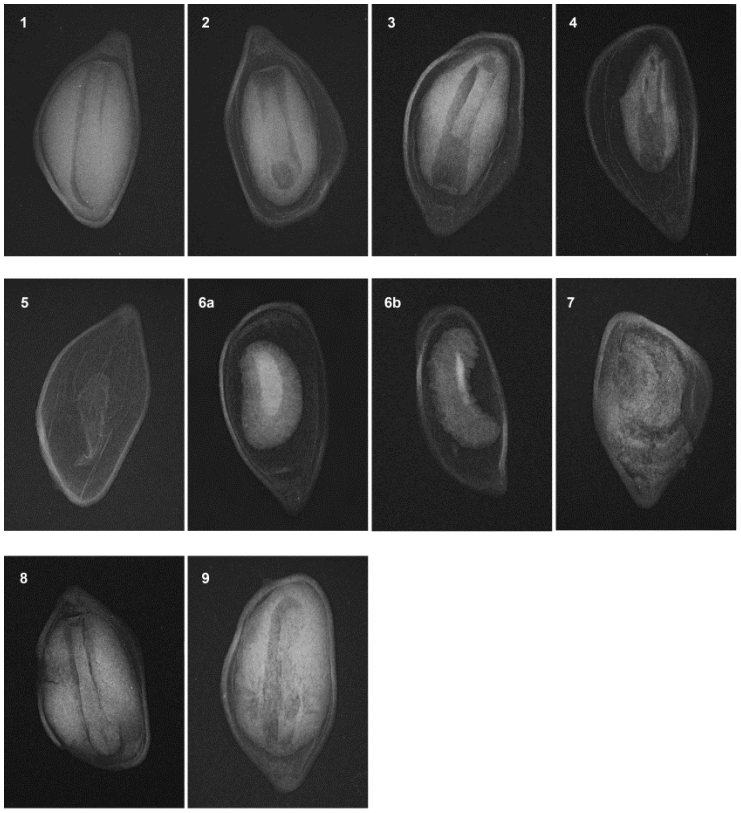

Figure 2. Norway spruce seed quality classes according to radiography. 1, full; 2, anatomically immature; 3, severely anatomically immature; 4, development stagnated; 5, empty; 6*a* and 6*b*, *Megastigmus strobilobius* larvae and *Plemeliella abietina* larvae, respectively; 7, *Cydia strobilella* damage; 8, mechanically damaged; 9, unidentified damage.

2.5 Data analysis

Versions 13 and 15 of the GenStat software were used for all the analysis apart from the time-to-event-analysis and the analysis of seedling height and diameter in study III. These were carried out using the SAS (version 9.2) software package. In the hierarchical generalized linear models, generalized linear mixed models and linear mixed models, the goodness-of-fit of the different models was evaluated on the basis of deviance and normality of standardized residuals. The individual model terms were assessed by comparing the log-likelihood statistics of two nested, candidate models, as well as the Akaike information criterion and Wald-significance tests for parameter coefficients. Fixed model terms were considered statistically significant with $p \leq 0.05$ in all studies and analysis.

In study I the effects of treatment and seed lot on germination and occurrence of disease symptoms were studied in a 3 (treatments) x 3 (seed lots) factorial layout. Germination data and occurrence of disease symptoms were analyzed using hierarchical generalized linear models with a binomial response and logit transformation of the odds. Treatment and seed lot effects were set as fixed model terms and hierarchical block factors (shelves within germination cabinet or replicate within shelves) were set as random. The probability of the occurrence of disease symptoms was analyzed on the basis of the data logged on days 21 (agar) and 28 (peat).

The percentage of emerged seedlings, as well as the percentage of the seedlings that survived the growing season and the cull proportion at the end of the growing season were analyzed using generalized linear mixed models in study II. The emergence data was grouped binary, in which an experimental unit was a batch of seeds and the counts of emerged seedlings were based on either the total number or the number of viable seeds sown in a container. The treatment and seed lot were used as fixed model terms and the block was set as a random term. Data on the seedling survival and the proportion of culled seedlings was binary, and the experimental units were individual, emerged seedlings. The means of treatment, seed lot and time to emergence were used as fixed independent variables and the block was used as a random term.

Data for time to seedling emergence was analyzed using a non-parametric time-to-event analysis with adjusted observation times as suggested by McNair et al. (2012) for interval data. The homogeneity of the time to emergence curves between different treatments was tested using log-rank statistics (Allison 2010). A univariate procedure was used to determine whether individual seedling height and diameter data could be approximated with either a normal or a two or three-parameter Weibull distribution. The goodness of fit was tested with the Anderson–Darling test. Furthermore, the mean and coefficient of the variation for diameter and height at the end of the growing season, as well as the means of the shoot and root dry mass and shoot/root-ratio were analyzed with a two-way ANOVA.

In study III the seedling height, stem diameter, shoot and root dry mass after both the first and the second growing season, as well as the relative height and diameter growth were analyzed using linear mixed models. The seed lot (A and B), the treatment (control, bottom fraction, and surface fraction), and their interaction were used as fixed model terms, and seedling nested inside the block was used as a random term. The experimental unit was an individual, emerged seedling. The time of emergence was analyzed using a non-parametric ANOVA (Friedman's test) because the assumption of normality for the residuals was not met. This analysis was done separately for the two seed lots.

The effect of the soaking treatments and seed lot on the percentage of emerged seedlings and the proportion of saleable seedlings at the end of the second growing season was analyzed using generalized linear mixed models. The emergence data was grouped binary, where a batch of seeds was designated as an experimental unit and the counts of emerged seedlings were based on either the total number or the number of viable seeds sown in a container. The data on the proportion of saleable seedlings was binary, and an experimental unit was an individual emerged seedling.

In study IV the proportions of different quality seeds in each tree or graft were analyzed with generalized linear mixed model using a logit link function. In this analysis, a tree (or clone) was taken as a fixed effect and individual cone nested within a tree was considered a random term. The seed weight data was analyzed using a mixed linear model to estimate and partition the total random variability between and within individual trees and cones. In this analysis, the tree and cone effects were considered random. An analysis of variation was carried out separately for the data including all seeds, all full seeds with a weight over 2.5 mg and for empty seeds. A weight limit of 2.5 mg was chosen as very small seeds would be removed in commercial seed lots to ensure the proper functioning of the sowing machines in the nurseries. In a separate analysis, differences in seed weight between the trees were analyzed with a mixed model, but a tree was considered as a fixed factor.

Additionally, hypothetical scenarios of full seed loss due to weight-based sorting were created. For these, the average full seed weights of all stand and orchard seeds were calculated. The upper and lower weight limits for seed sorting scenarios were set to 1 mg and 2 mg from the average weights, and the proportions of lost seeds were determined in the different scenarios.

3 RESULTS

3.1 Soaking effect on germination and seedling emergence

Seed soaking hastened germination or seedling emergence in all experiments (I–III). In study I the soaking treatment lasted for 24 h and in II and III the length of the soak (with aeration) was 15 h. In studies II and III the soaked seeds were separated into bottom and surface fractions, which behaved differently. Seedling emergence synchrony was investigated in two nursery studies with no favourable effect in study II, while seedling emergence was more synchronous as a result of the soaking in study III. The seed lots used in the experiments thus showed similar, but not identical responses to the soaking treatments.

In study I both soaking treatments increased the germination percentage on day 10 (germination energy) in all three seed lots, but the soaking effect was more pronounced in the germination test on water agar than in peat. For the water agar samples, the difference in the germination energy between the control and the soaking treatment in which the water was changed was 16.0–34.9 percentage points depending on the seed lot. On both substrates the seed lot effect was also statistically significant but with no interaction effect with the soaking treatment. In the model for germination capacity (germination percentage on day 21 or day 28) neither soaking nor the seed lot had a statistically significant effect on either growth media.

According to a time-to-event analysis in study II, the median time of emergence in the nursery was just over 2 days and the mean time of emergence was approximately a day and a half shorter in the bottom fraction compared to the control seeds and the surface fraction (Table 2). The temperature at the seedling level remained under 20 °C for the first weeks after sowing with two temperature peaks reaching 30 °C at the most (Figure 3A). Comparisons of the emergence curves revealed that the difference between control and bottom fraction seeds was statistically significant in both seed lots, while the emergence curves of the control and the surface fraction seeds were similar. In seed lot A, the interval between emergence percentage of 5% and up to 95% was 8 days in all treatments. In seed lot B this time was 8 days for the control seeds and seeds of the bottom fraction, while seedling emergence was spread over 10 days in the seeds of the surface fraction.

In study III almost all the seedlings from both soaked fractions emerged during the first 2 weeks, while seedling emergence extended over a longer period of time in the control seeds (Figure 4). The temperature at the seedling level was 15–25 °C for the first two weeks after sowing, after which there was a temperature peak reaching over 35 °C (Figure 3B). The mean time of emergence was 2.5–3.5 days shorter in seeds from the bottom fraction than for the control seeds, while the difference between the surface and control seeds was smaller. The treatment effect and the time of emergence was statistically significant in both seed lots.

Table 2. Proportions (model predictions) of 1-year-old Norway spruce seedlings emerged by the end of the growing season, median and mean time of seedling emergence (days after sowing) and interval between 5 and 95% seedling emergence in peat filled containers. (Study II).

Seed lot	Treatment	Emergence percentage (± approximate SE)		Time to seedling emergence, days ± SE		Time of 5–95 % germination
		n = 81	n = viable seed	Median	Mean	Interval, days
A	Control	94.7 (±0.9)	93.3 (±0.7)	15.6 (±0.17)	16.7(±0.15)	14–22
A	Bottom fraction	97.0 (±0.7)	97.7(±0.6)	13.5(±0.08)	15.0 (±0.11)	12–20
A	Surface fraction	79.2 (±1.9)	92.9 (±1.2)	15.9 (±0.20)	16.8 (±0.15)	14–22
B	Control	96.9 (±0.6)	96.5 (±0.7)	15.7 (±0.20)	16.6 (±0.14)	14–22
B	Bottom fraction	98.3 (±0.4)	97.8 (±0.6)	13.5 (±0.09)	15.5 (±0.18)	14–22
B	Surface fraction	88.4 (±1.5)	93.2 (±1.1)	16.2 (±0.16)	17.2 (±0.19)	12–22

The emergence percentage is calculated in relation to all the sown seeds in each block (n = 81) and on the basis of the viable seeds (full and anatomically immature) according to radiography (n = viable seed). The bottom and surface fractions were separated after a 15 h soak.

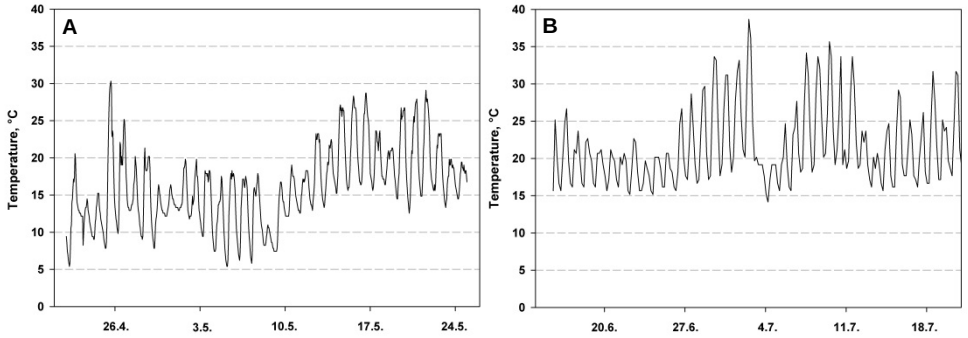

Figure 3. The temperature at the seedling level in the nursery during the first 5 weeks after sowing in experiments II (A) and III (B). The seeds were sown on April 22 in study II and on of June 14 in study III.

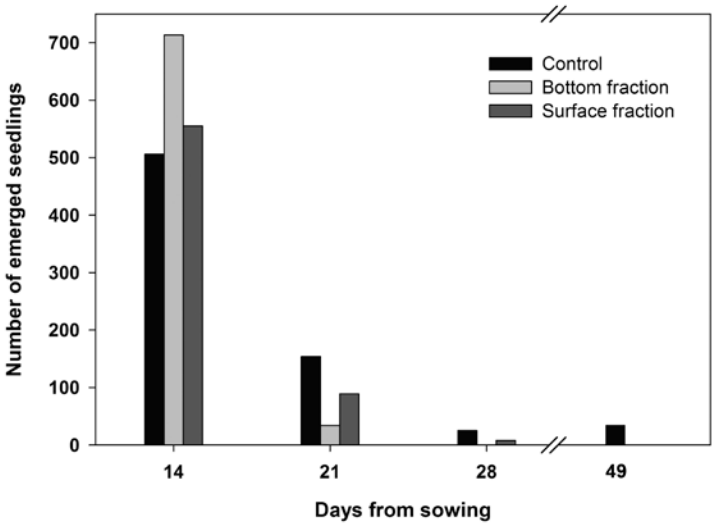

Figure 4. The number of newly emerged seedlings on each day of observation. Containerized Norway spruce seedlings originating from soaked (bottom and surface fraction) and control seeds were sown on June 14. Study III.

3.2 The effect of soaking on seed and seedling quality

Soaking effectively separated the good seeds into the bottom fraction in the two seed lots studied as this fraction contained only viable and full seeds. The effect was consistent in the two experiments (II & III). In study II 95.3% and 94.9% of the seeds sank during the soak in the two seed lots respectively. All the empty and larva infested seeds floated, although the surface fraction also contained a large number of viable seeds (Table 3) as confirmed by the percentage of emerged seedlings in the nursery. The most typical poor-quality seeds in the surface fraction were infested with spruce seed chalcid *M. strobilobius* larvae.

Table 3. The mean percentage of different quality seeds according to radiography and the percent mean of the water content (on fresh weight basis) of Norway spruce seeds after a 15 h soak. (Study II).

Seed lot	Treatment	Full	Larva	Immature	Empty	Other	Water content, % (± SE)
A	Control	98.6	0.8	0.6	0	0	5.4 (±0.01)
A	Bottom fraction	100	0	0	0	0	25.5 (±0.17)
A	Surface fraction	77.8	15.6	5.8	2.5	0.4	22.2 (±0.40)
B	Control	99.2	0	0.8	0	0	5.9 (±0.03)
B	Bottom fraction	100	0	0	0	0	26.5 (±0.29)
B	Surface fraction	86.2	3.7	9.5	3.7	0	missing value

The class “immature” includes anatomically immature, but viable seeds. Seeds with mechanical or undetermined damage were classified as “other”.

The soak-sorting affected the seedling morphology differently in the experiments for 1 and 1.5.-year-old seedlings although the same seed lots were used in the studies. In study II 1-year-old seedlings originating from the surface fraction were the shortest in both seed lots and the effect of soaking was statistically significant for seedling height. The mean seedling height differed in the two seed lots, with seedlings from seed lot A being on average taller than those from seed lot B. The seed lot x treatment interaction was not statistically significant. Despite these differences, the treatments did not affect the height uniformity of the seedlings. The differences in coefficients of variation (CV) were statistically insignificant. With respect to the stem diameter at the end of the growing season, neither the seed lot, treatment or their interaction had a statistically significant effect. Furthermore, none of the variables affected the uniformity of stem diameter – the CVs did not differ from each other.

In study III the treatment × seed lot interaction term had a statistically significant effect on the final height of the 1.5-year-old seedlings (Table 4). Seedlings from the bottom fractions showed the greatest height. Seedlings from seed lot A were taller than those from lot B in the control and bottom fraction seedlings. The stem diameter, however, was affected solely by the treatment: seedlings originating from the bottom fraction had the largest final stem diameter.

The final shoot dry mass in the 1.5-year-old seedlings showed a statistically significant increase as a result of the soaking treatments: the shoots were heaviest in the bottom fraction seedlings. The shoot dry mass was also higher in the seedlings from seed lot A than in those of lot B. The root dry mass was higher in for the seedlings of both soaked fractions than for the control seedlings. The seed lot effect was almost statistically significant, with heavier root systems in seedlings of seed lot A. The relative height and diameter growth (relationship between the final height or diameter and the height or diameter after the first growing season) was not influenced either by the treatment or the seed lot.

Table 4. Tests for fixed effects for measurements of 1.5-year-old containerized Norway spruce seedlings after the second, final growing season according to linear mixed models. A block was set as a random term (test not shown). Denominator degrees of freedom for approximate F-tests were calculated, using algebraic derivatives. (Study III).

Response variate	Fixed model term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Height, 2nd year	Treatment	96985.46	2	48492.73	1383.0	<0.001
	Seed lot	27131.88	1	27131.88	1383.0	<0.001
	Treatment × seed lot	9808.60	2	4904.30	1383.0	<0.001
Diameter, 2nd year	Treatment	21.62	2	10.81	1342.6	<0.001
	Seed lot	1.06	1	1.06	1341.6	0.304
	Treatment × seed lot	0.87	2	0.44	1340.7	0.647
Shoot dry weight, 2nd year	Treatment	11.31	2	5.65	654.8	0.004
	Seed lot	6.24	1	6.24	656.7	0.013
	Treatment × seed lot	0.37	2	0.18	655.6	0.833
Root dry weight, 2nd year	Treatment	13.86	2	6.93	654.7	0.001
	Seed lot	3.57	1	3.57	656.6	0.059
	Treatment × seed lot	0.47	2	0.24	655.2	0.789
Relative height growth	Treatment	2.41	2	1.20	1224.4	0.300
	Seed lot	0.87	1	0.87	1203.2	0.352
	Treatment × seed lot	0.02	2	0.01	1195.9	0.990
Relative diameter growth	Treatment	5.46	2	2.73	1205.6	0.066
	Seed lot	1.04	1	1.04	1182.7	0.308
	Treatment × seed lot	0.43	2	0.22	1177.0	0.806

Soak-sorting affected the seedling survival and had a varying effect on the proportion of saleable seedlings between the two studies. The probability of a seedling surviving to the end of the growing season decreased in relation to the time of emergence in the study on 1-year-old seedlings (study II). Neither the seed lot nor the seed lot x treatment interaction significantly affected survival. The treatment effect, however, was statistically significant; the survival of seedlings originating from the surface fraction was lower than in other treatments and the difference between the treatments was more pronounced in seedlings which emerged slowly. Overall 1.4% of the control seedlings, 1.3% of the seedlings originating from the bottom fraction and 2.9% of the surface seedlings died during the nursery growing.

The seedling quality was also strongly related to the emergence time in the 1-year-old seedlings, with a decreasing probability of good quality seedlings as the emergence time increased, rendering the emergence time the main effect statistically significant (Figure 5). There was no statistically significant difference in the proportion of saleable seedlings between the treatments or the seed lots. The proportion of cull seedlings was 4.2%, 3.3% and 4.9% for the control, bottom and surface fractions respectively.

The bottom fraction seeds produced the highest proportion of saleable seedlings, 94.0%, calculated as the number of saleable 1.5-year-old seedlings in relation to all the emerged original seedlings (study III). The proportion of saleable seedlings was 88.9% in the seedlings originating from the control and 90.1% of the surface fraction seedlings. The treatment effect was hence statistically significant, while the seed lot effect was not.

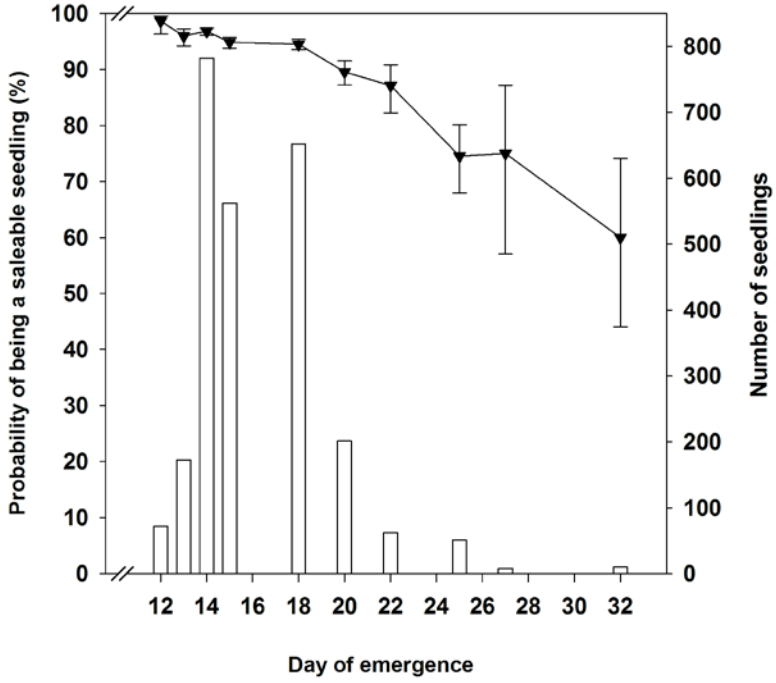

Figure 5. The probability of a 1-year-old Norway spruce seedling being saleable (filled triangles ± SE) in relation to the emergence time according to model predictions. The histogram shows the number of emerged seedlings on each observation day. The seeds were sown in peat filled containers on April 22 and the seedling emergence was observed in a nursery for 32 days. The final count and measurement of the seedlings was done in September. The proportion of saleable seedlings was counted on the basis of the seedlings which emerged during the first 32 days. (Study II).

3.3 Fungi present in seed lots, the effect of soaking and symptoms of damping-off

Fungal species from 14 genera were isolated from seeds of the three commercial seed lots tested in study I (Table 5). Soaking treatments did not have a systematic effect on fungal diversity or their abundance in the seeds. Fungi from genera *Penicillium* and *Trichoderma* were isolated from all seed lots and seeds in all treatments. Other species were isolated and the fungal abundance differed among seed lots. For example, *S. conigenus* was found on seed lots A and B, but not on C, and *Thysanophora penicillioides* (Roum.) W.B. Kendr. was isolated only from seed lot A. Sequence comparisons of our isolates and those in GenBank failed to yield 100% matches except for *S. conigenus* (AY437756.1), *Trichothecium roseum* (Pers.) Link (EU552162.1), *Fusarium avenaceum* (Fr.) Sacc. (syn. *Gibberella avenacea* R.J. Cook) (DQ093676.1), *Acremonium strictum* W. Gams (AY625058.1), *Phoma herbarum* Westend (AY805589.1) and *Penicillium namyslowskii* K.M. Zalessky (AF033463.1). Two species (‘‘x’’ and ‘‘y’’), could not be identified using morphological or molecular techniques.

When the germination and seedling emergence as well as the emergence of disease symptoms was followed after the soaking treatments, the portion of disease cases was considerably larger for the germination test on water agar compared to peat, and differences between the seed lots were also more pronounced. On water agar, seed lot B had approximately four times as many seeds or germinants with disease symptoms compared to the other two seed lots (Figure 6). The seed lot was statistically significant and treatment was almost so ($p=0.07$) in the model for disease symptoms in water agar, with no significant interaction effect. In the model for the cases of disease symptoms in peat, seed lot, treatment, or their interaction had no statistically significant effect. The mean percentage of germinants displaying disease symptoms seed lots and treatments was 5.29 ± 0.7% (SE) in peat.

Table 5. The presence of fungi in three Norway spruce seed lots plated on malt agar plates in three treatments: 1) Control (no soaking), 2) Seeds soaked for 24 h, and 3) Seeds soaked for 24 h, with the water changed 3 times. The inspection of the seeds and fungal isolations were done under a stereo-microscope after one, two and three weeks incubation. (Study I).

Seed lot	A			B			C		
Treatment	1	2	3	1	2	3	1	2	3
Penicillium spp.	99.3 (0.5)	100.0	92.7 (1.5)	85.0 (10.0)	38.3 (2.8)	90.0 (10.4)	69.7 (15.3)	68.0 (20.1)	57.3 (13.3)
Trichoderma spp.	3.7 (1.1)	6.0 (5.2)	13.0 (1.9)	16.7 (2.2)	21.7 (2.4)	27.0 (10.1)	19.3 (2.3)	10.3 (1.8)	14.0 (7.4)
Fusarium spp.	1.0 (0.6)	0	4.0 (1.1)	1.0 (0.6)	4.0 (1.1)	5.0 (1.3)	0	0	0
Acremonium spp.	0.7 (0.5)	1.0 (0.6)	0	0.7 (0.5)	2.7 (0.9)	0.3 (0.3)	0.3 (0.3)	3.0 (1.0)	0
Trichothecium roseum	0	0	0	7.0 (1.5)	2.0 (0.8)	3.3 (1.0)	0.7 (0.5)	0	0
Cladosporium spp.	1.0 (0.6)	0	3.0 (1.0)	0	0.7 (0.5)	0	0	0	0.7 (0.5)
Alternaria spp.	0.7 (0.5)	0	2.7 (0.9)	0	0	0.7 (0.5)	0	0	0
Sirococcus conigenus	1.3 (0.7)	2.0 (0.8)	0	0.3 (0.3)	0	0	0	0	0
Thysanophora spp.	2.3 (0.9)	1.0 (0.6)	0	0	0	0	0	0	0
Aureobasidium spp.	0.7 (0.5)	0	0	0	0	0	0	0	0
Apiospora spp.	0	0	0	0	0	0	0.7 (0.5)	0	0
Phoma spp.	0	0.3 (0.3)	0	0	0	0	0	0	0
x	0	0	0	0	0	0	6.0 (1.4)	6.0 (1.4)	6.3 (1.4)
y	0	0	0	0	0	0	10.3 (1.8)	0	0

Note: The percentage values and their standard errors (in parentheses) are based on 300 seeds (100 seeds per replicate) from each seed lot x treatment combination. Individual species identified by their morphological features or by DNA sequencing are presented in separate rows, whereas rows with only genus include several species or when identification on species level was not possible. ‘‘x’’ and ‘‘y’’ indicate fungi which were not identified.

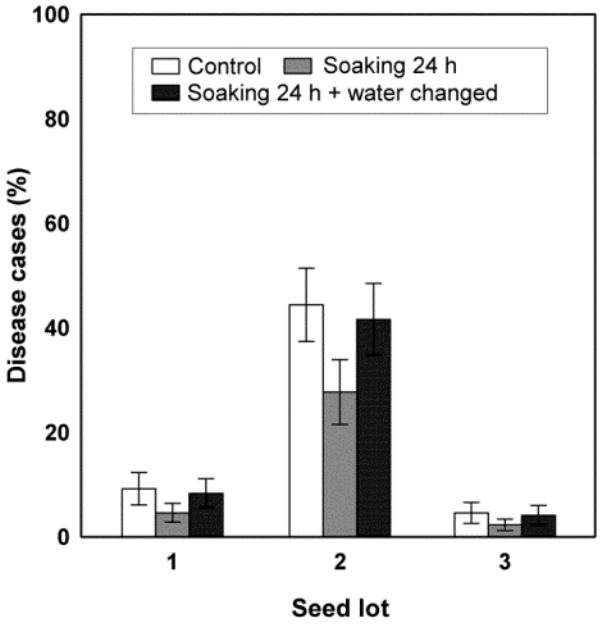

Figure 6. The percentage and approximate standard error of seeds and germinants of Norway spruce with disease symptoms on day 21 on water agar. (Study I).

3.4 Reduction in seed yield caused by empty seeds and insects feeding directly on seeds

On the basis of radiography (study IV), spruce seed chalcid (*M. strobilobius*) larvae infested and spruce seed moth (*C. strobilella*) damaged seeds were found on all trees in the forest stand material, with maximum proportions of 14.5% and 6.9% respectively (Table 6). 1–3 cones per 5 studied cones in each tree included *C. strobilella* damage. Spruce seed gall midge (*P. abietina*) infested and developmentally stagnated seeds were observed in some trees in small proportions. Thus, the empty seeds were the most prominent reason leading to a reduction in the yield of full seeds as the proportion of empty seeds varied from 8.0% to 59.3% between the individual trees in the forest stand studied.

In the cones collected from a seed orchard, *M. strobilobius* and *P. abietina* infested seeds were absent, whereas all the studied cones apart from one cone in clone E456D had *C. strobilella* damage, with 3.3%–11.3% of the seeds damaged in the different clones (Table 7). As in the material in the forest stand, empty seeds formed the largest class of poor-quality seeds in most clones.

In the cones from the forest stand there was statistically significant variation between the individual trees in the proportions of full, empty and immature seeds, seeds with stagnated development, as well as all the three categories of insect damaged seeds (Table 6). In the seed orchard, statistically significant variations between the clones were observed only in the proportions of full and empty seeds (Table 7).

Table 6. Observed proportions (%) of different quality seeds extracted from cones collected from seven Norway spruce trees in central Finland, in addition to the total number of seeds evaluated in each tree and the p-value of the tree (genotype) effect on the proportion of seeds in each quality class. (Study IV).

Seed quality class	Tree							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Tree effect, P-value**
Full	29.2	56.4	60.3	56.7	84.5	86.4	64.7	**<0.001**
Empty	59.3	35.2	29.5	24.1	9.9	8.0	32.8	**<0.001**
Immature	3.1	0	0.7	0.9	0.3	1.1	0.8	**0.002**
Severely immature	2.7	0	0.6	1.3	0.1	0	0.4	**0.002**
Development stagnated	2.5	0	0	0.6	0.2	0.3	0.1	**0.029**
***Cydia strobilella* damage**	2.1	0.1	6.9	0.3	0.5	1.8	0.1	**0.006**
***Megastigmus strobilobius* larva**	0.2	8.0	0.9	14.5	3.8	0.7	0.3	**<0.001**
***Plemeliella abietina* larva**	0	0	0.6	0	0.3	0	0.3	test not possible*
Mechanical damage	0.1	0.2	0	1.1	0	1.2	0.1	0.175
Unidentified damage	0.8	0.1	0.4	0.6	0.4	0.5	0.4	test not possible*
Total number of seeds	891	855	691	698	1127	941	1153	

* Number of observations too low.

Table 7. Observed proportions (%) of different quality seeds extracted from cones collected from five clones in a Norway spruce seed orchard (Sv 403) in central Finland, in addition to the total number of seeds evaluated in each clone and the p-value of the clone effect on the proportion of seed in each quality class. (Study IV).

Seed quality class	Clone					Clone effect, P-value
	E11	E246	E252	E456D	E1549	
Full	63.8	63.9	79.2	79.1	33.2	**<0.001**
Empty	25.7	20.7	8.6	14.6	52.0	**<0.001**
Immature	0.6	0.5	0.2	0.1	0.3	0.427
Severely immature	0.1	0.5	0	0	0	0.712
Development stagnated	0.5	0.3	0.2	0	0	0.574
***Cydia strobilella* damage**	6.2	8.6	10.7	3.3	11.3	0.118
***Megastigmus strobilobius* larva**	0	0	0	0	0	-
***Plemeliella abietina* larva**	0	0	0	0	0	-
Mechanical damage	1.1	2.2	0.7	1.6	0.4	0.138
Unidentified damage	2.0	3.3	0.4	1.4	2.3	**0.023**
Total number of seeds	991	970	1215	1159	922	

3.5 Variation in seed weight

When the seed weights between individual trees or clones were compared, statistically significant differences were found to be present in all major quality classes (Tables 8 & 9). In the stand, tree number 3 produced the lightest and tree number 7 produced the heaviest full seeds. The average weights of full seeds in the orchard material also varied by more than 1 mg between the lightest and heaviest clones. In addition, the ranges of seed weights, comparing the seeds from all quality classes, were different between trees and clones (Figures 7 & 8).

However, examination of the source of the seed weight variation revealed that the intracone variation was larger than that between cones, trees, or clones (Tables 10 & 11). A variance component analysis showed that the intracone variation explained 85.1% of the total variance in the seed weight in the stand seeds. An examination of full seeds with a

weight greater than 2.5 mg revealed that the intracone and intertree variations accounted for 53.0% and 28.6% of total variance in seed weight, respectively. In an analysis of the orchard seed material, the intracone variation accounted for 80.2% of the total variation in seed weight. An analysis of just full seeds with a weight greater than 2.5 mg showed that the intracone variation explained 51.7% and the interclone variation explained 24.3% of the total variation.

Concerning losses of full seeds in different hypothetical scenarios of weight-based sorting, the losses between trees or clones can be seen to vary with fixed weight limits (Tables 12 & 13). If acceptable weight limits are set at 1 mg upwards and downwards from the mean full seed weight in the forest stand material (5.85 mg), 21.0% of full seeds would be lost. If the weight limits are set 2 mg from the mean, 1.8% of all full seeds are lost.

Using the same weight limits for the seed orchard material, 33.4% or 4.9% of full seeds would be lost, respectively. These scenarios for the seed orchard seed present a situation in which seed sorting would be based on some previous settings, not the average weight relating to this seed lot. By setting the weight limits 1 mg or 2 mg apart from the average weight (6.35 mg) of this seed lot, 25.4% or 2.5% of full seeds would be lost.

Table 8. The average observed seed weight (mg) (±SE) of different quality seeds from seven individual Norway spruce trees in central Finland and the p-value of the clone effect on the seed weight in each quality class.

	Tree							
Seed quality class	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Tree effect, P-value**
Full	6.12 (±0.049)	6.09 (±0.034)	5.22 (±0.030)	6.51 (±0.037)	5.60 (±0.021)	5.368 (±0.029)	6.37 (±0.029)	**<0.001**
Empty	1.40 (±0.020)	1.89 (±0.038)	1.57 (±0.026)	1.86 (±0.039)	1.61 (±0.029)	1.49 (±0.082)	1.61 (± 0.041)	**<0.001**
Immature	4.34 (±0.126)	-	3.74 (±0.079)	4.55 (±0.238)	3.92 (±0.707)	4.29 (±0.347)	5.05 (±0.190)	**0.038**
Severely immature	3.01 (±0.118)	-	2.972 (±0.293)	3.68 (±0.211)	3.00 (0)*	-	3.78 (±0.376)	**0.026**
Development stagnated	2.76 (±0.095)	-	-	2.71 (±0.550)	2.43 (±0.314)	1.51 (±0.522)	2.85 (0)*	**0.005**
***Cydia strobilella* damage**	3.46 (±0.228)	2.40 (0)*	3.26 (±0.089)	5.40 (±1.218)	2.94 (±0.707)	3.46 (±0.198)	3.12 (0)*	**0.007**
***Megastigmus strobilobius* larva**	2.76 (±0.354)	3.19 (±0.124)	2.44 (±0.194)	3.50 (±0.081)	3.00 (±0.108)	2.59 (±0.117)	2.67 (± 0.327)	**<0.001**
***Plemeliella abietina* larva**	-	-	2.90 (±0.280)	-	2.68 (±0.117)	-	2.28 (±0.114)	0.197
Mechanical damage	1.91 (0)*	4.26 (±2.118)	-	2.66 (±0.492)	-	2.48 (±0.306)	0.55 (0)*	0.268
Unidentified damage	3.40 (±0.677)	3.59 (0)*	5.161 (±0.323)	5.89 (±0.265)	5.64 (±0.332)	4.23 (±0.437)	5.42 (±0.318)	**0.011**

Table 9. The average observed seed weight (mg) (±SE) of different quality seeds in five clones in a Norway spruce seed orchard in central Finland and the p-value of the clone effect on the seed weight in each quality class.

Seed quality class	Clone					Clone effect, P-value
	E11	**E246**	**E252**	**E456D**	**E1549**	
Full	6.66 (±0.039)	5.54 (±0.029)	6.76 (±0.025)	6.25 (±0.023)	6.39 (±0.050)	**<0.001**
Empty	2.49 (±0.038)	1.69 (±0.026)	2.07 (±0.054)	1.94 (±0.034)	2.36 (±0.014)	**<0.001**
Immature	5.85 (±0.324)	3.71 (±0.104)	4.97 (±0.623)	6.03 (0)*	5.50 (±0.624)	**0.006**
Severely immature	5.18 (0)*	3.63 (±0.375)	-	-	-	0.167
Development stagnated	2.77 (±0.124)	2.69 (±0.929)	3.59 (±0.939)	-	3.26 (±0.358)	0.632
***Cydia strobilella* damage**	4.68 (±0.218)	3.78 (±0.123)	4.38 (±0.075)	4.45 (±0.236)	4.28 (±0.082)	**<0.001**
***Megastigmus strobilobius* larva**	-	-	-	-	-	-
***Plemeliella abietina* larva**	-	-	-	-	-	-
Mechanical damage	4.27 (±0.662)	3.87 (±0.377)	5.33 (±0.518)	4.04 (±0.455)	3.36 (±1.407)	0.387
Unidentified damage	5.85 (±0.510)	4.53 (±0.253)	6.43 (±0.340)	5.79 (±0.385)	5.62 (±0.222)	**0.007**

- no observations
* only one observation

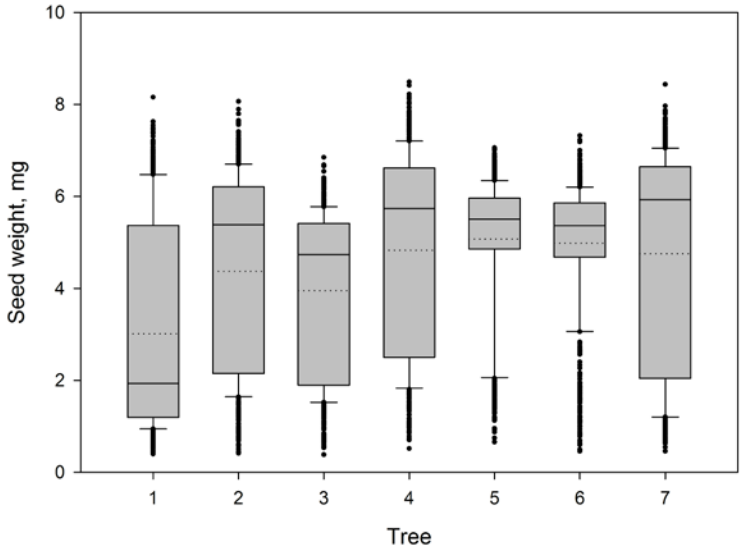

Figure 7. A box plot of the seed weights of Norway spruce seeds collected from a forest stand in central Finland. Each seed from five cones from each tree was individually weighed. The box includes 50% of the observations, and the whiskers indicate the 90th and 10th percentiles. The solid line inside the box indicates the median, and the dashed line indicates the mean. (Study IV).

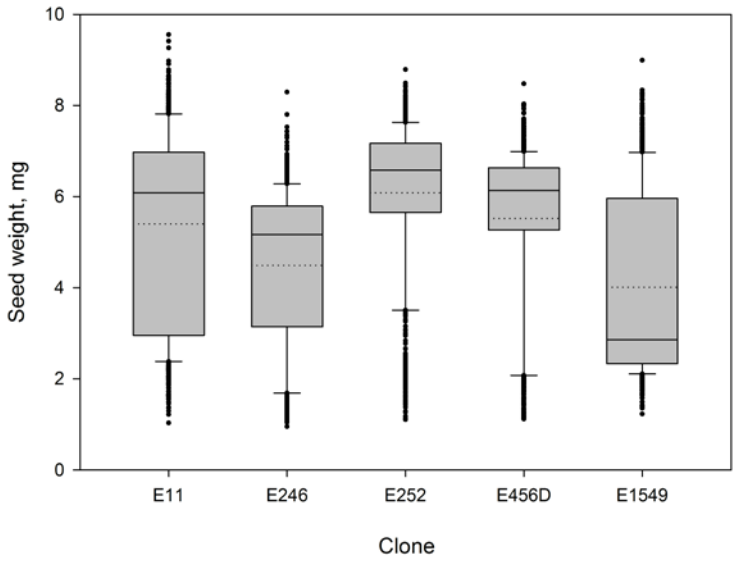

Figure 8. Range of seed weights of Norway spruce seeds collected from a seed orchard located in central Finland. Each seed from four cones from each clone was individually weighed. The box includes 50% of the observations, and the whiskers indicate the 90th and 10th percentiles. The solid line inside the box indicates the median, and the dashed line indicates the mean. (Study IV).

Table 10. Variance component estimates of seed weight between individual trees, between cones (within the same tree) and within cones. The proportion of variance of each of these components is compared to the total variance in the seed weight. The cones were collected from seven trees in a Norway spruce stand in central Finland. (Study IV).

Term	Variance estimate	SE	Proportion, %
All seeds			
Intertree	0.4910	0.3080	11.3
Intercone	0.1530	0.0530	3.5
Intracone	3.6830	0.0650	85.1
Full seeds (>2.5 mg)			
Intertree	0.1949	0.1314	28.6
Intercone	0.1250	0.0395	18.3
Intracone	0.3620	0.0081	53.0
Empty seeds			
Intertree	0.0277	0.0200	7.4
Intercone	0.0199	0.0082	5.3
Intracone	0.3280	0.0111	87.3

Table 11. Variance component estimates of seed weight between clones, between cones (within the same clone) and within cones. The proportion of variance of each of these components is compared with the total variance in the seed weight. The cones were collected from five clones in a Norway spruce seed orchard in central Finland. (Study IV).

Term	Variance estimate	SE	Proportion, %
All seeds			
Intertree	0.6870	0.5070	17.1
Intercone	0.1090	0.0440	2.7
Intracone	3.2150	0.0630	80.2
Full seeds (>2.5mg)			
Intertree	0.2164	0.1924	24.3
Intercone	0.2138	0.0793	24.0
Intracone	0.4610	0.0112	51.7
Empty seeds			
Intertree	0.0948	0.0715	32.9
Intercone	0.0215	0.0090	7.5
Intracone	0.1720	0.0071	59.7

Table 12. The proportion of lost full seeds in each tree in a forest stand in two hypothetical scenarios of seed sorting: A) seeds with a mass of 1.0 mg lighter and heavier than average (4.85–6.85 mg) included in the final seed lot, B) seeds with a mass of 2.0 mg lighter and heavier than average (3.85–7.85 mg) included in the final seed lot. The average full seed weigh in the forest material is 5.85 mg.

			Tree							Mean
			1	2	3	7	8	9	10	
Loss of full seed, %	A	Range 4.85–6.85 mg	19.6	14.8	24.5	32.3	12.5	21.9	27.5	21.0
	B	Range 3.85–7.85 mg	1.9	1.3	0.7	3.0	1.3	3.1	1.5	1.8

Table 13. The proportion of lost full seeds in each clone in a seed orchard in four hypothetical scenarios of seed sorting. Scenarios A and B use the ranges adopted from the forest stand material and scenarios C and D use ranges adjusted according to the average weight of the seed orchard material. A) Seeds with a mass of 4.85–6.85 mg included in the final seed lot, B) seeds with a mass of 3.85–7.85 mg included in the final seed lot, C) seeds with a mass of 1.0 mg lighter and heavier than average (5.35–7.35 mg) included in the final seed lot. D) seeds with a mass of 2.0 mg lighter and heavier than average (4.35–8.35 mg) included in the final seed lot. The average full seed weigh in the seed orchard material is 6.35 mg.

			Clone					Mean
			E11	E246	E252	E456D	E1549	
Loss of full seed, %	A	Range 4.85–6.85 mg	42.7	18.4	48.5	20.2	37.3	33.4
	B	Range 3.85–7.85 mg	13.4	1.10	5.9	0.7	4.2	4.9
	C	Range 5.35–7.35 mg	32.3	36.1	26.3	13.5	21.9	25.4
	D	Range 4.35-8.35 mg	4.3	5.0	0.8	1.1	1.9	2.4

4 DISCUSSION

4.1 Soaking effects on germination and seedling emergence

The findings in studies I–III that soaking increased the initial, but not the final germination or emergence percentage, is in line with observations for similar treatments for conifers (James 1985, Campbell & Landis 1990, 1987, Kolotelo et al. 2001) including Norway spruce (Löyttyniemi 1969, Himanen et al. 2010).

In studies II and III where the mean and median time of emergence were determined, both were shortened more that of the length of the soaking. This suggests that the effect of the soak is not limited to physical imbibition only, but metabolic activity is also resumed in the favourable conditions of the soak.

The slower emergence rate of the surface fraction seeds compared to the bottom fraction may be explained through their slower imbibition rate: the floating seeds had imbibed less water than the sunken ones during the 15 h soak (Table 3), so their metabolism was probably also less activated than the seeds in the bottom fraction. It remains uncertain as to whether the late emergence of floating seeds was due to this inefficient imbibition or whether the slow germination indicates that some other factor may also be causing the slow imbibition and subsequent floating.

Interestingly, in study II on the growing of 1-year-old seedlings the emergence of the seedlings did not become more synchronous as a result of the soaking, but in the experiment with 1.5-year-old seedlings (study III) soaked seeds emerged in the first two weeks, while the control seeds emerged over a longer period. In study II the differences observed in the emergence curves was due to the difference in the timing of seedlings' emergence, rather than due to the difference in the *shape* of the curve. The observation is particularly interesting as the same seed lots were used for both studies.

The difference is probably related to the difference in the temperature during the emergence phase (Figure 3), as the optimum temperature for Norway spruce seed germination is around 20–22 °C (Leinonen et al.1992). In study II no temperature peaks detrimental to the germination process took place during the first 3 weeks after sowing, although the temperature was slightly sub-optimal for this time period. In study III the overall temperature was higher, as is typical for midsummer, and a four day spell of temperatures reaching over 30 °C hit the seeds after 2 weeks from sowing. Most seeds had emerged by this time, but those that had not were faced with the high temperatures. The proportion of non-germinated seeds at this point was greater for the control seeds than the soaked seed. Thus in this experiment the soaking helped the seedlings emerge prior to the harsh conditions creating a more synchronous emergence pattern.

The effect of soaking on the germination or emergence rate is therefore likely to vary according to temperature or other environmental conditions affecting germination. A more simultaneous germination is often cited or thought to be a result of soaking treatments (e.g. Toymey & Durland 1923, Kolotelo et al. 2001, Feurtado et al. 2003). The current data both agrees and disagrees with these claims.

4.2 The effect of soaking on seed and seedling quality

Soaking resulted in viable and full seeds in the bottom fraction, while the floating surface fraction contained larvae-infested, anatomically immature and empty seeds in both seed lots. However, the surface fraction also contained a large number of viable seeds (Table 3). The results were essentially the same in both studies (II and III). Fractioning during aerated soaking can therefore be expected to give a rather consistent result in Norway spruce within a seed lot.

Commercial seed lots with high levels of purity and germinability were chosen for these studies as they are representative of seed lots typical in commercial seedling production in Finland. Their properties are, however, limiting in terms of studying the effects of soak-sorting, because they contain a low percentage of empty, immature or larvae infested seeds,

which was demonstrated by the small size of the surface fraction. Soak-sorting may therefore be more useful for upgrading seed lots with poor quality, atypical to the most commonly used and most desired seed lots in nurseries.

Similar to the results reported here, soaking is known to separate empty and damaged seeds of other conifer species as well (Barnett and McLemore 1970, Kolotelo et al. 2001). In IDS treatment, good seeds are separated from empty or dead seeds by incubating, drying and finally separating them, typically by soaking (Bergsten 1987). As in the current study, in IDS the undesirable seeds float (Bergsten 1987, Sweeney et al. 1991, Downey and Wang 1992, Tillman-Sutela 1997), although the mechanism of this treatment is different to the current research. In the present study, sorting the seeds after surface drying was not studied. It is possible that further fractioning of the surface fraction, for example, in which a large percentage of full seeds remained after the soak-sorting, could function similarly to IDS treatment.

In studies II and III, it was observed that the number of emerged seedlings in the nursery agreed with the number of viable seeds in each fraction as determined by radiography. The results suggest that careful examination of radiographs can provide an accurate prediction of seedling emergence for a particular seed lot.

The effects of soaking on seedling morphology were different in the two studies (II and III). The height of the seedlings was the greatest in the bottom fraction seedlings in study II for the 1-year-old seedlings while the other morphological features – stem diameter, shoot and root dry mass – were unaffected. Furthermore, no differences in the uniformity of seedling height or stem diameter, indicated by coefficients of variation, could be detected between the treatments. In the study of the 1.5-year-old seedlings, however, the treatment affected all the morphological features measured: the seedlings originating from the bottom fraction were the largest.

As seedling height is not a good indicator of out-planting success (Grossnickle 2012), the increase in this attribute as a result of the treatment does not have a clear interpretation. In study II, the 1-year-old seedlings from both of the seed lots were generally taller than recommended for this type of Norway spruce container seedlings (Rikala 2002), indicating supraoptimal conditions (irrigation, fertilization etc.) in the nursery.

Stem diameter is known to be a better indicator of tree seedling survival and performance after out-planting than seedling height (e.g. Aphalo and Rikala 2003, South et al. 2005, Ritchie et al. 2010, Tsakaldimi et al. 2013). The results in this study indicate that the treatment increased the seedling quality for 1.5-year-old seedlings but not for 1-year-old seedlings.

Again, the results both agree and contradict some similar studies with other conifer species. Boyer et al. (1985) found that seed treatments that shortened the germination time subsequently increased the average seedling diameter of loblolly pine (*Pinus taeda* L.). In another study, loblolly pine seedlings that germinated earliest achieved the largest stem diameter (Boyer et al. 1987). Mexal and Fisher (1987) also reported a decrease in shoot biomass in relation to emergence times for loblolly and ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.).

The explanation of the difference between the two studies (II and III) may be in the different reduction in the emergence time due to the soaking treatment. In study II the mean emergence time was 1.1–1.7 days shorter for the bottom fraction seedlings compared to the control. In study III, the reduction was 2.3–3.5 days. This lengthening of the growing season in the first year of the seedling development of the 1.5-year-old seedlings may have

been sufficient to cause an increase in the seedling size, whereas the smaller change gained in study II was too short to cause any major changes in seedling morphology.

In study II the survival of seedlings originating from the surface fraction was lower than in other treatments, but the treatment did not alter the proportion of saleable seedlings. In study III the bottom fraction seeds produced the largest number of saleable seedlings. Again, the differences between study II and III may be due to the difference in the effects of the soak on the emergence timing.

The time of the seedling emergence was directly related to the seedling mortality and cull percentage for the 1-year-old seedlings where it was studied (study II). The later a seedling emerged, the more likely it was for it to die during the growing season or to be rendered as a cull seedling. The effect of the time of emergence was more pronounced in the surface fraction than in other fractions. This suggests that the individuals in the surface fraction are indeed genetically or physiologically inferior causing them not only to germinate slowly but also to be more susceptible to diseases or to die for other reasons. This possible effect is difficult to separate from increased mortality caused for example by more intense light competition that the slowly emerging seedlings experience. However, it is unlikely that competition is the sole explanation given that the growth resources are effectively unlimited for all seedlings during the first weeks after emergence and no root competition exists in containerized production.

These findings on seedling survival and quality are similar to results on other conifer species (Boyer et al. 1987, Mexal and Fisher 1987) and may be more widely applicable. In a study on loblolly pine and ponderosa pine, the increase in mortality between seedlings emerging first and last in bare-root nurseries was 19 and 30 percentage points, respectively (Mexal and Fisher 1987). In Boyer et al. (1987) loblolly pine mortality increased approximately 7 percentage points when comparing early and late-emerging seedlings in a bareroot seedbed (Boyer et al. 1987).

The curve showing the decreasing proportion of saleable seedlings in relation to the time of emergence (Figure 5) is likely to vary according to the seed lot, sowing time (spring, summer), growing conditions and tree species. However, such curves could be used to determine the latest point in time when it is no longer economically sensible to wait for the rest of the seedlings to emerge. According to the present data, nurseries seeking a minimum rate of 90% seedlings suitable for sale could plant empty cells with a substitute seedling after 21 days sowing.

4.3 Fungi present in seed lots, the effect of soaking on their prevalence and symptoms of damping-off

Seed lots differed in the abundance and the species diversity of the isolated fungi, that in turn remained unaffected by the soaking treatments. The species isolated are typical for conifer seeds (Mittal et al. 1990, Motta et al. 1996, Vujanovic et al. 2000, Sutherland et al. 2002, Talgø et al. 2010). However, common, fungi from genera such as *Penicillium*, *Cladosprorium*, *Acremonium* and *Aureobasidium* are considered a sign of low quality seeds when abundant (Mittal & Wang 1993, Sutherland et al. 2002). Certain species of genera *Trichoderma* are known to be antagonistic (Finch-Savage et al. 2003, Grodnitskaya & Sorokin 2007) but some are found to be harmful (Mittal & Wang 1993).

Among the isolated fungi in this study were *T. roseum* and members of genera *Fusarium*, *Alternaria* and *Phoma*. These genera are known to include pathogenic species

causing diseases in seedlings, such as damping-off (Lilja 1979, Mittal & Wang 1993, Lilja et al. 1995). Apart from *S. conigenus*, for which the primary infection of cones and seeds occurs in the tree, all the isolated fungi are soil-borne (Sutherland et al. 2002). These fungi produce airborne spores in abundance during the autumn, when cones are collected. This suggests that seed contamination occurred during cone collection, storage or processing (Domsch et al. 1980, Mittal & Wang 1987, Fraedrich & Miller 1995). Infected branches or ground vegetation may have possibly been collected with the cones, or the cones could have been stored in contact with the ground, or the seeds may have been contaminated via seed-handling machinery contaminated by earlier processed material. Since *S. conigenus* is often encountered on conifer cone scales, inclusion of the previous year's cones in collections may be a source of infection for this species (Sutherland et al. 1981, 2002). To reduce the level of contamination in seeds, avoiding contact with soil is often mentioned to be among the most effective measures (Willan 1991, Thompsen & Schmidt 1999, Sutherland 2002).

Differences between the seed lots with respect to the incidence of disease were apparent when the seeds were germinated on water agar plates. In peat, the incidence of disease was in general lower than on water agar and differences between the seed lots disappeared. *Sphagnum* peat has a low pH and it contains antagonistic microbial flora (Tahvonen 1982). These qualities make peat an unfavourable growth medium for many pathogens, reducing the emergence of disease symptoms even though harmful fungi may be present. As there is pressure to change the growth media in seedling production from *Sphagnum* peat into media considered more ecologically sustainable, such as coconut mulch or un-humified *Sphagnum* medium, the importance of seed borne microbes may increase. Germination conditions in general play an important role in the development of damping-off (Thomsen & Schmidt 1999). Therefore, the realization of the risk caused by seed-borne microbes under nursery conditions will be affected by the growing measures: over-irrigation, low temperature or insufficient lighting will increase the risk of disease.

Soaking treatments did not have a statistically significant effect on the emergence of disease symptoms in this study. For other conifers, rinsing has at times reduced (James 1985, 1987) and sometimes increased the fungal contamination of seeds (James 1986). The negative effect of rinsing in a study by James (1986) was possibly caused by the adverse effect that rinsing had on beneficial fungi such as *Penicillium* (James 1986), which have also been reported to protect seeds of Sakhalin spruce (*Picea glehnii* F. Schmidt) from damping-off (Yamaji et al. 2001). In the case of this study, it may be that changing the water was simply ineffective in causing changes in the fungal community, because little change could be noticed in the frequencies in prominent genera such as *Trichoderma* and *Penicillium*.

4.4 Reduction in seed yields caused by empty seeds and insects feeding directly on seeds

Seed quality, especially the proportion of full seeds, varied among individual trees and clones. In trees or clones with low numbers of full seeds, in contrast, the proportion of empty seeds was large. The proportions of empty seeds are in line with findings by Heikinheimo (1937) and Sarvas (1968).

The large proportion of empty seeds indicates that either pollination or fertilization was unsuccessful in certain genotypes. The susceptibility of the female strobili of these

genotypes may have been nonsynchronous with the peak pollen dispersal; however, this is speculative, as no observations on the flowering phenology of the trees and grafts were conducted. The results concur with studies in which the uneven contribution of genotypes in conifer seed crops is well documented (Eriksson et al. 1973, Byram et al. 1986, Stoehr and Farmer 1986, Saarsalmi et al. 1994, Nikkanen and Ruotsalainen 2000). As the empty seeds cause such a dramatic decrease in the yield of full seeds – up to 59% of seeds were empty – and have an effect on the effective population size, measures to ensure pollination, such as supplemental mass pollination (El-Kassaby & Reynolds 1990, Eriksson et al. 1995) should be considered in seed orchard management of Norway spruce.

Sampling of the cones for the study IV cannot be considered random with regard to all pest damage, as cones with cone rust infestations and clear external signs of insect damage were excluded. However, the presence of spruce seed moth larvae *C. strobilella* or seed infesting insects (*M. strobilobius*, *P. abietina*) is not visible on the surface of the cone. Therefore, the results on their frequency can be regarded as quite reliable. The prevalence of these insects is again comparable to the findings in previous studies (Nikula & Jalkanen 1990, Rosenberg & Weslien 2005, Rosenberg et al. 2012). It is, however, interesting that although *C. strobilella* larvae were found commonly in the study material, the proportions of damaged seeds were relatively low. In the seed orchard material, the species was present in all but one cone, yet the damage covers 11.3% of the seeds at the most. This is rather low for one of the most severe cone pests known to infest Norway spruce. In the evaluation of the significance of a pest, the level of damage must be studied instead of mere observation of the presence of the species.

The evaluation of the importance of the production environment (stand vs. orchard) concerning *C. strobilella* damage is difficult with the limited study material, although interestingly, the damage was more common in the seed orchard seeds than in the stand seeds. The study material was collected in different years (2011 and 2013), which may explain the difference, as spruce seed moth populations fluctuate annually (Annila 1981). Because the collection sites are approximately 90 km apart, spatial variation in insect population density can also be expected.

In the forest stand, the proportion of *C. strobilella* damaged seeds differed in a statistically significant manner between the trees. Glynn and Weslien (2004) also reported a genotypic effect on the infestation rate of *C. strobilella* in Norway spruce. However, in the seed orchard material of this study, no statistically significant differences were observed between the clones. However, as the cones were collected from one graft per clone only, the data in this study is limited.

Rosenberg et al. (2012) reported that as the number of *M. strobilobius* infested seeds in Norway spruce cones increased, the seed weight decreased. The findings in the present study do not support this. In the stand material, tree number 4 showed the highest proportion of *M. strobilobius* infested seeds, but it also contained the heaviest full seeds. The average full seed weight also showed similar variation in the orchard material as for the stand seeds, although no *M. strobilobius* were found in the orchard seeds.

4.5 Variation in seed weight

Previous studies of conifers have shown that seed weight is dependent on the mother tree (Hellum 1976, von Weissenberg 1981, Lindgren 1982, Stoehr and Farmer 1986, Clair and Adams 1991, Matziris 1998). This is explained by the large proportion of maternal tissue in

conifer seeds and also due to environmental factors such as nutrient availability being mediated by the mother tree (Clair and Adams 1991).

Examination of the *average* weights of full seeds revealed that statistically significant differences between trees and clones were present in the present material as well. However, analyzing the source of the variation in seed weight – either for all seeds or full seeds – showed that the maternal effect was not the most important factor, because the intracone variation in seed weight was larger than that between trees or clones or between cones. In the forest stand 85.1% and in the seed orchard 80.2% of the variation in the weight of all seeds was due to intracone variation. This is a novel finding. In the previous studies mentioned, intracone variation has not been tested, due to the measurement of seed weight from bulked samples including seeds from several cones.

It is also notable that the proportions of the variation components were similar in both the stand and orchard material. This suggests that the effect on seed weight caused by the different sources of variation was not a random phenomenon or dependent on the production environment. Due to the limited number of mother trees and data from 1 year only in the present study, further evidence is needed to confirm this. It must also be noted that the study material was collected from a first-generation orchard. This may be a source of the similarity between the results of the stand and orchard material.

These results apply when sorting is done on a weight basis only. Although seed weight and seed size are strongly correlated, they should not be viewed as synonymous. Although various conifer taxa share traits with regard to seed weight, the applicability of our results to other conifers species needs to be confirmed.

4.6 The effect of weight-based seed sorting on the genetic base of Norway spruce seed lots and seed availability

4.6.1 Genetic diversity

The maintenance of high genetic diversity in forest management is key in adaptation to climate change and for a fully functioning forest ecosystem (Alfaro et al. 2014). Forest management practices, including many steps in forest regeneration, significantly impact the genetic diversity of forest trees (Edwards & El-Kassaby 1996, Ratnam et al. 2014), and therefore, care must be taken in composing seed material for regeneration. The results give reason to re-evaluate current conceptions of weight-based sorting of Norway spruce seed, which is believed to lead to the exclusion of certain maternal genotypes (Hellum 1976, Lindgren 1982). The lightest and heaviest seeds in a given seed lot are likely to lack seeds from certain mother trees also according to the present data, indicating that these extreme fractions are genetically narrow and should not be used as regeneration material alone. However, as sorting begins with all the seeds extracted from the cones, the sorting effect on the genetic diversity of the main seed lot is far less dramatic.

As the present data shows, not all maternal genotypes contribute equally to the pool of full seeds, as the full seed proportion varies. The proportion of full seeds was 29.2%–86.4% in the forest stand and 33.2%–79.2% in the seed orchard depending of the tree or clone. The number of cones produced by each tree or graft was not determined, and, therefore, the actual contribution of individual trees or clones to the entire full seed production of the stand or seed orchard cannot be assessed with the present data. However, it is known that both the abundance of flowering, as well as the number of full seeds, vary on a yearly basis

in Norway spruce (Eriksson et al. 1973, Nikkanen & Ruotsalainen 2000). The effect of this phenomenon on genetic diversity is mitigated in seed orchards by using sufficient numbers of clones. The current results emphasize the need for this. In a natural forest stand, the non-uniform contribution of each mother tree would be evened out by this yearly variation, as the new stand is not the result of seeds from a single year. In contrast, the seeds and eventually seedling lots in artificial forest regeneration present a snap shot of this continuum, including the genetic variation of just 1 year. As artificially regenerated clear-cut areas are typically only a few hectares in size in northern Europe and the forested landscape is cut and regenerated mosaically, this practice helps to ensure that the landscape-level genetic diversity remains high.

It must also be noted that even the trees or clones contributing minimally to the production of full seeds have participated in the genetic composition of the seed lot via pollen dispersal. If we were to ensure that the genetic diversity at a given site is as high as possible, pooling seeds over several years for a single seed lot could provide acceptable results. For Norway spruce, however, the cone production of individual clones correlates over years: the clones producing cones abundantly tend to do so in successive seed years (Ruotsalainen & Nikkanen 1989). Therefore, a more effective way of evening out clonal differences in seed lots would entail clonal cone collection and a subsequent mixing of the clonal material in the desired proportions.

Based on this study, seed sorting is not the main culprit in the narrowing of genetic diversity by exclusion of certain maternal genotypes. Instead, we should take into account the natural processes occurring in Norway spruce seed production. As shown in this study and in previous studies (Eriksson et al. 1973, Saarsalmi et al. 1994), yearly variation in the genetic construction of seeds produced in a seed orchard is so large that it may be necessary to take this also into account in planning seed use maps, guidelines for seed transfer, and in determining the sufficient number of clones in new seed orchards.

4.6.2 Seed availability

From the seedling production point of view, uniform seed weight in seed lots is favourable as the seed weight affects the germination rate (Dunlap & Barnett 1983, Sorensen & Campbell 1993) and the seed weight also correlates positively with seedling size (Reich et al. 1994). Pneumatic sowing machines also require seeds of similar weight and size because large variations increase the chance of malfunction resulting in container cells with too many or zero seeds. This encourages sorting out large fractions from both ends of the distribution in each seed lot, i.e. excluding the smallest and the largest seeds.

From the society point of view this is, however, problematic. According to the different scenarios presented in Tables 12 and 13, the weight limits set for seed sorting have a substantial impact on the loss of full seeds. From the perspective of society, serving the needs of nurseries in providing seed lots with a narrow range of seed weight may cause economic losses, especially if seed orchard seeds are lost in addition to losing some genetic diversity. Seed orchard establishment and breeding programmes are supported by public funding and losing viable seeds leads to insufficient use of these subsidies. In the case of shortages of seed orchard seeds, the use of stand seeds increases. This means that the genetic gain of seed orchard seeds and the subsequent benefits for silviculture and the public economy are lost. Therefore, care must be taken in balancing different preferences in seed sorting.

4.7 Seed quality

4.7.1 Demands for quality by the different operators

The quality of seed is perceived differently by the seed producers and users since the purpose for the seeds differs. The primary objective of the seed producer is to produce saleable seed lots with the largest profit. The seedling producer on the other hand seeks seed lots with qualities resulting in a high percentage of saleable seedlings, while society as a whole is mostly interested in the genetic quality of the regenerated forests, although economically viable seed and seedling companies are relevant for society as well.

From the seed producer's point of view, it is not in its direct interest to maximize the technical quality (purity, germinability etc.), if the excess quality is not financially compensated for by the customers or from other sources of income. The level of quality they produce is therefore driven by what can be marketed legally and what the customers are willing to purchase. Equally, seedling producers are not interested in maintaining "excess" genetic diversity of the seeds and eventually seedling lots in case the customer acquiring the seedlings is not willing to compensate for the extra work required to produce and maintain the diversity.

In the case of seed weight, as described previously, the demands from society and the seedling producers can be contradictory. As the legislation does not give precise or particularly strict directions for seed sorting (Decree of the… 2002), the practices conducted by seed producers are driven by the wishes of the customers (i.e. nurseries) in preference to the needs of society at large.

In a highly developed market for forest reproductive material, some seedling producers and forest service providers could use the level of genetic diversity as a selling argument for the forest owners. In that case the demands for for example seed weight uniformity could be different to the current situation, and thus the seedling producers' and the demands of society at large would meet better. This would, however, require a higher level of expertise in the forestry field and an increased interest and willingness to pay for forest regeneration by the forest owners to compensate for the more laborious seedling production caused by more heterogeneous seed material.

When it comes to other seed quality attributes, such as germinability and the potential for seed-borne disease in seed lots, the demands of the seed and seedling producers are fairly uniform. In a fully functioning, free market situation suitable seed lots could be chosen from lots with different quality attributes. This would create a situation in which the seed buyer could choose the level of quality they want and what they are willing to pay for it, i.e. this would be an optimization task for the customer. Since there is a chronic lack of seed orchard seeds, and sometimes even suitable seeds all together, free competition is not possible. The nursery manager has typically one or a couple of seed lots with the appropriate provenance to choose from with varying quality attribute combinations. Therefore, the pressure for work on seed quality comes in part from the goodwill of seed producers or their owners and the willingness to work for the good of the field of forest regeneration rather than in reaction to monetary pressure from the customers.

4.7.2 Quality management in seed production and use

There are several ways in which the different operators in seed production can and do promote the quality attributes they prioritize, as well as the availability of seeds. Seed

producers have many management tools to increase seed production especially in seed orchards. The establishment of the orchards on suitable agricultural land, and the management of nutrient and moisture availability for the grafts, as well as their appropriate cutting is the basis for seed production (Almqvist et al. 2007, Antola et al. 2009). Gibberellic acid applications can be used to promote the flowering of the grafts (Bonnet-Masimbert 1987, Helenius 2010, Rosenberg et al. 2012), in addition to supplemental mass pollination (El-Kassaby & Reynolds 1990, Eriksson et al. 1995), or simply enhancing the movement of pollen in the orchard that can help to ensure the fertilization of ovules. Integrated pest management methods including the use of pesticides are available to reduce the loss of full seeds to insects (Rosenberg et al. 2005, 2012, Helenius et al. 2015), although further development of methods is necessary. Increasing the level of hygiene during cone collection and storage are advised, for example, in Kolotelo et al. (2001) and Helenius et al. (2015).

In nurseries, quality improvement is possible with the methods studied in this thesis. Soaking treatments may be used to exclude empty and larvae infested seeds, as well as slowly emerging seeds. Under suboptimal or supraoptimal germination conditions soaking may increase germination energy resulting in enhanced seedling morphology and a reduction in the proportion of cull seedlings and mortality. Although the risk of seed-borne disease is determined during the various phases of seed production, germination and growth conditions play a major role in the control of the emergence of disease symptoms.

Society may influence the seed trade and quality through legislation and the allocation of resources for forest breeding, research and by providing subsidies for seed companies, nurseries and forest owners (e.g. Council Directive 1999, Decree of the… 2002). In Finland tree breeding and providing instructions on the establishment of seed orchards are defined as an official task to the Natural Resources Institute (Luke). Seed companies also receive subsidies for the establishment and early management phase of the seed orchards and the two seed producers (Tapio Silva Oy, Siemen Forelia Oy) currently operating in this field are government owned, although they function as companies. Quality, for example genetic diversity, of seed lots can be managed through these channels.

4.7.3 Framework for quality management

Quality management can also be approached through a more theoretical framework. Lillrank (1998) presents a construction in which quality control is divided into two axis. On the horizontal axis we move from centralized administration to a decentralized one, and on the vertical axis we start from norm-based control to a customer oriented one. Society may choose any of the combinations formed to manage quality of any given product or service. In a free-market context, quality control is decentralized and customer oriented. In this case customers may have different objectives and the market chooses how the different quality attributes are emphasized. In many key functions of society – for example flight or drug security – quality is controlled by norms in a centralized fashion.

At the moment, seed quality is on the one hand quite strictly government managed in Finland due to several economic arrangements presented above. On the other hand, legislation or the control of the breeding programmes etc. are not particularly precise and seeds are produced by companies with expectations of making a profit rather than operating merely for societal benefit. It can be debated whether the current situation is actually able to control or drive quality development in an efficient manner. In Sweden the society has chosen a free-market-based approach, in which forest companies may have their own

breeding programmes and the seed orchard management establishment does not receive government subsidies. In British Columbia, for example, in Canada, forest seed production is provided by government owned seed centers and thus society can to a large extent dictate the level of genetic diversity, for example, in the seed lots. The assessment of any need for changes to the Finnish system is a political decision.

To conclude, although the objectives of the different operators in the field of production of forest reproductive material may differ in some regards, a compromise or a balance between the different objectives can be found if the quality attributes of different seed lots are known and controlled. If the seed and seedling production processes, which affect quality attributes or the availability of genetically improved seed material, are poorly known and controlled, conscious choices cannot be made. Therefore, more research is needed to gain knowledge and control of seed quality in the production of Norway spruce seeds and of the reproductive biology of this species with high societal and ecosystem importance.

CONCLUSIONS

1. Seed soaking has an effect on the timing of germination and in some cases on seedling size. It may also make the emergence more synchronous.
2. The rate of seedling emergence in the nursery affects the probability of a seedling developing into a healthy, saleable seedling. This may be due to slowly germinating seeds being genetically inferior, or because growing measures are ill-suited for the slowest individuals.
3. Soak-sorting can be used to upgrade a seed lot by excluding the surface fraction. In this study the bottom fraction included only full, viable seeds.
4. Commercial seed lots of Norway spruce contain some pathogenic fungi, but the most common fungi found on their surface are saprophytic or even beneficial taxa. The source of fungal contamination can be traced to a certain extent. Improving collection and cone and seed handling hygiene can be beneficial in preventing seed-borne diseases in seedling production.
5. The seed lots differ in their potential to cause damping-off-type symptoms. The realization of the risk of disease due to seed-borne fungi depends on the growth measures at the time of the seedling production. If production methods, for example, the growth medium are changed, the importance of seed microbes may increase. With the current production methods their impact on seedling production is not great.
6. Due to different proportions of full seeds from individual trees and clones, collecting equal numbers of cones from each tree / clone does not ensure their equal representation in seed lots. This phenomenon thus affects the effective population size of seed lots.
7. Seed feeding insects show genotypic, maternal preferences, but the present data on this is limited.
8. Weight based sorting has a smaller impact on the genetic diversity of a seed lot than previously thought.
9. The chosen weight limits in seed sorting have an effect on the loss of full seeds.

10. Seed producers, seedling producers and society at large have different preferences regarding seed quality, and they have different tools for influencing the quality attributes relevant for them.

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