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Intracone variation explains most of the variance in *Picea abies* seed weight: implications for seed sorting

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Abstract

Norway spruce seed is collected from both forest stands after final felling and from seed orchards. To produce high-germinability seed lots easy to use in nursery sowing machines, empty, insect-damaged, and other poor-quality seeds are culled. Sorting is done typically by weight or size. Previous studies of conifer seed have indicated wide variation in seed weight between individual trees or clones. However, the intratree or intracclone variation has rarely been taken into account, and intracone variation in seed weight has not been examined. We collected cones from a forest stand and from a clonal seed orchard in central Finland. Each seed from each cone was extracted, weighed, x-rayed to assess their quality. Trees and clones differed in terms of the proportions of different quality seed. Variance component analysis showed that the intracone variation explained a larger proportion of the total variation in seed weight than did the intercone or intertree, or interclone variation. Thus weight-based seed sorting has less effect on the genetic diversity of a seed lot than previously believed. We also conclude that the large differences in proportion of full seed among trees and clones impact the contribution of genotypes in seed and eventually seedling lots.

Keywords

Cone insect, Norway spruce, radiography, seed orchard, stand seed

50 **1 Introduction**

51

52 In Finland, approximately 1100 kg of Norway spruce (*Picea abies* (L.) H. Karst.) seed is used
53 annually in nurseries to produce seedlings for forest regeneration. Recently (2002–2012), 15–
54 78% of this has originated from seed orchards (Finnish Statistical Yearbook 2013). The role
55 of genetically improved forest regeneration material is more prominent in the other Nordic
56 countries, but in general seed collections from forest stands after final felling still play a
57 substantial role in seedling production of Norway spruce in Northern Europe (Mullin et al.
58 2011; Swedish Statistical Yearbook of Forestry 2014). This also holds true for many other
59 conifer species with economic importance.

60

61 Conifer seeds are sorted after seed extraction and seed wing removal to discard empty, insect-
62 infested, and damaged seed, that would otherwise reduce the germination capacity of a seed
63 lot. Sorting is usually done by sieves, gravity tables, and blowers (Kolotelo et al. 2001;
64 Karrfalt 2008). The former sorts seeds by size and the latter two mostly by weight. Light
65 weight as well as the largest seeds are often removed to ensure proper functioning of sowing
66 machines in nurseries.

67

68 Seed weight can also affect the germination rate. Sorenson and Campbell (1993) reported
69 faster germination in heavy Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seeds than in
70 lighter seeds, as did Dunlap and Barnett (1983) in a study of Scots pine (*Pinus sylvestris* L.).
71 Seed weight also correlates positively with seedling size in conifers (Dunlap and Barnett
72 1983; Reich et al. 1994), although the effect is typically temporary (Mikola 1980; Sorenson
73 and Campbell 1993).

74 The main storage tissue of conifer seeds, the megagametophyte, which comprises the major
75 part of the seed weight, is haploid maternal tissue (Reich et al. 1994). Therefore, there is a
76 genetic relationship between the mother tree and seed weight (Roach and Wulff 1987; Castro
77 1999). In several conifer species, including Norway spruce, individual trees or clones in a
78 seed orchard produce seed of varying weight (Andersson 1965; Hellum 1976; von
79 Weissenberg 1981; Lindgren 1982; Stoehr and Farmer 1986; Clair and Adams 1991;
80 Chaisurisri et al. 1992; Matziris 1998; Glynn and Weslien 2004). In addition to a genetic
81 component, seed weight is affected by the temperature sum of the ripening year. Therefore
82 seed weight differs among years in the same location (Sorensen and Franklin 1977; Borgman
83 et al. 2014) and in general the lightest seeds originate from the northern and the heaviest
84 seeds from the southern latitudes in the Northern Hemisphere (Khalil 1986). The nutritional
85 status of the mother plant also affects the seed weight (Bazzaz et al. 2000; Karlsson and
86 Örlander 2002). Due to the individual or clonal differences in seed weight, seed sorting by
87 weight is thought to lead to a decreasing proportions or even exclusion of individual maternal
88 genotypes in a given seed lot.

89

90 Previous studies of the effect of genotype on seed weight in conifer seed lots have been done
91 by bulking together the cones from individual trees or clones (Andersson 1965; Hellum 1976;
92 von Weissenberg 1981; Lindgren 1982; Stoehr and Farmer 1986; Clair and Adams 1991;
93 Chaisurisri et al. 1992; Matziris 1998; Glynn and Weslien 2004). The effect of maternal
94 genotype on seed weight has been assessed by measuring all seed in the bulk samples or by
95 taking subsamples from these, with the result that the seed weight is determined by the
96 mother tree. However, this approach does not take into account the variation in seed weight
97 within a tree or within an individual cone. It is, in fact, unclear how much variation in seed
98 weight is explained by intratree factors in comparison to genotypic maternal variation.

99 In this study, we considered the following: (i) does there exist genotypic maternal (intertree
100 or interclone) variation in seed quality, (ii) how much variation in seed weight is explained by
101 intertree/graft, intercone and intracone variation, and (iii) is the variation in seed weight in a
102 forest stand similar to that in a seed orchard? With this information, we aimed to assess the
103 effect of weight-based sorting on the genetic base of Norway spruce seed lots.

104

105

106 **2 Material and Methods**

107 ***2.1 Cone collection in a forest***

108

109 Cones of seven randomly chosen individual trees were collected from a mature, naturally
110 formed Norway spruce dominated stand in Kuopio (Puijo Hill), central Finland (62°54'N,
111 27°39'E), 190–215 m above sea level (a.s.l), on March 14, 2011 (Table 1). The seven trees
112 were located at a range of 470 m. The site is a fertile Oxalis-Myrtillus-type forest, with an
113 average temperature sum (baseline temperature +5 °C) of 1300 degree days (d.d.) and annual
114 precipitation of 549 mm (years 1981–2010 in Kuopio; 10 x 10 km data; Venäläinen et al.
115 2005). In the year of seed maturation (2010) the temperature sum in Kuopio was 1522 d.d.
116 and the precipitation 570 mm.

117

118 Ten liters of cones were collected from the tree crowns of each tree with a boom lift. Cones
119 were collected from three points, i.e. from different side and height, in each crown to get a
120 representative sample. Cones with clear rust infection or visible insect damage were not
121 collected. Tree height and diameter at breast height were measured at the site. A core sample
122 was drawn from each of the trees at breast height by an increment borer and the age of the
123 trees was counted from the tree rings.

Of the cones collected, three from each tree were drawn randomly for water content analysis. By measuring the water content we ensured that the seed development had been completed normally and that our seed samples were thus representative. The water content of the cones was measured from one fourth of each of these cones (cones cut along both length and width) on a fresh weight basis, using constant temperature oven method (103 °C, 17 h) (International Seed Testing Association 2007). A minimum on 50 seeds were removed from the rest of each cone, and the water content of the seeds was measured similarly. The mean water content of both cones and seeds for each tree was counted as the average of the three cones measured.

2.2 Cone collection in a seed orchard

The cones of five clones - from one graft in each clone - were collected from a first generation Norway spruce seed orchard in Joroinen, in central Finland (Sv403 Suhola 1, 62°15'N, 27°42'E, 90 m a.s.l.) on January 14, 2013 (Table 2). The orchard was established in 1994 on old agricultural land with 35 different clones at a density of 360 grafts per hectare. The total area of the seed orchard is 4.7 ha and the average height of the grafts at the time of cone collection was 9.5 m. The grafts in the seed orchard were originally to be planted in a more southerly location in Finland. The plus trees from which the grafts originate are located approximately 2° south (latitude) and from 100–200 d.d. warmer conditions than where the orchard is located. The average temperature sum in Joroinen is 1260 d.d. and mean precipitation 563 mm (years 1981–2010; 10 x 10 km data; Venäläinen et al. 2005). In the year of seed maturation (2012), the temperature sum in Joroinen was 1297 d.d. and precipitation 737 mm. Two liters of cones were collected from each graft. The cone and seed water contents were measured as described for cones collected from the forest stand.

2.3 Seed extraction, measurement, and evaluation

After the collection and water content measurements, the cones were cleaned of debris (twigs and needles) and stored at -15 °C. Five cones from each stand tree and four cones of each orchard 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 to 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 placed on an adhesive film in a cardboard frame so that each seed could be monitored. The seeds were then x-rayed (Faxitron MX-20; Faxitron Bioptics LLC, AZ, USA; CEA orthochromatic mammography film, Agfa Healthcare NV, Mortsel, Belgium, exposure 18 kV, 4 s) to evaluate their quality.

The seeds were classified as 1) full (the megagametophyte fills the seed coat and the embryo fills the embryo cavity), 2) anatomically immature (the megagametophyte and / or the embryo do not entirely fill the seed coat or the embryo cavity), 3) severely anatomically immature (the megagametophyte fills less than 60% of the seed coat and / or embryo is missing or shorter than 50% of the total length of the seed), 4) development stagnated (the megagametophyte and embryo development have commenced but stopped, creating dried remnants of these tissues), 5) empty (seed coat entirely empty or only remnants of membrane tissue are visible), 6) larvae-infested, 7) insect damaged, 8) mechanically damaged, or with 9) unidentifiable damage (Fig.1). 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 (Vötsch VCL 0003; Vötsch Industrietechnik GmbH, Balingen Germany) at 20 °C

and relative humidity of 35% for at least 48 h prior to weighing each seed individually (Mettler-Toledo MT5 microscale; Mettler-Toledo, Greifensee, Switzerland).

2.4 Statistical analysis

To compare the proportions of different quality seeds tree-wise, a generalized linear model, using a logit link function, was fitted separately for each seed quality class. Tree or clone was taken as a fixed effect. Due to correlated structure of the data, an overdispersion parameter was estimated so as not to underestimate parameter standard errors and p-values. The logit model was:

$$\text{logit}(p_i) = b_o + b_i T_{Li} \quad (1)$$

where $\text{logit}(p_i)$ is the proportion of a given quality class in each tree; b_o is the intercept; T_i is the i th tree effect.

Individual model terms (including an overdispersion parameter) were assessed by comparing the log-likelihood of two nested, candidate models as well as the Akaike information criterion and Wald-significance test for parameter coefficients.

The seed weight data were analyzed with linear mixed 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 as random. The variance component model was:

$$y_{ijk} = \mu + a_i + b_{ij} + e_{ijk} \quad (2)$$

where y_{ijk} = observation on seed k in cone j in tree i ; μ is the intercept; a_i is the random tree effect; b_{ij} is the random cone effect, and e_{ijk} is the random seed effect.

In separate analyses, the differences in seed weight between trees and clones were analyzed also with a linear mixed model, but here tree or clone was taken as a fixed factor for tree-wise comparison. The fixed model terms were considered statistically significant at $p \leq 0.05$. For the analyses, we used GenStat 15 software (VSN International Ltd, Hemel Hempstead, UK) (VSN International 2012).

3 Results

3.1 Seed quality

The cone water content was under 30% in all samples and the seed water contents were still lower (Tables 1 and 2). The proportion of full seed varied among the individual trees of the forest stand (Table 3). The cones of tree no. 1 had less than 30% full seed, while trees no. 5 and 6 had approximately 85%. Spruce seed chalcid (*Megastigmus strobilobius* Ratzeburg) larvae-infested and spruce seed moth (*Cydia strobilella* (L.)) damaged seeds were found on all trees in small numbers. Spruce seed gall midge (*Plemeliella abietina* Seitn.) infested seeds were present in three trees out of seven. Developmentally stagnated seeds were observed in five trees.

In addition to the proportions of full seed, the percentage of empty and spruce seed chalcid infested seed showed wide variation between the mother trees; e.g. in the latter, the proportion of infested seeds was over 10-fold in tree no. 4 than in trees 1 and 7. The

proportions of full, empty, immature, and spruce seed chalcid-infested seed, as well as seed with spruce seed moth damage and with development stagnated varied in a statistically significant manner between trees (Table 3).

In the material collected from the seed orchard, the differences in proportions of full and empty seeds between clones were statistically significant as well (Table 4): Clone E1549 produced only 33.2% full seed, which is notably less than in the others. In contrast to the seed from the forest stand, spruce seed chalcid and spruce seed gall midge infested seeds were completely absent in the orchard cones, while spruce seed moth damage was more common.

3.2 Seed weight

When the seed weights between individual trees or clones were compared, statistically significant differences were present in all major quality classes (Tables 5 and 6). In the stand seed, tree no. 3 produced the lightest and tree no. 7 the heaviest full seed. The average seed weights of full seed in the orchard material also varied by over 1 mg between the lightest and heaviest clones. In addition, the total ranges of seed weights, considering the seed from all quality classes, were different between trees and clones (Figs. 2 and 3).

However, examination of the source from which seed weight variation derived from, revealed that the intracone variation was larger than that between cones, or between trees or clones (Tables 7 and 8). Variance component analysis showed that the intracone variation explained 85.1% of the total variance of seed weight in the stand seeds. Examination of full seed with mass greater than 2.5 mg revealed that the intracone variation accounted for 53.0% of total variance in seed weight and intertree variance accounted for 28.6%.

In analyzing all the orchard seed material, the intracone variation accounted for 80.2% of the total variation in seed weight. Analysis of only full seed with mass greater than 2.5 mg showed that the intracone variation explained 51.7% and the interclone variation 24.3% of the total variation.

4 Discussion

4.1 Seed quality and insect damage

Both the cone and seed water levels were indicative of normal maturation in both the stand and seed orchard material (Tables 1 and 2). Seed quality, especially the proportion of full seed, varied among individual trees and clones (Tables 3 and 4). In trees or clones with low numbers of full seed, the proportion of empty seed was, in contrast, large. This indicates that either pollination or fertilization was unsuccessful in certain genotypes. The susceptibility of the female strobili of these genotypes may have been non-synchronous with the peak pollen dispersal, but this is speculative, since no observations on the flowering phenology of the trees and grafts were conducted. Our 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).

Sampling of the cones for the study cannot be considered random with regard to insect damage, since cones with cone rust infestation and clear external signs of insect damage were excluded. However, the presence of spruce seed moth larvae or seed-infesting insects spruce

seed chalcid and spruce seed gall midge are not visible on the surface of the cone. Therefore the results on their frequency can be counted as reliable.

Spruce seed moth damage was more common in the seed orchard than in the stand seed. The study material was collected in different years (2011 and 2013), which may explain the difference: spruce seed moth populations fluctuate annually (Annala 1981). Since the collection sites are approximately 90 km apart, spatial variation in insect population density can also be expected. In addition, the difference may have been related to the seed production environment (stand vs. orchard), although our study material was from one stand and from one orchard only. In the forest stand, the proportion of spruce seed moth- damaged seed differed in a statistically significant manner among the trees. Glynn and Weslien (2004) also reported a genotypic effect on the infestation rate of spruce seed moth in Norway spruce. However, in our seed orchard material, no statistically significant differences were observed among the clones.

Rosenberg et al. (2012) reported that as the number of spruce seed chalcid-infested seed in Norway spruce cones increased, seed weight decreased. Our findings do not support this. In the stand material, tree no. 4 showed the highest proportion of spruce seed chalcid-infested seed, but it also contained the heaviest full seed (Tables 3 and 5). The average full seed weight also showed similar variation in the orchard material as in stand seed, although no seed chalcids were found in the orchard seed.

4.2 Seed weight

Previous studies in conifers have shown that seed weight is dependent on the mother tree (Hellum 1976; Lindgren 1982; von Weissenberg 1981; Stoehr and Farmer 1986; Clair and Adams 1991; Matziris 1998). This is explained to be due to the large proportion of maternal tissue in conifer seed 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, e.g. of full seed, revealed that statistically significant differences among trees and clones were present in our material as well (Tables 5 and 6). However, analyzing the source of variation in seed weight – either of all seed or full seed – showed that the maternal effect is not the most important factor (Tables 7 and 8), because the intracone variation in seed weight was larger than that between trees or clones, or between cones. This is a novel finding. In the previous studies mentioned, intracone variation has not been tested, due to measurement of seed weight from bulked samples, including seed from several cones.

It is also notable that the proportions of the variation components were similar in both the stand and orchard material (Tables 7 and 8). This suggests that the effect on seed weight from the different sources of variation was not a random phenomenon or dependent on the environment. Due to the limited number of mother trees and data from one 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 can be a source for the similarity between results in stand and orchard material. Our results apply when sorting is done on weight basis only. Although seed weight and seed size are strongly correlated, they should not be used as synonyms. Although various conifer taxa share traits with regard to seed weight, the applicability of our results to other conifers species needs to be confirmed.

4.3 Implications for seed sorting and genetic diversity in seed lots

The maintenance of forest genetic resources is paramount for adaptation to climate change and other environmental changes and for forest ecosystem functioning (Alfaro et al. 2014). Forest management practices, including many steps in forest regeneration significantly impact genetic diversity of forest trees (Ratnam et al. 2014) and therefore care must be taken in composing seed material for regeneration.

Our results give reason to re-evaluate current conceptions on the weight-based sorting of Norway spruce seed, which is believed to lead to exclusion of certain maternal genotypes (Hellum 1976; Lindgren 1982). The lightest and heaviest seed in a given seed lot are likely to lack seed from certain mother trees also according to present data, indicating that these extreme fractions are genetically narrow and should not be used as regeneration material alone. However, since sorting begins with all the seed extracted from the cones, the sorting effect on the genetic diversity of the main seed lot is far less dramatic.

As the present data show, not all maternal genotypes contribute equally to the pool of full seed via their different proportions of them. We did not determine the number of cones produced by each tree or graft and therefore cannot assess the actual contribution of individual trees or clones to the entire full seed production of the stand or seed orchard. However, we know that both the abundance of flowering, as well as the amount of full seed, vary on a yearly basis in Norway spruce (Eriksson et al. 1973; Nikkanen and Ruotsalainen 2000). The effect of this phenomenon to genetic diversity is counteracted 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, since the new stand is not a result of seed from a single year. In contrast, the seed and eventually seedling lots in artificial forest regeneration present a snapshot of this continuum, including the genetic variation of just one year.

Since 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 genetic diversity remains high on landscape level. It must also be noted that even the trees or clones contributing minimally to the production of full seed 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 seed over several years to a single seed lot could provide acceptable results. In 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 and 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 narrowing of genetic diversity by exclusion of certain maternal genotypes, but we should instead take into account the natural processes occurring in Norway spruce seed production. As shown in this and 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.

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Table 1. Height, diameter and age, both determined at breast height (1.3 m), of individual Norway spruce trees used for cone collection in a forest stand in central Finland. Cone and seed water content (on fresh weight basis) are calculated averages ($\pm$ standard error of the mean) of measurements from three cones in each tree at time of the cone collection March 14, 2011.

Tree	Height, m	Diameter (1.3m), cm	Age, a	Water content, % ($\pm$ SE)	
				Cone	Seed
1	29.5	68	165	23.1 ($\pm$ 0.30)	11.2 ($\pm$ 0.17)
2	27.0	53	129	23.1 ($\pm$ 0.25)	11.4 ($\pm$ 0.04)
3	24.5	46	154	25.2 ($\pm$ 0.23)	12.9 ($\pm$ 0.74)
4	24.0	41	90	20.9 ($\pm$ 0.37)	10.8 ($\pm$ 0.15)
5	24.5	45	124	21.6 ($\pm$ 0.35)	10.6 ($\pm$ 0.12)
6	25.0	38	133	21.9 ($\pm$ 0.30)	10.6 ($\pm$ 0.44)
7	27.5	51	135	21.8 ($\pm$ 0.13)	10.9 ($\pm$ 0.17)

Table 2. Origin of the clones used for cone collection in a Norway spruce seed orchard in central Finland. Cone and seed water content (on fresh weight basis) are calculated averages ($\pm$ standard error of the mean) of measurements from three cones in each clone / graft at the time of collection, January 14, 2013.

Clone number	Plustree origin		Water content, % ($\pm$ SE)	
	Municipality	Co-ordinates	Cone	Seed
E11	Miehikkälä	60°45'N, 27°30'E	29.0 ($\pm$ 1.34)	20.7 ($\pm$ 1.38)
E246	Somero	60°42'N, 23°30'E	29.8. ($\pm$ 0.64)	19.5 ($\pm$ 0.15)
E252	Mäntsälä	60°40'N, 25°20'E	25.5 ($\pm$ 0.38)	17.7 ($\pm$ 0.54)
E456D	Iitti	60°45'N, 26°25'E	26.8 ($\pm$ 0.61)	19.1 ($\pm$ 2.10)
E1549	Pöytyä	60°43'N, 22°51'E	28.6 ($\pm$ 0.87)	19.5 ($\pm$ 0.36)

Table 3. Observed proportions (%) of different quality seed extracted from cones collected from seven Norway spruce trees in central Finland, total number of seeds evaluated in each tree and p-value of tree (genotype) effect on the proportion of seed in each quality class.

	Tree							Tree effect, P-value
	1	2	3	4	5	6	7	
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
<i>Cydia strobilella</i> damage	2.1	0.1	6.9	0.3	0.5	1.8	0.1	0.006
<i>Megastigmus strobilobius</i> larva	0.2	8.0	0.9	14.5	3.8	0.7	0.3	<0.001
<i>Plemeliella abietina</i> 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 seed	891	855	691	698	1127	941	1153	

* Too low number of observations

Table 4. Observed proportions (%) of different quality seed extracted from cones collected from five clones in a Norway spruce seed orchard (Sv 403) in central Finland, total number of seeds evaluated in each clone and p-value of clone effect on the proportion of seed in each 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
<i>Cydia strobilella</i> damage	6.2	8.6	10.7	3.3	11.3	0.118
<i>Megastigmus strobilobius</i> larva	0	0	0	0	0	-
<i>Plemeliella abietina</i> 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 seed	991	970	1215	1159	922	

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

Table 6. Average observed seed weight (mg) ($\pm$ SE) of different quality seed 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.

	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
<i>Cydia strobilella</i> damage	4.68 (± 0.218)	3.78 (± 0.123)	4.38 (± 0.075)	4.45 (± 0.236)	4.28 (± 0.082)	<0.001
<i>Megastigmus strobilobius</i> larva	-	-	-	-	-	-
<i>Plemeliella abietina</i> 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 observation

* only one observation

Table 7. 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 seed weight. The cones were collected from seven trees in a Norway spruce stand in central Finland.

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

Table 8. 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 seed weight. The cones were collected from five clones in a Norway spruce seed orchard in central Finland.

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

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

Figure 2. Box plot of 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 the mean.

Figure 3. 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 the mean.