Working Papers of the Finnish Forest Research Institute 198

http://www.metla.fi/julkaisut/workingpapers/2011/mwp198.htm

ISBN 978-951-40-2296-8 (PDF)

ISSN 1795-150X

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The papers published in the series are not peer-reviewed.

The papers are published in pdf format on the Internet only.

http://www.metla.fi/julkaisut/workingpapers/
ISSN 1795-150X

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Publisher
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Year	Pages	ISBN	ISSN
2011	20	978-951-40-2296-8 (PDF)	1795-150X

Regional unit / Research programme / Projects

Eastern Regional Unit, Punkaharju / 3537 Vegetative propagation of forest trees

Accepted by

Katri Kärkkäinen, professor, 4.4.2011

Abstract

Testing of candidates as clones would greatly benefit breeding of Scots pine (*Pinus sylvestris* L.), but has not been applied because vegetative propagation is difficult. With a common interest in Scots pine breeding, forest research institutions from Sweden, Finland and Latvia (Skogforsk, Metla and Silava, respectively) joined in a collaborative project to develop pine cutting propagation for breeding purposes. The main objective of this effort was to find protocols for sufficient shoot production and at the same time maintain a high rooting response. Secondly, the aim was to increase the knowledge on the influence of different rooting agents, watering regimes and substrates on the rooting of cuttings. Both local and shared materials were used in studies of five propagation methods performed in all participating countries. Separate experiments were performed with rooting environment factors. According to the results, the average production of cuttings with propagation models including two harvests in consecutive years on the same donor plant can be predicted to be 10-15 cuttings/donor plant, with a substantial variation among families and donor plants. Even though rooting responses above 50% can be achieved, this could not be repeated for a large number of propagations. The generally low and erratic rooting responses leads to the conclusion that the project was unsuccessful in developing improved and reliable protocols for Scots pine cutting propagation. However, a propagation model enabling two harvests on a 1-year-old plant within the same year is a promising option but needs further studies.

Keywords

Clones, donor plants, propagation models, vegetative propagation

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1 Introduction

Scots pine (*Pinus sylvestris* L.) is one of the key species of forest ecosystems over large areas of the boreal forest zone in Europe, and is of considerable economic importance. In the Nordic countries, Scots pine has been included in forest tree breeding activities since the beginning of the 1940's, but it has only recently reached the second breeding cycle. The present pine breeding programmes apply a strategy where candidates are selected by testing their progeny. The progeny is produced by the polycross method, where each candidate is pollinated by a specified mixture of fathers. However, if the candidates could be propagated vegetatively a considerable time gain could be achieved. Instead of waiting for the candidates to flower, they can be tested directly and thus saving 10-15 years of the breeding cycle. Furthermore, more candidates can be tested which enables a stronger selection and a higher genetic gain with the same economic effort (Haapanen & Mikola 2004, Danusevicius & Lindgren 2002).

Of the Nordic conifers, clone testing after cutting propagation is possible for Norway spruce *(Picea abies* (L.) Karst.). This is applied since more than 15 years in Sweden (Karlsson & Rosvall, 1993). However, for Scots pine this option has not been available because of low rooting frequencies (Strömquist 1979). There are reports and experiences (suggesting that sufficient rooting response can be obtained for Scots pine in certain promoting conditions (Whitehill & Schwabe 1975, Högberg 2005, Hajek, J pers. comm.). There are also examples of improved rooting with other *Pinus species*. Loblolly pine was considered to be difficult to root until procedures were developed that substantially raised the rooting response (Frampton *et al*. 1999). Another example of cutting propagation development is jack pine (*P. banksiana*) that can reach good rooting when procedures are optimized (Browne *et al*. 1997).

From older trials with Scots pine cuttings, we know that cuttings show normal growth and are able to express genotypic differences (Aronen & Ryynänen, 2006; Niskanen *et al*. 2008). However, further development is still needed before cutting propagation can be utilised and included as a standard activity in the breeding programmes.

According to several scientific results, a climate change towards higher temperatures in the Nordic-Baltic region will take place. In forestry, there are many ways to adapt to such a situation. When it comes to tree breeding, ways to decrease the time needed for selecting trees in the next generation is essential to maintain and increase future wood production of this important species. With a common interest of Scots pine breeding and programmes running for the species, Skogforsk, Sweden, Metla, Finland and Silava, Latvia decided to join in a collaborative project on pine cutting propagation. Two research stations in Sweden (Sävar and Ekebo), two in Finland (Haapastensyrjä and Punkaharju) and facilities at the main office in Latvia (Salaspils) were included as experimental locations. The donor plants in Latvia were grown in research station Kalsnava. At this location the cuttings were collected and then transferred to Salaspils for rooting.

The main objective of the present collaborative project was to find protocols for sufficient shoot production from the Scots pine donor plants and at the same time maintain a high rooting response of cuttings. A second objective was to increase the knowledge on the influence of environmental factors, such as effect of different rooting agents, watering regimes and substrates on rooting. The wider aim of this application is to develop pine cutting propagation to a repeatable and reliable level with the prospect of including this operation in pine breeding.

2 Material and methods

2.1 Plant materials

From Finland and Latvia, respectively, 15 full-sib families from parents with high breeding values were included. From Sweden, 15 families from each of two research stations, one northern and one southern, were included. These sets of the families were used locally. Another collection of five families was shared among the participants, which means that 5 families extra were included at each location. The common five families consisted of two Swedish (one of Norhern and one of Southern origin), two Finnish, and one Latvian family. At Sävar, Ekebo, Haapastensyrjä, Punkaharju, 5-12 donor plants from each family and treatment combination, were included in the cutting production and rooting experiments. At Kalsnava, 20-39 donor plants per family were included.

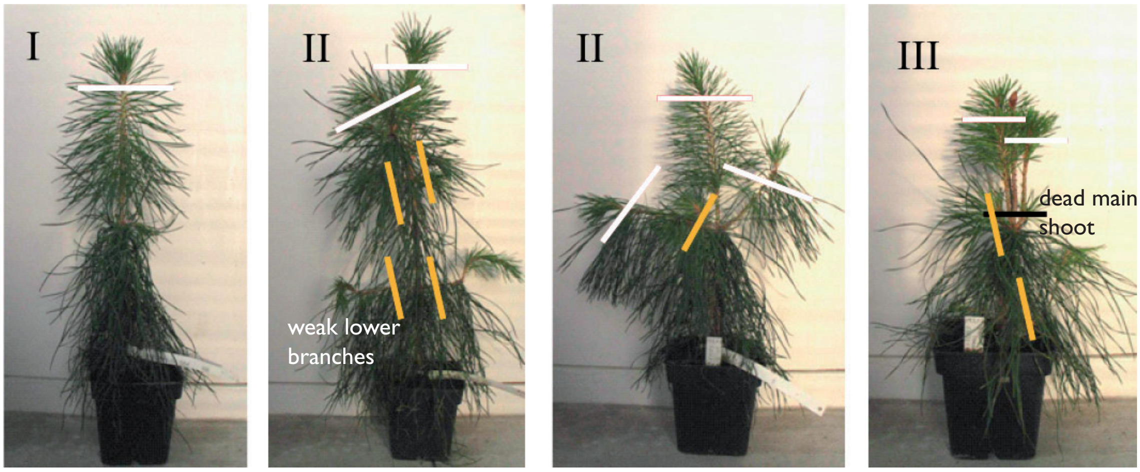
Donor plants in Haapastensyrjä and Punkaharju were severely damaged during the winter 2007/2008, probably caused by root injuries due to a lower hardiness than expected when winter frosts appeared. The lost material that was meant for studying the effects of environmental factors on rooting of cuttings was replaced by other families. Five Finnish families, four having a high breeding value and one being inferior to them, were used for comparing effects of rooting agents. From each family, 19-42 donor plants were used for experiments. For testing different watering regimes and rooting substrates, 110 Swedish families were used. The families belonged to a Northern breeding population, and a single genotype per family was used.

2.2 Donor plant cultivation

Seeds were sown in early spring of 2007 in medium-size containers. Germination and first growth took place in greenhouses with supplemental light (20 000 lux) to prevent bud set. In early summer, the donor plants were transplanted to 0.3 l-containers prior to growth outdoors during mid-summer. In order to achieve strong plants, the growing season was extended by transferring the plants back to greenhouse with supplemental light of 12 000 lux and heating (up to 20°C) in the autumn. For the donor plants in the propagation model C, "Turbo line", the extension of the growing period in the first year was approximately one month longer than in the other propagation models, and prior to that, the plants were transplanted into 1 l containers. Bud set and winter hardening of the donor plants was achieved by decreasing the the greenhouse temperature gradually from +20°C to +5°C, first under long-night (8h /16h day/night) and then under natural light conditions. Winter hardening was performed according to the common timetable at different locations. Winter storage was chosen individually after consideration of the prerequisites at each location, aiming for a low risk of frost damage to the roots. In the beginning of the second growing season, all donor plants were transplanted into 3 l containers.

2.3 Propagation models

All donor plants were pruned according to the same principles as shown in Figure 1. However, the timing of the pruning varied in the different propagation models. The tops of the donors, and likewise the tops of strong side branches were cut at the point in which the needle type changes from long to short needles. In addition, small side branches with short needles were removed totally.



No branches, terminal bud removed

Buds of strong upper shoots or branches removed, potentially existing weaker lower branches removed as whole (-1 cm left)

Bad/dead main shoot, or its 2^{nd} growth removed, buds of strong upper shoots removed, weak lower branches removed as whole

Fig. 1. Principles for pruning the donor plants demonstrated with examples representing the donors of the C propagation model "Turbo line"..

A. One-year-old donor plants pruned to yield 2x winter cuttings / 4-year method

Pruning of the donors took place in the beginning of June 2008, followed by the development of shoots during summer, natural winter hardening, storage in conditions for keeping the plants in dormancy (temperature controlled room, unheated greenhouse or outdoors), and finally excision of the shoots in late January/early February 2009 followed by immediate rooting. The harvested donor plants were moved from dormancy-keeping conditions to greenhouse in the beginning of June 2009 for a second shoot production during the summer after. The donor plants were exposed to natural winter hardening and were stored in dormancy-keeping conditions. A second shoot harvest and rooting was performed in February 2010.

B. One-year-old donor plants pruned to yield winter cuttings and late summer cuttings/ 3-year method

As treatment A, but the harvested donor plants were transferred to growing conditions in May 2009 for a shoot production during summer. The second shoot harvest was made in August 2009 followed by immediate rooting.

C. "Turbo line" i.e. one-year-old donor plants pruned 2 x cuttings in one year/ 2-year method

This treatment can be regarded as stretching pine biology towards the edge. In this propagation model the donor plants were taken to growing conditions into a heated greenhouse with assimilation light already in January 2008. This enabled two shoot harvests during the second year, presuming less physiological ageing during the process. Pruning of the donor plants was carried out in January 2008. Prior to excision of shoots and rooting in May 2008, three weeks of long-night treatment (6h/18h day/night) were applied. A second shoot harvest and rooting were made in August 2008.

D. One-year-old donor plants pruned to yield 2x late summer cuttings/ 3-year method

Pruning of the donors was done in May 2008, followed by development of shoots during summer, and excision of shoots in late August 2008 followed by immediate rooting. The harvested donor plants were stored in conditions for keeping the plants in dormancy and were transferred to growing conditions in May 2009 for a second shoot production during summer. The second shoot harvest was made in late August 2009 followed by immediate rooting.

E. Two-year-old donor plants pruned to yield winter cuttings/ 4-year method

Pruning of the donors was done in the beginning of June 2009, followed by development of shoots during summer, then natural winter hardening and storage in conditions for keeping the plants in dormancy (temperature controlled room, unheated greenhouse or outdoors), and finally excision of shoots in February 2010 followed by immediate rooting.

Experiments with these propagation models were distributed among locations according to following pattern:

Treatment	Sävar	Haapastensyrjä	Punkaharju	Ekebo	Salaspils
A	X	X	X	X	X
B	X	X			
C	X		X		
D	X			X	
E	X				X

2.4 Propagation of additional families for rooting environment experiments

Donor plants from additional five Finnish families used for rooting environment experiments were sown in 2006 and grown and treated according to propagation model C (“Turbo line”) in 2006-07. In 2008, these donors were grown outside in the Punkaharju nursery according to natural growth rhythm. In January 2009, these donors were brought inside and gradually warmed up to +5°C, followed by shoots excision for comparing the effects of rooting agents. The set of the additional 110 Swedish families followed propagation model A.

2.5 Treatment of cuttings and rooting environment

The general plan for performing the experiments was set up as follows. Excised shoots were dipped in 8000-10 000 ppm IBA (indole-butyric acid) solution for 10 seconds and then inserted in Jiffy-pots ™ with coconut fibre substrate. In Salaspils, a pure peat substrate but with the same size as the Jiffy-pots were used at the second propagation. Minimum cutting length was set to 3 cm, and the maximum cutting base diameter to 5 mm. The pots with inserted cuttings were put in a chamber or greenhouse with soil heating equipment and a target soil temperature of 22-25°C. The air temperature during rooting was kept at 15-17°C when possible i.e. during winter rooting and cool days during late summer rooting. During rooting, the relative air humidity was aimed to be kept in the interval 70-100%. Assimilation light equipment was applied during rooting, set to 18h/6h day/night photoperiod and 12 000 lux during the day period. Rooting in this environment was scheduled to take approximately 12 weeks, with weak fertilization starting 4-5 weeks after insertion. After rooting in late winter the cuttings were subjected to normal greenhouse conditions.

If rooting was done during late summer/autumn the cuttings were subjected to a temperature of 5–10°C and no soil heating during six weeks after the rooting period. Thereafter, the cuttings were stored in conditions for keeping the plants in dormancy.

2.6 Experiments on rooting environment factors

Originally, four different factors potentially affecting rooting of cuttings were planned to be tested: substrate temperature, watering regime, light quality and photoperiod, as well as growth regulators applied. Except for the factor under study, all the other conditions were according to previously described rooting environment. Due to the loss of donor plants the plan was revised and restricted to watering regime and gibberellin inhibitor experiments.

2.6.1 Watering regime and substrate quality

An intricate result from a rooting experiment of Scots pine cuttings (unpublished) is that the wetter part of a rooting bench had clearly better shoot development than a part subjected to moderate water supply. However, the rooting showed the opposite. This means that the watering regime needs to be optimised to get the best rooting result. An experiment was set up in Haapastensyrjä where two different watering regimes, normal and generous, were tested. Within both watering regimes, two different rooting substrates were tested: Jiffy-pots ™ with coconut fibre, and Kekkilä Spruce-Rhododendron Soil™-perlite mixture (75:25). For comparing watering regimes and substrates, donor plants from 98 Swedish families, a single genotype per family, were randomly divided into four watering-substrate treatments. In addition, a small experiment was performed to compare 1% (10 000 ppm) IBA application either as solution or as powder. The material used for this experiment consisted of 12 Swedish families, a single genotype per family, and these cuttings were rooted in Kekkilä Spruce-Rhododendron Soil™-perlite mixture with normal watering regime.

2.6.2. Gibberellin inhibitors applied together with IBA

Auxin treatment, i.e. a short dipping of cutting bases in IBA solution is known to be necessary for achieving satisfactory rooting response in Scots pine. The formation of root primordia is, however, an outcome of the synergistic action of different growth regulators present in the cutting. Gibberellins are considered as substances inhibiting the root formation, and e.g. in *Pinus caribea* the application of gibberellin inhibitor, paclobutrazol, has been found to beneficial for rooting of cuttings (Henrique et al. 2006). In the present experiment, executed in Punkaharju, the effect of gibberellin synthesis inhibitor on rooting of Scots pine cuttings was tested by comparing 10 000 ppm IBA and 10 000 ppm IBA together with 100 mg/l paclobutrazol treatments. The experiment was performed by dividing the donor plants from each of five Finnish families into these two treatments, 9-21 donors per treatment-family combination.

2.7 Donor plant loss

Due to suspected root injuries during the first winter a substantial donor plant loss occurred in Haapastensyrjä. This meant that no cutting production data were available from this location. Also in Punkaharju, the donor plants intended for the propagation model A were severely damaged and no cutting production data were available. In order to execute a propagation in Haapastensyrjä, cuttings from model B (first propagation) were collected in Sävar and transferred to Haapastensyrjä. Donor plant loss severely affected the experiments on environment factors.

In Punkaharju, three year old donor plants originating from additional five Finnish families replaced the intended material. Cuttings were harvested from these plants also the year before, as explained. In Haapastensyrjä, rooting environment experiments were performed with cuttings transferred from Sävar on a separate set of families. Moreover, the experiments planned to test the effect of substrate temperature in Sävar and light conditions in growth chambers were cancelled.

2.8 Statistical analysis

Effect of propagation round (first or second) was found to be insignificant. Cutting production in propagation 1 and 2 was thus pooled for each donor plant, except for model E that included one propagation round only.

Cutting production for the common five families, with donor plant as experimental unit, was subjected to variance analysis according to following model:

1) $Y_{ijkl} = m + loc_i + f_j + d_k + lf_{ij} + fd_{ik} + e_{ijk}$

where Y_{ijkl} = observed value of a donor plant, m = mean value, loc_i = fixed effect of location i, f_j = random effect of family j, d_k =effect of propagation model k, lf_{ij} = random interaction effect of location i and family j, fd_{ik}=random interaction effect of family j and propagation model k and e_{ijkl} = random error term.

Restricted to Sävar only:

2) $Y_{jkl} = m + f_j + d_k + fd_{ik} + e_{jkl}$

with factors as in model 1.

Cutting production for the local families were analysed separately at Sävar, Ekebo and Salaspils, respectively, according to the statistical model 2:

For Haapastensyrjä and Punkaharju, the model was reduced to:

3) $Y_{jl} = m + f_j + e_{jl}$

with factors as in model 1

Significance levels of fixed effects were determined by F-tests.

Pearson correlations were calculated among propagation models in Sävar.

Due to propagation failures, rooting response was analysed separately for each propagation model and location using model 3 and only for propagations that exceeded 5 % rooting frequency. Rooting response (p), expressed as probability of rooting, was transformed prior to analysis of variance:

$log(p) = ln(p/(1-p))$

For analysing effects of watering regimes, different substrates, and IBA application either as solution or powder on binary response (rooted or not) of Scots pine cuttings, a logistic regression model was used:

4) $log(p) = c + a_1w + a_2s + a_3i$

where: p = propability of rooting, c = constant, w = watering regime, s = substrate type, i = IBA application form, and a_1, a_2, a_3 = coefficients

The effect of gibberellin inhibitor on rooting of cuttings was subjected to analyses of variance using the following model:

5) $Y_{ijk} = m + g_i + gf_{ij} + e_{ijk}$

where m = mean value, g_i = fixed effect of gibberellin inhibitor treatment i, gf_{ij} = random interaction effect of gibberellin inhibitor treatment i and family j, e_{ijk} = random error term

In addition, also reduced models were used:

6) $Y_{ik} = m + g_i + e_{ik}$

where only the fixed effect of gibberellin inhibitor treatment was analysed.

and

7) $Y_{jk} = m + f_j + e_{jk}$

where only the random effect of family was analysed.

Student-Newman-Keuls multiple comparison test was performed to analyse differences in shoot production among the families used for gibberellin inhibitor experiment at Punkaharju.

3 Results

3.1 Propagation models

3.1.1 Cutting production

For the five common families, the average across all production events reached 10.9 cuttings per donor plants. The production varied significantly among families, locations and propagation models, as well as the interaction between families and locations (Fig. 2). The p-values obtained after ANOVA with statistical model 1 fell below 0.001 for all main effects, as well as the interaction effect between family and location. However, a strong discrepancy was found between the outcome of propagation model C in Sävar and Punkaharju, respectively. The average for model C in Sävar was 8.6 cuttings/donor plant compared with 20.5 in Punkaharju. Because of this, model 1 was also run after excluding data for model C but this did not change the results.

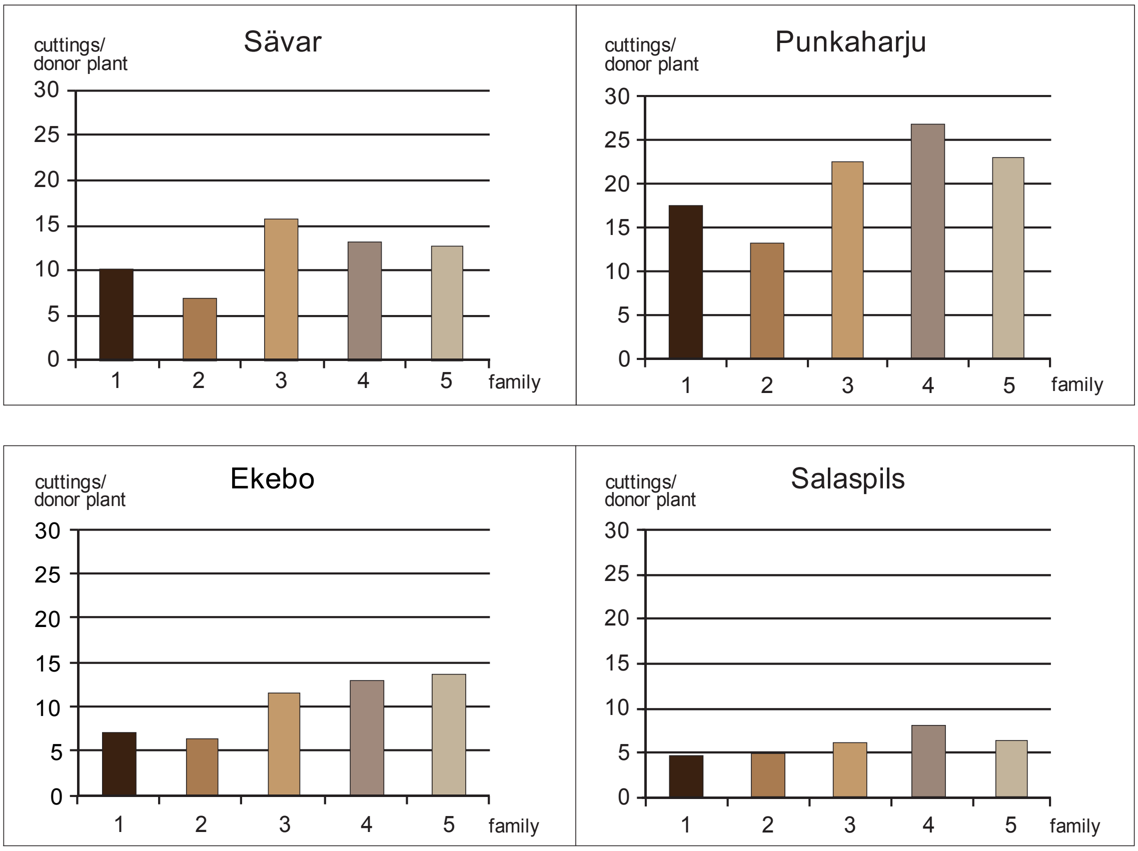


Fig. 2. Production of cuttings for five common families at four locations.

When only Sävar data was analysed (statistical model 2), both family and propagation model effects were significant as well as the interaction between family and propagation model (Fig.3). Propagation models A and D appeared to be most productive but as a reminder the very good production of model C in Punkaharju is included in the figure.

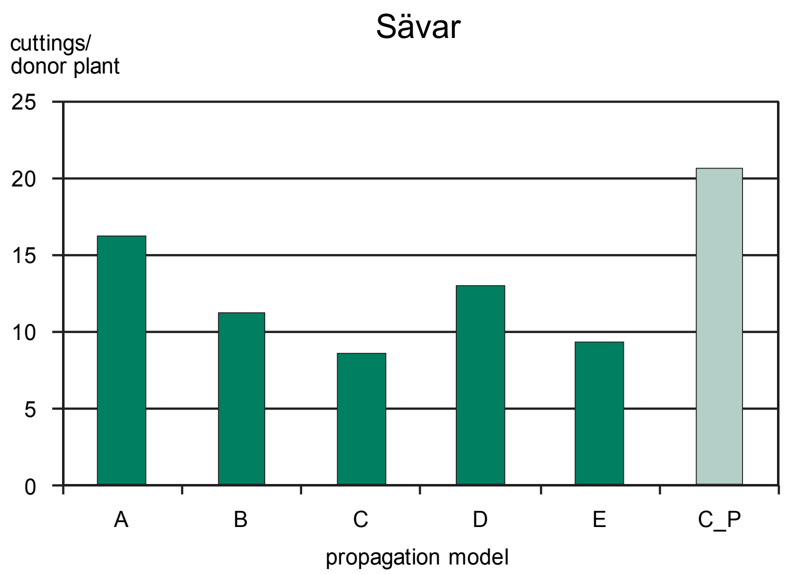


Fig.3. Comparison of cutting production for the different propagation models in Sävar with the five common families. The figure is complemented with the production result of model C in Punkaharju (C_P).

The average production for the propagation events with the 15 local families was 12.3 cuttings/donor plant. However, the production varied considerably among the different propagation events (Fig. 4). There was no clear pattern in production. Also in this case, the production with model C in Punkaharju gave the highest mean cutting production, 21.5 cuttings per donor plant, while the same model in Sävar only produced 7.8 cuttings per donor plant.

Variance analyses within three locations showed that propagation model effects were significantly different in Sävar and Salaspils, but not in Ekebo. Family effects were significantly different from zero in Sävar and Salaspils but not in Ekebo.

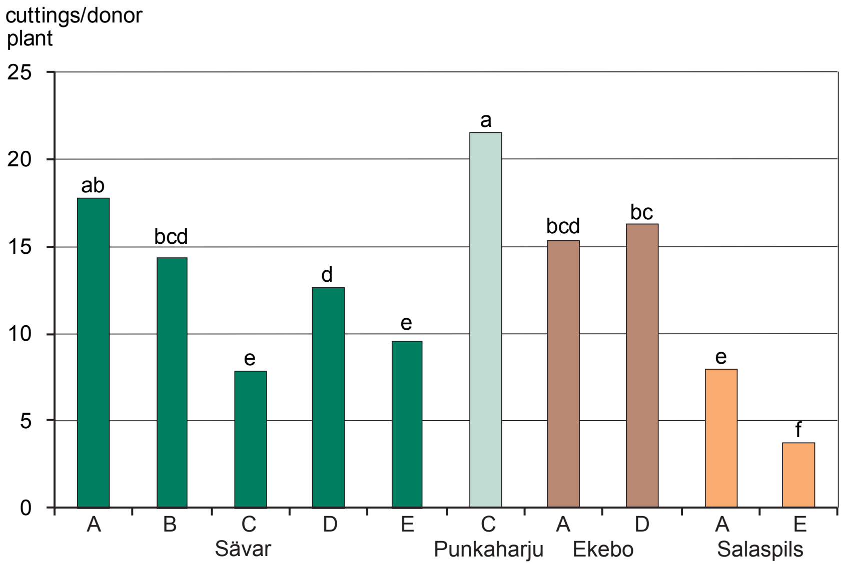


Fig. 4. Production of cuttings for the 15 local families at different locations and with different models. The same letter indicates no significant difference among entries (p<0.05).

As an example of the variation among families in one propagation event, the cutting production with model A in Sävar is shown in Fig. 5.

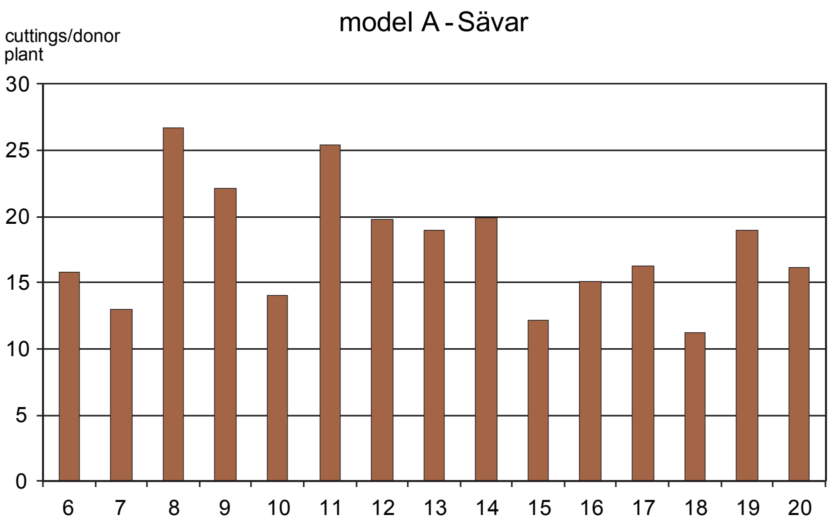


Fig. 5. Average cutting production for the local families in Sävar, with propagation model A

The family correlations across models in Sävar showed positive and fairly strong correlations (Table 1). There was a tendency to lower correlations when model C was involved.

Table 1. Correlations among propagation models on family level in Sävar.

	B	C	D	E
A	0.64	0.46	0.72	0.78
B		0.65	0.63	0.59
C			0.52	0.31
D				0.70

Clonal variation within families (=variation among donor plants within families) were substantial and for the best producing propagation event, Punkaharju model C, the number of cuttings produced per donor plant varied from 1 to 42. Cutting production underway with this propagation model in Punkaharju is illustrated in Fig.6.

Fig. 6. Shoot production in the "Turbo line" donor plants following the first pruning.

3.1.2 Rooting response

The average rooting response for the five common families was in general low and erratic and at nine propagation occasions the rooting failed completely or fell below 5%. For the other propagations the average response varied from 5.7% to 32.1% (Table 2). The best response was obtained for propagation model C in Sävar closely followed by propagation model B in Haapastensyrjä.

Table 2. Rooting response for the five common families, nr=no rooting response, nm=no material available.

location	model	Propagation 1	Propagation 2
Sävar	A	2.1%	nr
	B	nm	3.0%
	C	32.1%	3.0%
	D	2.1%	8.5%
	E	nr	
Haapastensyrjä	B	27.6%	nm
Ekebo	A	9.8%	8.9%
	D	14.8%	nr
Salaspils	A	5.7%	0.3%
	E	nr	

A similar erratic rooting pattern was found also for the 15 local families but with generally higher responses. At seven propagation occasions the rooting failed completely or fell below 5 % (Table 3) and were not analysed. The best response with local families was 53.2% (Sävar, model C). Rooting response in the other propagation events ranged from 9.8% to 22.6%.

Table 3. Rooting response for the local families. nr=no rooting response, nm=no material available.

location	model	Propagation1	Propagation 2
Sävar	A	18.0%	nr
	B	nm	19.5%
	C	53.2%	nr
	D	1.7%	16.6%
	E	nr	
Haapastensyrjä	B	22.6%	nm
Ekebo	A	11.2%	14.9%
	D	12.9%	nr
Salaspils	A	9.8%	0.3%
	E	0.2%	

Analyses according to statistical model 3 on the local families showed that family effects were significantly different from zero ($p<0.05$) in five propagations out of nine. No significant family effects were obtained for two highest rooting responses, Sävar propagation model C: propagation 1 and Haapastensyrjä propagation model B: propagation 1. The rooting response among families in the propagation with best rooting response (Sävar propagation model C, propagation 1) varied from 29% to 81%.

The difference in rooting response between first and last propagation was inconsistent and there was no obvious pattern of the second propagation having a lower response than the first.

3.2 Experiments on environment factors

3.2.1 Watering regime and substrate quality

The 110 Swedish Scots pine families, each represented by a single genotype and used for studying watering regime and substrate quality differed in their shoot production. The average number of shoots harvested per donor plant was 9.5 (±0.42 SE), the minimum number of the harvested shoots being 3 and the maximum 25. These families/genotypes also varied in the rooting success, the average rooting percentage being 16.1 (± 1.83). In the best genotype, 73% of the cuttings rooted, but on the other hand, 48 families/genotypes out of the 110 tested did not show any rooting.

When comparing the tested watering regimes and substrates, no significant differences between normal and generous water supply, or between Jiffy-pots ™ with coconut fibre and Kekkilä Spruce-Rhododendron Soil™-perlite mixture as substrates could be observed (p-values for these effects 0.703, and 0.575, respectively). It could be observed, however, that for successful rooting Jiffy-pots ™ seemed to need more generous watering than Kekkilä Spruce-Rhododendron Soil™-perlite mixture as substrate (Fig. 7). The IBA application forms, as solution or as powder were also compared with each other, but no significant difference ($p = 0.686$) between them could be found (Fig. 7).

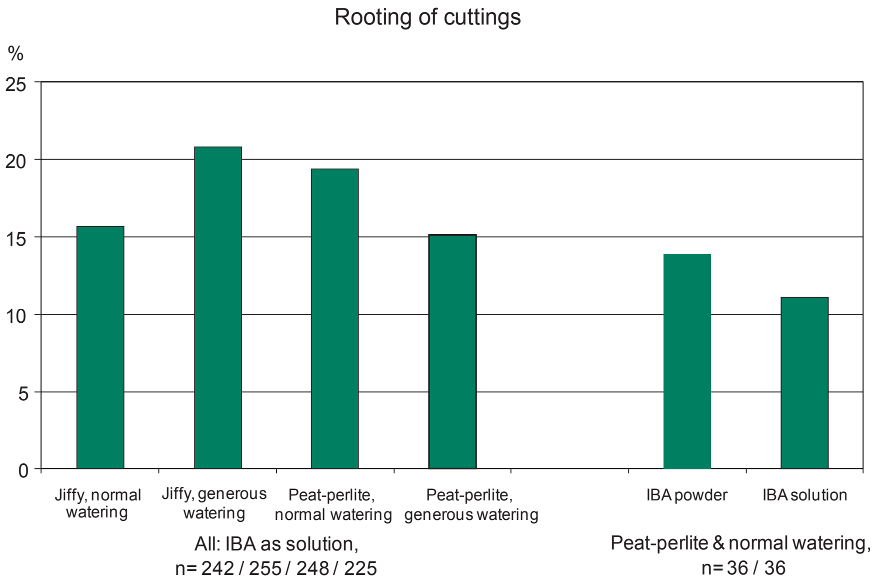


Fig. 7. Average rooting percentages of the cuttings subjected to IBA solution treatment and either to normal or generous watering, inserted into Jiffy-pots ™ with coconut fibre or into Kekkilä Spruce-Rhododendron Soil™-perlite mixture (=peat-perlite); together with the average rooting percentages of the cuttings treated either with IBA solution or IBA powder, and watered normally in Kekkilä Spruce-Rhododendron Soil™-perlite mixture.

3.2.2 Effect of Gibberellin inhibitors applied together with IBA

The five Finnish families used for testing the effect of gibberellin inhibitor on rooting of Scots pine cuttings differed significantly in their shoot production (Fig.8). The family had also a significant effect on the rooting of cuttings when analysed using a reduced model (p <0.001). Likewise, the application of gibberellin inhibitor had a significant effect on rooting in reduced model (p <0.001). When the same data was analysed having both gibberellin inhibitor application and family as factors, only their interaction was found to be significant with p=0.001 but the main factors having p-values of 0.130 and 0.126, respectively. As seen in Fig. 9, however, the application of IBA together with gibberellin inhibitor resulted, on an average, in a lower rooting response than application of IBA alone.

Fig. 8. Average shoot production (± SE) in the three-year –old donors of five Finnish families used for testing the effect of gibberellin inhibitor on rooting of Scots pine cuttings. The families marked with differing letters differ significantly from each other (Student-Newman-Keuls p= 0.05).

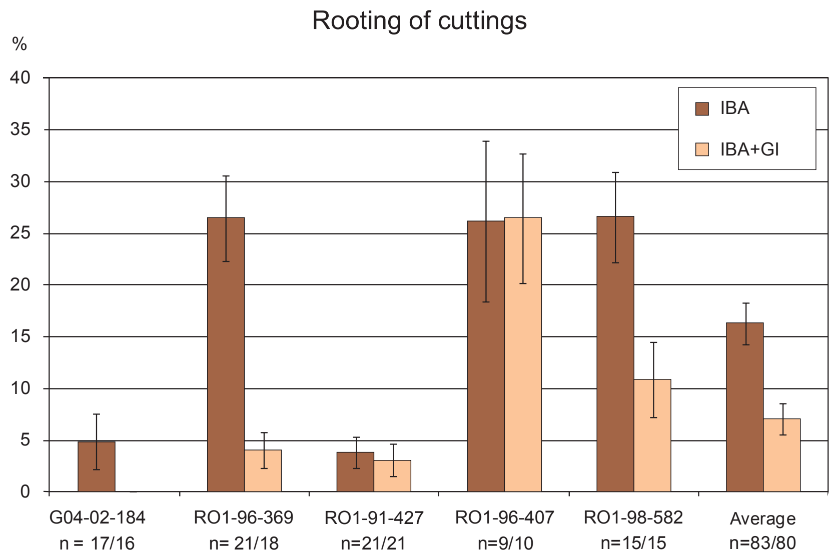


Fig. 9 Average rooting percentages (± SE) of the cuttings of five Finnish families subjected either to IBA treatment or to treament with IBA + gibberellin inhibitor Paclobutrazol.

4 Discussion

4.1 Cutting production

The variation in all main effects and interaction effects for the five common families can be interpreted in many ways. However, the Finnish families were less productive on all locations and this indicates that the family x location interaction rather was dependent on the three other families. Family 3 was a local family to Sävar and were the most productive at that location. It could mean that local adaptation plays a role in shoot production.

Differences among locations were probably connected to differences in conditions when cultivating the donor plants. For example, donor plant size differences at the pruning occasion could have affected the production. The very high production for model C in Punkaharju may have been caused by large size donor plants enabling shoot production also on side branches. It should be noted that the same model in Sävar produced small numbers of cuttings with theoretically the same cultivation scheme. Obviously local growing conditions have differed in some way, but there is no exact information that can give an explanation. One potential reason for observed differences could be that more space was allocated per plant in Punkaharju than in Sävar.

At Sävar, where all propagation models were tested, models A and D worked best (15 and 12 cuttings/donor plant), while model C and E produced least.

Slightly higher production was obtained with local families, a logical result considering the outcome with the five common families. With the assumption that local families were equivalently sampled from respective populations, the differences displayed in Fig. 4 shows the difficulty of keeping consistent production over locations using the same model.

Obviously, model E can be ruled out as a low-productive alternative. The split results of propagation model C in Sävar and Punkaharju remain for the local families. Based on all information, a mean number of cuttings after two harvests with propagation models A, B or D, can be predicted to be around 10, possibly higher. The Punkaharju result with propagation model C with an average above 20 cuttings/donor plant can be seen as a goal to aim for but needs confirmation.

Families correlated well across propagation models in Sävar, indicating that the model x family interaction is not of decisive importance.

4.2 Rooting response

Among the studied species of the genus *Pinus*, Scots pine has been considered as one of the most recalcitrant ones for vegetative propagation through rooted cuttings. As reviewed by Ragonezi *et al*. (2010), in many pines, such as *P. banksiana, P. caribaea, P. contorta, P. radiata, P. strobus*, and *P. taeda*, rooting percentages up to 80-100 % can be achieved. In Scots pine, the best rooting success reported so far was on an average 54% (Högberg 2005).

In the present study, the rooting response for the five common families was very disappointing as there were only two propagation events that exceeded 20 % rooting success. The same pattern appeared for the local families but with higher average. The low, erratic responses make it difficult to draw any wider conclusion from this study.

It is striking that a very good cutting production was achieved with propagation model C in Punkaharju but the rooting failed. However, the best rooting response was obtained with model C in Sävar, where the production on the other hand was rather low.

The rooting conditions applied in the present study were based on the previous experience on Scots pine cuttings, and also well in agreement with ones generally used for rooting *Pinus* cuttings (Ragonezi *et al*. 2010). There is, however, a suspicion that no location could provide technical solutions that enabled optimal rooting conditions. The balance between high air humidity and oxygen availability in the substrate is difficult to regulate and it is likely that either drought or excess of water in the substrate lies behind the low response. Another factor that in some cases were suspected to have affected rooting negatively is that the donor plant itself had an inoptimal water status. However, there are no data available that could prove these explanations and they should be seen as speculations.

It is tempting to suggest that development of model C is the way to go from here, assuming that the two successful results at two different locations can be made at the same place. However, such a conclusion cannot be made with the contradicting outcomes within locations.

The shifting degree of significance of families for rooting response is difficult to interpret. However, no family differences in the two highest rooting responses may indicate that all families will root when conditions are good. This goes in line with the results reported by Högberg (2005).

It is encouraging that there was no pattern in rooting response, in propagations where neither of the propagations failed, between first and second propagation with the same donor plants. This means that two harvests might be realistic from rooting point of view and will help to achieve a sufficient number of cuttings per clone after further development of the rooting protocol.

4.3 Rooting environment

Two watering regimes (normal and generous) and rooting substrates (Jiffy-pots ™ with coconut fibre and Kekkilä Spruce-Rhododendron Soil™-perlite mixture in 75:25) tested in the present study as rooting environment factors did not differ from each other. Soil or peat mixed with perlite or vermiculite is very often used for rooting pine cuttings (Ragonezi *et al*. 2010). The commercial Jiffy-pots™, on the other hand, were tested because of their convenient handling prior to cutting immersion, and to utilise the possibility of easy observing of emerging adventitious roots from a compact pot. When comparing the watering regimes, it seemed, however, that for successful rooting Jiffy-pots™ need more generous watering than the tested soil-perlite mixture as rooting substrate. Obviously, the ability of different substrates to remain moist varies, and watering should be adjusted accordingly.

IBA is the most commonly used auxin for induction of adventitious roots in coniferous cuttings, although for some pines also NAA (naphthalene acetic acid) has successfully been used. As treatment, a quick dip of cut shoot base in IBA solution or in IBA mixed with talk, as also tested in the present study, is most often applied (Ragonezi *et al*. 2010). In the small-scale experiment of the present study, no difference between IBA application either as solution or as powder was found.

Synthesis of gibberellins has been found to inhibit rooting (Hartman & Kester 1983, Davis *et al*. 1988). In a study by Henrique *et al*. (2006) in another pine species, *Pinus caribea*, the application of gibberellin inhibitor Paclobutrazol was reported to be beneficial for rooting of cuttings. Application of this gibberellin inhibitor together with the rooting agent IBA did not, however, improve the rooting response in Scots pine cuttings. On the contrary; in three of the tested five families Paclobutrazol treatment reduced the rooting percentage.

Thus, variation of the tested factors in the rooting environment, i.e. watering regime, substrate type, or addition of gibberellin inhibitor could provide no substantial improvement to rooting success of Scots pine cuttings.

4.4 Conclusions

The average production of cuttings with models including two consecutive harvests on the same donor plant can be predicted to be 10-15 cuttings/donor plant. The variation among families and donor plants will be substantial. A propagation model allowing the donor plants to grow for two years before cutting production do not appear to be an alternative. Propagation model C is an interesting option but the reason for the high production in Punkaharju and the low production in Sävar is not quite clear.

This study has not been successful in the development of improved protocols for Scots pine cutting propagation. It is annoying and frustrating to know that rooting responses above 50% can be achieved but not be able to repeat this over a larger number of propagations.

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