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Adventitious root formation and early growth of *Pinus contorta* cuttings

A study in genetics and methodology

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Degree project in biology, Master of science (2 years), 2013
Examensarbete i biologi 30 hp till masterexamen, 2013
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Abstract

In breeding it is important to have a reliable way to propagate the offspring. If the offspring from crosses are propagated in a good way, then the testing becomes more reliable. The use of rooted cuttings is the preferred propagation method for testing candidate trees in Skogforsks lodgepole pine (*Pinus contorta* var.*latifolia*) tree breeding program. In this thesis four questions were raised. The first question was if there are genetic differences in the formation of adventitious roots and early growth between full-sib families? This was tested by using 30 families, for recording root number and early growth of cuttings. A three-year-old field experiment with records of root number and height was used for comparison. Both experiments showed differences between the families in rooting of the cuttings. There was also a difference between families in height during the first year, but that difference did not remain after three years in the field. There were large variations between clones within the same family. The second question asked was which synthetic auxin, and in which concentration would result in the best rooting. Indole-3-butyric acid and Naphthalene acetic acid were tested with a concentration of 8000 ppm and 1000-8000 ppm, respectively. 8000 ppm Indole-3-butyric acid and 1000 ppm Naphthalene acetic acid was observed to be the best for rooting. Naphthalene acetic acid should be tested again with lower concentration. Also, Indole-3-butyric acid and Naphthalene acetic acid were tested in solid form mixed with talc. They gave in general a good rooting, but was considered unpractical to work with and thus not feasible for operational use today in Skogforsks breeding program. Each ortet produces different amount of shoots that can be used as cuttings. This is a problem when many and an even number of cuttings are desired. The two last experiments were designed to solve this problem. The first of the two experiments was designed to test if the bottom part of a shoot could root and grow as well as the top part when long shoots were divided in two parts. The different parts rooted equally well, but the top part of the shoot did grow better. The last experiment was to root needle fascicles. It did not work out well, because rooting and survival rates were very low.

Terminology

Auxin – A plant hormone.

BAP – 6-Benzylaminopurine, a synthetic cytokinin.

Clone – One genotype that may be represented by several individual cuttings.

Cytokinin – A plant hormone.

Full-sib families – The clones in the family have both the same mother and father. Compared to half-sib families where only one of the parent is the same.

IBA – Indole-3-butyric acid, a synthetic auxin.

Meristem – A part in plants with undifferentiated cells that divide and form new tissues.

NAA – Naphthalene acetic acid, a synthetic auxin.

Needle fascicle – Needles enclosed in a sheath and with a resting meristem.

Ortet – The plant from which clonal cuttings are taken.

Contents

1 Introduction

1.1 General background and objectives

Lodgepole pine (*Pinus contorta* var.*latifolia*)was introduced in Sweden around the 1920s and is today the most used exotic tree species in Sweden. It is native to the north western US and western Canada. Generally it has a higher growth rate than Scots pine (*Pinus sylvestris*) and is therefore of interest for the forest industry (Norgren 1996). To increase the growth and quality, Skogforsk (the Forestry Research Institute of Sweden) is managing a tree breeding for lodgepole pine in Sweden. The breeding of lodgepole pine is in an active phase. The best trees were selected in Swedish progeny trials and the trees have been crossed in order to establishing candidate populations, which will be tested in new field trials. Rooted cuttings are a preferred strategy for genetic field testing of candidate clones in tree breeding. The C2 breeding population were put out in field trials in 2010. The C4 breeding population will be planted in 2014. The plant material for this thesis originate from the breeding activity in those breeding populations. When rooted cuttings is used for genetic testing, it is important to make sure that all cuttings get an equal start as possible, to avoid unwanted selection inferred by the propagation method. A critical step for the success with rooted cuttings is the formation of adventitious roots, meaning post-embryonic roots that emerge from cuttings (Ragonezi et al. 2010). The difference between adventitious roots and ordinary roots is that adventitious roots are initiated close to vascular tissue, while ordinary roots is formed from a root apical meristem (Pijut et al. 2011, Goldfarb et al. 1998).

The objective of this master thesis was to investigate three factors that can affect the success of using rooted cuttings: 1) the genetic factor, 2) hormonal treatment and 3) the amount of shoots the ortet produce. An experiment to evaluate the genetic factor was carried out and another one to test the optimal hormone treatment. In this study two different experiments were carried out to deal with the problem that the ortet produce different amounts of shoot. The experiments were to divide long shoots and to use needle fascicles.

1.2 Genetic difference in the formation of adventitious roots

One thing that may affect the ranking of families in the breeding program is if different families root differently as cuttings. The problem is that seedlings, not cuttings are used for reforestation in Sweden. If the rooted cutting method would give some families a higher ranking in the breeding program, the selection of the best families would not be based on representative data. Therefore it was evaluated whether some families root better than the others and if this can be related to the early growth of the cuttings.

1.3 Hormones and the development of adventitious roots

The plant hormone auxin has for long been used to induce a better rooting in cuttings. There are different auxins, some natural and some synthetic. The ones used to improve rooting in

cuttings are different synthetic varieties. A quickly dip (approx. 5 sec) of cuttings in Indole-3-butyric acid (IBA) 8000 ppm diluted in 95 % ethanol before insertion is in practical use for propagation of lodgepole pine with rooted cuttings. However this concentration for "quick dip" with IBA, has been tested and developed for Scots pine cuttings. There is an another auxin that is used to improve rooting in pines, Naphthalene acetic acid (NAA). NAA has been used to promote rooting in Jack pine (*Pinus banksiana*) dwarf shoot with better result than IBA (Browne et al. 2000). Both IBA and NAA have been used in solid form mixed with talc in some experiments (Ragonezi et al. 2010). The questions are which concentration, state and auxin is the better for rooting lodgepole pine cuttings with a "quick dip" method.

1.4 Needle fascicles and shoots divided into halves

Another problem faced when using cuttings is that ortets do not produce equal amount of shoots that can be used as cuttings. In this master thesis two ways to circumvent this problem was tested. The first approach was to take needle fascicles and the other was to divide long shoots.

It has been shown that needle fascicles can be rooted, but usually with a low rate of success (Mergen and Simpson 1964, Larsen and Dingle 1969). At Skogforsk they have observed that fallen needle fascicles sometimes develop roots. From this observation came the idea to test how well they can root and if this could become a good method for vegetative propagation. In this master thesis the rooting of needle fascicles in different media and with different hormonal treatment was tested. The question asked is if a method for propagation of needle fascicles could produce plants for operational use in breeding.

The ortet often produces either many short shoots, or a few long ones. If it is possible to divide the long shoots and double the amount of cuttings, it would even out the differences between ortets. The divided shoots method is based on the combined principles that needle fascicles can form shoots and that cuttings can root. The upper part of the shoot is exactly like a normal cutting but the lower part does not have a top bud. Therefore the bottom half of the divided shoot has to rely on the needle fascicles to form a bud that can sprout a new shoot. The lower part will also have to form adventitious roots just like a normal cutting. The question asked is if the top part and the bottom part can root and grow equally well. If they do, dividing shoots could become a good method to get a similar amount of cuttings from each ortet.

2 Materials and methods

There are several experiments in this master thesis, to make it simple and keep them separated abbreviation are used (table 1).

Table 1: Experiment abbreviation

Abbreviation	Experiment description
G-experiment	The main genetic experiment conducted. A greenhouse experiment, with 30 full-sib families from the C4 breeding population.
C4-testing	The breeding programs testing. Used as a reference for rooting success.
C4-comp	Complementary cuttings to the breeding programs testing. Uses as a reference for rooting
F-experiment	The field rooting experiment of C2 cuttings that was planted by Skogforsk in 2010.
C2-field	The planted field trial of the C2 breeding population. Used as a reference to the F-experiment. Planted by Skogforsk in 2010.
H-experiment	Hormone experiment.
D-experiment	Divided shoot experiment.
N-experiment	Needle fascicle experiment.

2.1 Plant material

2.1.1 Greenhouse experiments

Cuttings for the three cutting experiments (G-,H- and D-experiment) were collected in connection to the regular propagation of the C4 breeding population within the lodgepole pine breeding program. The cuttings originated from ortets generated by controlled crosses. The ortets were two years of age when the cuttings were harvested. The ortet had been pruned once, in June 2012, to produce more shoots. Cuttings were harvested the same year in end of November and beginning of December. The cuttings were put in plastic bags and sprayed with water before storage at around -3 °C. Insertion of cuttings in connection to the regular propagation were made at three different times with start in January in 2013. After the first insertion of the C4-testing cuttings with standard protocol, the cuttings were stored in the fridge at 2 °Cto be used for the thesis experiments. An additional insertion was made of cuttings not used in the thesis experiments, but could be used as complimentary cuttings (C4-comp) to the breeding program. There were also some extra cuttings from C4 plants that had lost their label. These cuttings were harvested three days before the propagation in February and cuttings were kept in the fridge at 2 °C. There were no statistically significant differences between these extra cuttings and the others, in the experiments they were used and the parameters measured. The testing results are in the appendix, tables 10 and 11. A flowchart of which plant material used where can be seen in figure 1. The ortet for the N-experiment were also two years old and originated from seeds. They were brought up to become the root stocks for grafting.

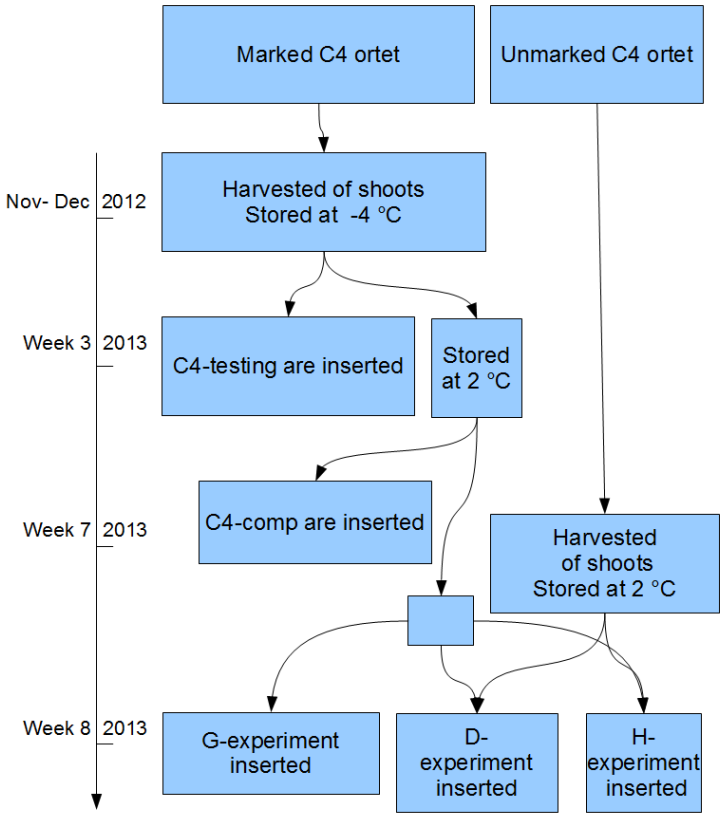

Figure 1: Division of the plant material

2.1.2 Field experiments

The plants in the field were from the C2 population breeding program. There were ten families and 280 cuttings in the F-experiment. As with the G-experiment there was also a breeding program testing (C2-field) conducted at the same time. The C2 plants were planted in the field in 2010 in random block designs. The field experiments was located approximately 10 km Northwest of Sävar (coordinates for Sävar, see section Growing conditions). The cuttings in this experiment were also collected from two-year-old ortets and treated in the same way as the C4 cuttings in the G-experiment. There is no inherited relationship between the C2 and C4 breeding populations.

2.2 Rooting scheme

2.2.1 The propagation protocol

First the standard protocol used by Skogforsk is presented and then the differences made for the cutting experiments. The first step in the propagation of the cuttings was to give them a

new cut surface and remove the lower needles, approximately 2.5 cm from the bottom. The length of the shoot was adjusted to approximately five centimetres. The second step was to place them in water for at least 30 minutes. The third step was to give them a quick dip in the hormone solution (IBA 8000 ppm in 95% ethanol for 5 seconds). Before they were dipped in the hormonal solution they were dried off with a paper towel, in order to not dilute the solution. The cuttings were put on the bench to air dry before insertion in approximately one minute. After this they were inserted in the trays. A schedule for the planting , replanting and evaluations of the experiments can be seen in table 2.

My experiments(G-, H- and D-experiment) were inserted in trays (Aero NT 84, Omni solutions) with 84 positions (7 rows and 12 columns). The cuttings were placed in pre-made holes in the substrate and it was made sure that the substrate was closed around the cutting.The substrate was AeroNT Substrate, with pH value: 5.2-5.8, EC: 0.1-0.6 mS, light fertilization mix and micronutrients. After insertion the trays were placed in the greenhouse. The cuttings that were replanted, were planted in fertilized peat with some sand. The trays, they were replanted in were Hiko 150ss. The F-experiment and C2-field were also replanted in a similar manner.

Both the C4-testing and the C4-comp cuttings were propagated in Plantek 49F trays with 80% unfertilized basic lime peat mixed with 20% perlit. Other than that, the standard protocol was followed when they were propagated. The C4-testing and the C4-comp cuttings were not replanted, because they were inserted in large pots to begin with.

Table 2: Propagation, evaluation and replanting schedule. C4 Comp. = complementary P = propagation, E = evaluation, R = replanting

Experiment	Week 3	Week 7	Week 8	Week 14	Week 15	Week 17	Week 25	Week 29
C4 testing	P			E				
Genetic			P		E	R	E	E
Divide			P			E,R	E	E
Hormone			P		E			
Needle pair			P			E,R		E
C4 Comp.		P		E				

2.2.2 Genetic experiment

The G-experiment was propagated in the standardized way described above. Thirty full-sib families and 157 clones were used. Each clone received one column, so the maximum number of cuttings per clone was seven. The position of the clones was randomized by mixing the bags with the cuttings in a large box. The families used were selected from the families with many cuttings left over from the C4-testing. Each family used was also represented by at least three clones.

2.2.3 Hormone experiment

In the H-experiment six different treatment were used together with a negative control (table 3). Except for the difference in hormonal treatment the propagation followed the standard protocol. In the negative control, the hormone dip stage was skipped. A quick dip for the hormonal solutions and a one centimetre dip for the talc powder mixtures. 47 clones were used with one cutting per treatment. Each clone was assigned to one column in the tray.

Table 3: Hormone experiment schematic. For each treatment 47 clones were used. Brands of the chemicals in parentheses. In the control the hormone dip step was skipped completely

Hormone	Concentration	Diluted with
IBA (Sigma)	8000 ppm	95% ethanol (Kemetyl)
NAA(Sigma)	1000 ppm	95% ethanol (Kemetyl)
NAA(Sigma)	4000 ppm	95% ethanol (Kemetyl)
NAA(Sigma)	8000 ppm	95% ethanol (Kemetyl)
IBA (Sigma)	4000 ppm	talc powder (Dialon,Hardford)
NAA(Sigma)	4000 ppm	talc powder (Dialon,Hardford)
Control		Without hormonal treatment

2.2.4 Divided shoots experiment

The propagation of the D-experiment mostly follow the standard protocol. The difference was that before the hormonal treatment, the shoot was divided in an upper and lower part. Three shoots from each clone were used and 34 clones. Each clone receive one column, so the last position was left empty. The clones that were used were selected based on their long shoots, at least eight centimetres in total.

2.2.5 Needle fascicle experiment

For the N-experiment, two types of growth media were used (tap water and pure sand). Needle fascicles were plucked from the mother plant to the water bath and cut out from the mother plant for the sand. The harvesting methods were different in order to make sure the needle fascicles could remain upright. They were taken from both the upper part and the lower part of the top shoot. 24 needle fascicles were taken from each mother plant and divided equally between the sand and the water, and the different hormone treatments. For each of the media 120 needle fascicles were used. Before propagation, the needle fascicles were treated with IBA of different concentrations (figure 2). The water in the water bath was changed directly after propagation and three more times after that. The changes were to avoid having any residue of IBA in the water and to reduce the growth of algae. In the water bath an air pump was placed to make the water move around and keep it aired. The N-experiments were placed in the greenhouse with the three other experiments(G, D and H). A plastic hood were placed on top of both the water bath and the sand to keep a high moisture in the air. The set up can be seen in figure 2.

(a) (b) (c)

Figure 2: N-experiment set up The flow chart shows how the needle fascicles were divided in to the different treatments. The IBA concentration is in ppm and in 95% ethanol. The B stands for that the needle fascicle is taken from the lower part of the shoot and T stands for it to have been taken from the top part. The other two pictures are of the needle fascicles after propagation, (b)=in water and (c)= in sand.

The N-experiment were replanted in Starpot 120-28 tray with peat mixed with 10% perlit. The needle fascicles chosen to be replanted were all needle fascicles with roots, three needle fascicles that had a visible formation of a bud and seven needle fascicles that still had fresh callus. Before replanting the ten needle fascicles without roots, were dipped in IBA 250 ppm in 95% ethanol for 10 minutes. The IBA used here was the same dilution that was used in the upstart of the needle fascicles experiment. It had been stored in a cold storage room at 2 °C. The tray was placed in the large greenhouse. Two days after replanting, the needle fascicles with roots were treated with 6-Benzylaminopurine (BAP) 0.2 g/l. This was to promote the development of shoots. The other needle fascicles were not treated, because BAP can be negative for the formation of roots. The dilution of the BAP was based on an old mixture that Skogforsk used for promoting the development of needle fascicles into shoots on seedlings. To make the mixture, 10 mg BAP(Sigma) was mixed with 30 μl HCL 0.5 M (LabService). After that 1 ml dH_2O and 5 μl Tween 20 was added and it was mixed until it had been diluted. The rest of the dH_2O was added to a total volume of 50 ml. When the mixture was finished, the BAP mixture was applied to the lower part of the needles with a cotton swab.

2.3 Growing conditions

All of the thesis experiments (G, H, D and N) were placed in the same greenhouse. Air temperature was 13 °C and in substrate 23 °C. Air moisture was 60 % with nozzles showering the cuttings every 15 minute. The light was approximately 5000 lux and included a six hour night. The lights were 400 W metal Halide. The greenhouse was located in Sävar, Sweden. Coordinates in WGS 84 (lat, lon): N 63°53.730', E 20°32.893'

The growing conditions for the C4-testing and the C4-comp cuttings in the large greenhouse: substrate temperature of 23 °C and air temperature 10-14 °C. The light regime included a six hour night. Watering was done through nozzles and by hand, in order to keep a suitable moisture level.

After replanting the G- and the D-experiments were placed in together with the conventional propagated cuttings the larger greenhouse with air temperature approximately 20 °C. Also

the replanted needle fascicles was placed in the large greenhouse. In week 26 the C4-testing, C4-comp G- and the D- experiments were moved outdoors. The needle fascicles stayed in the greenhouse.

2.4 Hormonal evaluation

The rooting evaluation of the H-experiment was performed slightly different in comparison to the other experiments. It was the first evaluation that was performed and therefore the most extensive. All measurements that were repeated and the same in several experiments have been given the number of the measurement and the letter code for the experiment (G = G-experiment, H = H-experiment, D = D-experiment, F = F-experiment and F also for C2-field). A complete table of all coded variables can be seen in appendix, tables 17, 18, 19 and 20. First the number of visible roots on the outside of the substrate was counted, then the root system was cleared from substrate and the total number of roots were counted. All roots longer than two millimetre were counted when cleared from substrate. The roots on the outside was counted in this experiment as a reference to the other experiments were the substrate could not be cleared, without hurting the roots. Only the primary roots were counted, not the branch roots. The longest root was measured and it was noted if the roots had started to branch. Two other qualities were noted, top vitality status and the vitality status of the part that has been in the substrate. The vitality was recorded on a scale of three: 0= dead, 1= alive and 2 = sprouted/roots. The vitality was recorded in the same way for the G-, the H- and the D-experiments at all evaluations. Example of how the cuttings looked like during the first evaluation can be seen in figure 3.

(a) **(b)** **(c)**

Figure 3: Example of cuttings during the first evaluation. Picture (a) is from the H-experiment and picture (b) is from the G-experiment. The last picture (c) is form the D-experiment and show one of the bottom part cuttings.

2.5 Genetic and divided shoot experiment evaluation

2.5.1 First evaluation

Both the G- and D-experiments were evaluated in the same manner during the first evaluation. To avoid hurting the plants, only the number of roots (GRoot, DRoot) seen on the outside of the substrate was recorded. After making a correlation on the H-experiments root count, it became clear that it is a good approximation on the true number of roots, to only count the ones on the outside. The height of the cuttings in the G-experiment (GHeight 1) and in the D-experiment (DHeight 1) and also the vitality was recorded. The vitality was recorded on a scale of three: 0= dead, 1= alive and 2 = sprouted. After the measurements were taken, the cuttings were replanted as described in section 2.2.1.

2.5.2 Midway and final evaluation

A midway evaluation and a final evaluation of the G- and D- experiments were conducted at week 25 and week 29, respectively. In both evaluations almost the same measurements were taken. The measurements taken for the G-experiment were: height (GHeight2,GHeight3) measured on the highest tip, number of shoots (GShootnumber1,GShootnumber2), vitality, dominance of a shoot (GDominance1, GDominance2,) and whether a bud on the cutting was inactive. The lower number indicate the midway evaluation and the higher number is the final evaluation in the variable names. The dominance was assessed by looking at the shoots of a cutting and evaluate if any shoot looked clearly dominant over all the others (scored 1 = dominant or 0 = not dominant). The inactive bud was taken as possible explanation to why some cuttings had a dominant shoot and others did not. Some of the shoots branched into more shoots and these branches were counted as shoots, but mostly the shoots counted came from pre-existing buds on the cutting. Growth (GGrowth) was calculated by taking the final height (GHeight3) and subtract it with the first height (GHeight1). The measurements for the D-experiment were almost the same. The inactive bud parameter was changed to a not sprouted parameter. The not sprouted parameter was in the midway evaluation restricted to just cuttings with a top, but for the final evaluation it was expanded to include those that lacked a top as well.

2.6 The C4-testing and the C4-comp cuttings evaluation

Evaluation of the C4-testing was done by recording which cuttings that were still alive. Those alive were counted as rooted. Only the same families that was used in the G-experiment was evaluated. Another method to evaluate the rooting of the C4-comp cuttings was used. The cuttings were pulled carefully to see if they had rooted. Non rooted cuttings were counted and then removed. No direct measurement of rooting could be done in this material. The C4-comp cuttings had been treated in the same way of storage as the G-experiment and inserted one week before. This made them good a reference with respect to the effect of the extra storage that the cuttings had to endure in comparison to the C4-testing cuttings.

2.7 Field experiments evaluation

The F-experiment and the C2-field were measured during week 28. The measurements taken were vitality, last years plant height (FHeight3) and this years plant height (FHeight4). Vitality was measured in a scale of zero to three, were zero is dead and three is full health. The height was measured with a measuring stick in the centimetres. This years height may not be the final height that the small pines will reach this year, but the growth would not have continued much longer according results from Norgren (1996).

2.8 Needle fascicles evaluation

The rooting and the health of the needle fascicles were recorded. All needle fascicles with roots were picked out to be replanted. Three needle fascicles showed a visible formation of a bud and were also picked out together with seven needle fascicles that still had fresh callus. The final evaluation of the needle fascicles was done week 29 and the number of needle fascicles that had survived and sprouted was counted.

2.9 Statistics and calculations

The descriptive statistics were performed with Libre office 4.0 and Open office 3.3. For the analysis R 2.15.0 with the Deducer was used. All test were performed on the 5% significance level. The test used were: Welch two sample t-test, ANOVA, Pearson's product-moment correlation, χ^2-test and Fishers exact test. To create a Linear mixed effect model for the G-experiment the lme4 package in R was used. The model for the GGrowth was built with data from the last measurement, discounting all cuttings that were dead or that had no shoots. All linear mixed effect models was fitted with maximum likelihood. Each variable in the models were tested by using a null model without the variable and comparing it with a model with the variable using a likelihood ratio test. The broad sense heritability was calculated with the data from the first measurement and a linear mixed effect model fit by REML with the formula: GRoot=Clone+ϵ was used to calculate the variance. The variance components from the linear mixed effect model for clone was used as the genetic variance and the ϵ was used to calculate the total variance, by adding it with the clone variance. Formula used to calculate the broad sense heritability: H = Vg/Vt, H= Broad sense heritability, Vg= genetic variance, Vt= total variance.

3 Results

3.1 Genetic variation in rooting and early growth

3.1.1 Root number and rooting

The question asked in this project was if there are any differences between families in the formation of adventitious roots. The result of the ANOVA performed on GRoot with and without dead cuttings, showed significant differences between families at a 5% level. Figure 4 shows GRoot recorded for the cuttings in different families. The number of rooted/non rooted cuttings between families were different when tested with a χ^2-test (Df 29, $p<0.001$). In the start of the experiment there were 34 cuttings in average per family and it ranged between 19 and 60 cuttings per family. The rooting percentages of the families can be seen in figure 5. It differed from 19.2% as the lowest to 87.5% as the highest. In twelve of the clones, all the cuttings had died before the final measurement and eleven were dead before replanting. The broad sense heritability for the number of roots was calculated with the data from the first measurement and was H = 0.28 (genetic variance 17.9 and total variance 63.9)

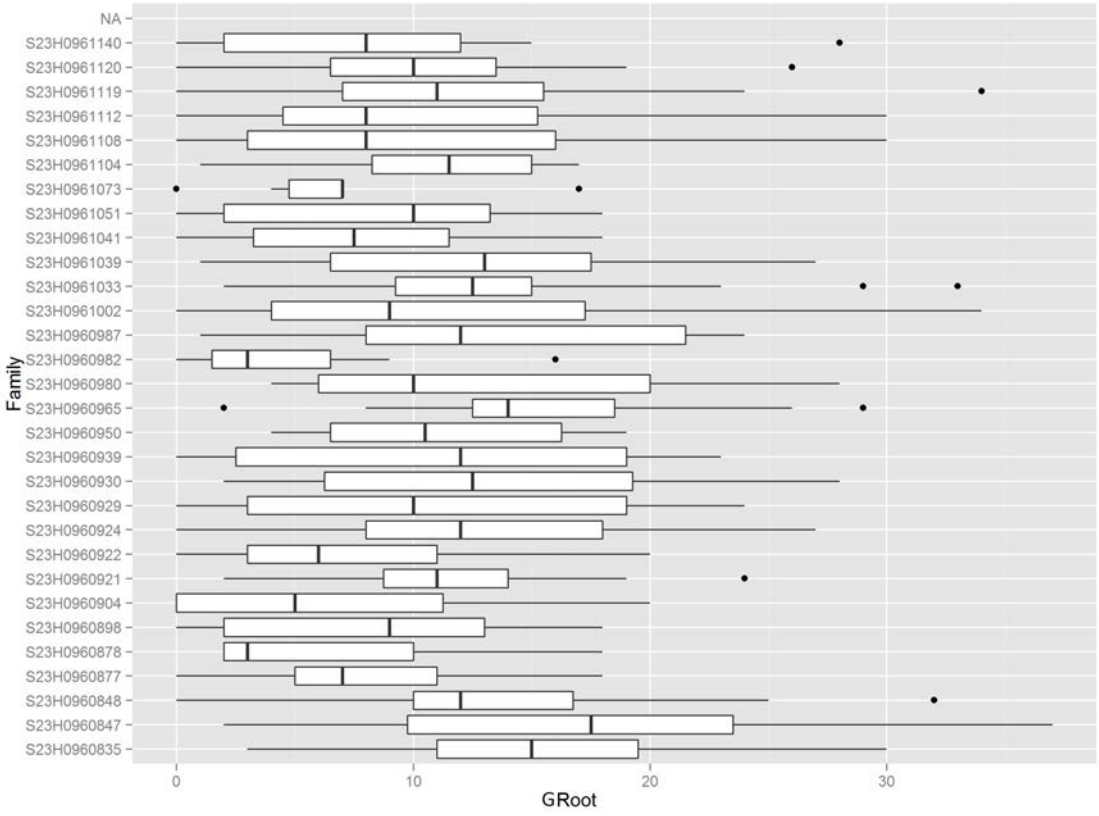

Figure 4: GRoot divided into families The dead cuttings are excluded.

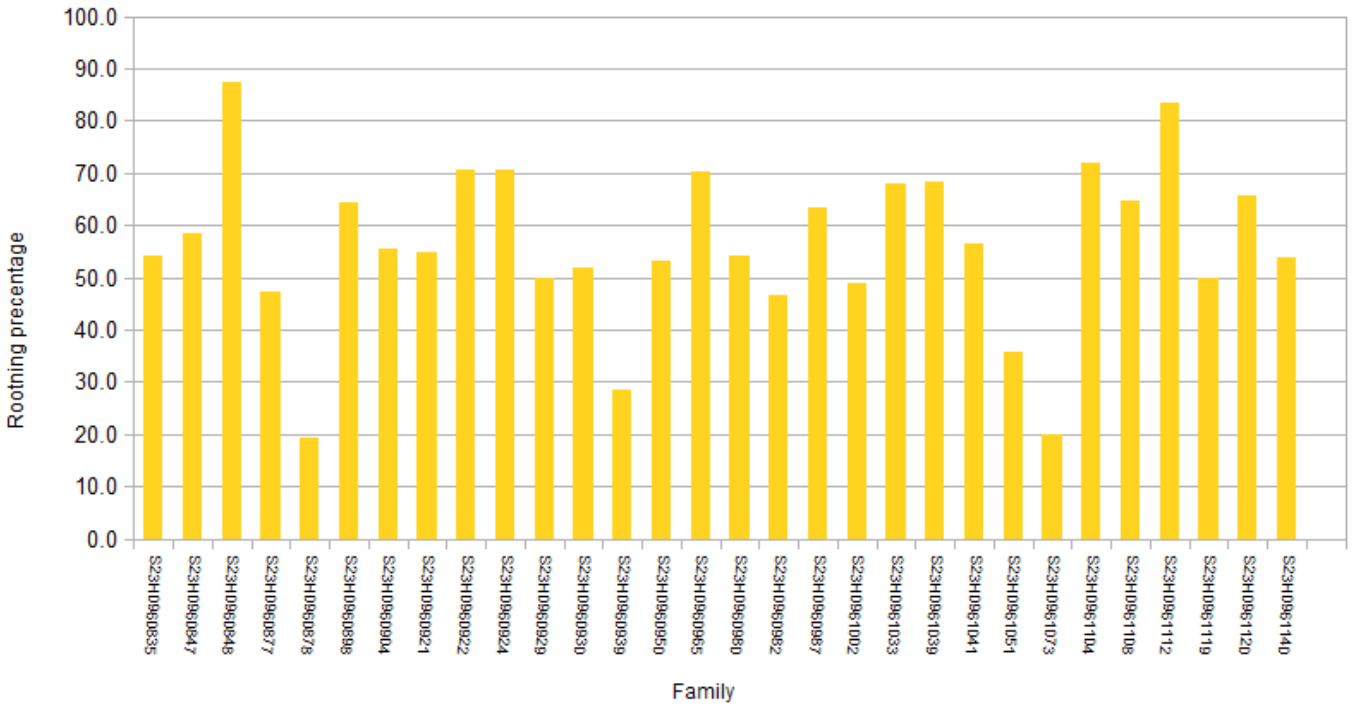

Figure 5: Rooting percentage The percentage of rooted cuttings per family. It ranged from 19.2% as the lowest to 87.5% as the highest.

3.1.2 Roots, shoots and growth

It was not only GRoot that differed between the families, but also GShoot2. The data used for the ANOVA with the GShoot2 were with the dead cuttings excluded. The test showed a significant difference in the GShoot2 between families at a 5% significance level (Df 29, p-value $1.1*10^{-7}$). A correlation between GShoot2 and GRoot could be found only when the dead cuttings were included (cor. 0.09533, p-value 0.0180), not when they were excluded (cor. 0.07757, p-value 0.0617). A correlation between GRoot and GGrowth could be found both when tested with and without the dead cuttings. All negative GGrowth was adjusted to zero. It was also tested if there was a correlation between GHeight3 and GGrowth, there was. Graphs on the correlations made without the dead cutting can be seen in figure 6. Correlations between clone means and between family means were also tested for all variables. Except for a correlation between GGrowth and GRoot with clone mean and dead cuttings included, no significant correlations were found (results not shown). All correlations were performed on the cuttings that were replanted.

3.1.3 Rooting in different data sets

The rooting in the C4-testing differed between 64.4% at the lowest and a 100% at the highest between the families. When tested with a χ^2-test if there were any difference between C4-

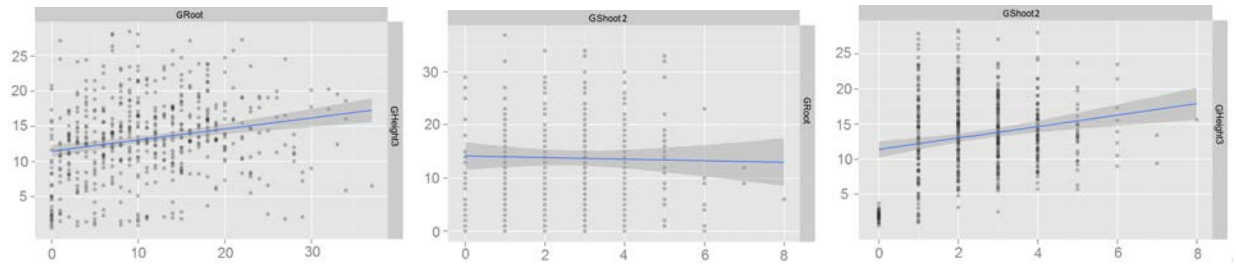

Figure 6: Correlation without dead cuttings The line represent a regression line and the grey field on both sides of the line represent the confidence interval. Each dot represents a cutting. Graph (a) show the correlation between the GRoot and GHeight3. The correlation had a coefficient of 0.2262 and a p-value<0.05 tested at a 5% significance level. Graph (b) is the correlation of GRoot and GShoot2 that is not significant at the 5% significance level (cor. 0.07757, p-value 0.0617) when the dead was excluded. Graph (c) show the correlation between GHeight3 and GShoot2 for each cutting. A significant relationship was found: cor. 0.2552 and p-value<0.05.

testing and the G-experiment, no significance was found at the 5% significance level. In the families from the C4-testing some extra clones, compared to the G-experiment, had been included to give a better estimate of family mean. A χ^2-test was also used to see if there were some families that differed from one another in rooting. Only two families got a significant result (Appendix, table 12). This means that only two families showed independent rooting results from one another, all the other rooted approximately the same in the G-experiment and in C4-testing. When the rooting from the G-experiment was compared with rooting data from the C4-comp, the χ^2-test was not significant at 5% level (table 4).

Table 4: χ^2-test of rooting The different data sets tested against one another. The test show that there is no difference in rooting between the different data sets.

Data 1	Data 2	χ^2	Df	p-value
G	C4-testing	0.236	1	0.627
G	C4-comp	0.602	1	0.438
C4-testing	C4-comp	1.082	1	0.298

3.1.4 Field experiment

In this F-experiment there were ten families. From plant height and rooting data collected earlier by Skogforsk and the height data collected during this summer (2013) several results were obtained. First, it was tested if there was a correlation between FRoot and the plant height. For all the different height measurements a significant correlation was found at 5% significance level with the Pearson's product-moment correlation test (table 5). The correlations were based only on the living cuttings.

The family data was used to see if there were any family differences in rooting and plant height. The ANOVA performed for the FRoot was significant at the 5% level. The ANOVA analysis was also significant for FHeight1, but with the other height measurements (FHeight2, FHeight3, FHeight4) it were not significant. The ANOVA results are shown in table 6. The range difference of the height between families is shown in figure 7.

Table 5: Pearson's product-moment correlation on FRoot vs. different heights The correlation is not strong, but significant for all of the different heights. The difference in degree of freedom is because between the measurements some of the cutting had died. CI = confidence interval.

Var.	Cor.	N	p-value	t	Df	CI
FHeight 1	0.1624	279	0.0065	2.74	277	0.04587, 0.2746
FHeight 2	0.157	270	0.0098	2.602	268	0.03831, 0.2713
FHeight 3	0.2174	266	$4*10^{-4}$	3.619	264	0.09974, 0.3291
FHeight 4	0.1749	266	0.0042	2.886	264	0.05577, 0.2891

Table 6: Family difference tested with ANOVA for the F-experiment The heights are chronologically arranged.

Variable	f-value	Df	p-value
FRoot	4.03	9	0.0002*
FHeight 1	3.32	9	0.0014*
FHeight 2	1.18	9	0.3142
FHeight 3	0.93	9	0.5068
FHeight 4	0.98	9	0.4638

3.1.5 Linear mixed effect models

Linear mixed effect models were constructed and tested for GGrowth and GShoot2. The best model for GGrowth and GShoot2 was the same: GGrowth/GShoot2 = GRoot + GDominance2 + Family + Clone $+\epsilon$. Different models were tested using a likelihood ratio test to see which gave the best explanation to the tested variable. GRoot and Dominance2 were fixed effects and Family and Clone were random effects. ϵ is the residual that account for all the variation, that none of the other parameters can explain. The Clone part explained 22.3% of the total variation and Family explained 8.0% of the total variation in the GGrowth model. GRoot affected GGrowth ($\chi^2(1)$=14.614, p=0.0001)by increasing with 0.10 cm (Sd=0.027) and GDominance2 affected GGrowth($\chi^2(1)$=21.746, p=$3.1*10^{-06}$) by increasing it 2.25 cm (Sd=0.48). In this model with GShoot2, the Clone part explained 14.8% of the total variation and Family explained 6.2% of the total variation. GRoot affected GShoot2 ($\chi^2(1)$=6.9985, p=0.008)by increasing it 0.2 shoot per root (Sd=0.14) and GDominance2 affected GShoot2 ($\chi^2(1)$=172.98, p=$2.2*10^{-16}$) by increasing it 0.4 if it was dominant (Sd=0.01). A summery of the models can be seen in table 7. Analysis on the genetic variance was performed on all

Table 7: Linear mixed effect models Two different models that has been tested and fit by maximum likelihood. One model per row is described, with the predicted variable to the left. The data used for the GGrowth was from the final measurement and cuttings without shoots has been excluded. The data for the GShoot2 are from the final measurement and dead cuttings excluded.

	Fixed effect estimate			Random effect variance		
Predictor	GRoot	GDominance2	Intercept	Family	Clone	ϵ
GGrowth	0.10	2.25	7.96	1.81	5.09	15.86
GShoot2	0.02	0.04	2.19	0.12	0.28	1.48

height measurements for both the G-, F-experiment and C2-field using mixed linear effect models fitted with maximum likelihood. To the C2-field both the full data set and a smaller

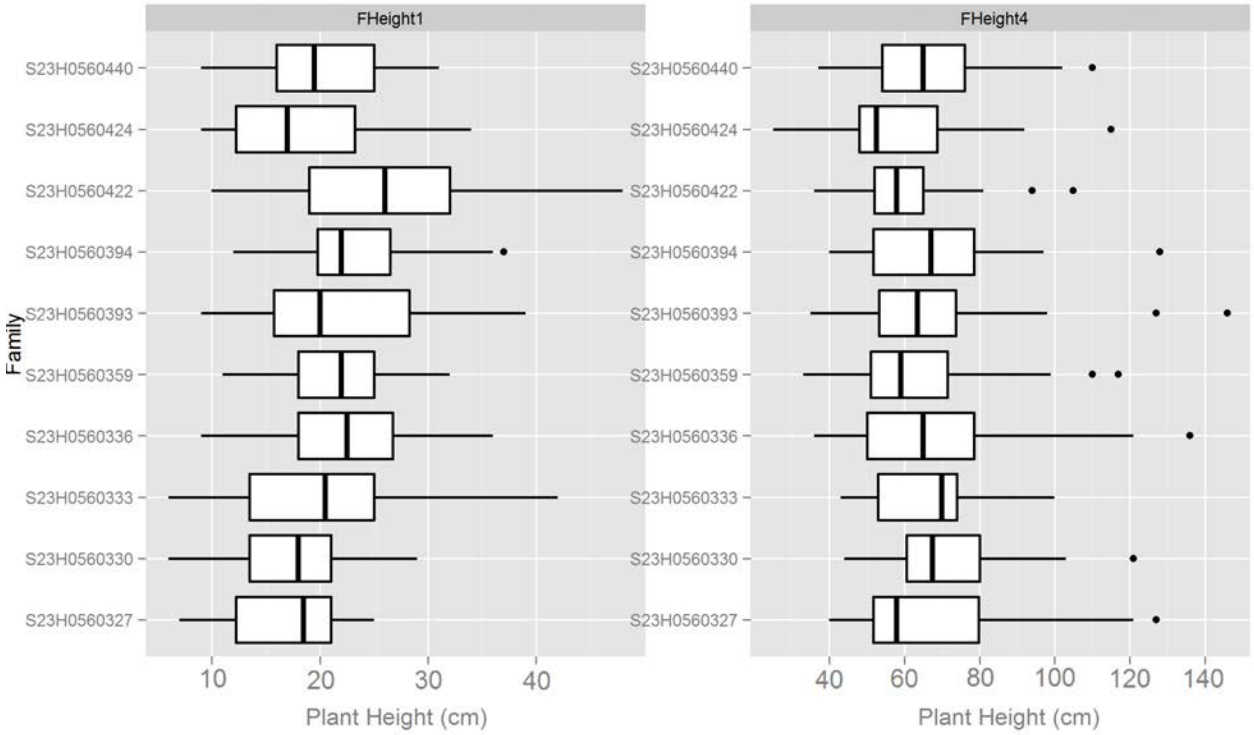

Figure 7: F-experiment plant height Results from the first and the last of the height measurements. The first measurement was taken before replanting to larger pots (FHeight1) and the last height measurement (FHeight4) was from the summer of 2013.

proportion containing the same families as in the F-experiment. The the genetic variance was divided into a family part and a clone part. The significance of the family and clone part in the model were tested with likelihood ratio test by comparing a null model (with either family or clone) against the full model (both family and clone included). For the G-experiment the the clone part was significant for all heights, but not the family part. For the F-experiment there were significance for clone in FHeight1 and FHeight2 only. When looking at C2-field complete data, both family and clone were significant for all heights. After sorting out only the same families that was used in the F-experiment from the C2-field data, significance could not be found for any height. The result of the likelihood ratio test can be seen in Appendix, tables 14, 15 and 16.

3.2 Optimal hormonal treatment

The question asked when testing the different hormones was which treatment that is best for the quick dip method. When comparing the rooting with a Fisher's exact test it became apparent that the rooting was concentration dependent, rather than depending on the hormone type. Those treatments that significantly differed from the control in rooting were IBA 8000ppm, NAA 1000ppm and IBA talc. When comparing against IBA 8000ppm (Skogsforsks standard), only NAA 8000ppm was significantly different. Figure 8 shows the rooting percentage of the treatments and it revealed NAA 1000ppm and IBA talc to induce

the strongest rooting. There were statistically significant differences in the number of roots

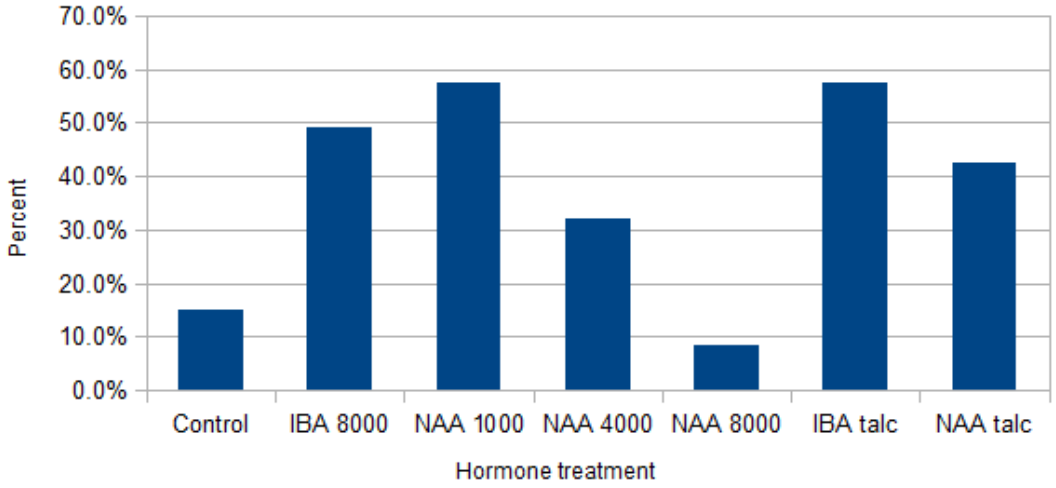

Figure 8: H-experiment rooting Rooting percentage built HRootB. The differences is large between the treatments from 8.5% as the lowest up to 57.4% as the highest. NAA stand for Naphthalene acetic acid and IBA stands for Indole-3-butyric acid. The number after the abbreviations is the concentration in ppm and the talc has the concentration 4000 ppm of hormone.

between the different hormone treatments. The ANOVA performed on all the cuttings gave the p-value $8.72*10^{-8}$ and the ANOVA performed on the same data but with non rooted cuttings excluded, gave the p-value of 0.0057 at the 5% significance level. The mean and standard deviation for each treatment on the HRootB is shown in table 8. To see where the differences where, t-tests were performed on all combinations of treatments and both with and without unrooted cuttings in the data. Those that did not significantly differ from IBA 8000 were NAA 1000 and NAA 4000 in both calculations. The two talc treatments did not differ from each other. The mean value of HRootB can be seen in figure 9 and the complete table with all the t-test results is shown in the appendix, table 13.

Table 8: HRootB per hormonal treatment Marked with [a] are from data where the cuttings without roots have been excluded. Concentration of hormone is after the abbreviation and the talc has a hormone concentration of 4000 ppm.

	All	Control	IBA 8000	NAA 1000	NAA 4000	NAA 8000	IBA talc	NAA talc
Mean	4.15	1.26	7.47	7.30	4.62	0.68	3.66	4.09
Std	7.62	3.41	9.62	9.35	9.18	3.23	6.45	6.57
Mean[a]	4.24	5.90	15.26	12.70	14.47	8.00	6.37	9.60
Std[a]	8.90	5.38	8.31	9.13	11.15	9.06	7.47	6.98

3.3 Divided shoot experiment

The χ^2-test(Df 1, p-value 0.157) of rooting data at 5% significance level showed that no difference in rooting between the top and the bottom cutting could found statistically. The t-test on DGrowth, DShoot2 and DRoot comparing the top and the bottom, were all significant

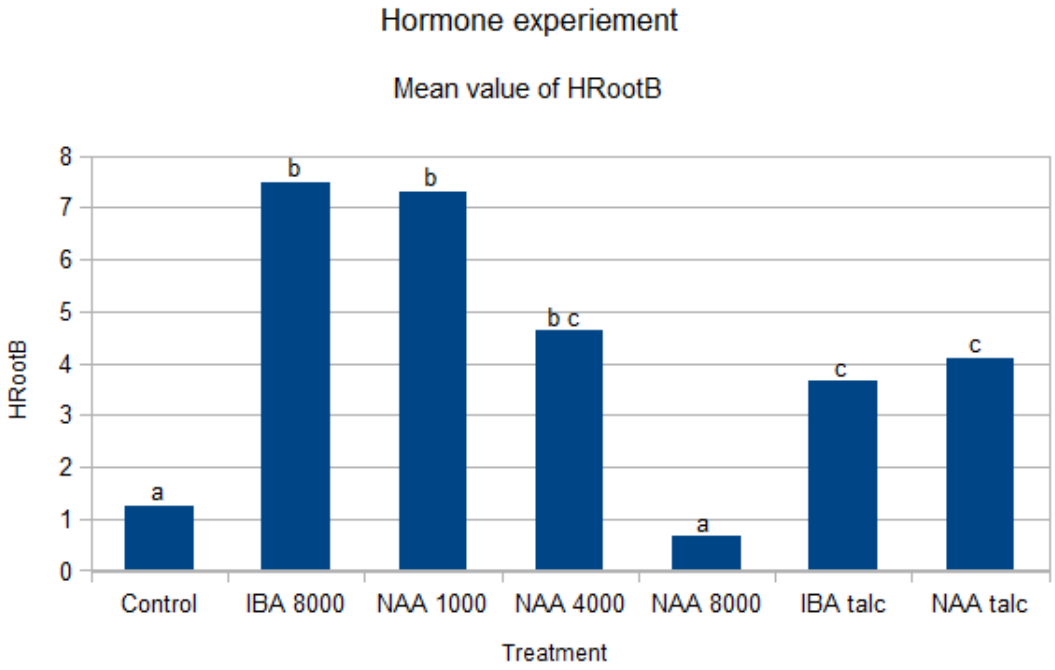

Figure 9: The mean value of HRootB per treatment All cuttings included. The letters on top represent which treatment that are not significantly different form each other.

at 5% level (table 9). The top cuttings showed higher mean values for all of these three variables. The DRoot data was from the first measurement and DShoot2 from the last measurement.

Table 9: T-test results of the D-experiment The top part has a higher mean for all the variables. Superscripts: a the dead cuttings excluded, b dead cuttings included from that measurement, c dead cuttings excluded and those that had not sprouted

	Top		Bottom				
Variabel	Mean	Std	Mean	Std	t-value	df	p-value
DRoot[a]	13.64	7.65	9.70	6.95	-2.612	91.90	0.010515
DRoot[b]	6.69	7.27	4.19	6.38	-2.312	188.87	0.021866
DShoot2[b]	3.04	2.28	2.09	1,58	2.299	82.59	0.024023
DGrowth[c]	8.38	3.48	6.05	2.83	-3.210	73.73	0.001968

3.4 Rooting and growth of needle fascicles

At the first evaluation, only 5 of 120 needle fascicles had developed roots. These needle fascicles were all from the water bath treatment. Four out of the five that developed roots had long roots. In the sand treatment none of the needle fascicles survived. At the final evaluation, only one had developed a shoot (figure 10 and it was one of those with roots. None of the hypotheses were tested because there were too few survivors of the needle fascicles.

Figure 10: The only sprouted needle fascicle

4 Discussion

4.1 Genetic variation in rooting and early growth

In this study a difference in the formation of adventitious roots between the families was observed. It was more than 68 percentage units difference between the lowest and the highest family in the G-experiment (figure 5). But there were also a large variation of rooting between clones within a particular families. In the C4-testing data set difference between the families could also be found. To this it can be added that in the F-experiment, there were also differences in rooting between families. All tested data sets in this thesis suggest a genetic difference in rooting ability. Other studies have also shown family differences in rooting. Foster (1990) for example, found that differences were mostly inherited additively in loblolly pine (*Pinus taeda*). Like in this study, he also used full-sib families, but he had larger experimental set-up and used the same genetic material in all his three replicates. In this study two groups of families has been used instead. The calculated broad sense heritability for the G-experiment here was 0.28 for rooting. Foster (1990) had a broad sense heritability of 0.13. Both heritabilities are low.

The correlation between GShoot2 and GRoot on individual cuttings was weak in the G-experiment (figure 6). A significant correlation was found only when dead cuttings were excluded form the data. An explanation for this is that also the dead cuttings had many roots, but no shoots. The GShoot2 is probably also affected by the number of buds that the cutting had in the beginning. A significant correlation between GHeight3 and GRoot was found in the G-experiment, both with and without dead cuttings. When the correlation between the different height measurements and FRoot were tested for the F-experiment it were significant, but low for all heights (table 5). In the F-experiment the correlation were calculated only with the living cuttings. The difference between families in height went from significant to non significant between FHeight1 and FHeight2 in the F-experiment, when tested with a ANOVA. This suggest that even if the rooting is family related and the rooting and the early growth is correlated, there may not be a relationship between the the first years growth in the field and the family. When the family and clone part were tested, if significant in different models for height measurements, the result were very different between the experiments. It became clear that the size of the data set may affect the result. In the largest data set (C2-field) significance for clone and family were found for all heights, but when the data set was reduced to fewer families no significance was found. It would be interesting to have the G-experiment in the field together with existing one, to see if the effects of difference in rooting that remains after 5-10 years. It is expected from the result in this study, that the differences in growth linked to the rooting will become undetectable.

Even if the correlation between GRoot and GShoot2 was weak, the GRoot strengthen the linear mixed effect model for the GShoot2. Interestingly the GDominance2 was an important factor in the model too. It would had been easy to think that if there was dominance of one shoot, there would be fewer shoots in total. This is because a dominant shoot often inhibit lateral shoot development(Raven et al. 2005). In the best model for the GGrowth, GDominance2 was the most important of the fixed effects. If the cutting has a dominant

shoot it is logic that it will be higher than a cutting with no dominant shoot. A cutting without a dominant shoot would put the resources into several shoots instead.

When tested, there was no significant difference in rooting between the C4-testing and the G-experiment cuttings. Even so, the C4-testing cuttings tended to exhibit a slightly better rooting. Interestingly when the families were tested against each another, only two families showed a significant difference in rooting. This can be due to mass significance, because many tests were performed. This result can also be because the cuttings used in the G-experiment were leftovers from the breeding program. Unconsciously people may choose the best looking cuttings first, therefore a lesser number of good cuttings may have remained. The fact that there was no difference between the G-experiment and the C4-comp, suggests that the difference in propagation method has no influence on rooting in this study.

4.2 Optimal hormonal treatment

In the H-experiment it became apparent that IBA 8000, NAA1000 and IBA talc showed better results with respect to rooting compared with control cuttings. When comparing the rooting with IBA 8000, that is used as a standard, only NAA 8000 rooted significantly different. If looking at the rooting percentage NAA 8000 rooted the least of all treatments, this is probably due to the high concentration. A too high concentration maybe contra-productive as was found by Grönroos and von Arnold (1987) when testing IBA concentration on lodgepole pine hypocotyls. From the ANOVA we can see that there are differences in the number of roots (HRootB) produced by the different treatments. The average number of roots were highest for IBA 8000 and NAA 1000 when all cuttings are counted. This makes them among the better hormone solutions tested. When looking at both the result from the rooting and the HRootB, both IBA 8000 and NAA 1000 are among the best of the tested treatments. The talc treatments were not evidently worse when it came to the formation of roots, but they were more difficult to handle. It had to be handled in a fume hood for safety. The hormone solved in ethanol could be used outside the fume hood on a bench top and only protective gloves were necessary for protection. Therefore it will not be recommended to use the hormones in solid state even if they gave good results. The experiment needs to be performed a second time with lower concentrations of NAA. It cannot be ruled out that the optimal concentration of NAA was not used, because it was most effective in the lowest concentration tested. A combination of both IBA and NAA together could also be useful to test in the future, because Bowen et al. (1975) obtained good result with the combination on lodgepole pine cuttings. They used a 50:50 mixture but I would suggest to take a the optimal concentration for each of IBA and NAA and lower it slightly. This because it has been shown that IBA and NAA induce optimal rooting at different concentrations(Bowen et al. 1975).

4.3 Divided shoots experiment

The top and the bottom cuttings developed roots at a similar rate. In the DRoot, DShoots and DGrowth, the top part has a higher average. The bottom part may not grow as well as the top part but it was not growing badly. A continued testing over the first years in the field would give a better picture. It may happen that after a few years, there is no clear differences in performance. Not having a developed bud may explain why the bottom part grew less well. Except from the obvious that the bud is already a non-sprouted shoot. In the bud exists a shoot apical meristem, that is one of the primary producer of auxin. Auxin is important for many developmental processes in the plant(Raven et al. 2005). To help the bottom cutting it is possible, but maybe not practical, to pre-treat the needle fascicles with cytokinin before harvest of the shoot. This treatment would make the needle fascicles develop into shoots(Browne et al. 2001). Cytokinin is also an inhibitor to root development, so there has to be time between the treatment and the harvest of the shoots(Raven et al. 2005).

4.4 Rooting and growth of needle fascicles

The rooting of needle fascicles was in this study not very successful, with only five out of 120 rooted in the water bath and none in the sand. Larsen and Dingle (1969) had higher numbers of rooted needle fascicles with *Pinus contorta*. They tested several more different factors that influence the rooting and they also had problems with making the rooted needle fascicles develop shoots. In this study, the only rooting media that lead to successful root growth was water. Interestingly all five needle fascicles that formed roots were situated close to the air pump. If it was the higher flow of oxygen or water is impossible to say without further experiments. Some other studies suggest that the status of meristem in the needle fascicle at harvest, is important for the ability to form shoots (Giroard 1971). In this experiment the application of 6-Benzylaminopurine (BAP) was to improve the formation of shoots. If it worked as intended or not could not be tested. The needle fascicle that formed a shoot may have done so without BAP. It is known that BAP can promote shoot development of needle fascicles, when used prior to harvest of the needles fascicles (Browne et al. 2001). So a pretreatment may give more needle fascicles that form shoots. Four out of five needle fascicles that developed roots had long roots. This suggest that keeping the experiment on longer would probably not have improved the number of rooted needle fascicles. Using the water bath had a clear advantage over propagating the needle fascicles in any substrate. It could easily be seen when they formed roots. To quickly replant the needle fascicles shortly after they have formed roots, may improve their chances to form shoots. This is because the needle fascicles would get access to new nutrients, while still having some reserves left in them.

4.5 Conclusions

There were genetic differences in the formation of adventitious roots and it affects the early growth. If the growth effect remains after some years in the field is still uncertain. Skogforsk

is already using one of the better hormone solutions that was tested here. Additional testing may give a more optimal hormone solution to use. Using the hormones in solid state was ruled out, because of practical reasons. The problem with the ortets producing different amount of cuttings, appears to be solvable by dividing long shoots. Some further testing is required to see if the difference in performance between top and bottom part of the shoot evens out with time. The needle fascicles experiment failed to give a solution to the problem with the ortets producing different amount of shoots.

5 Acknowledgements

First I wish to thank everyone at Skogforsk in Sävar for all the help and support that has been given me. For the help with the analysis I wish to thank Johan Kroon, my supervisor, and Erika Åhlander. I wish to thank my supervisors, opponents and friends for their help with the report.

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A Appendix

A.1 Statistical tests results

Table 10: T-test with the extra cuttings. Cold stored are the extra and the frozen are the leftover from the C4-testing. The first table if for the D-experiment's data and the second is for the H-experiment on the HRootB data. As can be seen there are no significant difference between the two types of cuttings.

Variable	Storage 1	Storage 2	t-value	Df	95%CI Lower	95%CI Upper	p-value
DRoot+dead	Cold	Frozen	0.170	98.25	-2.307178	2.740512	0.8650798
DShoot2-dead	Cold	Frozen	1.323	42.29	-0.3147179	1.512579	0.1930566

Treatment	t-value	df	95% CI lower	95% CI upper	p-value
Control	1.454991	45.00	-0.2586766	1.604997	0.1526124
IBA 8000	-0.275719	19.44	-8.245154	6.323077	0.7856728
NAA 1000	-1.089174	16.96	-11.60356	3.703123	0.2913143
NAA 4000	-2.12555	14.83	-15.76991	0.0296527	0.05076035
NAA 8000	32	1.452016	-0.3906238	2.330018	0.156232
IBA talc	22.96	-0.4160408	-5.326299	3.542749	0.6812429
NAA talc	18.48	-0.7859218	-7.018888	3.192048	0.4418755

Table 11: χ^2-test on D-experiment data. The the marked C4 cutting against the extra cuttings

Column	Row	Df	χ^2	p-value
Rooted/unrooted	Cold/frozen	1	1.451	1.451

Table 12: χ^2-test on families The rooting of the G-experiment against the C4-testing.

Family	χ^2	DF	p-value	est.	Lower (2.5%)	Upper (97.5%)
S23H0960835	0.58	1	0.446	0.129	0	0.459
S23H0960847	3.017	1	0.082	0.228	0	0.485
S23H0960848	0	1	1	0	0	1
S23H0960877	3.441	1	0.064	0.314	0	0.645
S23H0960878	1.418	1	0.234	0.234	0	0.618
S23H0960898	2.593	1	0.107	0.304	0	0.675
S23H0960904	0.831	1	0.362	0.175	0	0.552
S23H0960921	0.492	1	0.483	0.108	0	0.41
S23H0960922						
S23H0960924	2.443	1	0.118	0.268	0	0.604
S23H0960929	0.269	1	0.604	0.092	0	0.434
S23H0960930	0.453	1	0.501	0.125	0	0.487
S23H0960939	4.103	1	0.043*	0.32	0	0.63
S23H0960950	1.489	1	0.222	0.223	0	0.581
S23H0960965	0.437	1	0.508	0.127	0	0.503
S23H0960980	1.233	1	0.267	0.227	0	0.627
S23H0960982	0.238	1	0.626	0.089	0	0.441
S23H0960987	0.616	1	0.433	0.18	0	0.629
S23H0961002	0.611	1	0.435	0.112	0	0.391
S23H0961033	1.02	1	0.312	0.191	0	0.561
S23H0961039						
S23H0961041	1.359	1	0.244	0.243	0	0.652
S23H0961051	7.958	1	0.005*	0.435	0.133	0.738
S23H0961073	0.26	1	0.61	0.102	0	0.489
S23H0961104						
S23H0961108	3.133	1	0.077	0.318	0	0.67
S23H0961112	0.15	1	0.699	0.079	0	0.467
S23H0961119	2.136	1	0.144	0.228	0	0.534
S23H0961120	0.102	1	0.749	0.043	0	0.292
S23H0961140	1.706	1	0.191	0.256	0	0.641

Table 13: T-test on the HRootB data from the H-Experiment The first table is with all cuttings counted and the second with only rooted cuttings.

Treatment 1	Treatment 2	df	t-value	95%CI lower	95%CI upper	p-value
Control	IBA 8000	57.39	-4.1745	-9.1925	-3.2330	0.0001
Control	NAA 1000	58.02	-4.1622	-8.9486	-3.1365	0.0001
Control	NAA 4000	58.45	-2.3527	-6.2214	-0.5020	0.0220
Control	NAA 8000	91.73	0.8384	-0.7865	1.9354	0.4040
Control	IBA talc	69.82	-2.2578	-4.5281	-0.2804	0.0271
Control	NAA talc	69.11	-2.6208	-4.9838	-0.6758	0.0108
IBA 8000	NAA 1000	91.93	0.0870	-3.7155	4.0559	0.9309
IBA 8000	NAA 4000	91.81	1.4700	-1.0011	6.7032	0.1450
IBA 8000	NAA 8000	56.26	4.5868	3.8233	9.7512	$2.56*10^{-05}$
IBA 8000	IBA talc	80.46	2.2544	0.4469	7.1702	0.0269
IBA 8000	NAA talc	81.26	1.9914	0.0031	6.7629	0.0498
NAA 1000	NAA 4000	91.97	1.4024	-1.1159	6.4776	0.1642
NAA 1000	NAA 8000	56.83	4.5855	3.7272	9.5068	$2.54*10^{-05}$
NAA 1000	IBA talc	81.73	-2.1953	-6.9354	-0.3412	0.0310
NAA 1000	NAA talc	82.52	1.9273	-0.1030	6.5285	0.0574
NAA 4000	NAA 8000	57.22	2.7720	1.0929	6.7794	0.0075
NAA 4000	IBA talc	82.54	-0.5848	-4.2142	2.2993	0.5603
NAA 4000	NAA talc	83.32	0.3230	-2.7437	3.8076	0.7475
NAA 8000	IBA talc	67.69	2.8291	0.8775	5.0799	0.0061
NAA 8000	NAA talc	67.02	-3.1876	-5.5359	-1.2726	0.0022
IBA talc	NAA talc	91.97	-0.3167	-3.0938	2.2427	0.7522

Treatment 1	Treatment 2	df	t-value	95%CI lower	95%CI upper	p-value
Control	IBA 8000	25.92	-3.8539	-14.3544	-4.3673	0.0007
Control	NAA 1000	27.56	-2.7807	-11.8193	-1.7881	0.0097
Control	NAA 4000	21.43	-2.5606	-15.5158	-1.6176	0.0180
Control	NAA 8000	3.88	-0.4341	-15.6921	11.4921	0.6872
Control	IBA talc	22.45	-0.2111	-5.0860	4.1452	0.8347
Control	NAA talc	22.85	-1.6015	-8.4811	1.0811	0.1230
IBA 8000	NAA 1000	46.17	1.4253	-1.3914	8.1439	0.1608
IBA 8000	NAA 4000	23.97	0.2363	-6.1426	7.7310	0.8152
IBA 8000	NAA 8000	3.93	1.4978	-6.2922	20.8140	0.2098
IBA 8000	IBA talc	44.77	3.9497	4.3562	13.4248	0.0003
IBA 8000	NAA talc	40.96	2.4271	0.9505	10.3713	0.0197
NAA 1000	NAA 4000	22.93	-0.7819	-9.4143	4.2502	0.4422
NAA 1000	NAA 8000	3.81	0.8081	-9.7365	17.5057	0.4665
NAA 1000	IBA talc	50.07	-2.5500	-9.8575	-1.1710	0.01388
NAA 1000	NAA talc	43.53	1.0167	-2.2454	6.8146	0.3149
NAA 4000	NAA 8000	5.72	1.2051	-6.8220	19.7554	0.2757
NAA 4000	IBA talc	21.14	-2.5154	-14.7873	-1.4053	0.0200
NAA 4000	NAA talc	22.04	1.4855	-1.9269	11.6602	0.1516
NAA 8000	IBA talc	3.63	-0.3431	-15.3648	12.1055	0.7505
NAA 8000	NAA talc	3.75	-0.3341	-15.2579	12.0579	0.7562
IBA talc	NAA talc	42.52	-1.5216	-7.5114	1.0521	0.1355

Table 14: Results from the likelihood ratio test of the G-experiment

	Family	Clone
GHeight1	0.06, 0.8036	72.02, $2.2*10^{-16}$
GHeight2	2.35, 0.125	43.76, $3.717*10^{-11}$
GHeight3	0.13, 0.7221	59.28, $1.366*10^{-14}$

Table 15: Results from the likelihood ratio test of the F-experiment

	Family	Clone
FHeight1	0.12, 0.734	17.67, $2.63*10^{-05}$
FHeight2	0, 0.9995	11.75, 0.0006
FHeight3	0.76, 0.382	0.7645, 0.382
FHeight4	0, 1	0, 1

Table 16: Results from the likelihood ratio test of C2-field The table contains the result from both form when the complete data set was tested and the reduced. The reduced contain only the families that is the same as in the F-experiment.

	Full data set		Reduced data set	
	Family	Clone	Family	Clone
FHeight2	41.84, $9.884*10^{-11}$	17.85, $2.395*10^{-05}$	1.11, 0.293	0, 1
FHeight3	24.18, $8.762*10^{-07}$	4.02, 0.045	0, 1	0, 1
FHeight4	16.12, $5.94*10^{-05}$	6.58, 0.010	0, 1	0, 1

Table 17: Variable explanation the G-experiment

Variable	Unit	Measured	Explanation
GRoot	Count	Week 15	Number of roots seen on outside the substrate.
GHeight1	Centimetre	Week 15	The height of the cutting, measured from the substrate to the top.
GHeight2	Centimetre	Week 25	The height of the cutting, measured from the substrate to the top.
GHeight3	Centimetre	Week 29	The height of the cutting, measured from the substrate to the top.
GDominance1	Scored	Week 25	One shoot clearly dominant over the others scored 1, no dominant shoot scored 0.
GDominance2	Scored	Week 29	One shoot clearly dominant over the others scored 1, no dominant shoot scored 0.
GShoot1	Count	Week 25	Number of shoots were counted.
GShoot2	Count	Week 29	Number of shoots were counted.
GGrowth	Centimetre	Calculated	GHeigth3 - GHeight1 = GGrowth

Table 18: Variable explanation for the F-experiment

Variable	Unit	Measured	Explanation
FRoot	Count	Before replanting	Number of roots seen on outside the substrate.
FHeight1	Centimetre	Before replanting	The height of the cutting, measured from the substrate to the top.
FHeight2	Centimetre	The autumn it was planted in the field	The height of the cutting, measured from the ground to the top.
FHeight3	Centimetre	Week 28	The height of the plant was measured from the ground to the beginning of this years shoot.
FHeight4	Centimetre	Week 28	The height of the plant was measured from the ground to the top of this years shoot.

Table 19: Variable explanation for the D-experiment

Variable	Unit	Measured	Explanation
DRoot	Count	Week 17	Number of roots seen on outside the substrate.
DHeight1	Centimetre	Week 17	The height of the cutting, measured from the substrate to the top.
DHeight2	Centimetre	Week 25	The height of the cutting, measured from the substrate to the top.
DHeight3	Centimetre	Week 29	The height of the cutting, measured from the substrate to the top.
DDominance1	Scored	Week 25	One shoot clearly dominant over the others scored 1, no dominant shoot scored 0.
DDominance2	Scored	Week 29	One shoot clearly dominant over the others scored 1, no dominant shoot scored 0.
DShoot1	Count	Week 25	Number of shoots were counted.
DShoot2	Count	Week 29	Number of shoots were counted.
DGrowth	Centimetre	Calculated	DHeigth3 - DHeight1 = DGrowth

Table 20: Variable explanation for the Hormone experiment

Variable	Unit	Measured	Explanation
HRootA	Count	Week 15	Roots on the outside of the substrate counted.
HRootB	Count	Week 15	The true number of roots longer than 2 mm counted.