Self-pollination – Is it negative to have yourself as a neighbor?

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SKOGFORSK

Sammanfattning

I det här projektet har 10 av 67 kloner från fröplantagen T12 I Gnarp, Sverige, valts ut för självpollineringsstudier. Avsikten med detta är att undersöka om utplaceringen av klonerna har en effekt på graden av självpollinering, där klonerna i denna studie antingen växer på rad med kopior från samma klon eller i en blandning av kopior från alla 67 kloner. För att undersöka effekten på graden av självpollinering har frövikt, antal frön per kotte, groningstid, groningsprocent, groningsenergi, antalet kotyledonbarr per fröplanta samt höjden på fröplantorna studerats.

Tio kopior valdes ut från var och en av de tio klonerna, där fem kopior växte i en rad med kopior av samma klon och de resterande fem kopiorna växte i en blandning av kopior av alla andra kloner. Signifikanta skillnader hittades mellan de olika klonerna och även mellan de olika positionerna när det gällde frövikt och antal matade frön per kotte. Vid plantering av klonkopior i rad innebär detta 12 % färre matade frön per skördad kotte med ökad självpollinering som trolig förklaring. För en hög fröproduktion är det därför fördelaktigt att plantera kopior i mix bland andra kloner. Om det finns skillnader mellan positionerna i ökad abortering av kott har ej studerats, men en ökad aborteringsfrekvens i klonrader är trolig. Någon skillnad i graden av inavlat frö i det skördade matade fröet kunde inte påvisas i groningsanalyser och vid odling. Att ställa kloner på rad kan underlätta t.ex. vid särplockning och om man vill ersätta en klon men en annan. Det är därför upp till plantageägaren att väga kostnaden mot nyttan. Den signifikanta skillnaden mellan klonerna antyder att några av klonerna är mer mottagliga för självpollinering än andra och det kan således vara en god idé att ta hänsyn till vilka kloner man väljer att ha med i sitt fröplantage för att minska sannolikheten för inavel.

Summary

In this project, 10 of 67 clones from the seed orchard T12 in Gnarp, Sweden, have been selected for an self-pollination studies. The intention is to investigate if the positioning of the copies in clonal rows or in clonal mix with other clones have an effect on the level of self-pollination by studying the seed weight; seed numbers per cone; germination time, percentage and energy; cotyledon needle numbers, as well as seedling height.

Ten copies from each of the 10 clones were selected for this study, where five of each copy grew in a long row with copies of the same clone (20-83 in a row) and the remaining five copies grew in a mixture of all 67 clones in the orchard. Significant differences was found between the different clones, indicating that some of the clones are more susceptible to self-pollination than others. A significant difference between the seed weight and the number of filled seeds per cone was found as well. For a seed orchard, this mean that in order to reduce the probability of inbreeding, the copies should be positioned with other clones, since the row position tend to have a 12% less filled seeds per harvested cone with an increase in self-pollination as a plausible explanation. Due to this it is more favorable to position the clones in a mixture with other clones in order to gain a high seed production. If there are differences between the positions in an increase of cone abortion have not been studied, but an increase in the abortion rate in clonal rows is probable. Any differences in the degree of inbred seeds in

the harvested filled seed could not be detected in the germination and seedling analysis. To position clones in a row could however facilitate i.e. when picking the cones clone wise and if one clone is to be replaced with another. Therefore it is up to the orchard owner to weigh the cost against the utility. The significant differences between the clones suggest that some of the clones are more susceptible to self-pollination than others, hence it can be a good idea to consider which clones to use in order to reduce inbreeding in the seed orchard.

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Introduction

Purpose and goals

The aim of this project has been to study if there is a difference in the degree of self-pollination in Scots pine (*Pinus sylvestris L.*) clones grown in rows and grown in mixtures of other clones, i.e. investigate if it brought a negative effect in having yourself as a neighbor. This has been done by performing different analyses and the results have been analyzed and compared between the different growth conditions as well as interpreted with the knowledge about seed formation and seedling development gained from literature.

Background

Seed orchards, such as the grafted clonal seed orchards, can be seen as a way for mass propagation of seeds for Scots pine and Norway spruce, which are the major species in Sweden [1] and as such, the orchards can have a central role in reforestation programs [2]. In year 2013, about 80% of all planted trees had their origin in seed orchards [3].

There are however some problems when it comes to seed orchards. Examples of these problems can be pollen contamination and inbreeding, affecting the seed production and quality in a negative manner [1].

Scots pine (*Pinus sylvestris L.*) bloom from May to June, depending on where the tree is located and the origin. As a protection mechanism from self-pollination, the female flower is receptive for pollen a couple of days up to a week before the first male flowers are ready to release its pollen. An additional protection mechanism is that the female flower is positioned at the upper part of the tree crown, while the male flower resides on the lower parts of the crown. It takes about three years from the initiation of the flowers to a mature cone releasing the seeds. The delay is partially caused by the fact that when the pollen tube has grown for a while, it stops and the entire cone enters senescence. The pollen tube continues to grow the following spring and the fertilization occurs in the early summer. The seeds and the cone are developed during the summer after fertilization. For a pine cone to be able to develop in a normal manner, several of the embryos should be fertilized, or the cone can be aborted [4].

Seed quality and quantity is a measure of several traits, such as if the seeds are filled, partially filled or aborted, and the quality can be reduced at any time of the developmental period, which is a 16 months-long-period from the pollination to a mature seed. Developmental studies in pine have shown that self-pollination often results in ovule abortion at various developmental stages. Most often the abortion appears to occur the first two weeks after the fertilization and this leaves the megaspore wall as a collapsed brown sac. These “empty seeds”, tends to look like seeds but do not contain any solid storage products or embryos [5].

The seed yield might decrease over time in the case of extreme inbreeding compared to hybridization. This is believed to be due to the duplication of recessive lethal genes causing the seed to abort [6].

Inbred seeds which are not aborted tend to germinate to a lesser degree compared to cross-pollinated seeds. They also have a lower germination energy, which means that they have a slower growth and will produce smaller seedlings. The seedlings brought up from inbred seeds have in turn a higher mortality, slower growth and will most likely be more damaged by pests and deceases after plantation in the forest compared with non-inbred seedlings [1].

It is also likely to believe that as a result of inbreeding and reason for the lower and slower germination with the inbred seeds could be that the seeds have not developed properly, where either the endosperm or the embryo is in a less developed state and hence affect the germination percentage [7].

Objective

In this project, 10 copies from 10 different clones from the seed orchard T12 in Gnarp has been chosen for an analysis of the seeds and seedlings, where the purpose is to investigate the possibility of a higher amount of inbreeding in copies of the same clone growing next to each other in long clonal rows compared to copies of the same clones grown in a mixture with other clones. If a significant different could be suggested from the results, this knowledge could be used to see if a high level of inbreeding is to be expected when using seeds from orchards with the copies growing in clonal rows, as well as facilitate the organization of the planting of trees in new seed orchards. In this study the number of filled and empty seeds derived from the cones of each copy has been calculated and weighed, anatomical potential have been estimated, germination and a seedling analysis have been performed and from the gained knowledge from the calculations and the analysis a possible difference between the two different growth positions has been evaluated.

Materials and Methods

The seed orchard

The Scots pine seed orchard FP-620 Gnarp in northern Sweden was selected for this study. This orchard was first established in 1988 and was completed in 1994. The orchard includes 8481 copies of 67 clones planted in 7 x 2.5 m spacing and covers an area of 21 ha [8]. The 67 clones were selected among 322 phenotypically chosen plustrees based on the results from a freezing test [9 & 10]. In every second row in the orchard the trees are planted in clonal rows, in row between the trees are randomly mixed with no clones next to each other.

Ten of the 67 clones were selected (table 1) for this study. Due to low number of filled seeds in trees from clonal rows the clone 133 was excluded from seed analyzes and seedlings studies.

Table 1. The ten clones and their origin as well as the survival rate of the frost and field trials.

Clone	Orchard part*	Local	Survival rate in freezing test trial after 10-15 weeks (%)	Survival rate field trial year 20 (%)
82	94	Svedjelandet, Stugun	88.9	72.2
92	94	Hede flygplats	71.7	73.5
100	94	Hede flygplats	92.8	70.2
133	94	Skallsjön	92.1	74
136	94	Skallsjön	81.3	71.6
139	94	Skallsjön	92.5	73.8
336	94	Storåbränna	100	74.1
262	88	S.V Råsjövallen	61.4	67.6
272	88	S.V Råsjövallen	96.1	64.1
289	88	Malsjöbodarna	86.1	64.1

*) Orchard part = establishment year (1988 and 1994).

The pollen production has been studied since 2003, in four test plots with 28 trees each. Eight of the selected clones (table 2) have copies within these areas. No difference could be seen in the pollen production year 2011 between the 88 and 94 orchard parts. Hence, we do expect any effects of part in further analyzes.

Table 2. The pollen production (g) for eight of the studied clones in the pollination year 2011.

Clone	Pollen (g)	Copies
82	36.4	5
92	54.0	2
100	78.7	1
136	5.7	1
139	50.7	1
272	4.3	2
289	32.9	3
336	91.0	4

Ten copies of each selected clone were chosen and cones were picked during the winter 2012/2013 (a subsample of approx. 20 cones/tree). The seeds were extracted from the cones and put in -4°C until further analysis.

Dewinging and cleaning

The seeds were dewinged by gently rubbing the material in a small fabric pouch in order to break the wings into smaller fragments. The small wing fragments were sifted out before the material was soaked for approximate 20 minutes (Appendix 1, fig. A1-1), allowing the hook of the wing to release the seed. The material was dried (Appendix 1, fig. A1-2) before rubbed once again to break the remaining wings into smaller fragments and to help the hook to release. The material was strained to remove bigger objects, such as parts of the cone, and thereafter strained once again to remove smaller fragments.

The seeds were separated from the remaining wing fragments by the use of weak air flow in a gravity separator. This allowed the seeds to fall straight through the airflow, since they were heavier compared to the wing fragments. The wing fragments got caught by the air flow and hence separated from the seeds (Appendix 1, fig. A1-3). A stronger air flow was used to separate the filled seeds from the empty seeds. The heavier filled seeds fell against the direction of the air flow, whereas the lighter empty seeds got caught by the air flow and sorted away from the filled seeds. This was repeated three times, so no empty seed should be able to mix with the filled seeds.

Samples were X-rayed in order to tell if the settings were good enough to separate the filled and empty seeds. When the results of the X-ray suggested that this was the case, no change in the settings was done.

Seed calculations

The seeds were calculated and weighed (Appendix 2, table A2-1) before calculations were performed in order to determine the possible effects of the different growth positions for the different copies of the clones. The amount of seeds per cones was calculated for each copy, as well as the average weight for a single seed. Of the figures gained from these calculations, means were calculated for each clone for respective position (Mixed and Row).

X-ray analysis

After the random selection of 4 copies for each clone of which 2 from mix and 2 from rows (Appendix 2, table A2-1), 50 seeds were selected for each copy. The seeds were analyzed by X-ray (16 s, 16 kV) to see if all the selected seeds were filled or not. In the case of an empty seed (<2‰), this was replaced with a filled seed.

In the different endosperm classes (table 3), the A class has got an endosperm that almost fills the seed coat to capacity, whereas the B class has got an endosperm which does not fill the seed coat completely. The embryo class contains embryo class 0, which contains neither embryo nor endosperm (the empty seed); class I, which contains endosperm but no embryo; class II containing endosperm and one or more embryos which are not fully developed in the embryo cavity; class III contains one or several embryos in the endosperm, where the longest

embryo often measures between half and three quarters of the embryo cavity, and class IV, which contains endosperm with a fully developed embryo in the cavity [7].

Table 3. Expected germination percentage. Different stages of the seeds have different germination percentage based on their endosperm and embryo. (Adapted from Simak 1957)

Species	Endosperm class	Embryo class				
		0	I	II	III	IV
Scots pine	A	-	-	(50)	88	99
	B	-	-	(5)	(43)	(68)

Germination analysis

The seeds for the random selected copies were put on the Jacobsen's apparatus (Appendix 1, fig. A1-4), which consists of heated metal rails on top of a water bath. On the apparatus, the seeds were placed on a filter paper on an additional filter paper with a paper stocking leading water from the water bath to the filter papers. The seeds were covered by a transparent plastic cup and were incubated at a constant light at +20°C for 16 hours and +30°C for 8 hours to germinate.

The seeds were moved to peat filled containers (Plantek 49F, 155 cc and 330 cells/m^2) and put in a greenhouse (+20°C, half-light, 18/6 h day and night regime), once they had germinated (Appendix 3, table A3-1). The seeds which did not germinate were dissected to determine why they had not germinated (Appendix 3, table A3-2).

The germination energy was calculated by dividing the number of germinated seeds day eight with the total number of germinated seeds, and the average germination time (MG) was calculated according to following:

$$MG=((x*T1)+(y*(T2 – T1))+…+(z*(Tn – T(n – 1))/(x+y+…+z)$$

Where Tn represents the different time points for calculation and the other variables represent the number of germinated seeds at the specific time point.

Seedling studies

The seedlings were fertilized with NPK fertilization. Low dosage of 2 mS to begin with in order to allow the seedlings get adjusted to the extra nutrients before increasing the concentration to 3 mS after one week.

The number of cotyledon needles for each seedling was calculated in order to see if there was a significant difference between the numbers of needles (Appendix 4, table A4-2), since the number of cotyledons also indicate the quality of the seedlings.

The number of seedlings which were somewhat dried, skewed and/or not thoroughly rooted due to possible transplantation errors was registered to see if this could have an effect on the numbers of seedlings included in the height measurements.

The height of the seedlings were measured from the base of the stem to the apical meristem, 15 days after germination onset and 67 days after the last germinated seed had been transplanted (Appendix 4, table A4-4).

Significance calculations

MS Excel was used to perform a two-sided T-test to calculate if there were any significant difference between the different means of the copies grown in a row and the copies grown in a mix together with other clones. To determine whether equal or unequal variance was to be used, a F-test was performed and based on the two-sided probability that the variance of the different datasets were significantly different from each other.

The statistical analysis software SAS procedure GLM was used to determine possible significant differences between the two different growth positions when considering all the clones and copies as well as for a statistical analysis of the differences between the different clones. Following model was used.

$y = \mu + C + G + CxG + e$

Where μ is the overall mean, C is effects of clone, G the effects of growth position (row and mixture) and e the standard error.

Results

Seed calculations

Seed weight

There is a great variation between clones concerning seed weight of the filled seeds ($p \leq 0.0001$), as clone 100 has seeds that weigh 5.00 mg while seeds from clone 133 weigh 8.85 mg on average.

There is no overall effect of the positioning on the seed weight within the individual clones, but for clone 133 which have a significant ($p=0.005$) negative effect of the mixture, i.e. seeds from trees in clonal rows have higher seed weight (9.52 mg) than seeds from trees growing in mixture (8.19 mg) (fig.1). Looking at the seed weight for all the clones in respective position, there is a significant ($p=0.0057$) effect favoring the clonal rows, where the average weight for filled seeds is 7.00 mg for the copies grown in a row, whereas the average weight for the copies grown in a mixture with other clones is 6.75 mg.

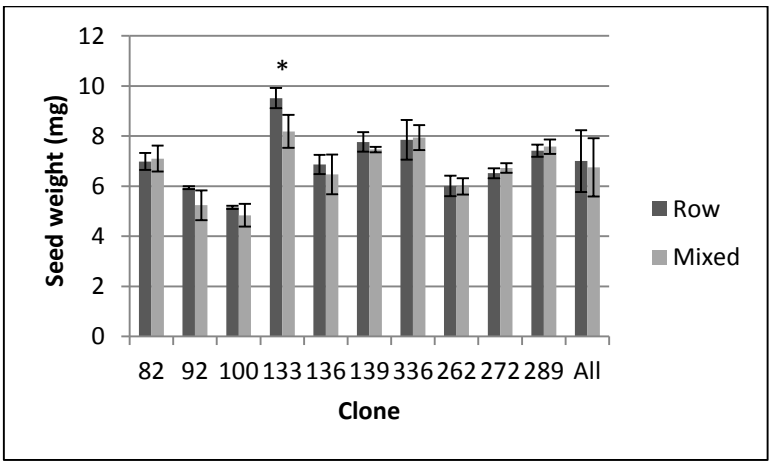

Figure 1. The average of the calculated seed weight (g) for each clone in respective position and an average of the seed weight for all the clones combined. The asterisk represents the samples where there is a significant difference ($p<0.05$) between the means for the different positions for each clone, and the error bars represents the standard deviation.

Seeds per cone

There is a significant difference in the number of filled seeds per cone between the different clones ($p \leq 0.0001$), where clone 100, 92 and 133 have a significantly lower number of seeds/cones compared to the other clones. Here the highest mean of filled seeds is 20.2 seeds/cone for clone 336 and the smallest mean is 4.5 filled seeds/cone for clone 133.

For the average number of filled seeds per cone (fig. 2), there is a significant positive effect of mixture in clones 92, 133 and 289, where the copies grown in a mixture with other clones have a significantly higher production of filled seeds per cone compared to the ones grown in a clonal row, whereas the differences in the other clones remain insignificant. Clone 92 had 4.4 filled seeds/cone for the row growing copies and 9.6 filled seeds/cone in the copies grown in a mixture ($p=0.0144$). Clone 133 had only 2.3 seeds/cone in the row, whereas the clone produced 6.7 filled seeds/cone grown in a mixture ($p=0.0023$). The final clone with a significant difference between the positions, clone 289, had 16.0 filled seeds/cone in the

samples grown in a row and 21.8 filled seeds/cone in the mixed ones (p=0.0147). When including all the clones in the calculations, there is a significant difference between the positions (p=0.0127) that shows a positive effect for the mixed position, where the average number of seeds per cone was 15.8 for the mixture and 14.4 for the copies grown in a row.

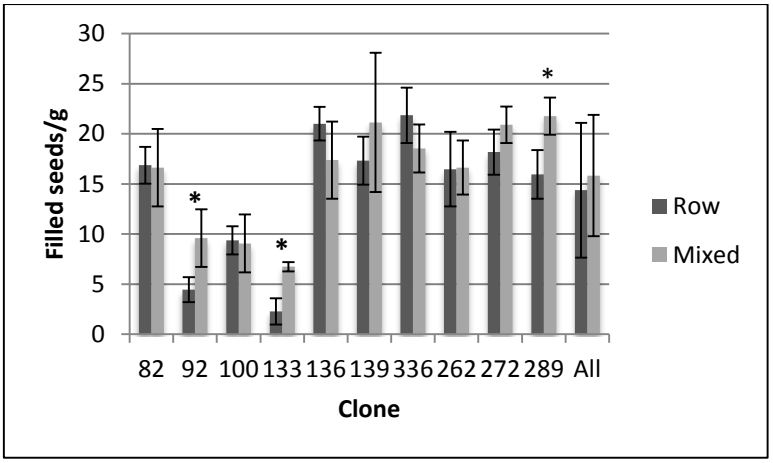

Figure 2. The average of filled seeds per cone for each clone in respective position, as well as an average of all filled seeds for all clones. The asterisk represents the samples where there is a significant difference ($p<0.05$) between the means for the different positions for each clone, and the error bars represents the standard deviation.

For the average number of empty seeds per cone (fig. 3) there is a significant difference between the clones ($p\leq0.0001$), where clone 100 has got an average of 18.5 and thereby has got the highest average of empty seeds per cone , whereas clone 262 has got the lowest average of empty seeds per cone (3.1 seeds per cone).

For the average number of empty seeds per cone, there is a significant positive effect for the row position in clone 136 compared to when the clone is positioned in a mixture with other clones (p=0.0003), where the row position had an average of 7.4 empty seeds per cone while the mixed position had 4.8 empty seeds per cone. A significant difference in the average of empty seeds per cone can be seen between the positions including all the clones in the calculations (p=0.0111), where the mean value is 7.8 empty seeds per cone for the row position and 7.1 for the mixed position.

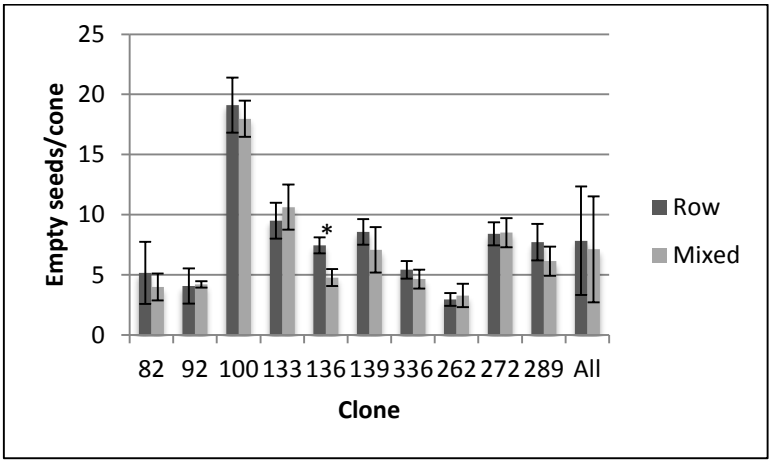

Figure 3. The average of empty seeds per cone for all clones in respective position. The asterisk represents a significant difference between the positions within a clone and the error bars represents the standard deviation.

The total number of filled and empty seeds per cone was 23.0 seeds where the trees were growing in mixture and 22.2 seeds in rows and was not influenced by the position (p=0.2055), data not included.

Germination analysis

Germination time

The seeds germinated was calculated with the start from day 5 after the seeds had been put on the germination table. A test for significant difference within each clone was performed daily, and no significant difference could be noted, with clone 100 at day 5 as the only exception.

The average germination time was calculated and no significant difference can be seen neither between the positions within the clones (fig. 4) nor between the positions only (p=0.6296). The average germination time for all the clones is 6.2 days for the row condition, whereas the average germination time for the mixed condition is 6.3 days. There is however a significant difference between clones (p≤0.0001), where clone 272 have the shortest germination time and clone 262 have the longest with 5.2 and 8.0 days, respectively.

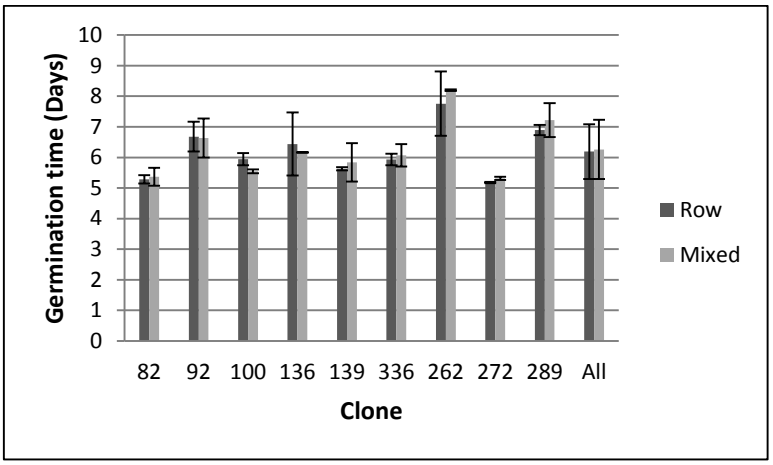

Figure 4. The average germination time for the clones in respective position. The error bars represents the standard deviation.

Germination percentage

The germination analysis indicate a high germination percentage for most of the clones and the germination percentage is rather similar within the clone. Clone 262 is the clone which have the lowest germination percentage, whereas clone 272 have the highest total germination percentage (fig. 5) Looking at all the clones, there is no significant difference in the germination percentage caused by the growth position (p=0.4373), where 94.6% of the seeds from the copies grown in a row as well as 93.4% of the seeds from the copies grown in a mixture had germinated. There is, however, a significant difference the germination percentage between the clones, where the small germination percentage of clone 262 is significantly lower compared to the other clones (p≤0.0001).

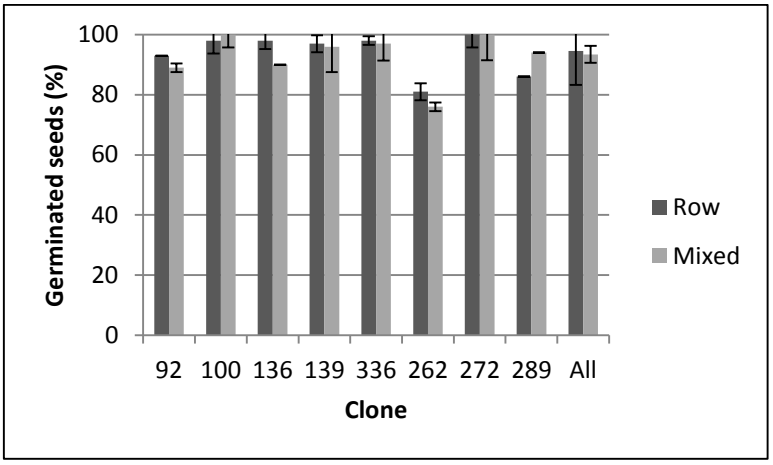

Figure 5. The germination percentage compared between the growth positions (row or mixed). The error bars represents the standard deviation.

The seeds which had not germinated (6%) were either fresh and not germinated (FEG) (5%), or dead (1%) (Appendix 3, table A3-2). The percentage of FEG (p=0.6047) and dead seeds (p=0.6034) were not influenced by the position, data not included.

Germination energy

The germination energy was calculated from the total amount of germinated seeds up to day 8 and the total number of germinated seeds on day 15 (fig. 6). No significant difference can be found between the positions within the clones, and when including all clones in the calculations, no significant difference can be seen between the two positions (p=0.6790), where the average germination energy is 89.7%. for the row position and 88.7% for the mixed position.

Clone 262 have an average germination energy on 63.3% after 8 days on the Jacobsen's apparatus, something which caused a significant difference ($p \leq 0.0001$) between the clones since clone 272, which is the clone with the highest germination energy after 8 days, have an average germination energy of 98.5%.

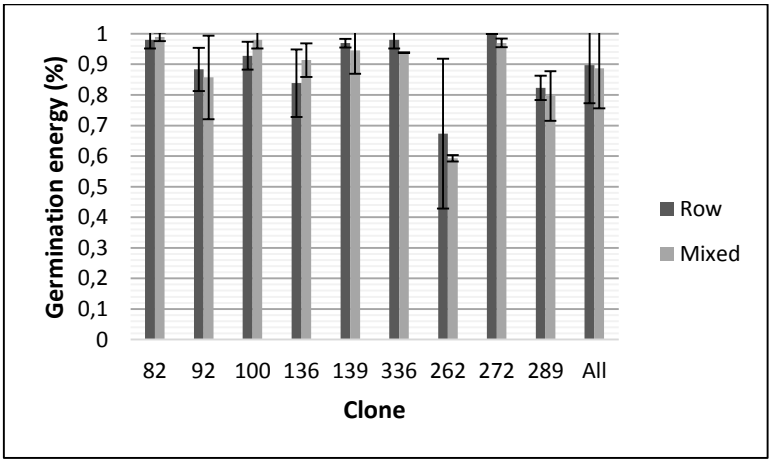

Figure 6. The germination energy for each clone in respective position, derived from the number of seeds germinated up to day eight and the total number of germinated seeds on day 15. The error bars represents the standard deviation.

Seedling studies

Cotyledon needles

The numbers of cotyledon needles for each clone and the respective treatment (row or mixed) was calculated (Appendix 4, table A4-1). The highest number of cotyledon needles for a seedling was 9 needles, whereas the lowest number was 4, not including the seedlings which did not grow and for which no cotyledon needles could be seen.

When looking at the average number of cotyledon needles for each clone at respective position (fig. 7), clone 82 have the largest average number of cotyledon needles (6.1 needles per seedling) and clone 100 have the smallest number of needles per seedling (5.3 needles per seedling) independent on which position the copies had. The differences in the average for these clones cause a significant difference between the clones ($p=0.0009$).

No significant differences can be seen between the positions with all the clones ($p=0.3403$), where the average number of cotyledon needles is 5.6 needles for the row position and 5.7 needles for the mixed position. No significant differences can be seen between the positions within the clones.

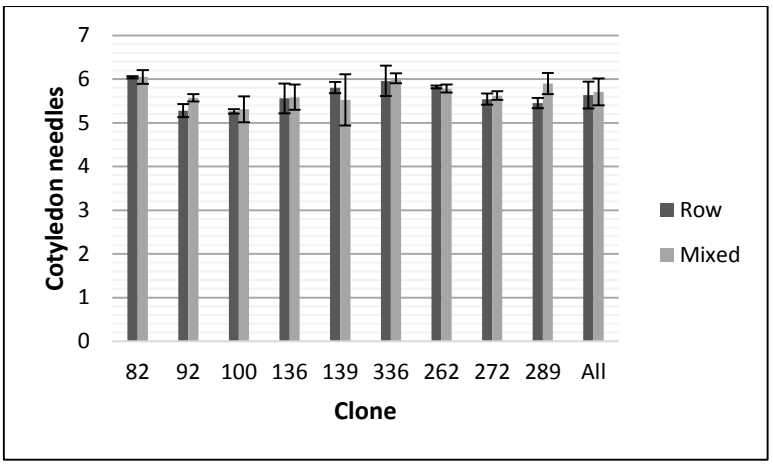

Figure 7. The average number of cotyledon needles for each clone and position. The error bars represents the standard deviation.

Seedling height

The height of the seedlings was measured 11.7 weeks after germination start (Appendix 4, table A4-3). The highest seedling was 134 mm whereas the shortest was 10 mm starting from the surface and up to the apical meristem.

There is a significant variance between the clones ($p \leq 0.001$), where the biggest difference is between clone 272 and 262; the average height for clone 272 is 78.1 mm whereas the average height for clone is 59.5 mm. There is no significant difference between the two positions ($p=0.4496$) with all clones in the calculations, where the mixed position have an average height of 68.7 mm and the row position have an average of 68.0 mm. There is also no significant difference between the different positions within the different clones, with clone 289 as the only exception ($p=0.0143$) where there is a positive effect for trees grown in a

mixture (70.6 mm) compared to trees grown in a row with trees of the same clone (65.8 mm) (fig. 8).

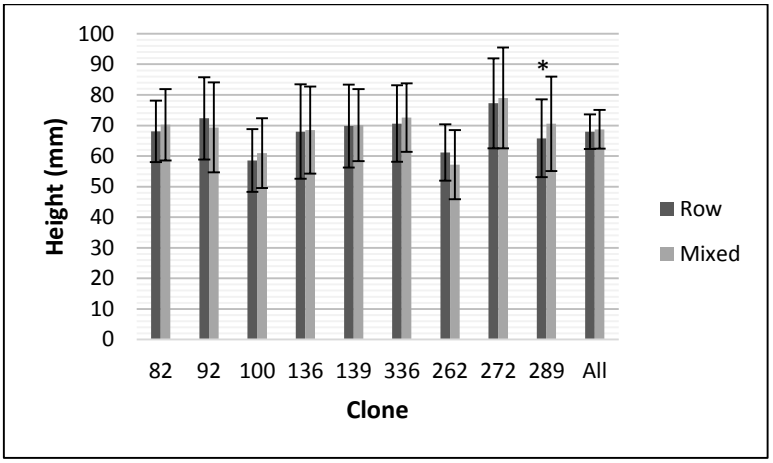

Figure 8. The average height of the seedlings for each clone and position, 11.7 weeks after germination start. The error bars represent the standard deviation and the asterisk represents a significant difference ($p<0.05$) in seedling height between the positions within a specific clone.

Discussion

Seed calculations

The weight of the seeds is an important factor since it tells something about the quality of the seed, where a higher seed weight often represents a better seed quality. Hence this factor is good to investigate in order to study the possibilities of inbreeding, since the quality of the seeds often is reduced at a high level of self-pollination [5]. There was a significant difference in the seed weight between the two positions according to GLM, where the seeds derived from the clonal rows had a 4% higher average weight compared to the seeds derived from the copies in the mixed position.

Comparing the seed weight between the two different positions within each clone did however show that the difference was insignificant for most of the clones, with clone 133 as the only exception.

The number of filled seeds per cone did also differ significantly between the different positions in the analyze including all clones. There are 12% more filled seeds per cone in the mixed position compared to cones in clonal rows, indicating that there is a difference in the amount of self-pollination between the different positions, since the self-pollinated seeds often is aborted and can create empty seeds [5]. There was a significant difference in the number of empty seeds per cone between the positions as well, where there are 10% more empty seeds per cone for the copies growing in clonal rows. The total number of seeds per cone, in other words: sum of empty and filled seeds, was not affected by the position. There were significant differences between the clones in both the seed weight and the number of seeds per cone. From these differences one can assume that the different clones are different susceptible against self-pollination. The significantly higher number of empty seeds and lower number of filled seeds per cone suggest a rather high level of self-pollination in the row position [5]. This indicates that this is not the position to favor, whereas one can assume that the high filled seed weight suggest the opposite. The seed weight can however be explained by the fact that since there are so few vital seeds, these can get more nutrients and are thereby able to get heavier. Hence, the results of the seed calculation suggest that the mixed position is the favorable position in order to minimize the self-pollination.

The significant difference between the number of filled and empty seeds per cone for the clones can be underestimated, since the number of aborted cones was not included in this study. A specific number of embryos have to be fertilized in order to produce a cone [4] and perhaps the cones with a high level of self-pollination have been aborted and therefore not accounted for in this study.

Germination analysis and seedling studies

The differences in the germination time, germinations percentage, germination energy, number of cotyledons and seedling height between the positions were insignificant, both when it came to the result including all the clones to the calculations and when looking at the positions for each individual clone, except for clone 289 in the height measurements, where the mixed position a 7% higher average height compared to the row position. The vast

majority of insignificant differences suggests that there are no difference in the amount of inbred filled seeds in rows and in mixture [1].

There was however a significant difference between the clones, where clone 262 was significantly different from the rest of the clones since it had a much lower germination percentage (fig. 5) and a longer germination time (fig. 4) in comparison to the other clones. The lower germination percentage and longer germination time of clone 262 could also be caused by self-pollination. Inbred seeds often have a lower as well as slower germination, since either the endosperm or the embryo might be in a less developed state [7]. There were also significant differences between the clones when it came to the height measurements (fig. 8), where clone 262 were shortest and clone 272 tallest.

There were significant differences in the germination energy (fig. 6) between the different clones, where the germination spanned from 63.3% up to 98.5% in different clones, as well as between the number of cotyledon needles (fig. 7), where the difference was 14% between the highest and lowest average.

When considering clones only and not the different positions, the shortest germination time and higher germination percentage and energy, as well as the seedling height suggest that clone 272 might be less susceptible against self-pollination compared to for example clone 262 which had the lowest germination percentage and energy, the longest germination time and the shortest seedlings [1].

One have to consider that there were germinated seeds already at day 5 (T1) and these could have germinated some days before T1. Hence, the result of the germination time can be somewhat misleading.

Conclusion

The seed calculations suggest that there are a significant difference between the two positions, with the mixed position as the favorable one, since the copies in this position produced 12% more filled seeds and 10% less empty seeds indicating a smaller level of self-pollination compared to the copies grown in the row position. The differences in the seed production can however be much greater than these numbers, since one do not know how many cones which have been aborted due to self-pollination.

There were nothing wrong with the seeds produced from the row position, since no significant differences could be seen in the germination an seedling studies, and therefore the seeds could still be used to produce healthy seedlings.

Different clones appear to be more or less susceptible to self-pollination and hence different clones gets a different quality of the seed and seedlings.

So, with the knowledge gained from this study, one can assume that it does matter were the copies from the different clones are positioned, and to be able to produce as much vital seeds per cone as possible in a seed orchard it is preferable to position the copies in a mixture of other clones in order to minimize the risk of inbreeding and seed abortion.

To put the copies in a mix can however increase the workload for the orchard owner, if the cones should be picked clone wise or if a clone is to be removed from the orchard. So either you sacrifice 12% of the vital seeds per cone by placing the copies in a row, or you make it more difficult to work clone wise by placing the copies in a mix with other clones. Which one that truly is the best position should therefore be determined by the orchard owner based on the means of the seed orchard.

Future prospects

In the seed orchard in Gnarp there are 57 other clones and several other copies. For this project, the copies were selected based on how many cones they had after the main cone production already had been picked. Therefore one could complement this study by choosing other copies and also use more clones in the selection another year, as well as study the number of flowers and the total number of produced cones. Perhaps then another conclusion can be drawn.

A DNA-analysis using micro satellites could be performed to establish an increased level of self-pollination, since it is quite difficult to determine differences in the level of inbreeding if the phenotypic effects are not substantial.

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I would like to thank Ulfstand Wennström, who was my main supervisor at Skogforsk and the one that had to read and correct my report oh so many times; Erik Walfridsson, who was my assistant supervisor when I was handling the seeds; the rest of the staff at Skogforsk, especially those who took care of my seedlings and gave them water, and Bengt Andersson, who was my first contact at Skogforsk and the one who forwarded my search for a degree project to Ulfstand. And thanks to Anders Nordström, for being my supervisor at the University.

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Appendices

Appendix 1 – Experimental setup

The winged seeds were soaked in a soaking apparatus (figure A1-1) in 20 minutes so the hook of the wing would release the seed.

Figure A1-1. **Soaking apparatus**. The apparatus where the seed material was soaked with water in order to make the wing detach from the seed.

The soaked seed and wing mixture was dried by a drying equipment using the ventilation systems (figure A1-2).

Figure A1-2. **Drying equipment**. The seed material was dried by the use of the ventilation system.

When separating the seeds from the remaining wing fragments, and separating the filled from the empty seeds, the strength of the air flow in the gravity separator (figure A.1-3) was regulated by two pinions determining the size of the openings for the air flow. To separate the seeds from the wing fragments, the openings were set to 7 and 10 mm, respectively, and to divide the filled seeds from the empty seeds, the openings were set to 10 and 21 mm, respectively

Figure A1-3. Gravity separator. The apparatus where the seeds were separated from the wing fragments and the filled seeds were separated from the non-viral seeds.

The seeds were placed upon soaked filter papers on Jacobsen's apparatus in order to germinate (figure A1-4).

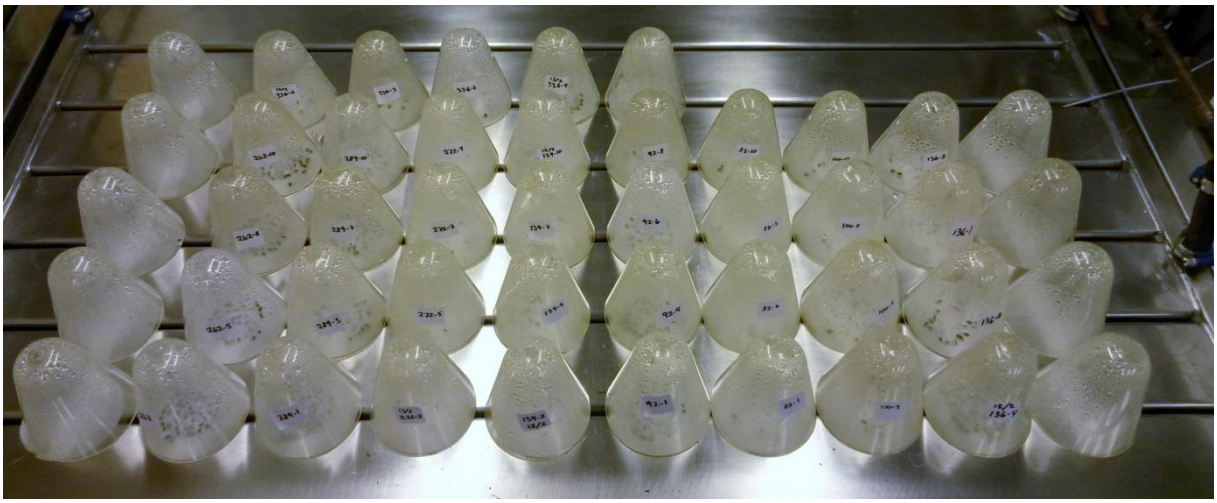

Fig A1-4. **Jacobsen's apparatus.** The seeds were put on filter papers on a germination table under translucent plastic cups in order to germinate. Here the conditions were 16h at 20°C and 8h at 30°C with a constant light exposure.

Appendix 2 – Seed data

Table A2-1. **Seed data.** The data of the number of cones, seeds as well as seed weight for each copy of each clone.

Clone	Position	Orchard part	Copy	Cones	Filled seeds	Empty seeds	Filled (g)	Empty (g)	Seeds/ cone (Filled)	g/seed (Filled)
82	Row	94	1[a]	20	373	188	2.533	0.39	18.65	0.00679
82	Row	94	2	20	312	55	2.251	0.118	15.60	0.00721
82	Row	94	3[a]	25	372	88	2.695	0.213	14.88	0.00724
82	Row	94	4	18	341	90	2.21	0.182	18.94	0.00648
82	Row	94	5	20	325	102	2.34	0.21	16.25	0.00720
82	Mixed	94	6[a]	26	444	123	3.207	0.253	17.08	0.00722
82	Mixed	94	7	26	411	124	2.943	0.251	15.81	0.00716
82	Mixed	94	8	24	537	50	3.346	0.096	22.38	0.00623
82	Mixed	94	9	19	309	75	2.242	0.171	16.26	0.00726
82	Mixed	94	10[a]	22	255	97	1.947	0.211	11.59	0.00764
92	Row	94	1[a]	20	125	82	0.745	0.129	6.25	0.00596
92	Row	94	2	24	86	62	0.505	0.106	3.58	0.00587
92	Row	94	3	20	64	125	0.378	0.196	3.20	0.00591
92	Row	94	4[a]	21	108	61	0.65	0.103	5.14	0.00602
92	Row	94	5	21	85	94	0.504	0.15	4.05	0.00593
92	Mixed	94	6[a]	19	133	87	0.783	0.152	7.00	0.00589
92	Mixed	94	7	21	150	87	0.755	0.135	7.14	0.00503
92	Mixed	94	8[a]	23	197	99	0.906	0.138	8.57	0.00460
92	Mixed	94	9	29	386	111	2.26	0.171	13.31	0.00585
92	Mixed	94	10	25	299	103	1.442	0.14	11.96	0.00482
100	Row	94	1	20	208	423	1.083	0.709	10.40	0.00521
100	Row	94	2	20	142	362	0.737	0.597	7.10	0.00519
100	Row	94	3[a]	21	209	459	1.084	0.767	9.95	0.00519
100	Row	94	4	20	178	330	0.901	0.5	8.90	0.00506
100	Row	94	5[a]	21	220	376	1.125	0.628	10.48	0.00511
100	Mixed	94	6	20	148	373	0.656	0.554	7.40	0.00443
100	Mixed	94	7	20	152	378	0.788	0.628	7.60	0.00518
100	Mixed	94	8[a]	21	186	331	0.99	0.566	8.86	0.00532
100	Mixed	94	9	12	88	233	0.378	0.316	7.33	0.00430
100	Mixed	94	10[a]	22	311	377	1.544	0.538	14.14	0.00496
133	Row	94	1	24	57	261	0.571	0.566	2.38	0.01002
133	Row	94	2	19	47	145	0.446	0.296	2.47	0.00949
133	Row	94	3	17	31	184	0.276	0.357	1.82	0.00890
133	Row	94	4	21	61	209	0.59	0.452	2.90	0.00967
133	Row	94	5	24	44	197	0.418	0.452	1.83	0.00950
133	Mixed	94	6	24	150	186	1.153	0.317	6.25	0.00769
133	Mixed	94	7	23	155	281	1.293	0.496	6.74	0.00834
133	Mixed	94	8	22	107	267	0.986	0.51	4.86	0.00921
133	Mixed	94	9	22	164	217	1.339	0.361	7.45	0.00816
133	Mixed	94	10	24	201	268	1.515	0.461	8.38	0.00754
136	Row	94	1[a]	21	489	137	3.159	0.245	23.29	0.00646

136	Row	94	2	23	456	160	3.087	0.35	19.83	0.00677
136	Row	94	3	23	501	181	3.369	0.364	21.78	0.00672
136	Row	94	4[a]	22	465	171	3.49	0.398	21.14	0.00751
136	Row	94	5	21	399	170	2.742	0.358	19.00	0.00687
136	Mixed	94	6	22	278	100	1.847	0.189	12.64	0.00664
136	Mixed	94	7	23	470	97	2.769	0.18	20.43	0.00589
136	Mixed	94	8[a]	22	391	130	2.247	0.262	17.77	0.00575
136	Mixed	94	9	22	477	93	3.019	0.163	21.68	0.00633
136	Mixed	94	10[a]	23	331	114	2.559	0.278	14.39	0.00773
139	Row	94	1	23	366	190	2.941	0.398	15.91	0.00804
139	Row	94	2[a]	20	375	153	2.805	0.253	18.75	0.00748
139	Row	94	3	23	418	220	3.386	0.487	18.17	0.00810
139	Row	94	4[a]	19	264	143	2.108	0.307	13.89	0.00798
139	Row	94	5	21	418	206	3.024	0.407	19.90	0.00723
139	Mixed	94	6	22	694	107	5.203	0.205	31.55	0.00750
139	Mixed	94	7[a]	23	415	179	3.124	0.315	18.04	0.00753
139	Mixed	94	8	22	543	132	4.05	0.259	24.68	0.00746
139	Mixed	94	9	22	375	152	2.832	0.315	17.05	0.00755
139	Mixed	94	10[a]	20	287	197	2.088	0.386	14.35	0.00728
336	Row	94	1	15	298	81	2.639	0.25	19.87	0.00886
336	Row	94	2[a]	20	428	124	3.478	0.352	21.40	0.00813
336	Row	94	3[a]	20	449	122	3.335	0.333	22.45	0.00743
336	Row	94	4	19	366	87	2.967	0.22	19.26	0.00811
336	Row	94	5	20	525	96	3.551	0.198	26.25	0.00676
336	Mixed	94	6	18	314	95	2.264	0.236	17.44	0.00721
336	Mixed	94	7[a]	22	469	100	3.817	0.26	21.32	0.00814
336	Mixed	94	8	22	458	74	3.934	0.202	20.82	0.00859
336	Mixed	94	9[a]	13	224	62	1.767	0.165	17.23	0.00789
336	Mixed	94	10	20	318	105	2.503	0.298	15.90	0.00787
262	Row	88	1	26	398	71	2.113	0.121	15.31	0.00531
262	Row	88	2[a]	20	419	66	2.628	0.142	20.95	0.00627
262	Row	88	3	20	219	55	1.385	0.11	10.95	0.00632
262	Row	88	4	20	336	73	2.036	0.141	16.80	0.00606
262	Row	88	5[a]	21	386	48	2.342	0.088	18.38	0.00607
262	Mixed	88	6	21	327	55	1.858	0.103	15.57	0.00568
262	Mixed	88	7	22	397	103	2.323	0.2	18.05	0.00585
262	Mixed	88	8[a]	21	434	75	2.834	0.168	20.67	0.00653
262	Mixed	88	9	20	284	43	1.665	0.082	14.20	0.00586
262	Mixed	88	10[a]	24	352	80	2.118	0.165	14.67	0.00602
272	Row	88	1	22	411	187	2.67	0.329	18.59	0.00653
272	Row	88	2[a]	20	361	184	2.34	0.335	18.05	0.00648
272	Row	88	3	20	306	189	2.077	0.35	15.30	0.00679
272	Row	88	4	19	331	139	2.065	0.236	17.42	0.00624
272	Row	88	5[a]	20	430	151	2.82	0.258	21.50	0.00656
272	Mixed	88	6	26	606	275	4.123	0.525	23.31	0.00680
272	Mixed	88	7[a]	22	475	186	3.263	0.281	21.59	0.00687

272	Mixed	88	8	21	384	164	2.596	0.248	18.29	0.00676
272	Mixed	88	9[a]	20	409	150	2.776	0.256	20.45	0.00679
272	Mixed	88	10	21	438	172	2.798	0.3	20.86	0.00639
289	Row	88	1[a]	25	425	180	3.253	0.438	17.00	0.00765
289	Row	88	2	20	277	166	2.114	0.301	13.85	0.00763
289	Row	88	3[a]	20	257	176	1.817	0.354	12.85	0.00707
289	Row	88	4	20	362	180	2.645	0.343	18.10	0.00731
289	Row	88	5	22	395	117	2.934	0.213	17.95	0.00743
289	Mixed	88	6	20	390	154	2.773	0.247	19.50	0.00711
289	Mixed	88	7[a]	20	458	128	3.584	0.244	22.90	0.00783
289	Mixed	88	8	20	467	112	3.6	0.215	23.35	0.00771
289	Mixed	88	9	21	420	93	3.25	0.13	20.00	0.00774
289	Mixed	88	10[a]	22	507	144	3.81	0.259	23.05	0.00751

a) Clone copies selected for germination and seedling studies. Note that no copy was selected from clone 133, since there were so few vital seeds in the copies grown in a row of the same clone

Appendix 3 – Germination data

Table A3-1. The number of germinated seeds. The number of seeds which had germinated after x numbers of days after placing the seeds on the Jacobsen's apparatus.

		Days after placing seeds on Jacobsen's apparatus										
Copy	**Position**	**5**	**6**	**7**	**8**	**9**	**11**	**12**	**13**	**14**	**15**	**Percentage**
82-1	Row	34	11	3	0	1	1					100
82-3	Row	41	5	3	1							100
82-6	Mix	44	2	3	1							100
82-10	Mix	21	19	5	3	1	0	0	0	0	0	98
92-1	Row	7	18	10	7	1	1	0	0	0	1	90
92-4	Row	2	9	18	11	3	5	0	0	0	0	96
92-6	Mix	4	11	17	3	4	4	2	0	1	0	92
92-8	Mix	11	10	11	9	2	0	0	0	0	0	86
100-3	Row	14	26	5	3	1	0	0	0	1		100
100-5	Row	12	17	13	1	3	2	0	0	0	0	96
100-8	Mix	25	13	9	1	2						100
100-10	Mix	28	11	8	3							100
136-1	Row	18	20	5	1	4	0	0	0	0	0	96
136-4	Row	7	7	17	7	3	7	2				100
136-8	Mix	8	16	10	6	0	2	0	0	0	0	84
136-10	Mix	14	14	13	1	3	3	0	0	0	0	96
139-2	Row	20	21	6	0	1	0	0	0	0	1	98
139-4	Row	21	18	6	2	0	1	0	0	0	0	96
139-7	Mix	23	21	6								100
139-10	Mix	10	17	11	3	3	0	0	1	1	0	92
336-2	Row	9	24	11	4	0	1	0	0	0	1	100
336-3	Row	9	23	13	3	0	0	0	0	0	0	96
336-7	Mix	12	24	8	2	3	0	0	0	0	0	98
336-9	Mix	1	22	17	5	1	2	0	0	0	0	96
262-2	Row	0	1	4	16	10	8	2	0	1	0	84
262-5	Row	0	6	15	12	5	1	0	0	0	0	78
262-8	Mix	0	2	9	13	7	8	1	0	1	0	82
262-10	Mix	0	0	7	14	7	4	2	1	0	0	70
272-2	Row	35	13	2								100
272-5	Row	42	4	4								100
272-7*	Mix	36	9	4	0	1						100
272-9	Mix	33	14	1	0	1	1					100
289-1	Row	0	9	15	7	5	3	0	0	0	0	78
289-3	Row	4	18	10	8	1	5	0	0	1	0	94
289-7	Mix	0	8	12	14	7	1	0	1	3	0	92
289-10	Mix	3	9	20	9	4	3	0	0	0	0	96
Total		548	472	331	170	84	63	9	3	9	3	94

* Day 5, germinated seeds: 35+1 (one missing)

Table A3-2. Non-germinated seeds. The table shows the number of seeds which did not germinate as well as how many of these were dead or simply just did not germinate although seemingly healthy (FEG).

Copy	Position	FEG	Dead	Total	Percentage
82-1	Row	0	0	0	0
82-3	Row	0	0	0	0
82-6	Mix	0	0	0	0
82-10	Mix	0	1	1	2
92-1	Row	5	0	5	10
92-4	Row	2	0	2	4
92-6	Mix	4	0	4	8
92-8	Mix	6	1	7	14
100-3	Row	0	0	0	0
100-5	Row	1	1	2	4
100-8	Mix	0	0	0	0
100-10	Mix	0	0	0	0
136-1	Row	2	0	2	4
136-4	Row	0	0	0	0
136-8	Mix	4	4	8	16
136-10	Mix	2	0	2	4
139-2	Row	1	0	1	2
139-4	Row	2	0	2	4
139-7	Mix	0	0	0	0
139-10	Mix	3	1	4	8
336-2	Row	0	0	0	0
336-3	Row	0	2	2	4
336-7	Mix	0	1	1	2
336-9	Mix	2	0	2	4
262-2	Row	5	3	8	16
262-5	Row	11	0	11	22
262-8	Mix	8	1	9	18
262-10	Mix	15	0	15	30
272-2	Row	0	0	0	0
272-5	Row	0	0	0	0
272-7	Mix	0	0	0	0
272-9	Mix	0	0	0	0
289-1	Row	11	0	11	22
289-3	Row	3	0	3	6
289-7	Mix	4	0	4	8
289-10	Mix	2	0	2	4
Total		93	15	108	6

Appendix 4 – Seedling calculations

Table A4-1. The number of seedlings and the total number of cotyledons for each clone and position, respectively.

Clone	Position	Seedlings	Cotyledons
82	Row	96	580
	Mixed	99	599
92	Row	91	480
	Mixed	89	496
100	Row	96	505
	Mixed	101	536
136	Row	97	540
	Mixed	90	504
139	Row	97	563
	Mixed	95	527
336	Row	93	553
	Mixed	97	584
262	Row	79	460
	Mixed	72	416
272	Row	98	543
	Mixed	98	551
289	Row	85	464
	Mixed	91	536
All	Row	832	4688
	Mixed	832	4749

Table A4-2. The number of seedlings, cotyledons as well as cotyledons per seedling for each of the selected copies.

Clone	Position	Orchard part	Copy	Seedlings	Cotyledons	Cotyledon/seedling
82	Row	94	1	49	297	6.06
82	Row	94	3	47	283	6.02
82	Mix	94	6	50	308	6.16
82	Mix	94	10	49	291	5.94
92	Row	94	1	44	237	5.39
92	Row	94	4	47	243	5.17
92	Mix	94	6	46	259	5.63
92	Mix	94	8	43	237	5.51
100	Row	94	3	49	256	5.22
100	Row	94	5	47	249	5.30
100	Mix	94	8	51	260	5.10
100	Mix	94	10	50	276	5.52
136	Row	94	1	47	250	5.32
136	Row	94	4	50	290	5.80
136	Mix	94	8	42	226	5.38
136	Mix	94	10	48	278	5.79
139	Row	94	2	49	280	5.71

139	Row	94	4	48	283	5.90
139	Mix	94	7	50	297	5.94
139	Mix	94	10	45	230	5.11
336	Row	94	2	49	280	5.71
336	Row	94	3	44	273	6.20
336	Mix	94	7	49	299	6.10
336	Mix	94	9	48	285	5.94
262	Row	88	2	40	232	5.80
262	Row	88	5	39	228	5.85
262	Mix	88	8	39	223	5.72
262	Mix	88	10	33	193	5.85
272	Row	88	2	49	276	5.63
272	Row	88	5	49	267	5.45
272	Mix	88	7	49	279	5.69
272	Mix	88	9	49	272	5.55
289	Row	88	1	38	204	5.37
289	Row	88	3	47	260	5.53
289	Mix	88	7	43	261	6.07
289	Mix	88	10	48	275	5.73

Table A4-3. The total number of seedlings and the total height for all seedlings for each clone and position.

Clone	Position	Seedlings	Height (mm)
82	Row	92	6264
	Mixed	92	6462
92	Row	89	6292
	Mixed	89	6133
100	Row	95	5564
	Mixed	99	6094
136	Row	94	6391
	Mixed	87	5957
139	Row	93	6508
	Mixed	91	6378
336	Row	91	6427
	Mixed	95	6892
262	Row	75	4649
	Mixed	71	4061
272	Row	93	7182
	Mixed	96	7582
289	Row	80	5263
	Mixed	89	6279
All	Row	802	54540
	Mixed	809	55838

Table A4-4. The height measurements for each copy, where the seedlings were measured from the peat surface to the apical meristem 62 days after the last germinated seed had been transferred to the peat.

Clone	Position	Orchard part	Copy	Seedlings	Height (mm)	Height/seedling (mm)
82	Row	94	1	48	3184	66.33
82	Row	94	3	44	3080	70.00
82	Mix	94	6	47	3170	67.45
82	Mix	94	10	45	3292	73.16
92	Row	94	1	44	3110	70.68
92	Row	94	4	45	3182	70.71
92	Mix	94	6	46	3132	68.09
92	Mix	94	8	43	3001	69.79
100	Row	94	3	48	2804	58.42
100	Row	94	5	47	2760	58.72
100	Mix	94	8	50	2967	59.34
100	Mix	94	10	49	3127	63.82
136	Row	94	1	45	3066	68.13
136	Row	94	4	49	3325	67.86
136	Mix	94	8	40	2511	62.78
136	Mix	94	10	47	3446	73.32
139	Row	94	2	45	3254	72.31
139	Row	94	4	48	3254	67.79
139	Mix	94	7	50	3556	71.12
139	Mix	94	10	41	2822	68.83
336	Row	94	2	46	3293	71.59
336	Row	94	3	45	3134	69.64
336	Mix	94	7	47	3332	70.89
336	Mix	94	9	48	3560	74.17
262	Row	88	2	38	2365	62.24
262	Row	88	5	37	2284	61.73
262	Mix	88	8	38	2217	58.34
262	Mix	88	10	33	1844	55.88
272	Row	88	2	47	3437	73.13
272	Row	88	5	46	3745	81.41
272	Mix	88	7	49	3984	81.31
272	Mix	88	9	47	3598	76.55
289	Row	88	1	35	2318	66.33
289	Row	88	3	45	2945	65.44
289	Mix	88	7	42	2945	70.12
289	Mix	88	10	47	3334	70.94