Identity verification of trees in the 61 years old common ash (Fraxinus excelsior) clonal seed orchard FP202 (Birkemarken, Humlebæk) by DNA genotyping with microsatellite markers

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By Lene Rostgaard Nielsen, Lea Vig McKinney, Ditte Christina Olrik, Viggo Jensen and Erik Dahl Kjær
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Identity verification of trees in the 61 year old common ash (*Fraxinus excelsior*) clonal seed orchard FP202 (Birkemarken, Humlebæk) by DNA genotyping with microsatellite marker

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Preface

Denmark has a long-standing tradition for working with applied forest genetics based on seed from selected, healthy, productive and straight elite trees. Over the previous 60 years this work has implied a close cooperation between the Arboretum in Hørsholm and The Tree Improvement Station in Humlebæk. The Arboretum (since 2004 a part of Forest & Landscape, University of Copenhagen) has over the years selected, developed and tested superior seed-trees, whilst The Tree Improvement Station (today a part of The National Forest & Nature Agency, Øresund) has worked with effective propagation and distribution of improved material on a national scale. Strategies as well as tangible plans are regularly discussed and coordinated. The cooperation has been a strong link between research on the one side and forestry on the other, and since 1947 the Forest and Nature Agency has established, managed and harvested seed from an extensive network of seed sources, which, as far as seed orchards are concerned, have all been established in interaction with Forest & Landscape for breeding purposes.

One of the first seed orchards in the systematic breeding work is FP202. Selection and grafting of particularly interesting ash trees was started in 1934 in the Forest Botanical Garden under C. Syrach-Larsen’s direction (Larsen, 1945). In 1945-46 there was a large scale propagation of 14 selected clones. Helmhuth von Barner was responsible for planting 977 of these graftings at an area close to the newly started Tree Improvement Station in 1947, thereby establishing the (to our knowledge) World’s first Ash seed orchard. FP202 has since developed into one of the most important seed sources of ash in Danish forestry. In later years it has been the preferred seed source in Danish Forestry due to its origin from straight trees, but also because FP202 is expected to have the highest level of genetic diversity among the three ash seed orchards on the Danish market.

Due to the recent problems with ash dieback, special interest for the clones in FP202 has arisen. Dieback is supposedly caused by a fungus ‘Chalara fraxinea’. Though there are still many unanswered questions concerning this serious illness (Thomsen et al., 2008), it is very interesting that some clones show considerable resistance. FP202 contains both resistant and susceptible clones, and since 2007 it has become particularly interesting to study progenies from FP202 in order to increase the understanding of the genetic aspects of the disease and the option for breeding to increase resistance (Olrik et al., 2007). This work has included selective harvest from healthy clones in 2008.

Many years have passed since the first elite ash tree was selected and grafted in the 1930’ies, and there are many steps where mistakes could have occurred since. It is not possible to discern clones from each other by their appearance, and correctness of the clone identity in FP202 is therefore completely dependent on the high degree of care taken by all those involved from 1930’ies up to today. Fortunately, it is now possible to control the clone identity by DNA markers, and with this knowledge we decided to
control the clone identity of FP202, 60 years later. This report deals with the technique, results and conclusions of the analysis. In the appendix we have selected documentation concerning FP202’s establishment and treatment material which has only partly been published earlier.

Helmuth von Barner and C. Syrach-Larsen (who founded and managed the seed orchard for more than 40 years) would have been delighted by the results. There are only very few technical errors in this the (maybe) oldest ash seed orchard in the world.

Hørsholm og Humlebæk, December, 2008

Erik Dahl Kjær, Lars Graudal og Bjerne Ditlevsen
# Content

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface</td>
<td>1</td>
</tr>
<tr>
<td>Content</td>
<td>3</td>
</tr>
<tr>
<td>Abstract</td>
<td>4</td>
</tr>
<tr>
<td>Danish summary</td>
<td>5</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>7</td>
</tr>
<tr>
<td>2. The Seed orchard</td>
<td>8</td>
</tr>
<tr>
<td>3. Methods</td>
<td>9</td>
</tr>
<tr>
<td>4. Results</td>
<td>10</td>
</tr>
<tr>
<td>5. Conclusion</td>
<td>13</td>
</tr>
<tr>
<td>6. References</td>
<td>14</td>
</tr>
<tr>
<td>Appendix 1: Description of the establishment of FP202</td>
<td>16</td>
</tr>
<tr>
<td>Appendix 2: Description of FP202, 1976</td>
<td>25</td>
</tr>
<tr>
<td>Appendix 3: Selection and propagation of clones in FP202 (1938-1944)</td>
<td>28</td>
</tr>
</tbody>
</table>
Abstract

The *Fraxinus excelsior* clonal seed orchard FP202 was established at Birke-emarken, Humlebæk, Denmark in 1947, and is to our knowledge the first *Fraxinus* clonal seed orchard established worldwide. 61 years after establishment we tested clonal identity of remaining ramets by application of 4 polymorphic SSR markers. FP202 consists of alternating rows of the predominant male clone V282, and 8 predominant female clones: V702, V703, V704, V710, V791, V792, V793, V797. However, unpublished observations suggest that V797 may have substantial contribution to the male gamete pool, and that the gender function of V793 also is uncertain.

Among ramets labelled as V702, V703, V704, V710, V791, V792, V793, V797 a single genotypic mismatch was observed (Tree 24-02, probably a rootstock). Further, one V791 ramet was mistakenly labelled V282 (Tree 09-03). An important additional finding was that V702 and V703 ramets had identical genotypes and therefore most likely originates from the same ortet. We cannot infer from the data if it is V702 or V703 that is the correct name. 60 of the ramets labelled V282 (male) had identical genotypes, but 5 ramets had an alternative genotype suggesting that they form an extra male clone represented by 5 ramets scattered in the V282 rows. The origin of this clone is unknown, but is probably introduced by mistake during the graftings in 1945-46. A single ramet labelled V282 is probably a root stock.

*To conclude:* 115 trees (93%) had a DNA profile that fits the documentation even though it shall be noted that V702 and V703 ramets probably form a single clone. 8 trees deviated from the expected: 2 trees are likely root stocks, 1 tree had an incorrect label, and 5 trees form an additional clone of so-far unknown origin.
Danish summary

Træer i aske klonfroplantagen FP202 (Birkemarken) blev undersøgt med DNA markører for at kontrollere deres klonidentitet. FP202 består (ifølge anlægsrapport og øvrig dokumentation) af rækker med (overvejende) hanlige træer (alle klon V282) som alternerer med rækker af overvejende hunlige træer (8 kloner rækkevis: V702, V703, V704, V710, V791, V792, V793, V797). Det skal dog bemærkes at Helmuth Barner i 1976 indikerer at V797 sandsynligvis er hanlig (♂?) og at V793 er tvilsom i forhold til hanlig/hunlighet (cf. Appendix 2, nedenfor), selvom begge kloner producerede en del frø i plantagens unge år (cf. Appendix 1, nedenfor).

Resultaterne af DNA analysen er sammenstillet i Figur 1 og Tabel 2. Baseret på DNA analyserne konkluderer vi, at der i hunrækkerne kun forekommer én afvigelse (vi antager der er tale om en grundstamme). Dog bemærkes at to af de hunlige kloner (V702 og V703) med stor sandsynlighed er identiske idet de har samme DNA profil. Blandt de 67 træer i hanrækker identificerede vi ét træ som ikke passede med de øvrige, men i stedet kunne identificeres som V791. 60 træer i hanrækkerne havde identisk genotype, som vi derfor antager er V282. 5 træer spredt i hanrækkerne havde identiske genotyper, som dog afvej fra øvrige, og vi konkluderer på den baggrund at froplantagen indeholder én ekstra klon repræsenteret med 5 rameter. Denne klon er sandsynligvis introduceret ved en fejl under podearbejdet i 1945-46, men oprindelsen af klonen er usikker. Endelig observerede vi ét træ i hanrækkerne, hvis genotype afveg fra alle andre træer, og vi derfor antager er en grundstamme. I alt 115 ud af de 123 undersøgte træer (93%) havde således en DNA profil som passede med det forventede ifølge dokumentationen. Evt. enkelt træ blev af tekniske grunde ikke undersøgt og indgår derfor ikke i analyserne.

Konklusion: Froplantagen indeholder 124 træer, hvoraf 8 træer har en DNA profil som afviger fra forventningen ud fra dokumentationen: 2 træer antages at være grundstammer, 1 træ antages plantet forkert i forhold til kortet (evt. forbyttet podekvist), 5 træer udgør én ekstra klon fordelt i hanrækkerne. DNA-analysen afslørede desuden at V702 og V703 må antages at være samme klon uden det er muligt at afgøre om det er V702 eller V703 som er den korrekte betegnelse.
1. Introduction

The *Fraxinus excelsior* clonal seed orchard FP202 located at Birkemarcken close to the Danish Tree Improvement Station in Humlebæk, is the second oldest existing clonal seed orchard in Denmark. Further, it is to our knowledge the first clonal *Fraxinus* seed orchard established world wise.

The seed orchard has been one of the main providers of *Fraxinus* seed to Danish Forestry since the 1960’ies and is still one of the most used seed sources in Denmark. This would be sufficient reason for investing time and effort in validating the clonal identity of the trees in this old seed orchard. However, a new severe disease which was first observed in 2003, has infected the Danish ash trees dramatically, threatening the use of this important species (Thomsen et al., 2008). In 2007 significant variation was observed between clones in their degree of susceptibility (Olrik et al., 2007) and these important differences have been maintained in 2008. The findings were based on a clonal trial with 40 clones tested at two locations (CSOs). Fortunately, the old *Fraxinus* FP 202 seed orchard includes some of the best performing clones, but also some of the inferior ones. For this reason, the verification of clonal identity in the seed orchard has become increasingly important.

In 2008 it was decided to collect seed exclusively from the healthy clones in the seed orchard. Seeds on these trees are presumably sired by the healthy clone (V282), and the expectation is therefore that such seed would have increased resistance against the disease. However, in order to be able to study such parent-offspring regression as well as in order to ensure seed collection from the correct trees, it was decided in 2008 to perform a full test of clonal identity of all trees in the seed orchard.

The present survey thus has three objectives: (i) to check the clonal seed orchard for “cryptic dysfunctions” due to grafting mistakes or seed/pollen production from root stock (cf. Hansen & Kjær, 2006), (ii) ensure seed collection from correct trees during exclusive seed collection, (iii) to facilitate precise comparison of parent-offspring regression in health and thereby allow studies on heritability and genetic background for the apparent resistance (to be studied in 2009).

The present report documents the applied methods and results of this survey with the three above objectives.
2. The Seed orchard

Establishment and management of the seed orchards are described in some detail in old reports and other written material mainly authored by Helmuth Barner, the director of the Tree Improvement station from its establishment in 1947 until 1988. Two key reports are reproduced in appendix 1 and 2, from where the below description has been extracted:

The seed orchard was established with graftings in 1947 on agricultural land. A total of 14 clones were included, 1 predominantly male clone and 13 predominantly female clones (Appendix 1). Brief description of plus trees and grafting work are included in Appendix 3, and pictures of the majority of the plus trees are included in Appendix 1. The establishment phase included mechanical weeding. Pruning of branches was applied in 1953 and 1954 to increase seed set, but with very limited effect. Application of fertiliser and removal of grass did increase the health and growth of the seed orchard trees, but did not lead to substantial seed yield (Appendix 1).

5 of the female clones were removed in 1961, leaving only 8 predominantly females (V702, V703, V704, V710, V791, V792, V793, V797), and one predominantly male (V282). However, in 1976, Helmut Barner questions if V793 and V797 are truely predominantly females (cf. Appendix 2), and these 2 clones may therefore also be important pollen donors in the Seed orchard. Further information on the selection and propagation of the individual clones are included in Appendix 3.
3. Methods

**Sampling**
Leaf material was collected on September 10th 2008. Two leaves were collected from each tree throughout the seed orchard. Each sample was labelled according to their position on the map (Figure 1). In total, 67 samples were collected from the male rows (presumed V282) and 56 samples from the female rows (presumed V702, V703, V704, V710, V791, V792, V793, V797). The material was stored at -20 °C until DNA extraction.

**Genotyping**
15-20 mg leaf tissue per individual was treated with liquid nitrogen and ground on a bead mill without any prior preparation. DNA extraction was carried out with the DNeasy 96 Plant Kit from QIAGEN following the manufactures protocol for frozen material.

The DNA-extractions were kept undiluted for the polymerase chain reaction. Eleven primer pairs developed for *Fraxinus excelsior* were initially tested. Ten of these (FEMSATL1, 2, 4, 5, 8, 10, 11, 12, 16, 19) were all developed by Lefort *et al.* (1999). FEMSATL12 has later been modified by Gerard *et al.* (2006) and it was the modified version that we tested. The last primer pair (M2-30) was developed by Brachet *et al.* (1999). Four polymorphic, interpretable microsatellite loci were identified and used for further genotyping. The chosen primer pairs were FEMSATL11, FEMSATL12, FEMSATL16, FEMSATL19.

PCR reactions were carried out using the Qiagen Multiplex PCR kit according to the manufacturer’s instructions except that the reaction volumes were scaled down to 15µl. PCR-amplifications were completed on Perkin Elmer Thermo cyclers (models 9700 and 2700) under the following conditions: an initial denaturation step of 15 min at 95 °C, 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s and extension at 72 °C for 60 s, and a final extension step at 60 °C for 30 min. Each amplified product was diluted with 30µl H2O and visualized with an ABI3130xl sequencer from Applied Biosystems.
4. Results

In Table 1 we see that the amplification of the 4 microsatellite loci in total resulted in 35 alleles. This level of polymorphism was highly sufficient to distinguish between the genotyped clones.

Table 1. Number of alleles per microsatellite locus. The alleles are given in base pairs

<table>
<thead>
<tr>
<th></th>
<th>Femsatl11</th>
<th>Femsatl12</th>
<th>Femsatl16</th>
<th>Femsatl19</th>
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<tbody>
<tr>
<td>1</td>
<td>183</td>
<td>172</td>
<td>186</td>
<td>174</td>
</tr>
<tr>
<td>2</td>
<td>185</td>
<td>174</td>
<td>188</td>
<td>176</td>
</tr>
<tr>
<td>3</td>
<td>191</td>
<td>176</td>
<td>196</td>
<td>180</td>
</tr>
<tr>
<td>4</td>
<td>193</td>
<td>178</td>
<td>200</td>
<td>182</td>
</tr>
<tr>
<td>5</td>
<td>197</td>
<td>190</td>
<td></td>
<td>186</td>
</tr>
<tr>
<td>6</td>
<td>201</td>
<td>196</td>
<td></td>
<td>188</td>
</tr>
<tr>
<td>7</td>
<td>203</td>
<td>198</td>
<td></td>
<td>190</td>
</tr>
<tr>
<td>8</td>
<td>205</td>
<td>200</td>
<td></td>
<td>192</td>
</tr>
<tr>
<td>9</td>
<td>213</td>
<td>204</td>
<td></td>
<td>194</td>
</tr>
<tr>
<td>10</td>
<td>206</td>
<td></td>
<td></td>
<td>198</td>
</tr>
<tr>
<td>11</td>
<td>211</td>
<td></td>
<td></td>
<td>202</td>
</tr>
</tbody>
</table>

Of the 67 examined male trees (labelled V282) it turned out that 60 ramets had identical genotype (recognized as V282, Table 2). The remaining seven were of a different genotype. One (position 0905) turned out to have same genotype as V791 and may thus be a wrong grafting. One was not recognized elsewhere (position 0307) and may be a root stock. The last 5 (positions 0107, 0901, 0909, 1505, 1903) all had the same genotype (see Table 2) that did not match with any of the others.

Among the female rows, only 1 tree (labelled V702, position 2402) did not resemble any other genotype and may thus be a root stock. However, very interestingly trees labelled V702 and V703 turned out to have identical genotype, and it is therefore very likely that they originate from the same ortet (= the same clone).
Figure 1. Positions of trees with deviating genotypes compared to documentation. Marked in red: »Extra male« = five trees with identical genotypes that are not identical to the V282 genotype. The two squares show the trees with no match. V702 and V703 turned out to have identical genotypes.
Table 2. Summary of the genotypes of the clones of *Fraxinus excelsior* from Birkemarken (seed orchard FP202). Alleles are given in base pair lengths at each of the 4 loci.

<table>
<thead>
<tr>
<th>Clone ID</th>
<th>Femsat11</th>
<th>Femsat12</th>
<th>Femsat16</th>
<th>Femsat19</th>
</tr>
</thead>
<tbody>
<tr>
<td>V282</td>
<td>183</td>
<td>201</td>
<td>174</td>
<td>174</td>
</tr>
<tr>
<td>V702</td>
<td>191</td>
<td>205</td>
<td>176</td>
<td>196</td>
</tr>
<tr>
<td>V703</td>
<td>191</td>
<td>205</td>
<td>176</td>
<td>196</td>
</tr>
<tr>
<td>V704</td>
<td>193</td>
<td>213</td>
<td>190</td>
<td>206</td>
</tr>
<tr>
<td>V710</td>
<td>183</td>
<td>203</td>
<td>172</td>
<td>190</td>
</tr>
<tr>
<td>V791</td>
<td>185</td>
<td>191</td>
<td>174</td>
<td>178</td>
</tr>
<tr>
<td>V792</td>
<td>183</td>
<td>191</td>
<td>174</td>
<td>174</td>
</tr>
<tr>
<td>V793</td>
<td>183</td>
<td>191</td>
<td>200</td>
<td>204</td>
</tr>
<tr>
<td>V797</td>
<td>197</td>
<td>205</td>
<td>190</td>
<td>200</td>
</tr>
<tr>
<td>Extra male (0107, 0901, 0909, 1505, 1903)</td>
<td>183</td>
<td>191</td>
<td>174</td>
<td>190</td>
</tr>
<tr>
<td>Male with no match (0307)</td>
<td>193</td>
<td>205</td>
<td>174</td>
<td>211</td>
</tr>
<tr>
<td>Female with no match (2402)</td>
<td>197</td>
<td>201</td>
<td>190</td>
<td>198</td>
</tr>
</tbody>
</table>

Please note that V702 and V703 have identical genotypes and it is therefore highly likely that it is indeed the same clone.
5. Conclusion

In conclusion, the seed orchard corresponded to 7 clones in female rows and 2 clones in the male rows rather than 8 clones in female rows and 1 clone in male rows. Besides this, there were only few unexplained deviations (1 assumed wrong/misplaced grafting, and 2 likely root stocks). According to old records, 2 of the clones in the female rows may produce significant amount of pollen and the pollen production id therefore likely to be distributed on more than one clone.

The identity of the »extra male« remains unknown. We have genotyped 40 clones deployed in different Danish seed orchards, but none of these match (Nielsen, unpublished results). A screening of old clonal archives with *Fraxinus* may reveal the true identity of this »extra male«, but this will probably not be an easy matter. Studies of segregation in terms of health of progenies from FP202 will be performed in 2008-2009, and here we will look for progenies sired by this »extra male«. Based on this parental analysis we expect to be able to determine the health status (in term of breeding value) of the »extra male«. This will only be possible if the clone has been sufficiently male fertile to sire a fair number of progenies in our progeny sample. Based on the results it will be possible to recommend if the clone should be removed from the seed orchard or maintained.
6. References


Larsen, C.S. 1945.

Identification and characterisation of microsatellite loci in ash (*Fraxinus excelsior* L) and their conservation in the olive family (Oleaceae). *Molecular Ecology* 8, 1088–1091.

Klonforskelle i angreb af asketoptørre. SKOVEN 39, 522–525.

FP 202, December 2008. Pruning has been applied to increase fruiting and ease seed collection (cf. Annex 1 below). Many trees therefore have low twigs. However, today the clonal identity is correct on most trees and the DNA analysis only identifies two trees to be likely root stock. This result proves careful pruning and removal of root stock sprouts in the seed orchard.
Appendix 1: Description of the establishment of FP202

**Source:** Selected part of the unpublished document ‘Frøhaver Ask’, which is part of the old files at the Arboretum. The file is undated and the authorship is not stated. We believe it was written by Helmuth Barner, the former director of The Danish National Tree Improvement Station, who was responsible for the establishment and management of the seed orchard.

We are not sure about the date. The document refers to the flowering in 1960, and is likely to be prior to 1961, because it does not refer to the thinning of the clonal seed orchard that was performed in that year according to documents from 1976 (appendix 2 below). The document include numbers of seed harvested up till 1966/67, but these figures may have been added later as no references are given to flowering after 1960. We therefore assume that the document dates around autumn 1960.
Planteavlstationen
Birkemarken
Areal: 1,5 ha.

Ansk
Prehave 202.

Formål: Produktion af askefrø.

Isoleringsområde: Isoleringsområde er cirka 500 m gennem skov til nærmeste askebevoksning, der er gammel og nede blomstrer mere.

Avlsmateriale: Som hundremmer indgår i plantagen følgende, der er udvalgt af dr. Svenache Larsen:

<table>
<thead>
<tr>
<th>Plantegård</th>
<th>Afd.</th>
<th>Trænr.</th>
<th>Trænr. nr.</th>
<th>Udgave</th>
<th>Prøvetagning</th>
<th>Lyntagning</th>
<th>Sæsong</th>
<th>Prøvetagning</th>
<th>Lyntagning</th>
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<td>80 stk.</td>
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<td>okul. 1945</td>
<td>-</td>
<td>podet 1946</td>
<td>-</td>
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<td>Boller</td>
<td>Nørreskov</td>
<td>30</td>
<td>-</td>
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<td>podet 1946</td>
<td>-</td>
<td>-</td>
<td>podet 1945</td>
<td>-</td>
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<tr>
<td>Tåsinge</td>
<td>Horreskov</td>
<td>5</td>
<td>-</td>
<td>40 stk.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Søde II</td>
<td>Horreskov</td>
<td>63 a</td>
<td>-</td>
<td>80 stk.</td>
<td>okul. 1945</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Horreskov</td>
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<td>-</td>
<td>20 stk.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Horreskov</td>
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<td>60 stk.</td>
<td>podet 1946</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>Svenstrup</td>
<td>Dalby Skov</td>
<td>V 23 a</td>
<td>-</td>
<td>4 stk.</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Bregentved</td>
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<td>112</td>
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<td>2 stk.</td>
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<td>-</td>
</tr>
<tr>
<td>Bregentved</td>
<td>-</td>
<td>&quot;Jubileumsasken&quot;</td>
<td>5 stk.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Svenstrup</td>
<td>Kimmerelev, Hessel</td>
<td>11</td>
<td>-</td>
<td>2 stk.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Som han er anvendt V. 202 Stenderup Nørreskov.

Plantagen er anlagt i foråret 1947 i den østligste del af Birkemarken. V. 282 indgår med 2 rækker i hver anden dobbelt række, hundremerne indgår derimellom som sit på skitsen.

Alt materialet er leveret af Arboretet i Hersholm.

Planteafstand 4 x 4 m.

Plantagens udvikling: Plantagen blev anlagt på gl. agerjord, der lige efter overtagelsen blev behandlet med harvning, hvorefter askene blev udplantet. De første 3 år blev der til stadighed renholdt med harvning, hvorefter man indskrænkede sig til at holde greaset nede med slåning.

For at prøve dels at påvirke frøsætningen, dels at lette plukningen, klippede man første gang i vinteren 1953/54 i rækken med hundre, grende ind på hverandens ask i hver række (i forbindelse). Dette blev gentaget i 1954/55 med de samme træer. - Intet udslag!
Ash
Birkemorhau
m. 202
Efter at man i nogle år havde holdt arealet med gres, viste det sig, at askene hømmede i udviklingen. Sidstene blev gullige, og væksten var særlig på midterarealet ringe.

I oktober 1957 fræsede man derfor mellem alle askerækkerne (4 træk mellem 2 rækker ask). Følgende række mellemrum blev dog ikke fræsede:

\[ 25 - 26 = V. 282 \]
\[ 33 - 34 = V. 262 \]

Idet rækkerne regnes fra nord.

Umiddelbart herefter blev på tvers af rækkerne givet:

\[ \begin{align*}
7 \text{ rækker ask} & = 225 \text{ kg svovlur ammoniak ialt} \\
6 - - - & = \text{ intet} \\
7 - - & = 225 \text{ kg kalksalpeter ialt}
\end{align*} \]

I foråret 1958 blev følgende tilført:

\[ \begin{align*}
7 \text{ rækker ask} & = 250 \text{ kg svovlur ammoniak ialt} \\
6 - - & = \text{ intet} \\
7 - - & = 250 \text{ kg kalksalpeter ialt}
\end{align*} \]

Allerede tidligt på sommeren 1958 sås klart udslag for gødkningen, idet 2x7 gødede rækker fik mørkegrønne, mundne blade. Der konstateredes ikke forskel på de 2 gødningsarters virkning.

Føråret 1959 gødedes hele arealet med 375 kg svovlur an. og senere med 375 kg. Arealet blev i 1959 holdt med fræsning og er i 1960 blevet harvet. Gødkning + fræsning gav klart positivt udslag m.h.t. vækst.

I 1950/59 blev foretaget en kraftig besøring af hveranden ± træ. Virkningen var, bedstet sommeren 1960, ikke særlig gunstig. Der har som frygtet udviklet sig kraftige vanris på de beskårne træer og blomstringen foråret 1959 var ikke bedre på de beskårne træer end på de andre træer.

1960 synes at være godt blomstringsår for ask. Blomstringen i frehavnen er dog beskedent og i lighed med de 2 tidligere blomstringsår, er der flest blonster i de laveste dele mod S. og N. Der er dog en nogenlunde god blomstring på 8 rækkerne også på midterstykket, hvilket tyder på, at det ikke alene er de ydre forhold, der hindrer 9 klonerne i blomstring på midterstykket.
Amk.
Sørs I, Broby Vestervskov, afd. 63 a.
V. 791.
fot. den 29/1 -1954.

Ask.
Tåstinge, Horze skov, afd. 5.
træ mrk. 3 gule platt. V. 710.
fot.

Ask.
Sørs II, Skræsinge, st. Bogeskov, afd. 67 a.
V. 782.
fot. den 29/1 -1945.
Ask.
Bregentved, Sorte led afd. 112.
V. 695.
fot. den 13/1 - 1954.

Ask.
Svenstrup, Kimmerslev Hessel afd. 11.
V. 698.
fot. d. 22/1 - 1954.
AaK.
Stenderup Nørreskov, afd. 12.
træ nr. 1, v. 702.

AaK.
Boller Nørreskov, afd. 30.
træ (gul ring), v. 704.
fot. den 30/1-44.

AaK.
Stenderup Nørreskov, afd. 30.
træ nr. 2, v. 703.
Appendix 2: Description of FP202, 1976


PROVENIENSMEDDELELSE NR. 7
STAMDOGESBLAD

1. Identification
1. Træart: Ask, Fraxinus excelsior
2. Løbenummer: 262
4. Ansvarlig tilskuesførende: Planteavlsslstationen
6. Areal: 1.5 ha
7. Kort: A 263ø

1. Fæltes anvendelsesområde: Ingen særlige begrænsninger inden for danske askeklassifikationer.

3. Oplysninger
1. Komponenter | Antal | Udplantnings-
 | | ved |_Formering-
 | | anlag | |_år |_hast |_indtil |
| Kloner | 14 | 1951 | 5 | 1945-46 | 1947 | 1/1-76 1241 |
| familier | 0 | | | | | |
3. Plantningssæde: 4 x 4 m.
4. Isolering: Enkelt små askegrupper 200-300 m N.V. for plantagen, enkelt aske 450 m gennem skov N. og NV for plantagen, samt 2 små askebevoksninger ca. 550-600 m gennem skov NV for plantagen.

4. Grundmateriale
1. Udgangsbevoksninger, Flustrør velgt i serdels gode og velformede askebevoksninger.
2. Udvalgsselektion. Efter kun for at få hønlig og hønlig kloner samt efter ræthed og stammeværdi.
3. Udvalgsstyrke: Vanskelig at vurdere.
5. **Komponenterne og deres afstamning.**

<table>
<thead>
<tr>
<th>Betegnelse</th>
<th>Kun</th>
<th>Oprindelser</th>
<th>Herkomst</th>
<th>Antal træer i plantagen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Formentlig dansk lokalrace</td>
<td>Stenderup, Nørreskov afd. 12</td>
<td>30 20</td>
</tr>
<tr>
<td>V 703 træ 1</td>
<td>?</td>
<td>lokalrace</td>
<td>Stenderup, Nørreskov afd. 10</td>
<td>30 20</td>
</tr>
<tr>
<td>V 704</td>
<td>?</td>
<td>-</td>
<td>Boller, Nedereskov afd. 30</td>
<td>40 10</td>
</tr>
<tr>
<td>V 710</td>
<td>?</td>
<td>-</td>
<td>Tåsinge, Horsenskov afd. 5</td>
<td>40 10</td>
</tr>
<tr>
<td>V 791</td>
<td>?</td>
<td>-</td>
<td>Sore II, Droby Vestergaard afd. 63a</td>
<td>80 20</td>
</tr>
<tr>
<td>V 792</td>
<td>?</td>
<td>-</td>
<td>Sore II, St. Bæge- skov afd. 67a</td>
<td>20 9</td>
</tr>
<tr>
<td>V 793</td>
<td>?</td>
<td>-</td>
<td>Sore II, L. Bægeskov afd. 82a</td>
<td>80 20</td>
</tr>
<tr>
<td>V 797, træ 1</td>
<td>?</td>
<td>-</td>
<td>Harsholm, Stasevang afd. 274</td>
<td>40 10</td>
</tr>
<tr>
<td>V 869</td>
<td>?</td>
<td>-</td>
<td>Svenstrup, Dalby afd. 23a</td>
<td>4 0</td>
</tr>
<tr>
<td>V 894</td>
<td>?</td>
<td>-</td>
<td>Bregentved, Brevinde afd. 9c</td>
<td>4 0</td>
</tr>
<tr>
<td>V 895</td>
<td>?</td>
<td>-</td>
<td>- 112</td>
<td>2 0</td>
</tr>
<tr>
<td>V 896</td>
<td>?</td>
<td>-</td>
<td>Bregentved &quot;Jubilæumsask&quot;</td>
<td>5 0</td>
</tr>
<tr>
<td>V 898</td>
<td>?</td>
<td>-</td>
<td>Svenstrup, Kimmerslev afd. 11</td>
<td>2 0</td>
</tr>
<tr>
<td>V 282 træ 44</td>
<td>?</td>
<td>-</td>
<td>Stenderup, Nørreskov afd. 12</td>
<td>500 122</td>
</tr>
</tbody>
</table>

| Ialt | 977 | 241 |

6. **Forventninger til fremplantagens afkom.**

1. **Udgangspopulationens niveau:** Bevoksering over middel.
2. **Plustreumvalgets forventede effekt:** Usikker i en så stærkt kærlvsrikart.
3. **Forventet heterosisseffekt:** Ingen-ringe, event. provensival-hybrid-effekt.
4. **Forventet kombinationseffekt:**?
Denne proveniensseddelage aflæser den tidligere udsendte proveniensseddelage nr 7 af september 1963, der bedes udtages af samlingen.
Appendix 3: Selection and propagation of clones in FP202 (1938-1944)

Source: Scanned copies of original registrations made during 1938-1944. Unpublished records kept at Forest and Landscape, Faculty of Life Sciences, University of Copenhagen.

V282-1
Fremar 1941: Pedel 278 stk. af eksempelheverede 7 plante pr stk. på Clicht.


19. IV: 490 stk. være med middelhøj 18.3 cm, stivte hjørner 100 cm.

2 / IV: Pehier ophængt og sortert.

203 stk. småkø, nede i rådning

150 - mest småkø Svinj

835 - de nedrørdende

142 - de nedrørdende

15 - løsset

+ 4 - sårede for eneste (formen de tilbyg og Nykøben stavne)
2611-44: Material hentet af E. Maginus, der beskriver Træet:
  "Totalkjøerle lung, Bredkøje 15,9 m. Dyr, q. 0,60 m.
  (jfr. hér Nendens Korter). Traek har været jævnt
  og rigele beæst med Træker af hvilke en del end-
  mår stad tilbage i det sammenfald. Entrer
  Traek med hjælp af S.F. i Stige til klipper, med Stang-
  saks. Materiallet til 2-300 Pskerlde jg. af de ned-
  klipper, gennem instandtæt Det Træek.

26-44: Skilte i Maginus Beskrivning 5, 2.

16-44: 1000 cm. Vægt = 76, 29 g.

7/5-44: Potet i Sti 12 stk.
21-44: Beete 10 stk.

24/5-44: Potet paa Kemmeljode 32 stk.
12/6-44: Ryst 12 stk.

26-45: Potet i Æg af 20 stk.
7/6-47: Ryst 13 stk.

5/11-49: Træet faldt i Storm (mindst 13 år gamm).

Undertegnede 50
V. 703.

26/44: Materialhundel af S. Maginio, den skriver:
"Tolkehøjde 28.2 m, Rækhøjde 14.8 m, Øvem. 43 em
(Skr. Ludvigs Naturligv.). Også dette er en bland
ejævnt i rigtighed, men her var ikke et sikkert
højde og det fandt vi med meget lidt, men det er det faalde
af vore gennemførte led, vaar det rette faalde
af vore gennemførte led.

84/44: 1000. Røm Veg: 76.25 g

4/5-44: Røde: 15 Stk.

17-44: Røde: 15 Stk.

24/5-44: Røde: paa nembohole: 30 Stk.

10-7-44: Røde: 21 Stk.

24/6-45:  Røde: 8 gr. 20 Stk.

7/6-47: Røde: 3 Stk.

Ind [??/62]
V. 704.

7 stk.

Boller Redskor, ejt. 30.

30-44: Materialte hentet af E. Majorus, der skrevet:

"Takskæbe 23 cm, Alskeby 14 cm, Buthjede 15 cm.
Omkv. 18 cm, 76 cm. Et meget smukt trik.
Stammen meder fuldstændigt ret, stammen afsormet, men
ikke ret stor. Kan meget lidt bi, som kan i det lidt
jævnt fordel over fraks. 

... Fraks var ældre
Forestår, men med et stammens tæ i det syvii
emn fra firk. Vegeti ..."

Skifte Foto i Majorus Retning 56-4.

½-44: 1000 km. Vgt.: 98.09.

½-44: Vedet i Hus. 12 stk.
½-44: Alle i Liv.

2½-44: Vedet paa Hummerfelt. 29 stk.
½-44: Rist. 27 stk.

½-47: Mem de Carl Calnings med tilbage i stort og
Underør. 134 (27 stk.) små u. 28 medere indeni
Ver. 7043 (2 stk. mindre) 3 cm. 7 cm. de kan sætte enige de
har meget Rihet. Bl.a. flere Dende af 3.

Disse blaa Plante kan nu haaret i cel i godt vid.
Indført 1950
V. 710. 2 Sek, 19.5.
Stene Skor og Tuninge
Koldemaarbejtd. D.

14/2-44: Materialite herled af E. Skjoldebo, der skrevet:
- Totaltjede 21 m³. Kædetræs
- Stenskjede 12,3 m³.
Onp. 78 cm. Skammene ikke helt re, men indej
Forekommer. Hanen selftørret, jernalg sigetog bent
med Br. Trede skæb i en besværlighed med mange
mægt. småbt. tørr, som dog forbiusvis tættes af vores
Han Trede. En ét. der med hjælp af Jomfru
Klipper med skæbde. Undersøkt af ca. 300 pod
Hvile og indsmulatt. Fremskrift af de udsikkeder.

44-44: 100 kg Vande / 100,0 kg

434: Podde i tværs 12 Stk.
49-44: Ret 12 Stk.
44: - 10 -

245-44: Podde fra Ummelninger 38 Stk.
107-44: Ret 32 Stk.

Frem: Podde fra Ummelninger 15 Stk. (Indsevning)

246 45: 20 Stk. podde i årsrap.
76 47: Ret 15 Stk.
V791

Act.

Dusty Veldecker, I Soro, Apr. 63 as

2015-16: 100 x 16 x 60
11-14: 100 x 60 x 60

ind 1/80
V793

8.9.43.  

Røk, "Frisk"  
Lille Bøgeskov dpt. 82°, Dpt. FK  
Indsendt og tol. 24.12.1944.

24/5-44: Pølse paa sammenløse 34 slk.  
19/6-14: Røst 29 slk.

24/45: Pølset i kæggepl. 20 th.  
7/6 47: Røst 16 thl.

Undf 1/50
V797

V797

Page 11

1953-44:  Punkt i Bliss 13 stk.
19-44:  Rest 17 stk.

245.44:  Punkt på Vemmeloft 20 stk.
10.7-44:  Rest 12 stk.

Und (not handwritten)
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