Betula pendula and Betula pubescens

Recent genetic Research

Gösta Eriksson
The cover picture originates from the *Betula pubescens* tree show planted at The Genetic Center, Ultuna, Uppsala, Sweden. Photograph Hartmut Weichelt
Preface

My Summaries of recent achievements in forest genetics in form of the books *Pinus sylvestris* Recent Genetic Research and *Picea abies* Recent Genetic Research were appreciated by many colleagues. Therefore, I was encouraged to summarize genetic research related to the two birch tree species in Fennoscandia, *Betula pendula* and *Betula pubescens*. Since there was a summary presented in 1985 I have searched for papers from 1985 and onwards. As in the preceeding books papers written in languishes that are not understood by the scientific society are not treated. Around 200 papers are included in this summary, most of them originate from Finnish scientists.

The outline is somewhat different from the previous books owing to the type of genetic papers published. Many papers treat resistance or tolerance to pests and diseases, which deserved a separate chapter. Similarly, a large number of papers treat tolerance against abiotic factors such as heavy metals and ozone. Therefore, this topic is presented in an own chapter.

I have tried to illustrate major achievements by graphic illustrations and tables. It should be remarked that none of the illustrations were taken from the original papers.

A generous grant from Föreningen Skogsträdsförädling, The Tree Breeding Association in Sweden, made this printing possible. Dr Anneli Veherä-Aarnio, METLA, Finland, has supplied me with valuable information, which I am most grateful for. Librarian Lars-Olof Hansson has given me important support in literature searching. Sincere thanks to Dr Björn Nicander for solving several computer problems.

Uppsala November 2011
Gösta Eriksson
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1.1 Field studies

Viherä-Arnio and Velling (2008) reported on growth and quality traits at age 22 of 21 *B. pendula* populations growing in two Finnish field trials at latitudes 60.35 and 63.18°N, respectively. Nineteen of the populations originated from the latitudinal and longitudinal ranges 54.84-63.18°N and 22.45-29.30°E. Finally, two populations originated from Novosibirisk in Siberia at the longitude 84°E and latitude 54.50°N. The latter two populations were not included in the studies of the relationship between population origin and performance. Some of the populations consisted of crosses or open-pollinated progenies.

In Table 1-1 I have summarised the main results for the relationships between population origin and trait values for the five traits studied. Only once, DBH at the northern trial, the relationship was non-significant. At the northern trial survival increased with latitude while there was a maximum of survival close to the test locality latitude at the southern trial. The population means for survival percentage varied between 30 and 65 in the northern trial while the corresponding figures for the southern trial were 58 and 87. At both trials the Finnish plus tree progenies were among the tallest. The progenies with the longest northward transfers, Siberia and Lithuania, were among the shortest in both trials. For all growth traits there was a maximum for a transfer a few degrees in northern direction. All curvilinear relationships for growth traits had their maxima at transfers a few degrees towards north (see Fig. 1-1). Populations transferred from south to north will have a longer growth period than the local population. This results in a better growth until the growth period becomes so long that the plants are damaged by autumn frosts. The increase for volume per hectare for a 2-degree-transfer to the north was estimated at 10m³/ha. However, this might be connected with an increase of damage. Stem tapering decreased slightly with latitude in the northern trial but increased with latitude in the southern trial. The stem defects, vertical branches and forking, decreased linearly with latitude in both cases, with a steeper slope at the northern trial.

In conclusions there was a large variation among populations for the six traits studied. The relationships between latitudinal origin and trait performance were significant in most cases but the degree of determination was never strong. The results suggest that transfers a few degrees of latitude northwards would result in increased yield. The results indicated that the selection of Finnish plus trees had been successful.

![Figure 1-1. The relationship between latitudinal transfer and tree height at age 22 of B. pendula populations in a Finnish field trial at 60.35°N. Minus means transfer to a northern (T) locality and plus means a transfer to a southern (S) locality. Viherä-Arnio and Velling 2008.](image)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Latitude 60.35°N</th>
<th>Latitude 63.18°N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree height</td>
<td>0.019 curvilinear</td>
<td>0.020 curvilinear</td>
</tr>
<tr>
<td>DBH</td>
<td>0.001 curvilinear</td>
<td>ns</td>
</tr>
<tr>
<td>Volume/hectare</td>
<td>&lt; 0.001 curvilinear</td>
<td>0.011 curvilinear</td>
</tr>
<tr>
<td>Stem tapering</td>
<td>&lt; 0.001 linear</td>
<td>0.001 linear</td>
</tr>
<tr>
<td>Survival</td>
<td>0.042 curvilinear</td>
<td>&lt; 0.001 linear</td>
</tr>
<tr>
<td>Vertical branch forking %</td>
<td>0.002 linear</td>
<td>&lt;0.001 linear</td>
</tr>
</tbody>
</table>

Table 1-1. The p-values and type of relationship between *B. pendula* population origin and six traits in two trials in southern and central Finland, respectively. Viherä-Arnio and Velling 2008.
Tree growth in a Norwegian field trial with three *B. pendula* and four *B. pubescens* populations at latitude 60°N was reported by Heide (2003). In this paper Heide also reported on bud flushing in three tree species after various combinations of low temperature treatment and forcing temperatures. The latter experiment did not treat genetic differences. In the field trial recordings of bud flushing was carried out annually.

As seen from Fig. 1-2 there is a strong relationship between duration of the growth period and breast height diameter or tree height at age 9 of three *B. pendula* and four *B. pubescens* populations growing in a field trial at 60°N in Norway. Heide 2003.

As seen from Fig. 1-2 there is a strong relationship between population origin and the two growth traits, breast height diameter and tree height. The fit to the curves is exceptionally good, $R^2 = 1.00$. With only three *B. pendula* populations I did not calculate any relationship between origin and growth in this species. Generally, there was a strong relationship between duration of the growth period and the growth traits (Fig. 1-3). The *B. pendula* population from latitude 63.84°N had a better growth than all populations. However, the estimated length of the growth period of this population did not deviate from the general pattern of a continuous shortening of the period with increasing latitudinal origin (Fig 1-4). The variation in flushing within years was small while it was large among years. For the above mentioned population it varied between day 105 and day 126 and was strongly significant. The author tried to relate bud flushing to different temperatures during previous year and to current year temperatures. Several of the relationships were significant but the degree of explanation did not reach 30% in any of this relationships. Therefore, the title of the paper that *High autumn temperature delays spring bud burst* is not fully justified with 70% of the variation explained by other conditions.

Two field trials with three populations of *B. pendula* in southern Finland, latitudes 60.24 and 60.65°N, were included in a study of growth, water content, freezing tolerance, and content of abscisic acid, ABA (Li et al. 2003b). Assessments were taken at age 3 in the southern trial and at age 10 in the northern trial. Water content and freezing tolerance was estimated in lateral buds. The latter trait was assessed by exposing buds in freezing baths and subsequent measurement of electrolytes. Freezing temperatures were not given but it was stated that samples were taken at 5 degree intervals during the lowering of the temperature. The temperature at which 50% electrolyte leakage, $LT_{50}$, occurred was used as estimate of frost tolerance. The paper contains several instructive illustrations of the development during the annual cycle of some of the traits. The difference in bud flushing between the two extreme populations was only 4-7 days. The corresponding difference for growth cessation was estimated at 40 days. In Fig. 1-5 I have preferred to illustrate results from one date when the differentiation was largest among the three populations. As seen from this figure there is a clear difference in performance; the northern population has low-

**Figure 1-2.** The relationship between breast height diameter and tree height at age 9 of three *B. pendula* and four *B. pubescens* populations growing in a field trial at 60°N in Norway. Heide 2003.

**Figure 1-3.** The relationship between length of the growth period and breast height diameter or tree height at age 9 of four *B. pubescens* populations growing in a field trial at 60°N in Norway. $R^2 = 0.99$ for both curves. Heide 2003.

**Figure 1-4.** The relationship between population latitudinal origin and duration of the growth period for three *B. pendula* and four *B. pubescens* populations growing in a field trial at latitude 60°N in Norway. Heide 2003.
Differences in performance of birch populations and species hybrids in two series of field trials in central Germany were reported by Rau (1991). Each series had four trials, in which survival, tree height, DBH, and several quality traits were assessed.

The offspring from seed orchards and individual clones in seed orchards of both species had the best height growth and a satisfactory survival (Fig. 1-7). The three B. pumescens seed orchard and clonal progenies ranked highest with respect to stem form.

Except for commercial standards from former Soviet Union the survival was good in the species hybrid trial. The three B. pumescens x B. pumescens families showed the best growth amounting to 13-52% superiority compared to the standards. The hybrids between B. pumescens and the exotic species seemed to confer improvement compared to the exotic species both in growth and survival. The B. pendula x B. papyrifera hybrids had a satisfactory survival but poorer growth than B. pumescens progenies. The stem form was also better in B. pumescens progenies than in B. pendula offspring.

I agree with the conclusion that these series of experiments clearly indicated that selection of seed tree stands, selection of plus trees, establishment of seed orchards, and crosses of genetically superior clones will lead to substantial improvement. A reservation for the young age of the trials is justified.
Scottish 450 masl
Scottish 50 masl
Scottish 450 masl
Scottish 50 masl
Scottish 450 masl
Scottish 50 masl
Scottish 450 masl
Scottish 50 masl
Scottish 450 masl
Scottish 50 masl

![Height and breast height diameter in two field trials at age 16 and 22 with Finnish polycross families and two different Scottish standards. B. pendula. Worrell et al 2000.](image)

Finnish polycross progenies were compared with unselected Scottish B. pendula in two field trials at ages 16 and 22 (Worrell et al. 2000). Juvenile recordings of growth and phenology in three field trials with Scottish and northern England populations were also reported. As a complement, four isozyme loci in nine populations from one of the young trials were determined. The site conditions in the two field trials differed considerably as illustrated in Fig. 1-8 with taller trees at age 16 in the Teindland trial than in the older Speymouth trial. Different Scottish standards were used in the field trials.

The inferior growth of the Finnish material is obvious and the poor survival of 36 and 59% makes the Finnish materials still more inferior. The Finnish material originates from more northern latitudes than the test localities. A southward transfer usually leads to reduced growth since the critical night length for growth cessation is shorter in northern populations. This in turn means that the growth period of such a material as the Finnish has a shorter growth period than the local standards and as a consequence of this it is shorter. The competition in the fairly old trials leads to suppression of the Finnish families. This is supported by the growth data from age 10. At this age the difference between the Finnish and Scottish materials was less pronounced.

In the juvenile material there was a significant variation in plant height with gradual decrease of growth from south west to north east. The equation for the relationship with geographic variables in one of the trials was:

\[
\text{Plant height in cm} = 146 - 1.81\text{Lat}^\circ\text{N} + 1.21\text{Longitude}^\circ\text{W}; \ p<0.01
\]

It should be noted that there was a considerable variation within the eight regions included in the study. In the two trials with more than 30 entries the range in height of the individual sources was 30.3-41.9 and 39.4-56.9 cm. There was a negative relationship between entry latitudinal origin and flushing date, \(R^2 = 0.49\). This is contrary to other results in other species with earlier flushing of northern than southern entries. The hypothesis is that the heat demand for flushing decreases with latitude. The unexpected relationship might be attributed, at least partly, to use of some non-autochthonous material. The difference in growth cessation among the populations was larger than for flushing. There was a tendency of positive correlation between flushing date and date for growth cessation. However, there was no clear geographic trend in growth cessation. The mean \(G_{st}\) based on the four isozyme loci studied was estimated at 0.062, which means that most of the marker variation is within populations. It was stressed that the simplistic view that local sources inevitably perform better than sources from other regions of Scotland is not substantiated by these preliminary growth data.

Four B. pubescens populations and one B. pendula population were planted in two trials at 50 masl and 450 masl at latitude 60.27°N in south western Norway (Skre 1993). One B. pubescens population originated from latitude 69.72°N and 200 masl. The four other populations originated from southern Norway and one of the B. pubescens populations originated from high altitude, 750 masl. The growth after two years in field was reported. The plant dry weights varied in the range 0.9-3.4 at the high altitude trial and in the range 2.8-43.5 grams/plant in the low altitude trial. To overcome this large difference in growth between the two trials in a comparison of performance of individual populations at the two trials, I have illustrated the percentage deviations from trial means for the individual populations in Fig. 1-9. It is somewhat surprising that the local population (LL LA) did not reach the trial mean at the low-altitude trial. However, at the high altitude trial this population had the highest dry

![Percentage deviation from dry weight trial mean of four B. pubescence and one B. pendula population (open columns) at two Norwegian test localities at 50 and 450 masl at latitude 60.27°N in populations originating from LL = low latitude 59-61°N, HL = high latitude 69.72°N, LA = low altitude 50 masl, IA = intermediate altitude 200 masl, HA = high altitude 750 masl. Skre 1993.](image)
weight. The poor performance of the high latitude population was expected since the photoperiodic conditions almost 10 degrees of latitude to the south will provoke an early growth cessation of this population since the critical night length for growth cessation is reached early at the southern latitude. Strongly significant differences between populations were noted for shoot length, leaf biomass, and number of leaves. There were large altitudinal differences in absolute and relative growth rates of the B. *pendula* population. The uptake rates of nitrogen of the B. *pendula* population were suggested as explanations for the differences. One of the conclusions from this paper was that competitions for light and nutrients are the selective agents at low elevation while temperature and nutrient availability are limiting factors at high altitudes.

A series of physiological-genetics papers on variation in growth and nitrogen metabolism were presented during the nineties by a group of Swedish scientists. Different B. *pubescens* populations were included in these studies that had a strong focus on physiological questions. Even phenotypic variation within populations was included in some cases.

Weih et al. (1998) carried out a detailed study of the response to temperature and fertilization of three B. *pubescens ssp. turtuosa* populations from northern Fennoscandia; latitudes and masl were 68.35 °N and 380 masl (Sweden), 69.37 °N and 95 masl (Finland), 70.67°N and 50 masl (Norway). The experiment, which lasted for two growing seasons was located at the locality of the southernmost population and had a factorial design. Detailed studies of growth, its components, uptake of nitrogen, and loss of nitrogen were carried out during the course of the study. Several instructive illustrations of the research data were presented. However, pooled data from the four treatments or pooled data from different assessment occasions were presented. This might be justified in absence of the interactions for genotype by treatment or genotype x assessment. The latter interaction was significant for a few of the traits.

Significant differences between the three populations were noted for most traits studied. As seen from Fig. 1-10 the Finnish population had the highest growth rate in all four treatments while the Norwegian (Lat. 70.67 °N) in three of the treatments had the lowest growth rate. The latter might be a consequence of its southward transfer of more than two degrees. The nitrogen productivity (Fig. 1-11) showed a fairly close relationship with the growth rate, R²=0.67. According to Fig. 4 in the Weih et al. 1998 paper the mean loss of nitrogen did not differ much in the two treatments with high fertilization but differed significantly in the low fertilization treatments. Only in two cases, the population x treatment interaction was significant; population x fertilization for root weight/total weight and for nitrogen loss.

It was concluded that the populations, which originated from different climatic regions, utilized nitrogen in different ways (Fig. 1-11).
Components of growth and nitrogen economy of young B. pubescens seedlings originating from two altitudes, 50 and 250 masl, at latitude 69.64°N in Norway were exposed to two temperatures and four levels of nitrogen fertilization in the range 1-10 grams per m² (Weih and Karlsson 1999). The study lasted for 11 weeks at two temperatures and 4 levels of nitrogen fertilization. Broken curves refer to the temperature treatments, ambient (LT) and 3° higher (HT). The fit to the curves was extremely good, all R² estimates > 0.97. Weih and Karlsson 1999.

There was a significant difference in biomass between the two populations but no difference in plant nitrogen concentration. For plant nitrogen concentration population x temperature and population x fertilization interactions were significant. For biomass only the population x temperature effect was significant. As expected the RGR of both populations were higher in high temperature treatment (Fig. 1-12). There was an extremely good fit to the polynomial curves of all four relationships, R² >0.97. The legends to figures 2 and 3 of the paper do not agree with the text of the X-axes. Therefore, I refrain from commenting on leaf area productivity, plant nitrogen productivity (PNP = plant biomass produced per unit nitrogen in the plant), and plant nitrogen concentration.

The Weih and Karlsson (1999) paper also contains a so-called transplantation experiment with seedling excavated from the ground at varying altitudes, 400-620 masl, at latitude 68.35°N. At each altitude one open and one closed locality were selected. After three years of acclimation the plants were exposed to two temperature regimes according to the above experiment. Unfortunately, the ANOVAs were run separately for the low- and high-altitude populations since the site effects were very different between low and high altitude; a strange argument. The authors focused on the leaf nitrogen productivity (LNP = plant biomass growth rate per unit leaf nitrogen) for LNP. Fig. 1-13 illustrates that the two low-altitude populations responded more strongly on elevated temperature than the two high-elevation populations. No significant differences were noted between populations within each altitude. Contrary to this, the RGR and PNP of the two high-altitude populations differed both at low and high temperature treatment (Fig 1-14). There was a stronger response to high temperature in both traits in the low-altitude population. Much of the discussion was devoted to plant physiology aspects but it was also speculated that the observed differences between altitudinal populations could be attributed to selection. Phenological differences between the altitudinal populations might limit or prevent gene flow between these populations. Finally, it should be

Figure 1-12. The relationship between relative growth rate (growth/week) of two B. pubescens populations from two altitudes, 50 (LA) and 250 masl (HA), at latitude 69.64°N in Norway grown for 11 weeks at two temperatures and 4 levels of nitrogen fertilization. Broken curves refer to the temperature treatments, ambient (LT) and 3° higher (HT). The fit to the curves was extremely good, all R² estimates > 0.97. Weih and Karlsson 1999.

Figure 1-13. The leaf nitrogen productivity (plant biomass growth rate per unit leaf nitrogen) of four B. pubescens populations from two different altitudes and two types of locality at each altitude, open or closed. LA = low altitude, HA = high altitude. Weih and Karlsson 1999.

Figure 1-14. The plant nitrogen productivity (biomass produced per unit plant nitrogen) of four B. pubescens populations from two different altitudes and two types of locality at each altitude, open or closed. LA = low altitude, HA = high altitude. Weih and Karlsson 1999.
mentioned that RGR does not necessarily inform about the total biomass production. Thus there may be a higher RGR in tiny plants than in tall plants.

Seven *B. pendula* populations from the entire distribution area in northern Italy were grown in a combined provenance and progeny trial (Avanzo 1994). The trial was established near Viterbo and growth and leaf characteristics were studied. The former was assessed at three occasions during the second year, July 4, July 29, and finally September 16.

As seen from Fig. 1–15 there is a weak tendency of a maximum for second year height (September 16) at elevations 600-800 masl. However, the ANOVA did not reveal any significant differences among the populations at any of the assessments. The plant height was significantly different among the families at the three dates when they were measured. There were no population differences for the leaf shape or phenology traits.

Three field trials with the five birch species *B. alleghaniensis*, *B. cordifolia*, *P. papyrifera*, *B. pendula*, and *B. pubescens* were established in Vermont, USA and measured at age 8 by Hannah (1987). The American seed sources were single tree progenies and the exotic sources mixes of single tree or multi-tree progenies. Therefore, the different materials are referred to as genetic entries. Unfortunately, only six seed entries were planted at all three sites. Except for one of the Finnish *B. pendula* entries there was a clear difference in growth between the three sites with yellow birch from Wisconsin being superior at the two trials with highest site indices (Fig. 1–16). The Latvian entry was the best performing *B. pendula* entry at the two test localities with highest site index (Fig. 1–17). It is surprising that the Latvian and Finnish entries performed so well in spite of the long transfer southwards, 14-16 degrees of latitude. Such a transfer is expected to result in an early growth cessation and thus a short growth period with less growth than the domestic entries.

Hubert and Cundall (2006) recommended firstly that seeds from five registered seed stands should be used for birch cultivation in Scotland, secondly that populations of *B. pendula* and *B. pubescens* from localities south of the planting site might be used. They advised that material from Finland and other localities with continental climate should not be used.

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**Figure 1–15.** The relationship between plant height at age 2 and altitude at population origin of seven Italian, Alpine *B. pendula* populations. Avanzo 1994.

**Figure 1–16.** Tree height at age 8 in one field trial in Vermont, USA, latitude 44°N and 400 masl. Four birch species were included in this trial. Hannah 1987.

**Figure 1–17.** Tree height at age 8 in three field trials at different elevations in Vermont, USA, approximate latitude 44°N (from left to right 400, 305, and 518 masl). The origin of the five genetic entries are indicated. Hannah 1987.
1.2 Phenotypic variation

Nordell and Karlsson (1995) focused on resorption of biomass and nitrogen in four *B. pubescens* populations from latitude 68.35°N and 380-670 masl. Each population was represented by ten trees. Two populations originated from dry sites at 380 masl and one originated from a rich site at this altitude. A fourth population grew close to the timber line, 670 masl, at a rich site. Biomass, leaf area, and nitrogen content were assessed during four years in green leaves and abscissed leaves in the four populations to enable estimates of resorption. It should be stressed that such a study is not a truly genetic study since only phenotypic variation can be revealed.

I have summarized the effects on population and within-population variation for several traits in Table 1-2. As seen from this table there was strongly significant differences among trees for all traits while the population effect was significant twice. If the strong phenotypic variation in the studied traits reflects genetic setup of the trees this should give good possibilities for change of the populations via selection.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Population</th>
<th>Individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf weight/leaf area</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Green leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abscissed leaves</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Leaf N content</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Green leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abscissed leaves</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Biomass resorption, BR</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>BR efficiency</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Nitrogen resorption</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>NR efficiency</td>
<td>ns</td>
<td>***</td>
</tr>
</tbody>
</table>

Four *B. pubescens* spp. *tortuosa* populations growing at latitude 68.35°N were examined with respect to nitrogen content, photosynthesis, and leaf morphology by Karlsson and Nordell (1988). The site conditions varied among the sites. The number of trees recorded at each site was not given but according to Fig. 1 in the paper many trees per site were included. Variation in nitrogen content and photosynthetic capacity varied significantly within and among populations. Leaf weight and leaf shape varied significantly among trees but not among populations.

Photosynthetic light response and photosynthetic nitrogen utilization were studied in three of the populations presented in the previous paragraph (Karlsson 1991). One population was located at timber line, 660 masl. Five trees per population were included in this study.

As regards maximum photosynthetic rate, the variation among trees within populations explained more of the variation (35%) than variation among the three populations (15%). There was a negative relationship between leaf nitrogen content and potential photosynthetic nitrogen efficiency but the correlation explained less than 20% of the variation. The relationship was somewhat stronger and positive between nitrogen concentration and photosynthetic rate but the degree of explanation did not reach 50%.

Leaves from 36 trees in three localities in England were collected and analysed morphologically and by molecular markers (Howland et al. 1995). Eight leaf traits were recorded and evaluated by principal component analysis (PCA). Ribosomal DNA and RAPDs were also studied. Leaf length and leaf width contributed most to the observed variation. A continuous variation in leaf traits was observed in populations composed of both *B. pendula* and *B. pubescens*. Trees from two localities showed a scattered distribution in the PCA while the trees from the third locality had less heterogeneous pattern in the PCA. No less than 15 length variants were noted in the rDNA analysis with the range 9.8-12.2 kb (kilo-base-pairs). Some of the variants were detected in one population only. Ten polymorphic bands were detected in the RAPD analysis. In the PCA all diploids formed a distinctive group while the tetraploids showed a scattered distribution. It was concluded that tetraploids are more variable than diploids both as regards morphology and molecular variation. The separation of two birch species in East Anglia was questioned since many intermediate types occur.

A morphology index based on nine quantitative leaf traits was used to classify 461 plants as *B. nana*, *B. pubescens*, or their hybrid in 14 Icelandic woodlands (Thórsson et al. 2007). The score for the morphology index varied from 0 to 12 with 0 being pure *B. nana* and 12 *B. pubescens*. The chromosome number was determined for each plant. Where possible, pairs of plants with *B. nana* phenotype and *B. pubescens* phenotype were studied. The percentages of diploids, triploids, and tetraploids were 38.2, 9.5, and 52.3, respectively. No aneuploids were detected. The majority of the triploids had a wide distribution of morphology indices and a broad maximum around a score of 4.1. The narrowest maximum was noted for the diploids and the mean score amounted to 1.3 while the corresponding score for the tetraploids was 8.3 and somewhat broader than for the diploids. Linear discriminant analysis (LDA) was also used for classification of the plants. By use of this model correct classification of *B. nana* and *B. pubescens* plants amounted to 96 and 97%, respectively. Contrary to this, triploids were correctly classified in 44% of the cases. The classification with the aid of the LDA was more precise than the morphological index method. A geographical structure was observed when the ploidy groups were analysed separately. There
were significant relationships between the LDA values of the localities and mean July temperature. However, the degree of explanation for the three ploidy levels did not exceed 40%. In conclusion, this study clearly showed that introgression between *B. nana* and *B. pubescens* in Iceland is commonly occurring.

1.2 Greenhouse and growth chamber studies

A detailed study of growth and physiological characters of four *B. pubescens* and one *B. pendula* population was reported by Skre (1991b). Seeds were mixed from four trees in each population. The *B. pendula* population originated from the same locality as one of *B. pubescens* populations, latitude 60.85°N and 200 masl. One population was southern, 59.75°N and 750 masl, another population was from latitude 60.27°N and 50 masl. Finally, one northern population from latitude 69.74°N and 200 masl was included. The material was cultivated at five different photoperiods, 12, 15, 18, 21, and 24 hours of light. At each photoperiod three temperatures, 9, 15, and 21°C were used. Three different light intensities were also used, 500, 250, and 125 μM/m² and sec. The plant growth was poor at the low light intensity.

In Figs. 1-18 and 1-19 I have illustrated the relationship between day length and growth for the two extreme temperatures, 9°C and 21°C, respectively. As seen from Fig. 1-18 there is a good agreement in performance of the *B. pendula* and *B. pubescens* populations from the same locality. The fit to the four curves is amazingly good. At the high temperature there is a tendency that continuous light results in less growth than at a day length of 21 hours. At a temperature of 21°C the northern population from latitude 69.74°N did not grow well under shortest day lengths tested, 12-18 hours (Fig. 1-19). This is not surprising since growth at such northern latitude takes place during the continuous light conditions. Contrary to this, southern populations might experience a stress from continuous light. It should be added that the plant dry weights at the intermediate temperature in some populations resulted in intermediate growth to the extreme temperatures while in other cases the dry weight at 15°C were largest. The total leaf area after cultivation during 6 weeks at three temperatures and continuous light are shown in Fig. 1-20. Especially, the high latitude population had a comparatively low leaf area in all treatments. The relationships between leaf area and total biomass were generally weak.

![Figure 1-18. Plant dry weight of one *B. pubescens* (dashed curves) and one *B. pendula* population cultivated for 6 weeks at two temperatures, 9°C (blue) and 21°C (red), and five day lengths. Skre 1991b.](image1)

![Figure 1-19. Plant dry weight of two *B. pubescens* populations cultivated for 6 weeks at two temperatures, 9°C (blue) and 21°C (red), and five day lengths. The light red and light blue curves refer to a population from latitude 59.75°N and 750 masl; the dark blue and red curves refer to a population from latitude 69.74°N and 200 masl. Skre 1991b.](image2)

![Figure 1-20. Leaf area per plant in four *B. pubescens* populations and one *B. pendula* population (open columns) after 6 weeks' cultivation at three temperatures, 9, 15, and 21°C (blue, purple, and red), respectively. LL = low latitude, HL = high latitude, LA = low altitude, IA = intermediate altitude, HA = high altitude. Skre 1991b.](image3)
I have illustrated the maximum net assimilation rate in Fig. 1-21, which shows that the differences among populations were smaller at 15°C than at 21°C. There was no clear relationship between this trait and biomass. Based on the ANOVA the following traits had a significant population effect:

- Dry weights of leaf, shoot, root, and whole plant
- Shoot/root ratio
- Leaf area
- Specific leaf area
- Leaf area ratio (area/weight)

The interaction effects population x temperatures, population x day length, and population x light intensity were significant for some of these traits. The three-way interactions were in most cases non-significant.

In conclusion this paper gives valuable information on responses to ambient conditions of populations from different origins. The results are worthy publication in a recognised journal rather than in its present publication.

The chemical composition of the same populations in the same treatments as described in Skre (1991b) was reported by Skre (1991a). Chlorophyll, proteins, carbohydrates, amino acids, and phosphorus were analysed.

The population effect was significant for phosphorus content, nitrogen content, non-structural carbohydrates, and the carbohydrate/nitrogen ratio. From Fig. 1-22 it is seen that there were strong relationships between day length and nitrogen or glucose content in the two populations. The water soluble protein content was particularly high in the northern population (Fig. 1-23), which was interpreted as adaptedness to the short growth period at latitude 69.74°N.

Three B. pubescens populations represented by four open-pollinated offspring were cultivated at different temperatures, 3-24°C and continuous light (Skre 1991c). The latitudes and altitudes of the three populations were: 59.72°N and 95 masl, 59.75°N and 750 masl, 62.22°N and 800 masl. Leaf development was the main focus of this paper.

The germination varied among trees in the range 12-65% and was highest in the low altitude population, 40.2% and lowest in the population from altitude 750 masl, 22.2%. In Fig.1-24 I have plotted the leaf growth against the cultivation temperatures based on Table 7 in Skre (1991c). The best polynomial fit to the data is shown for each population. These curves differ from the curves shown in Fig.
4b of the Skre 1991c paper, in which maximum growth is indicated for all three populations. The population effect was significant for all eight temperatures tested while the female effect was significant at 12 and 15°C only. The total growth showed a pronounced peak in the populations originating from highest level (Fig. 1-25). The two other populations had a broader maximum. It is likely that the highest temperatures 21 and 24°C execute a severe stress to the plants growing under continuous light. Plants originating from high latitudes do not experience continuous light during the growth period. The ANOVA showed a significant difference among populations for total plant growth as well as its components, leaf, shoot, and root weights. The effect of female was significant for leaf weight only. It was stated that the variation in growth was least in the population from altitude 800 masl. The larger variation in the low-elevation population was attributed to inbreeding. Introgression from B. nana to the 750-elevation population was suggested as a reason for high variation within this population. The shoot/root ratio differed significantly among populations and females. This ratio was lowest in the 800-masl-population. An increase with temperature was noted for the 750-masl-population. Net assimilation rates, NAR, were estimated at three temperatures, 9, 15, and 21°C, in the two high-altitude populations. During the early phase of growth the NAR dropped most at the highest temperatures and levelled off from day 20. The NAR of the 800-masl-population was much lower at 9°C than at the other two temperatures. The exponential leaf growth rate at low and medium temperatures was interpreted as a reflection of dark respiration. It was speculated that the low shoot/root ratio of the 800-masl-population was due to storing of assimilates in the roots. Doing so there was no need for a rapid growth response once favourable temperatures are available. Furthermore, the competition with other plants is less intense at the high altitude, which means that rapid juvenile growth does not contribute as much to fitness as under conditions with severe competition.

Figure 1-23. Water soluble proteins in three B. pubescens populations and one B. pendula population (open columns) after 6 weeks’ cultivation at two temperatures, 9 and 21°C, respectively. LL = low latitude, HL = high latitude, IA = intermediate altitude, HA = high altitude. Skre 1991a.

Figure 1-24. The relationship between temperature and leaf growth per day in three populations of B. pubescens cultivated under continuous light. The altitudes of the populations are shown. Skre 1991c.

Figure 1-25. The relationship between temperature and total biomass grams per plant in three B. pubescens populations growing under continuous light in the temperature range 3-24°C. Harvest took place at the 12-leaf stage. Skre 1991c.
The adaptedness of four B. pendula populations to different edaphic conditions was tested in hydroponic solutions with varying concentrations of aluminium (Kidd and Proctor 2000). The soil acidity and aluminium content of the four localities varied:

- pH = 3.2, monomeric aluminium = 3.7 mg/L
- pH = 4.3, monomeric aluminium = 21.1 mg/L
- pH = 4.8, monomeric aluminium = 5.3 mg/L
- pH = 6.1, monomeric aluminium = 2.0 mg/L

Root number and length, maximum length and breadth of leaves, seedling height and number of buds were recorded before and after the 84 day long treatment with aluminium concentrations, 2-35 mg/litre. The mineral content in roots and shoots of aluminium, calcium, iron, potassium, magnesium, and phosphorus separately for shoots and roots was determined.

The difference among populations was significant for all traits, except for number of roots. The population x treatment interaction was significant for all traits except root elongation rate. I have illustrated the relationships between aluminium concentration and root elongation rate in Fig. 1-26. The degree of explanation for all relationships and for all populations was above 60%. The root elongation rate for the population from the highest pH at the highest Al concentration is surprising (brown square in Fig. 1-26). Such a concentration is expected to be toxic. In all populations there was a drop in plant height between the two highest concentrations of aluminium Fig. 1-27. The population from the lowest pH, i.e. the most acidic soil, displayed a good fit to the polynomial relationship between aluminium concentration and plant height increment (dark blue squares in Fig. 1-27). The height growth of the pH-4.3 population at 2 mg Al/L deviated considerably from the general pattern. If this outlier is excluded from the relationship the R² becomes 0.72.

A priori it would be expected that there would be a relationship between root growth and plant height in the individual populations but there was no such relationship. Nor was there any relationship between leaf area and plant height. The relationships between different traits were not much discussed in the paper.

I have indicated the treatment with the highest content of Al, Ca, Fe, K, Mg, and P separately for shoots and roots in Table 1-3. As regards roots the control had the highest contents of iron, magnesium and potassium in all populations. Only calcium content in shoots had a common maximum in all four populations; treatment 2mg Al/L.

### Table 1-3. The treatment having the highest amount of six mineral nutrients in roots and shoots in four Scottish B. pendula populations originating from soils of varying acidity; pH 3.2, 4.3, 4.8, and 6.1. The aluminium treatments were 2, 5, 10, 15, 25, and 35 mg Al per litre. Kidd and Proctor 2000.

<table>
<thead>
<tr>
<th>Population pH</th>
<th>3.2</th>
<th>4.3</th>
<th>4.8</th>
<th>6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot Al</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Shoot Ca</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Shoot Fe</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shoot K</td>
<td>2</td>
<td>35</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Shoot Mg</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Shoot P</td>
<td>2</td>
<td>35</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Root Al</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Root Ca</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Root Fe</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Root K</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Root Mg</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Root P</td>
<td>2</td>
<td>35</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>
All other cases varied and sometimes considerably. It is evident from this table that the populations responded differently as regards uptake of some of the elements. The relationships between mineral element content and growth performance were not presented. The authors ranked the aluminium sensitivity in the following way pH 4.3 – 3.2 – 4.8 – 6.1.

1.3 Growth rhythm

A summary of the impact of external agents on seasonal responses in B. pendula was presented by Aalto and Palva (2006). In this summary there was a focus on the genetic regulation of basic processes for the different responses to signals from the environment. They also summarized results on growth cessation and frost tolerance in various populations.

Myking and Heide (1995) studied dormancy release, chilling requirement for bud flushing, and growth rhythm in three geographically widely separated B. pendula and B. pubescens populations from western Scandinavia. There was a strong focus on effects of different treatments, which means that there were a low number of plants per population and treatment. After sowing in May, the seedlings were growing under continuous light until August 31st. Growth cessation was induced by exposure to 16h night length at temperatures 20/10°C. At October 4 seedlings were transferred to three chambers with temperatures 0, 5, and 10°C. During the middle of the months November – February nine plants of each population and each chilling temperature were put in growth chambers with natural light conditions at a temperature of 15°C. During flushing treatment continuous light or 16h night lengths were applied. This treatment lasted for 60 days. The critical temperature for dormancy release was estimated by keeping 5 plants from each population at 12, 15, or 21°C under natural light conditions from October 4 until end of August next year. This was coined as effect of overwintering temperature on time of bud flushing. Flushing was recorded in a 3-degree scale. The growth of plants under natural photoperiodic conditions from latitude 59.67°N was followed the first season to study the variation in growth rhythm among the populations.

As expected the days to flushing was longest in the material that was exposed to shortest chilling treatment, 44 days, and dropped considerably to the 74-day chilling treatment. For the longest chilling treatment, 136 days, the differences between populations and chilling temperatures were small. The largest differences were found for the 44-day treatment, which is illustrated in Fig. 1-28. The northernmost B. pendula population required shorter time for flushing than the other two populations. It is also evident that the lowest chilling temperature resulted in the shortest time to flushing in both species. Continuous light during chilling resulted in shorter time to flushing than in the long-night treatment (Fig. 1-29). In spite of the low number of plants per population and treatment a strongly significant population effect was noted for bud flushing. The interaction population x duration of chilling and the 3-way interaction, population x duration of chilling x chilling temperature were also significant in both species.
A clear population effect was noted for number of days to flushing of the material exposed to +12°C immediately after the dormancy treatment, which ended on October 4 (Fig. 1-30). At +15°C there was no significant difference between the two southern populations. It was stated that plants from the +21°C treatment had a very late and irregular flushing. There was also a clear population effect on growth rhythm as is shown in Fig. 1-31. As expected the growth cessation took place in the sequence north to south.

Considering the wide geographic origin of the three populations, covering 13 degrees of latitudes, the observed differences in chilling requirement were not substantial although significant. The most pronounced population effects were noted for growth rhythm (Fig. 1-31) and number of days to flushing in the “overwintering” treatment.

Leinonen (1996) studied the chilling requirement for dormancy release and temperature requirement for growth initiation in three B. pendula populations from Scotland (Lat. 57.05°N), Russia (54°N), and Finland (61.82°N). Half of the material was kept outdoors in the Botanical garden of Joensuu, Finland and the other half under controlled conditions in a cold chamber at +3.8°C. The latter temperature was assumed to be optimal for breakage of dormancy. Both treatments started on October 4. Under forcing conditions the one-year-old seedlings were kept at a 12h photoperiod and a mean temperature of 15.8°C. Since the treatment took place in a greenhouse the temperature fluctuated between 10-25°C. An exponential function was used to estimate the relationship between temperature sum required for dormancy release and chilling, \( t_{50} = a + b \cdot e^c \). The parameters a, b, and c have to be determined and x is the previous chilling while c describes the slope of the curve. The chilling requirement of each origin was considered to have been met when the derivative attained a value of -2.14. The Scottish population had the highest chilling demand for dormancy release; both under outdoor and controlled conditions (Fig. 1-32) while the Finnish population had significantly lower chilling requirement under controlled conditions.

The temperature requirement for flushing was lower in all populations after treatment under controlled conditions (Fig. 1-33). As seen from Fig. 1-33 there was a different pattern in the two treatments such that the Russian and Scottish populations performed similarly in the outdoor treatment while the Finnish and Scottish populations performed similarly in the control treatment.
Cold acclimation after long night, water stress, and low temperature treatments was reported by Li et al. (2002). Seedlings of three origins, latitudes 58.17, 63.24, and 67.74°N, were included in a study in growth chambers. Plant growth, frost tolerance, and ABA concentrations were recorded. The ion-leakage method was used for assessment of frost injuries, LT50. In the water stress treatment the soil water was 50% of field capacity.

In a first experiment seedlings from 63.24°N were exposed to six different treatments as seen in Fig. 1-34. There was a drastic reduction of growth following exposure to low temperature, +4°C. In all cases the mild water stress applied enforced the plant response. In the water stress + continuous light treatment there was a slight tendency to growth cessation while the plants growing under continuous light without water stress did not show any sign of growth cessation. Fig. 1-35 reveals that there is a swift response to high frost tolerance after low-temperature treatment. Moreover, the effect is enhanced by long nights while long night treatment alone probably needs a longer time for building up frost tolerance. Water stress alone did not change the frost tolerance much but it enhanced the effect when combined with low temperature or long nights. In all treatments the populations ranked in the same way; the northern population had the highest frost tolerance and the southern had the least frost tolerance.

There was a large difference in ABA concentration among the populations after 4 days in the long night treatment. This difference did not remain after 14 days (Fig. 1-36). The central and southern populations even showed an increased concentration of ABA in contrast to the two other treatments. In the LN and LN+LT treatments the ranking was the same in both treatment and both occasions.

Figure 1-33. The temperature requirement for flushing after chilling treatment for various numbers of weeks outdoors (dashed) or under controlled conditions (unbroken) in three populations of Betula pendula. Leinonen 1996.

Figure 1-34. Height increment after one week in 6 different treatments of B. pendula seedlings. LT=low temperature, WS=water stress, LN=12h night, CL=continuous light. Cultivation took place in a growth chamber with one population from latitude 63.24°N. Li et al. 2002.

Figure 1-35. Frost tolerance of leaves after 2 weeks in 3 different treatments of B. pendula seedlings. LN=long night, LT=low temperature, +4°C. Cultivation took place in a growth chamber with three populations from latitudes 58.17, 63.24, and 67.74°N. Li et al. 2002.

Figure 1-36. ABA concentration 4 and 14 days after start of 3 treatments of B. pendula seedlings. LN=long night, 12h, LT=low temperature, +4°C. Cultivation took place in a growth chamber with three populations from latitudes 58.17, 63.24, and 67.74°N. Li et al. 2002.
Frost tolerance after 14 days spraying with ABA of seedlings belonging to two populations (latitudes 58.17 and 67.74°N) cultivated for 14 days under three different conditions in growth chambers was reported by Li et al. (2003a):

- Continuous light at +4°C, CL-LT
- 12 hours of night at +18°C, LN-HT
- Continuous light at +18°C, CL-HT

In a second experiment (Exp. 2) seedlings of the two populations were grown for 4 weeks under 12 hours night conditions, LN, followed by 7 weeks with low temperature, +4°C during these LN conditions. Another treatment consisted of cultivation under 11 weeks with continuous light conditions after the initial 4 weeks.

A third experiment was conducted with night lengths slightly shorter than the critical night lengths for growth cessation of the two populations, 1.5 h night for the northern population and 9.5 h night length for the southern population. Daily spraying of ABA was carried out during 8 weeks. Each growth condition in the three experiments had a corresponding control material.

Freezing temperatures were not given but it was stated that samples were taken during the lowering of the temperature at 2 degree intervals in experiment 1 and at 5 degree intervals in experiment 2.

The paper contains many instructive illustrations presenting the development of the different traits studied. I have preferred to illustrate the bud frost tolerance at the end of the treatments in experiment 1 in Fig. 1-38. This figure illustrates that the frost tolerance of the northern population was more pronounced than in the southern for the corresponding treatments. The acclimation was faster in the northern than in the southern population (not shown). The low temperature treatment provoked a better frost tolerance than high temperature in all cases (Fig. 1-38). ABA spraying improved the frost tolerance significantly in both populations. The frost tolerance in leaves and stems showed a similar pattern as in buds.

Elongation after ABA treatment retarded the growth under both light conditions (Fig. 1-39) but there was no tendency to growth cessation in any of the treatments. The continuous light treatment in experiment 2 resulted in a growth cessation in all treatments with a stronger response in the northern than in the southern population. In experiment 3 with its slightly shorter night length than the critical night length there was a strong response on growth of the ABA treatment in both populations resulting in an elongation of just one centimetre. Since the cultivation took place under a night length shorter than...
the critical it was expected that the untreated seedlings should continue to grow, which they did up to week 8. The ABA treatments resulted in earlier development of dormancy and a later dormancy release. The results indicate that ABA plays a great role in cold acclimation and that the northern population responded faster to ABA treatment. With two such distant populations included in these experiments it is expected that great population differences in response will occur.

Still another study of Li et al. (2005) used the three *B. pendula* populations from latitudes 58.17, 63.24, and 67.74°N for a study of ABA content, frost tolerance, dormancy development and release. Seedlings of the three populations were cultivated for 22 weeks in continuous light at 18°C. After that, two treatments were initiated, in the first the seedlings were exposed to 12 h long night treatment at 18°C for 12 weeks. In the second treatment, referred to as acclimation deacclimation, seedlings were cultivated for 10 weeks at 12 h nights; during the first 4 weeks the temperature was 18°C, followed by 3 weeks at 6°C and further 3 weeks at 0.5°C. After that, the seedlings were exposed to continuous light and 18°C. Frost tolerance was estimated as the temperature at which 50% of the cellular solutes are lost. Dependent on the expected stage of acclimation plants were exposed to minimum temperatures varying between -15°C and -50°C. Some frost tolerance was attained after 2 weeks and it was almost identical in the two treatments (Fig. 1-40) as it should be since the treatments did not differ the first two weeks. The difference between the three populations was significant. After 12 weeks in the first treatment the plants had attained a frost tolerance to approximately -14°C in the three populations. The northern population had earliest reaching of this frost tolerance. The low-temperature treatment increased the frost tolerance considerably compared to the +18°C treatment (Fig. 1-40) and the difference among populations was significant. As expected the highest frost tolerance was found for the northern population. ABA increased in long night treatment and fluctuated after this increase in acclimation deacclimation treatment.

![Figure 1-40. Frost tolerance of three *B. pendula* populations from latitudes 58.17, 63.24, and 67.74°N in two treatments and 2 occasions after onset of the experiment; the first with 12 h night at +18°C, and the other with a stepwise drop of the temperature from week 5 to +6°C and from week 8 to +0.5°C all the time at 12 h night. From week 11 the temperature was raised to +18°C and the plants were kept under continuous light. Significant differences are indicated. Li et al. 2005.](image-url)
Table 1-4. The performance of Northern (Lat. 67.67°N) Central (63.67°N), and Southern (61.34°N) Finnish B. pubescens populations with respect to bud flushing, water content, LT_{50} in stems and buds. Significant differences in bold. The approximate ranges of the studied variables are given. Only months with clear differences are shown. Welling et al. 2004.

<table>
<thead>
<tr>
<th></th>
<th>Order increasing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flushing %</td>
<td>January North Central South</td>
</tr>
<tr>
<td>Days to flushing</td>
<td>November South North Central 14-17</td>
</tr>
<tr>
<td></td>
<td>January North South Central 12-17</td>
</tr>
<tr>
<td></td>
<td>May South North Central 4-6</td>
</tr>
<tr>
<td>Water content</td>
<td>March North South Central 35-50</td>
</tr>
<tr>
<td></td>
<td>July South Central North 55-50</td>
</tr>
<tr>
<td></td>
<td>August South/Central North 50-38</td>
</tr>
<tr>
<td></td>
<td>September South North Central 43-39</td>
</tr>
<tr>
<td>LT_{50} stems</td>
<td>March North South Central -20°C to -60°C</td>
</tr>
<tr>
<td></td>
<td>April North Central South -10°C to -30°C</td>
</tr>
<tr>
<td></td>
<td>September South/Central North -55°C to -70°C</td>
</tr>
<tr>
<td>LT_{50} buds</td>
<td>South/Central North -17°C to -35°C</td>
</tr>
</tbody>
</table>

One half-sib family from each of three latitudinal populations of B. pubescens, 61.34, 63.67 and 67.67°N, were studied with respect to dormancy, frost tolerance, and gene expression of two dehydrin genes (Welling et al. 2004). Dormancy release was studied after collection of twigs every month, except December and June, from a field trial at latitude 64.88°N with one open-pollinated family per population. The twigs were taken from five trees and these twigs were exposed to continuous light at 18°C and flushing was recorded. Water content of buds from the trees in the field trial was determined. Frost tolerance in stems and buds were tested with temperatures down to -70°C. Frost damage was estimated visually for buds and stems and LT_{50} was calculated.

In Table 1-4 I have tried to extract differences in flushing, water content, and frost tolerance in the three populations. Only months with clear differences are shown. It is unclear whether the differences in frost tolerance were tested statistically. The a priori expectation is that there is a clinal variation from south to north in the traits studied. There was no such trend as regards flushing percentage. The limited differences among populations in percentage of flushing twigs were attributed to a strong regulation by temperature of this trait. The central population had the shortest time to flushing at the three occasions (months) when there were significant differences. According to the assumed clinal variation the shortest time to flushing was...
expected for the northern population. Nor did the water content follow the expected clinal variation at all occasions. This was also true for the LT50 in stems during winter while the expected order was noted for October freeze testing. The central population performed more as a northern population than as a central population. My conclusion from these observations is that the expected clinal variation in these traits was not verified except for a few occasions. The narrow base of the material, one open-pollinated family from each population might be one reason since the families might not be representative for the populations from the three latitudes. The number of samples per each test seemed to be low, which also might have influenced the deviation from the expected clinal variation.

The variation of the two dehydrin gene transcripts over the year are illustrated in Figs 1-40 and 1-41. When the transcript of DHN1 (dehydrin 1) decreases during winter and spring the DHN2 transcript increases to a maximum in January-March. The variation during the year of these two transcripts is rather similar for the three populations. The extended minimum of DHN1 during winter and spring of the northern population constitutes a slight deviation. Noteworthy is the good fit of all three populations to the third degree polynomials. The increase of DHN1 was interpreted as a response to increasing night lengths.

Plants from one _P. pubescens_ population from latitude 65.08°N were used to study the effect of a long night (12h), low temperature (+4°C), combinations of them, and water stress on the transcript levels of dehydrin 1 and dehydrin 2. Figure 1-42 reveals that low temperature treatment alone does not cause any rise of DHN1. The long night (12h) treatment for 9 weeks caused a slight increase of DHN1. The combined long night and low temperature treatments caused a pronounced increase of DHN1. The longer the low temperature treatment the larger the increase of DHN1. It is likely that there is a photoperiodic triggering of the DHN1 transcript which is enhanced by low temperature treatment. The level of DHN1 in leaves after one 3 weeks long night treatment or two temperature treatments did not cause any difference from the control. Similarly, water stress did not cause any rise of DHN1 level.

Fig. 1-43 reveals that the picture is the opposite for DHN2. All low temperature treatments resulted in high levels of DHN2 and so did the water stress treatment too. It was suggested that DNH2 had a role for transport of water to the apical part of the plant.

The combination of experimental geneecology and molecular genetics such as is the case in the present study is most useful for a basic understanding on dormancy and dormancy release. More representative samples of each population are desirable.
Two *B. pendula* populations, each represented by 10 open-pollinated families, from Scandinavia, latitudes 56.72 and 61.17°N were cultivated under two nutrient regimes (Black-Samuelsson and Eriksson 2002). One was referred to as High-N, in which there was free access to nutrients while the Low-N treatment was programmed for a growth rate of three percent. The plants were cultivated for 17 weeks under controlled conditions. Initial night length was 8 hours, night prolongation started at week 7 with 2h per 14 days. At week 13 the final night length of 16h was reached. No leaf colouring was noted in the High-N treatment at the termination of the experiment.

Figure 1-44 reveals that there were strong treatment effects for all traits but root dry weight. Under free access there is not the same need for the plants to develop roots as in the Low-N treatment, in which nutrients are limited. The results clearly indicate that the growth of birch plants is very plastic with such large difference between the two treatments. Since there were significant population x treatment interactions it means that these two populations responded differently to the treatments. The results of the joint analysis of the material considered both populations and families gave the significances presented in Table 1-5. It is obvious that the strong interactions for many of the traits result in non-significant family effects. This is also true to some extent also for the population effects. However, the low number of populations may also contribute to absence of population effects. The most striking information from this study is the large plasticity of many traits. The strong family x treatment interactions for several growth and phenology traits are also noteworthy.

Seeds from 7 Finnish *B. pendula* populations originating from the latitudinal range 60.24–66.87°N were sown at five occasions from May 29 to July 24 with 14 days intervals (Partanen 2004). The plants were growing in a greenhouse at latitude 61.81°N with natural light conditions. Day and night temperatures were programmed to be 20 and 10°C, respectively. However, full temperature control was not possible in the greenhouse at sunshine. Growth cessation was determined as the day when 95% of the annual growth was reached. At October 2 the plants were placed outdoors. Budset was defined as the date when 50% of the

Figure 1-45. Number of days from sowing to growth cessation of 7 Finnish *B. pendula* populations of different latitudinal origin. Sowing took place at 5 occasions (1-5) with 2-week intervals starting on May 29. The experiments were carried out in a greenhouse at latitude 61.81°N with natural light conditions. Partanen 2004.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Population</th>
<th>Population x treatment</th>
<th>Family</th>
<th>Family x treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Shoot DW</td>
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<td>Root DW</td>
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<tr>
<td>Shoot/Root</td>
<td>*</td>
<td>ns</td>
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</tr>
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Table 1-5. The significances of different effects in an experiment with two Scandinavian *B. pendula* populations each represented by 10 open-pollinated families in an experiment with controlled nutrient supply at two levels. Black-Samuelsson and Eriksson 2002.
seedlings had formed buds. Budset and growth cessation were followed up during the second growth period too. The number of days to growth cessation was lowest in the material from the latest sowing, July 24 and highest in the material from the first sowing, May 29. There was a fairly strong relationship between population latitudinal origin and growth cessation for the three first sowing dates (Fig. 1-45) while it was weak the last sowing date when the plant growth hardly exceeded 5 cm. The poor growth is probably responsible for masking any relationship with latitude for growth cessation in the material from the two last sowing dates. The relationships between plant height and population latitudinal origin were never strong (Fig. 1-46). For the first sowing date the 62.05°N population deviated strongly by higher growth than expected from the rest of the populations. If this population is omitted from the analysis, the R² value increases to 0.70. For the second growth period only mean values for budset and growth cessation were reported. Sowing date did not cause any significant difference in budset during the second growth period while there was a significant difference for growth cessation (Fig. 1-47). I assume that part of the growth during the second growth period is a result of stem units formed in the buds during the first growth period. Therefore, it is somewhat surprising that the large differences in growth between the sowing dates during the first growth period did not influence growth cessation or budset much during the second growth period. Partanen concluded that the results obtained are of significance in nursery practice. Thus sowing time and plant origin should be considered when designing cultivation regimes in nurseries.
Seeds from 7 *B. pendula* populations originating from the latitudinal span 58.17-67.74°N were sown at 8 occasions from May 21 to July 30 and their growth was measured every week until the same plant height was found at three consecutive measurements (Viherä-Aarnio et al. 2005). The experiments were carried out in greenhouse at latitude 60.52°N with natural light conditions. The first sowing date resulted in the longest growth period with a continuous reduction of the growth period until the last sowing date. As a consequence of this, there was a parallel decline in final seedling height. Thus, at the last sowing date no population reached a plant height of 5 cm. Regressions were used to estimate the dependence of population latitudinal origin (L) and sowing date (SD) on:

- Timing of growth cessation, TGC
- Night length at growth cessation, NLGC
- Duration of the growth period, DGP
- Final height of seedlings, FH

The following equations were obtained:

- \( TGC = 418.04 - 1.073SD - 0.05088L^2 + 0.02066SD \times L \)  \( R^2 = 0.92 \)
- \( NLGC = 25.78 - 0.0988 - 0.004686L^2 + 0.0019SD \times L \)  \( R^2 = 0.92 \)
- \( DGP = 418.04 - 2.073SD - 0.05088L^2 + 0.02066SD \times L \)  \( R^2 = 0.97 \)
- \( FH = 1787.4 - 10.678SD - 20.499L + 0.0098SD^2 + 0.099SD \times L \)  \( R^2 = 0.96 \)

The extremely good fit of all four relationships must be regarded as amazing. I have illustrated the observed plant final heights for the first and the fourth sowing dates SD=141 and 176 in Fig. 1-48. As can be seen from this figure the agreement between the observed values and the expected values for the populations is extraordinary good for these two sowing dates. The two curves in Fig. 1-49 show that there is a good fit to the observed data for duration of the growth period at both sowing dates.

In conclusion independent of the date of sowing there is a clinal variation of final plant height and duration of the growth period. Moreover, the fit to relations between population latitudinal origin and the traits studied was extraordinarily good.

Two Norwegian *B. pendula* populations from latitudes 60.72 and 67.12°N were cultivated in hydroponic cultures at four different root zone temperatures, +2, +6, +12, and +17°C for eight weeks (Solfjeld and Johnsen 2006). In the first experiment the seedlings were allowed to acclimate for three weeks at 17/11°C day and night temperatures before they were placed in the four treatments at above ground temperatures 19/12°C. The photoperiod was the local at latitude 59.67°N. In experiment 2 seedlings were taken directly from cold storage for a gradual temperature acclimation to the 20/15°C used in the 8 weeks cultivation with the four root temperatures. The photoperiod was 18 hours of light. In a third experiment only the southern population was included. Carbohydrate concentrations were measured in experiment 1.

In spite of the low number of treatments the fit to the curves in Figs 1-50 and 1-51 is surprisingly good. Moreover, the number of plants behind each point was four, which makes the fit still more remarkable. As regards leaf area increment the southern population did not show such a good fit to temperature as the other two traits. There were large differences in soluble carbohydrates and starch concentration in the two extreme root temperatures but there was no significant difference between the two populations.
In experiment 2 there was no difference in flushing between the two populations. The root temperature did not influence the flushing. The root zone temperature had a strong effect on formation of new roots. No root growth was noted at 2°C while root growth was noted after three weeks at 17°C. After four weeks new root formation was found at 12°C. After 8 weeks 27% of the plants had root growth both in actively growing plants and plants coming directly from cold storage.

The development of frost tolerance from September through winter of two B. pendula populations from latitudes 69.65°N and 50 masl (coastal) and 66.98°N and 100 masl (continental) was reported by Stuschnoff and Junttila (1986). The development of frost tolerance was faster in the coastal than in the continental but both reached the same maximum frost tolerance level in December, -80°C. The frost tolerance was high even in April, -40°C and there was no significant difference between the two populations.

Luomajoki (1986) studied the meiotic division in five populations of B. pendula and seven populations of B. pubescens from the entire range of distribution in Finland, latitude 60.04-69.07°N. There was a focus on the tetrad stage in this investigation. Most of the observations were carried out between 1964 and 1971. In addition to this, he made observations in 1983 too. There was a relationship between latitudinal origin and reaching of the tetrad stage.

Even if the study by Luomajoki (1999) on anthesis in Finnish B. pendula and B. pubescens stands is not a traditional genetic study it gives information of value for forest genetics. This study, initiated by Risto Sarvas, builds on pollen recordings in 10 B. pendula and 14 B. pubescens stands from 1963 to 1974. The date or temperature sums for 50% anthesis was estimated by Luomajoki. There was a strong relationship between latitude and 50% anthesis in both species, \( R^2 = 0.84 \) and 0.92 in B. pendula and B. pubescens, respectively. There were a few years with overlap in flowering of the two species in some stands. The northernmost B. pendula reached the tetrad stage at a temperature sum of 608-700 degree days while the corresponding values for the southern stands were 742-986 dd. The northernmost B. pubescens population, which is growing further north than northernmost B. pendula population, reached the tetrad stage at 429 dd. In southern Finland the corresponding values were 850-1031. There was a clinal variation in both species. The author developed a model which resulted in the same estimate for reaching of the tetrad stage in all populations. The equation was:

\[
\text{Photothermal sum} = \Sigma (\text{Daylight length})^2 \times \text{degree days}
\]

Luomajoki concluded that both temperature sum and photoperiod played a role in reaching of the tetrad stage.

1.4 Miscellaneous

Luomajoki (1986) studied the meiotic division in five populations of B. pendula and seven populations of B. pubescens from the entire range of distribution in Finland, latitude 60.04-69.07°N. There was a focus on the tetrad stage in this investigation. Most of the observations were carried out between 1964 and 1971. In addition to this, he made observations in 1983 too. There was a relationship between latitudinal origin and reaching of the tetrad stage.

Even if the study by Luomajoki (1999) on anthesis in Finnish B. pendula and B. pubescens stands is not a traditional genetic study it gives information of value for forest genetics. This study, initiated by Risto Sarvas, builds on pollen recordings in 10 B. pendula and 14 B. pubescens stands from 1963 to 1974. The date or temperature sums for 50% anthesis was estimated by Luomajoki. There was a strong relationship between latitude and 50% anthesis in both species, \( R^2 = 0.84 \) and 0.92 in B. pendula and B. pubescens, respectively. There were a few years with overlap in flowering of the two species in some stands. The northernmost B. pendula reached the tetrad stage at a temperature sum of 608-700 degree days while the corresponding values for the southern stands were 742-986 dd. The northernmost B. pubescens population, which is growing further north than northernmost B. pendula population, reached the tetrad stage at 429 dd. In southern Finland the corresponding values were 850-1031. There was a clinal variation in both species. The author developed a model which resulted in the same estimate for reaching of the tetrad stage in all populations. The equation was:

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\]

Luomajoki concluded that both temperature sum and photoperiod played a role in reaching of the tetrad stage.
It is assumed that phenols and nitrogen are defensive means against herbivory in *B. pubescens*. Severe outbreaks of damage by *Epirrita larvae* occur certain years and feeding can start once the buds are open. Three populations from different altitudes along a mountain slope at latitude 69.75°N were selected for determination of phenol and nitrogen content together with recordings of bud flushing (Haukkioja et al. 1985). Defoliation was carried out to simulate insect attacks. The annual growth of twigs was not impressive during the study period; varying in the range 2-6 cm. Only in one population one year there was a substantial death of defoliated trees, 45%. Four out of five years there was a significant difference in phenol content among the populations while nitrogen content differences were noted one year only. The phenol content varied more over years than nitrogen content. As stated elsewhere, this type of study informs about phenotypic variation and does not prove genetic differences. Only if the phenotypic differences reflect genetic differences such a study has genetic implications.

Seidler (1995) reported that the mean growth period of *B. pubescens* in one phenological garden in Germany, latitude 50.98°N and 360 masl, was estimated at 170 days. She noted that there was a large annual variation during the 20 years of observation. Flushing took place at March 30 in 1990 and at May 16 in 1980. Unfortunately, the biological mapping of parts of Europe by observations in all established phenological gardens remains to be done.

### 1.5 Markers

The location of the major ribosomal DNA family in *B. nana* and *B. pubescens* was determined by Ananthawat-Jönsson and Heslop-Harrison (1993). Four major and two minor sites were detected in the tetraploid *B. pubescens* while there were four major sites in the diploid *B. pubescens*. It could be added that the major sites were in nucleolus organizer regions. It was speculated that introgression between the two species has increased the adaptability and ability to survive under harsh conditions. Such a hypothesis can only be verified by studies of growth under varying growth conditions.

Five isozyme loci in seven *B. pendula* populations originating from the Nordic countries were used to study population differentiation (Rusanen et al. 2003). The latitudinal and longitudinal ranges covered were 59.48-69.74°N and 10.42-29.32°E, respectively. The northernmost population from Finland is a marginal population with a lower heterozygosity, $H_i = 0.096$, than in the other six populations, $H_i = 0.148$. The marginal population did not contain any private alleles. The $F_{ST}$ for the seven populations was low and estimated at 0.032. This result indicates that there is a strong gene flow among the populations studied. There was a trend to geographic differentiation with the four Finnish populations showing close relationship in the UPGMA (unweighted pair group method with arith-

### Figure 1-52

Number of trees with the 13 different haplotypes in 47 populations of *B. pendula*. Each of the haplotypes R, I, and V occurred in one population only. Palmé et al. 2003.

Nucleotide variation in the *BpMADS2* gene was studied in one population from latitude 61.82°N and one from 60.33°N, each was represented by 10 trees (Järvinen et al. 2003). There was limited variation between the two populations and particularly in the coding region. There was a large number of microsatellites in all three introns studied and it was suggested that the *BpMADS* gene is a hotspot for formation of microsatellites.

Palmé et al. (2003) utilized PCR-RFLP cp haplotypes in a study of 47 *B. pendula* populations from a large part of the distribution in Europe; latitudinal range 40.56-67.01°N and longitudinal range 4.83°W-60.35°E. Five of the primer-enzyme combinations tested were found to be variable. Thirteen haplotypes were found with two main groups of haplotypes. The gene diversity and molecular variance was estimated by AMOVA. A grouping of the populations was carried out by boundaries according to Monmonier’s (1973) maximum-difference algorithm. From Fig. 1-52 it is seen that haplotypes A and C were the most common while each of the haplotypes H, D, K, R, I, and V occurred in less than five of the 431 trees examined. The rarest haplotype, H, occurred in two German populations while the four trees with haplotype I originated from a French population. Five of the seven Polish populations had only haplotype C. All 12 trees with haplotype Q originated from populations east of the Ural Mountains. Seven of the ten trees with haplotype U originated from Val-
bonne in France, lat. 44.24°N and long. 4.55°E. There was a scattered distribution of the 15 trees with haplotype B. Fig. 1-53 reveals the negative relationship between haplotypes A and C.

Based on the haplotype pattern six groups of populations could be identified (see the legend to Fig 1-54). I have illustrated the distribution of gene diversity classes in the six regions in this figure. Two Norwegian populations with only two and three trees studied belonged to the group with 0 diversity. They were excluded from Fig. 1-54. After this exclusion nine populations had no gene diversity. The low number of populations means that any far-reaching conclusions cannot be drawn. The intermediate diversity class, 0.34-0.66, had the highest number of populations. No populations with zero diversity was found in the eastern Russian populations. One of these populations, Berezovka from lat. 56.27°N and long. 60.35°E, had 5 haplotypes and the highest genetic diversity, 0.85. Half of the populations in region 4 belonged to the highest gene diversity class.

The results were used for an analysis of migration routes after the last glaciation. It was stated

1. that *B. pendula* was thinly spread in Central Europe and that the species rapidly colonized new areas after the melting of the ice.
2. The southern populations did not migrate much after the temperature climate improved. The spreading northwards was also hindered by the east-west mountain chains in southern Europe.
3. The regions four and five were probably colonized from different refugia.
4. The restricted distribution of the rare haplotype T in northern Scandinavia was most likely the result of a mutation. A refugia in Fennoscandia during the glaciation was ruled out as a source for this rare haplotype.
5. The clear difference of region six from the other regions suggests that these populations originated from eastern refugia.

Parts of the above study were also presented by Lascoux et al. (2004), in which $G_{st}$ was estimated at 0.42. (It is not fully clear if this estimate was based on 23 populations as stated in Table 1 in the paper or on all 47 populations that were illustrated in Fig. 6 of the Palme et al. 2003 paper.)

A comparison of chloroplast haplotypes in *B. nana*, *B. pendula*, and *B. pubescens* was carried out by Palmé et al. (2004). *B. pendula* and *B. pubescens* were compared in 13 stands within the latitudinal range 47.93–66.49°N and six of the stands were in Russia. *B. nana* and *B. pubescens* were compared in six stands, all in Sweden and Norway, latitudinal range 62.33–68.27°N. All three species were compared in one Russian stand at 56.27°N. As in the above study, PCR-RFLP analyses were used. Ten haplotypes could be used in the study. It was noted that most haplotypes were closely related.

Haplotypes A and C were the most common haplotypes and they occurred in most of the populations. The percentage of trees with haplotype C varied in the range 50-65% in the three species. Three of the less common haplotypes were also found in all species. The rarest haplotypes were geographically restricted and occurred in one species only. Within regions there was a clear tendency that both or all three species shared the same haplotypes.
Thus haplotype T occurred in all three species but only in northern Fennoscandia. The below $F_{st}$ estimates for each of the species give information on the population differentiation within the three birch species:

- B. nana: 0.58
- B. pendula: 0.32
- B. pubescens: 0.26

In the joint analyses of two or three species in individual regions no significant differences between species were noted. However, an exact analysis showed that there was a significant difference between B. pendula and B. pubescens in two of the 14 stands included in the study. Significant differences between B. nana and B. pubescens were noted in three of the seven stands. The estimates of introgression ratios, which reflect the local sharing of haplotypes, were larger than zero. Another estimate of introgression was also estimated IG, which is the expected value if the sharing of haplotypes is not geographically structured.

Hybridization between B. pendula and B. pubescens was suggested as the main reason for the large sharing of haplotypes between the two species. The similar geographical pattern of common and rare haplotypes in the two species is one strong argument for hybridization. The finding that geography is more important for the haplotype pattern than species is another support for the hybridization hypothesis. This hypothesis is also supported by observations of hybridization reported elsewhere in this report.

It was claimed that introgression from B. pendula to B. pubescens is somewhat stronger than the opposite introgression. However, the support of this from the estimates of introgression rates was less convincing. It was stated that a full genetic picture of the species differences cannot rely only on haplotype patterns.

The Maliouchenko et al. (2007) paper is an extension of the three papers above with larger number of populations and studies of microsatellites from nuclear DNA as well. Fifty-three locations were included in the study with 41 B. pendula, 22 B. pubescens and 5 B. nana populations. In five populations less than five trees were sampled. $G_{st}$ and $N_{st}$ were estimated and compared to detect any phylogeographic structure. If $N_{st}$ is significantly larger than $G_{st}$, it means that there is a phylogeographic structure.

In all, 66 haplotypes were identified with a larger number in B. pendula than in B. pubescens. However, this difference could be attributed to the larger number of populations in B. pendula than in B. pubescens. The average gene diversities were almost identical, 0.47 and 0.49, respectively. Only 3% of the variation could be attributed to differences among species.

There was no indication of a phylogeographic structure in B. pendula and B. pubescens while there was such a structure in B. nana. The low number of populations tested and their wide distribution might explain the deviating pattern of B. nana.

The clustering was more pronounced in B. pubescens than in B. pendula with seven and two clusters, respectively. The main B. pubescens cluster comprised populations from France to Siberia. One northern Fennoscandia and Russia constituted another cluster. Still another cluster comprised three of the Siberian populations. Four of the seven B. pubescens clusters were marginal populations in Britain, Iceland, Siberia, and Spain. There was no evidence for any isolation by distance in this study.

The migration of the populations after the last glaciation was again discussed and the present study did not reveal any strongly deviating results compared to previous studies by this group.

### 1.6 Global warming

Billington and Pelham (1991) studied bud flushing at ages 2 and 4 in 7 B. pubescens and 3 B. pendula populations growing in a trial at latitude 51.85°N in Scotland. Each population was originally represented by 10 open-pollinated progenies and 16 seedlings per family. Scoring of developmental stage took place on April 25-26 at age 2 and on April 28-29 at age 4.

Flushing was much more advanced at age 4 than at age 2. It is not likely that the large difference can be attributed to the limited difference in date of observation. Rather flushing is much dependent on the prevailing weather conditions and they might be responsible for the observed difference at the two ages. The ANOVA showed strongly significant effects for the following factors:

- Year
- Species
- Populations within species
- Families within population
- Year $x$ site interaction
- Year $x$ population interaction

Contrary to this, the year $x$ family interaction was non-significant. The heritabilities for bud flushing showed a wide range over B. pubescens populations varying in the ranges 0-0.65 and 0-0.57 at ages 2 and 4, respectively (Fig. 1-55). The large difference in heritability estimates at the two ages in the same population is disturbing. This should
be born in mind when the potential for the individual populations to respond to a warming of 2°C is estimated. Such a temperature increase will corresponds to 40 days earlier bud flushing. This was the starting value for estimations of the selection differentials required in each population to match the increased temperature climate. Then the percentage of seedlings exceeding this selection differential was estimated for each population and year. The result is not impressive as demonstrated in Fig 1-56. Especially at age 6 there were altogether only 7 seedlings in the two species that fulfilled the requirements. The large variation between ages within populations renders these predictions a large degree of uncertainty. Moreover, the expectation that an increase of the temperature with 2°C leads to change of flushing with 40 days seems rather large. A smaller increase would result in a larger number of plants that fulfil the requirement to match a temperature increase.

Kramer (1994) used two models to predict the risk for frost damage in B. pendula and B. pubescens in The Netherlands and Germany at changed temperature climate. The first model comprised the shift of -2 - +8°C in steps of 1°C. The second model assumed higher temperature increase during the winter months than during the summer. Flushing dates were obtained from phenological gardens, i.e. the same genetic entries are studied at several localities. The occurrence of frost exposure during eleven days around the leaf unfolding was estimated. Temperature data from 40-46 years were used in the model for frost risk estimations. The probabilities for frost exposure of B. pubescens in Germany and The Netherlands under current conditions and according to the two models constitute the essence of this study. As seen from Fig. 1-57 the differences between the models and the unchanged conditions do not differ much. Thus, there is limited risks for increased frost exposure following global warming according to the model used.

Figure 1-56. The percentage of seedlings with a bud flushing matching a temperature increase of 2°C in 7 B. pubescens and 3 B. pendula populations at ages 2 and 4. The data emanate from recordings of flushing in a Scottish trial at latitude 55.85°N and 198 masl. Billington and Pelham 1991.

Figure 1-57. The probabilities for frost exposure around flushing of B. pubescens under present conditions and according to two models of temperature change (see text). Results for Germany and The Netherlands are shown separately. Kramer 1994.
Observations of flushing of birch by Finnish amateurs were used by Linkosalo et al. (2000) for projections of changes in flushing that may take place in case of climate warming. Two models were tested:

1. The chilling-triggered model (Ch-T-M)
2. The photoperiod-climate-triggered model (Ph-Cl-T-M)

Model 1 means that chilling is required for breaking of dormancy. Flushing will occur once a certain temperature sum has been reached after breaking of dormancy.

Model 2 means that flushing will not take place unless the photoperiodic conditions for flushing are met. Even if dormancy is broken and the temperature sum for flushing is reached, flushing will not take place if the photoperiodic conditions do not prevail.

Two climatic change scenarios were used. The first assumes a uniform increase of the temperature throughout the year. The second considered a variable increase of temperatures during the year according to Finnish predictions of climate change. For each scenario increases of +0.5, 1.0, 1.5°C… up to 10°C were simulated for 92 years. The mean change in bud burst date was calculated by subtracting the mean date of bud burst in a given warming scenario from the mean date of bud burst obtained with the same model run with unmodified climatic data. Frost damage risk was based on occurrence of exposures to temperatures below -5°C between flushing date and June 30. Based on the photoperiod-climate-triggered model mean bud flushing date was estimated for three threshold dates March 3, March 23, and April 10. For April 10 bud flushing was predicted to change by 2.6 days per degree of temperature increase. The change was still larger for the March dates, 4.1 days per degree increase. There was no difference between the two scenarios of warming. In contrast to this, there was a large difference between the two scenarios in the chilling-triggered model. For temperature increases in the range 2-10°C the dormancy release was gradually delayed from November 30 to end of January or early February with a stronger effect in the second scenario. Up to an increase of 2°C the two scenarios gave similar results. As regards bud flushing the second scenario had a broad maximum change of flushing date at temperature increases of 6-8°C. This change was larger than in the first scenario (uniform increase of the temperature during the year), which resulted in a gradual increase of the change. This change was also larger than obtained in the chilling-triggered model.

Up to +2°C increase of temperature, the chilling-triggered model did not project any risk of frost damage. A maximum at 52% risk for frost damage was noted around an increase of +7°C if the second scenario was used. In Fig. 1-58 I have illustrated the risk for frost damage at the maximum for an increase of +7°C and the highest increase analysed by the authors, +10°C. This figure shows that the risk for frost damage is far higher in the chilling-triggered model (Ch-T-M) than in the photoperiod-climate-triggered model (Ph-Cl-T-M). Only at large increase of the warming frost risks increase for the latter model. A threshold temperature of -5°C for occurrence of frost damage seems somewhat too low but was based on observations by Braathe (1995).

In an earlier report Linkosalo (1999) reported on date of flushing and flowering in *B. pendula* from a large number (44 and 31, respectively) of observations in Finland between latitudes 60 and 66°N. Latitude was the major factor determining the phenology of these two traits, \( r = 0.60 \).

Häkkinen (1999) tested three models for bud flushing based on the recordings between 1896-1955 (except for years 1912-1916):

1. Flushing starts when dormancy is completed
2. Ontogenesis begins during dormancy but at a slow rate
3. Ontogenesis does not begin until a certain photoperiod occurs

The three models were tested by the mean square errors of the methods. The mean square error was smallest for model 3 and there was almost no difference between models 1 and 2. There is some support for requirement of a certain photoperiod before flushing can take place. The earlier paper by Häkkinen et al. (1998) has essentially the same content as the Häkkinen (1999) paper.

The models for flushing were further developed by Linkosalo et al. (2006). Two new models were tested:

4. the nullifying model
5. the reversing model

The nullifying model says that whenever the temperature drops below the threshold temperature for flushing promotion, the accumulation of temperature has to start...
from zero again. According to the reversing model an additional developmental phase is assumed to occur between the phases rest and forcing. Sophistical mathematical calculations are required for estimates based on model 5. Contrary to model 1, low temperature interferes with the forcing in models 4 and 5. This leads to a later bud flushing. The testing of models 4 and 5 did not lead to any improvement of the predictions for bud flushing.

1.7 Conclusions

1.7.1 Growth and growth rhythm

For growth traits such as tree height and breast height diameter several investigations have shown a strong impact of population latitudinal origin. In Fennoscandia the maximum growth is noted for populations transferred a few degrees of latitude to the north. Under other conditions there was a decrease in growth from south to north. Good growth of a few Nordic-Baltic populations were noted in north-western USA. Population differences were also noted for components of growth such as root dry weights, shoot dry weights, leaf dry weight, and leaf area. A large phenotypic plasticity were observed. Populations from different soil pH and aluminium content revealed differences in response to increased aluminium for root growth and plant height in hydroponic experiments with variable aluminium levels. When population data were compared with seed orchard or plus tree offspring it was frequently shown that there may be a larger gain by individual tree selection than just selecting populations. As regards quality traits there was a linear decrease of stem defects from south to north. It has been observed that competition for light and nutrients are selective agents at low elevation while nutrient availability and temperature are limiting factors at high elevation. Several studies were carried out to study the effect of temperature, water availability, photoperiod, and light intensity on growth rhythm and frost tolerance. It was noted that the genetic variation in flushing was much less than variation in growth cessation. Chilling requirement for dormancy release and flushing varied significantly among populations. Northern populations responded more swiftly than southern populations on the ambient treatments. Flushing seemed to be independent of root temperature. Frost tolerance increased from south to north. The increased frost tolerance was accompanied by an increased ABA content four days after onset of treatment. It can be concluded that long nights initiate growth cessation, budset, cold acclimation, dormancy development, and increased freezing tolerance.

The role of two dehydrin genes was studied during over-wintering. When one of the transcripts of these two genes reached its maximum the other was approaching its minimum. This study was the only one with deviation from the expected clinal variation south - north. The limited material may have contributed to this deviation from the general clinal variation since each population was represented by just one open-pollinated family. Sowing date resulted in the same clinal variation for growth cessation and duration of the growth period.

1.7.2 Markers

$G_{ST}$ and $F_{ST}$ estimates based on isozymes were low, 0.06 and 0.03, respectively. Molecular markers showed much larger estimates of $F_{ST}$: $B.\ nana$ 0.58, $B.\ pendula$ 0.32, $B.\ pubescens$ 0.26. In joint studies of the three species it was disclosed that geography was more important than species belonging. This suggests that hybridization between the species had occurred. A larger differentiation was noted for $B.\ pubescens$ than for $B.\ pendula$ in one study.

1.7.3 Global warming

Some inconsistency in flushing data were noted in a phenological gaarden study with a large heritability variation between years of observation. The predictions for frost exposure during flushing after increased temperature varied from negligible to high risk for frost damage.

The misuse of the term ecotype coined by Turesson (1922) is widespread and a sign of lack of basic genetic knowledge.
2.1 Growth and growth rhythm

Viherä-Aarnio and Velling (1999) carried out a detailed study of growth and quality traits at age 32 in a progeny trial with B. pendula and B. pubescens OP-families at latitude 62.14°N in Finland. Besides, B. pendula and B. pubescens were represented by one stand each while 3 populations of B. papyrifera were included.

In Fig. 2-1 I have summarized some of the most important traits in this study. As seen from this figure the stem volume of the B. pendula OP-progenies was largest with a narrow range, 340-354 dm³. B. papyrifera was characterised by poorest growth and veneer quality. There were significant differences among the entries for all growth related traits, height, DBH, stem volume, present growing stock, and total yield. The veneer traits of the B. pendula OP-families were superior to all other progenies. Taper did not differ among the entries while percentage of taper, length of sweep, and length of stem defects differed. There was a tendency that the OP-families of B. pendula, which were obtained from plus trees, were somewhat superior to the B. pendula stand progeny. It can be concluded that the plus tree selection of these three plus trees was successful. However, the low number of plus trees does not permit general statements.

Seeds were collected from 42 B. pubescens trees of good form and health (Blackburn and Brown 1988). They originated from seven populations in Scotland. Each population was represented by 5-7 open-pollinated offspring per population. A progeny trial was established at a cold site at Shin in north-western part of Scotland. The plantation has 6-tree row plots with 6 replications of each family. The range of family mean heights at age 2 was 135-169 cm. As seen from Table 2-1 most genetic effects as regards tree height were significant. The weather conditions were adverse during the winter, which called for a scoring of damage. Six classes were used for such a scoring. Both family and origin were strongly significant as regards die-back of the young trees. It is evident that there are large genetic differences since most of the tests showed significant differences in spite of the low number of trees and the strict selection of the material.

Blackburn and Brown (1988) also reported on the performance of 20 open-pollinated progenies of B. pendula. The trial with this material was located approximately 17 km east of Glasgow in Scotland. The range for family mean height was 112-138 cm. Also in B. pendula the family effects for tree height at age 3 and die-back were strongly significant. The level of die-back in this trial was lower than in the B. pubescens trial. The estimated narrow sense heritability for die-back was 0.01 suggesting no genetic difference for this trait. It was speculated that the severe wind conditions at the trial dominated over any possible genetic effect.

Branches from 5 trees were taken on October 2 for a test of frost tolerance following exposure to temperatures in the range -3 - -19°C. The five trees represented a north-south gradient. Sections of the twigs were scored in three classes. Significant differences were found at temperatures -11 and -15°C.

The authors concluded that there seems to be good prospects for improvement of growth but admitted that the results were valid for these two test localities and for young material.

<table>
<thead>
<tr>
<th>B. pubescens</th>
<th>Origin</th>
<th>Family</th>
<th>Family in each of 7 populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>height age 2</td>
<td>*</td>
<td>***</td>
<td>6 of 7</td>
</tr>
<tr>
<td>B. pubescens</td>
<td>die-back</td>
<td>***</td>
<td>4 of 7</td>
</tr>
<tr>
<td>B. pendula</td>
<td>height age 3</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>B. pendula die-back</td>
<td>-</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2-1. The tree volume dm³/10, percentage of veneer log trees, and Length of Veneer Timber Part of the trunk in B. pendula and B. pubescens open-pollinated progenies at age 32 in a Finnish trial at latitude 62.14°N and 85 masl. One B. pendula, one B. pubescens, and three B. papyrifera stand offsprings (green latitude labels) were also included. Viherä-Aarnio and Velling 1999.
In her PhD thesis Baliuckiené (2009) presented growth and molecular data based on 100 open-pollinated families from 24 Lithuanian and 14 full-sib families from two Swedish populations of *B. pendula* planted at three sites in Lithuania; latitudinal range 54.92–55.97°N and 12–120 masl.

Fig. 2-2 reveals that family x site interaction is more important than the family effect for the two growth traits, tree height and DBH. Both the family and family x site interaction effects were strongly significant for the two phenology traits; bud flushing and leaf colouring. The family variance component was larger than the interaction component for these two traits. The deviations from trial means in percent in the three test localities illustrate the importance of the family x site interaction. As seen from Fig. 2-3 families 1, 3, and 5 perform above trial mean in some of the test localities and below in others. These families contributed significantly to the interaction. The coefficients of additive variance for tree height of individual populations in the three trials were in some cases extremely large and far above the permitted 25% for OP-families (Fig. 2-4). The large CVa’s suggest that there are good possibilities to improve the tree height in some of the populations. Impressive gains in growth and survival were noted after selection of the 30% best performing families in each breeding zone (Fig. 2-5). Height was given 50% higher weight than the other traits in the selection. This explains the slight drop of branch thickness in series 1.

Fig. 2-6 reveals that the coefficients of additive variance were highest for flushing and tree height while the coefficients were somewhat lower for the five quality traits. Except for branch number the family effect was significant for all other traits. The population effect was significant for one trait only, leaf colouring. The approximate ranges of population means for pilodyn penetration and tree height were 180–200 mm and 210–250 cm, respectively. Thus, the variation among populations was not particularly pronounced.

Figure 2-2. Variance components for family and family x site for height (age 6), DBH (7), bud flushing (7), and leaf colouring (7) in three progeny trials in Lithuania with 114 open-pollinated families of B. pendula. Baliuckiené 2009.

Figure 2-3. Deviation from tree height trial mean of six *B. pendula* families in percent. Families 1, 3, and 5 contributed significantly to the family x site interaction. Baliuckienė 2009.

Figure 2-4. The coefficients of additive variance, %, for tree height at age 6 in six *B. pendula* populations growing in three progeny trials in Lithuania with 100 open-pollinated Lithuanian families and 14 Swedish full-sib families of *B. pendula*. The trials are indicated. Baliuckienė 2009.

Figure 2-5. The percentage gain following the 30% best performing *B. pendula* families in three Lithuanian breeding zones. A weight of 1.5 was given to tree height. Baliuckienė 2009.
The strength of the genetic correlations between pilodyn penetration and the two phenology traits was weak, 0.07 and 0.15, respectively. This means that selection for either of the phenology traits would not reduce wood density. There was a strong positive correlation between pilodyn penetration and DBH, 0.71±0.08. Such a strong and positive correlation is rarely found in literature. Moreover, the precision is good thanks to the large number of parents tested.

The five polymorphic microsatellite regions studied did not indicate any geographic pattern, which agrees with expectation. Two populations were characterized by large genetic diversity and for large coefficients of additive variance for bud flushing. Whether this is a coincidence or not is impossible to say based on just two populations. In conclusion, this investigation presented important information on growth, phenology, and quality traits from well-designed experiments.

Two Finnish *B. pendula* progeny trials located at Lat. 60.24°N were used for estimates of genetic parameters for growth and growth rhythm at ages 14-15 (Wang and Tigerstedt 1993). Most of the families emanated from a reciprocal recurrent selection mating design. Part of the full-sib families were provenance hybrids; Southern Finland x Central Finland and Central Finland x Southern Finland. In addition polycross progenies were also included in one of the trials. Three stages of flushing and three stages of growth cessation were assessed at intervals of 2-3 days during the actual period. Data from these trials containing more than 400 genetic entries were analyzed as one single trial although planting took place two consecutive years.

As seen from Fig. 2-7 the family variance components for growth traits were low in spite of the large number of families studied. The family effect was non-significant for stage 3 during flushing, DBH, and stem volume. The mean values for type of family – Central Finland x Southern Finland, Southern Finland x Central Finland, and Polycross with mixed origin – did not vary much as seen from Fig. 2-8. In spite of this, there were a few cases with significant differences of the two phenology traits. For stage 1 of flushing there was a difference of 1-7 days among the three types of progeny; Southern Finland x Central Finland, Central Finland x Southern Finland, and Polycross of Central Finland. For stage 2 there was a time difference of 7-15 days between the three types of family. The corresponding differences for growth cessation were much larger, varying between 1 and 25 days. The latest growth cessation was noted for the Central Finland x Southern Finland families, which were the most southern material in these trials. These families were on an average taller than the other types of families.

It was speculated that the relatively low estimates of family variance for the growth traits might be attributed to a constant stress in these trials with 6-tree row plots and that random errors may be large in this type of progeny trials. However, increased competition in other progeny trials resulted in increased differences among families (Franklin 1979).

![Figure 2-6. The coefficient of additive variance for growth and quality traits based on 83 open-pollinated B. pendula families; Ht = tree height, DBH = breast height diameter, Fl = Bud flushing, Lc = Leaf colouring, Pil = pilodyn penetration, Str = stem straightness, BNo = branch number, BAn = branch angle, BTh = branch thickness. Baliuckiené 2009.](image1)

![Figure 2-7. The family variance components for 3 flushing, 3 growth cessation, growth period duration, and 3 growth traits based on two trials with B. pendula in southern Finland at Lat. 60.14°N. The two trials contained 421 entries; full-sibs and polycross families. Wang and Tigerstedt 1993.](image2)

![Figure 2-8. The mean values for stages 2 during flushing and growth cessation, for height (m) for breast height diameter (cm), and volume (dm³/10) based on two southern Finnish B. pendula progeny trials at latitude 60.24°N. The white arrows indicate significant differences between the types of cross. Wang and Tigerstedt 1993.](image3)
Some flushing traits showed significant correlations with growth cessation traits. The degrees of freedom in the analyses were 303-381, which means that the correlations may be significant without close relationships. As a consequence of this, the degree of explanation was always below 10%. The relationships between growth cessation and growth traits were somewhat stronger but the degree of explanation reached 30% at most. With such a large number of families it is a pity that genetic correlations were not estimated.

Table 2-2. Significant difference among families for stem volume and physiological traits as well as differences between 4 slow-growing families and 5 fast-growing B. pendula families in a field trial at age 8 located at latitude 60.70°N in southern Finland. The strengths of the polynomial relationships between stem volume and 8 carbon dioxide related traits are given. Wang et al. 1995a.

<table>
<thead>
<tr>
<th>Family variation</th>
<th>Relationship with stem volume R²</th>
<th>Difference fast &gt; slow growing families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem volume</td>
<td>***</td>
<td>+</td>
</tr>
<tr>
<td>Net photosynthesis</td>
<td>**</td>
<td>0.61</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>*</td>
<td>0.25</td>
</tr>
<tr>
<td>Intercellular CO₂ conc.</td>
<td>*</td>
<td>0.61</td>
</tr>
<tr>
<td>CO₂ resistance</td>
<td>-</td>
<td>0.12</td>
</tr>
<tr>
<td>Water transpiration</td>
<td>-</td>
<td>0.06</td>
</tr>
<tr>
<td>Stomatal density</td>
<td>***</td>
<td>0.76</td>
</tr>
<tr>
<td>Guard cell density</td>
<td>***</td>
<td>0.68 + slow &gt; fast</td>
</tr>
<tr>
<td>Total guard cell length</td>
<td>***</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Another genetic-physiological study with a similar approach of selecting fast growing and slow growing families was reported by Wang et al. (1995b). In this case photosynthetic rate in branches from the canopy and in low parts of the crown was analysed together with several leaf characteristics. Besides the fast- and slow-growing families there were three controls. I have summarized the most important results from this investigation in Table 2-3. There were significant family differences for six of the eight traits studied. For three traits the fast-growing families showed a higher value than the slow-growing families. The percentage family variance components for the traits studied varied in the range 21-55%. Some of these estimates are higher than the permitted 50% for full-sib families. However, no discussion of the high estimates was carried out. As in the previous study the most interesting relationships are those with stem volume. Two of them reached an $R^2$ of 0.80 or larger. In spite of the non-significant family effect for photosynthetic rate in the lower part of the crown, the relationship between this trait and stem volume reached such a high $R^2$ estimate as 0.60. The authors combined traits in a multiplicative way and related thus obtained values to stem volume. The leaf area index x photosynthetic rate in the canopy gave the strongest relationship with stem volume, $R^2=0.82$. Instead of multiplying traits I added the photosynthetic rate + leaf area index and related thus obtained sums to stem volume, which resulted in an $R^2$ estimate of 0.88 (Table 2-3). It should be added that the photosynthetic rate in the canopy and leaf area index were fairly strongly correlated, $R^2 = 0.77$. It is somewhat surprising that stem volume, which probably depends on many components, is so strongly related to two traits such as photosynthetic rate in the canopy and the leaf area index.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Family variation</th>
<th>Relationship with stem volume $R^2$</th>
<th>Difference fast &gt; slow growing families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthetic rate canopy, PRC</td>
<td>*</td>
<td>0.81</td>
<td>+</td>
</tr>
<tr>
<td>Photosynthetic rate low level</td>
<td>ns</td>
<td>0.60</td>
<td>Slow &gt; fast +</td>
</tr>
<tr>
<td>Leaf area index, LAI</td>
<td>**</td>
<td>0.85</td>
<td>+</td>
</tr>
<tr>
<td>Mean tilting angle</td>
<td>ns</td>
<td>0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Leaf size</td>
<td>*</td>
<td>0.19</td>
<td>ns</td>
</tr>
<tr>
<td>Leaf shape</td>
<td>***</td>
<td>0.36</td>
<td>+</td>
</tr>
<tr>
<td>Leaf fresh weight</td>
<td>**</td>
<td>0.04</td>
<td>ns</td>
</tr>
<tr>
<td>Leaf dry weight</td>
<td>***</td>
<td>0.33</td>
<td>Slow &gt; fast +</td>
</tr>
<tr>
<td>PRC + LAI</td>
<td></td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>
A detailed study of growth and growth rhythm was carried out in a field trial (Lat 60.95°N) at age 15 with full-sib families (Wang and Tigerstedt 1996). Four fast growing, three slow growing families, and one mixture of two stands were included in this study. Assessments of the increase of the girth were carried out at 10 occasions between May 10 and October 10. This made it possible to identify the period of the highest growth rate.

Total stem volume increased in the following sequence: slow growing families - stand - fast growing families. Only the difference between the slow growing families and the stand offspring was non-significant. As seen from Fig. 2-10 there was a large difference in stem volume between the four fast growing families and the slow growing families. Contrary to this, the variation in increment between the four fast growing families and the four slow growing families - stand - fast growing families was small, although slow growing families. According to the authors these differences were significant. It was found that the main reason for difference in growth between the two types of families was an increased growth rate of the fast growing families during the period June 16 – August 14; coined as growth efficiency (GE). It was suggested that selection for growth rate during the period of largest growth would be a better alternative than selection for an extended growth period in applied breeding. This would reduce the risks for exposure to late spring frosts or early autumn frosts. Fig. 2-11 shows that growth initiation was not correlated with total stem volume while annual growth, growth efficiency, growth cessation, and duration of the growth period were strongly correlated with total stem volume.

In their study of the effect of ozone and freezing of four open-pollinated families of *B. pendula* Silfver et al. (2008) reported on growth of the control material. I have illustrated the family performances for growth in Fig. 2-12, which shows that there is family variation for both traits. According to the authors these differences were significant.

Bud flushing at latitude 60.44°N of open-pollinated offspring covering the distribution area of *B. pubescens* in Finland was recorded during two years by Sulkinoja and Valanne (1987). The age of the trees studied varied but most of the trees were 6 and 12 years at the recording of flushing. Flushing was earliest in the offspring from southern Lapland, 66.55–68.33°N. The flushing date of the northern Lappish offspring from latitudes 68.77–69.75°N occurred simultaneous with the two OP-families from southern Finland, 59.92 and 61.78°N. The reason for the late flushing of the northern Lappish OP-families was attributed to introgression from *B. nana* into *B. pubescens*. Some support for this explanation was obtained from measurements of bud size.

![Figure 2-10. Stem increment and duration of growth period at age 4 in 7 full-sib families of *B. pendula* in a trial at latitude 60.95°N in Finland. *Wang and Tigerstedt* 1996.](image1)

![Figure 2-11. The family mean correlation coefficients between growth and growth rhythm traits in a Finnish *B. pendula* trial at latitude 60.95°N in Finland. *TStV* = total stem volume, *AG* = annual growth, *GI* = growth June 16-August 14, *GC* = growth cessation, *GPD* = growth period duration; *ns* = non-significant. *Wang and Tigerstedt* 1996.](image2)

![Figure 2-12. Total dry weight and relative height increment at age 3 of control plants belonging to four open-pollinated *B. pendula* families in an experiment with freeze testing and exposure to elevated ozone. *Silfver et al.* 2008.](image3)

![Figure 2-13. The number of days to flushing under different combinations of photoperiod (short and long night) and temperatures, 9, 15, and 21°C. *Heide* 1993.](image4)
Shoots of *B. pendula* and *B. pubescens* were collected monthly from November 15 to March 15 and exposed to continuous light or 16 hour-nights at different temperatures, 9, 15, or 21°C to study the bud flushing (Heide 1993). The shoots originated from three trees of each species. The focus of the paper was on species differences in response to the combinations of treatment offered to the shoots. In addition to the two birch species other species were also investigated. Except for an ANOVA table and a few comments on the results in this table, genetic variation was not discussed. According to this table variation in degree days to bud flushing was strongly significant in both species. As seen from Fig 2-13 continuous light provoked early flushing while long nights at low temperatures delayed bud flushing.

The impact of storage temperature and storage time on bud flushing of two *B. pendula* clones and two progenies were studied by Skuterud and Dietrichson (1994). Sampling of shoots from trees in a trial at latitude 60.20°N and 170 masl took place on November 25. The weather conditions prior to the collection of the shoots were such that dormancy was broken with high probability. After sealing of open cuts by wax and fungicide treatment the shoots were kept in cool-storage at 0, 3, and 6°C. At five occasions, shoots were taken out from the cold storage and exposed to forcing temperatures 9, 12, or 15°C. Breaking the bud scale of the earliest opening bud was the criterion for flushing, which was recorded daily. I will not elaborate on the three different methods used to estimate the threshold temperatures for bud flushing. They gave almost identical results.

The response to storage temperature and storage length of the two most extreme entries are visualised in Fig. 2-14. This figure illustrates that the storage time has a great impact on time to flushing and that the difference between storage temperatures 0 and 3°C do not differ much. For both entries the time to flushing was much shorter after storage at 6°C. Thus, at a storage time of 64 days at 6°C the flushing had already started. The higher the temperature after the shoots were exposed to flushing temperatures the shorter the time to flushing (Fig. 2-15). The ANOVA of this three-factor study revealed strongly significant effects for entry and significant effects of entry x storage temperature for all forcing temperatures and for entry x duration of storage for forcing temperatures 12 and 15°C.

The threshold temperatures varied among entries in the range 0.90-2.15°C. They were not affected by storage temperature or duration of storage. In Fig. 2-16 number of degree days for flushing of the individual entries are illustrated for three storage times.

In conclusion, it was convincingly shown that bud flushing in *B. pendula* is dependent on storage temperature, duration of storage, and threshold temperatures in forcing conditions genetics.
In three experiments Viherä-Aarnio et al. (2006) estimated the critical night length for budset in open-pollinated families from two Finnish populations from latitudes 60.45°N and 67.74°N. Night lengths were selected such that they should cover the most likely critical night lengths of the two populations. The major features of the 3 experiments conducted are presented in Table 2-4. The assessment of budset was done immediately after the termination of the long night treatment. The budset of each OP family was fit to an S-shaped curve and the critical night length was read graphically when the curve crossed 50%. The family variation in the two populations in the three experiments is shown in Fig. 2-17, which reveals that the variation among families was larger in the northern than in the southern. Especially, one northern family showed an exceptionally long critical night length in experiment 1. It was stated that the ranking of the families varied significantly between the experiments. In spite of this there was a significant difference in critical night length among families in the southern population. The variation in ranking might partly be attributed to differences in design of the experiments; variation in the duration of the night length treatments as well as numbers of night lengths tested. It should be added that the precision in the estimates of critical night length was good with limited 95% confidence intervals. At the population level there was a clear difference between the two populations.

The white stem character of *B. pendula* has attracted northern American horticulturists as an alternative to the domestic *B. papyrifera* (Miller et al. 1991). Ten open-pollinated Finnish *B. pendula* families and five full-sibs were followed over 12 years in a progeny trial in Michigan. The growth of *B. pendula* was inferior to *B. papyrifera* and the survival was catastrophic with almost no survival after attacks by the bronze birch borer at age 10. The poorer growth of the Finnish material must be attributed to a photoperiodic effect following the long southwards transfer. Before the severe attacks, the family heritability for tree height at age 9 was estimated at 0.66.

### 2.2 Inheritance

In Germany two purple *B. pendula* trees were used in several crosses to disclose the inheritance of the purple phenotype (Hattemer et al. 1990). In Fig. 2-18 I have summarised the segregation in different experiments, each involving several crosses. Based on the crosses it was suggested that the purple phenotype is caused by dominant alleles. It was also disclosed that homozygosity for one of these dominant alleles was lethal. The deviation from the 1:1 segregation shown in Fig. 2-18 is attributed to a lower vitality of plants carrying a dominant purple allele. The purple plants were characterised by a lower growth speed than normal plants. The offspring from crosses between early flowering plants and purple plants as males resulted in the normal growth habit of the male plant. Crosses between normal offspring from early flowering plants were carried out and the occurrence of male

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### Table 2-4. The design of 3 experiments on the photoperiodic control of budset in two geographically distant *B. pendula* populations in Finland. Viherä-Aarnio et al. 2006.

<table>
<thead>
<tr>
<th>Weeks until onset of night treatment</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night length for N pop.</td>
<td>2.5 - 4.0</td>
<td>1.5 - 6.0</td>
<td>1 - 4.5</td>
</tr>
<tr>
<td></td>
<td>4 treats</td>
<td>6 treats</td>
<td>6 treats</td>
</tr>
<tr>
<td>Night length for S pop.</td>
<td>6.5 - 8.0</td>
<td>0 - 8.5</td>
<td>5.0 - 8.5</td>
</tr>
<tr>
<td></td>
<td>4 treats</td>
<td>6 treats</td>
<td>6 treats</td>
</tr>
<tr>
<td>Duration of treatment</td>
<td>4 weeks</td>
<td>33 days</td>
<td>47 days</td>
</tr>
</tbody>
</table>

![Critical night length for 50% budset](image1.png)

**Figure 2-17.** The minimum, mean, and maximum critical night length for budset in 21 open-pollinated Finnish *B. pendula* families. The border between the open and filled box is the mean critical night length in each population. Viherä-Aarnio et al. 2006.

![% green and purple plants](image2.png)

**Figure 2-18.** The mean percentage of green and purple plants in different crossing experiments involving two purple phenotypes of *B. pendula*. Hattemer et al. 1990.
flowering at four months was recorded. The percentage of flowering plants varied in the range 5-59%. There was also a large variation in flowering intensity with some plants flowering extremely abundant. It was suggested that a small number of genes controlled early flowering. The inheritance of the GOT and LAP isozyme loci was studied in a cross involving one purple parent. There was a weak linkage between the two isozyme loci but no linkage with the purple locus.

Four families each represented by 30 offspring were used for mapping of the B. pendula genome by AFLP and microsatellite markers (Pekkinen et al. 2005). Two of the families shared one parent. The number of informative markers was 36, 40, 61, and 77 in the four families. Twenty of the markers were informative simultaneously in the two families sharing one parent. Seventeen markers showed strongly significant distortion from the Mendelian segregation, p<0.001. A still larger number showed distortion from expectation at the 0.05 level. The map obtained contained 16 linkage groups. Seven of the groups contained only 2-3 markers. The reason for the deviation from the expected number of linkage groups for B. pendula was attributed to ...the structure and small size of the mapping population. The deviation from the Mendelian segregation was discussed. It was assumed that the trees with the dominant AFLP markers were heterozygous, which might be wrong. It was noted that the markers showing distortion were clustered that might be attributed to some biological cause. Lethal genes were given as example of a biological cause. In conclusion many more markers are needed to get a saturated map for B. pendula.

2.3 Species hybridization

In a series of papers Icelandic researchers have reported on hybridization between the diploid B. nana and the tetraploid B. pubescens (Anamthawat-Jónson and Tomasson 1990, Anamthawat-Jónson and Tomasson 1999, Thorsson et al. 2001, Anamthawat and Thorsson 2003)

The chromosome numbers of 21 seedlings from crosses between B. nana and B. pubescens were determined by Anamthawat-Jónson and Tomasson (1990) with B. nana as female parent. One backcross of a hybrid with B. pubescens was carried out. Besides, the chromosome numbers of 24 open-pollinated offspring from trees in nature were determined.

Eleven of 15 F1 plants had 42 chromosomes and varied strongly with respect to morphological characters. The other four plants had 28 chromosomes. Four of the nine backcross plants had 56 chromosomes. Also these plants were very variable but with B. pubescens characteristics. All plants obtained from open-pollination had 56 chromosomes. Microsporogenesis was examined in 29 trees with pairing apparently normal in the majority of microcytes. Some irregularities during anaphase I and telophase I were observed.

This study revealed that triploids are not completely sterile and they can serve as a bridge for introgression, and mainly from B. nana to B. pubescens

Offspring from eight open-pollinated B. nana plants and offspring from 22 B. nana plants crossed with B. pubescens was examined with respect to chromosome number by Anamathawat-Jónson and Tomasson (1999). The open-pollinated seedlings originated from Reykjavik where there is no B. nana plants growing. Thus, the obtained seedlings are with high probability B. nana x B. pubescens hybrids. This was confirmed by the 42 chromosomes of these plants. The crosses were carried out without bagging of the inflorescences. Seven of the plants obtained from this cross were diploid and 15 were triploid. It was concluded that the high frequency of triploids found suggests that hybridization between B. nana and B. pubescens is frequent under Icelandic conditions.

Seventy-two plants of B. pubescens, B. nana, or their hybrids were selected in two Icelandic stands without concern of identity for a study of introgression between the two species (Thorsson et al. 2001). Growth form, growth habit, leaf shape, leaf tip, leaf base, and leaf margin were combined into a morphological index in 14 classes; 0 = B. nana and 13 being B. pubescens. Counting of chromosome numbers were carried out on metaphase cells from leaf tissues. The morphological index of the plants from the north-eastern population (latitude 64.75°N and 21.50°W) showed two peaks but with many plants with intermediate indices. The plants with 56 chromosomes had high morphological indices while plants with 28 chromosomes had low indices. The plants with 42 chromosomes had intermediate indices. A continuous variation of morphology indices from the other population (latitude 64.25°N and longitude 20.50°W) to plants with 28, 42, or 56 chromosomes was noted. Molecular evidence for introgression was also presented. It is somewhat surprising that hybridization between a diploid species and a tetraploid species seems to be so common.

Anamathawat-Jónson and Thorsson (2003) looked for spontaneous hybrids between the coexisting B. nana and B. pubescens in Icelandic localities. Counting of chromosome numbers was facilitated by introduction of the protoplast dropping method, which gives high quality metaphases from young leaf buds. A random sample of 61 trees resulted in seven triploid hybrids. It was noted that hybrids frequently are found at edges of birch stands suggesting that they might be outcompeted by the pure species in central parts of stands. Morphologically, there is an overlap between the hybrids and the parental species. Genomic in situ hybridisation (GISH) is the most useful technique for identification of hybrids. This technique allows the separation of chromosomes or chromosomal regions from different parental species. GISH results were presented for the first time for birch and it showed that species-specific DNA sequences occur in B. nana.
Fluorescence in situ hybridisation was used for analysis of ribosomal DNA, which revealed that B. nana has two pairs of ribosomal major loci. B. pubescens has also two pairs of major rDNA loci plus one pair of unexpressed minor loci. This investigation has given further insights to the understanding of introgression between B. nana and B. pubescens.

From 10 widely distributed stands in Iceland pollen samples were collected from 92 trees/shrubs by Karlsdottir et al. (2008). Images of 120-130 pollen grains per tree were taken by a digital camera with 400 x magnification. Grain diameter and pore depths were measured on each pollen grain. The pollen grains from the triploid trees were often of the same size as pollen from B. nana. The triploid pollen was significantly smaller than the B. pubescens pollen. The pollen from the hybrids had wider distribution of sizes than the pure species. The pore depth of the triploid pollen was almost identical with B. pubescens pollen and significantly different from B. nana. The frequency of abnormal pollen was much higher in the triploids than in the pure species. The frequency of pollen with deviation from the most common number of three pores was higher in the triploids. It was pointed out that the characteristics of the pollen from interspecific hybridisation may be utilised in analysis of past hybridization in fossil samples.

### 2.4 Inbred materials

Wang et al. (1999) presented growth and survival data at age 13 from a unique B. pendula field trial (latitude 60.50°N) containing three generations of selfing. It should be noted that the families did not constitute a lineage and that the number of families at the three levels was 6, 9 and 3 for S₁, S₂, and S₃. The material is not perfect for a study of the relationship between inbreeding depression and the coefficient of inbreeding. However, the data obtained for the growth traits showed a clear and significant decline between the levels of inbreeding (Fig. 2-19). There were significant linear effects for all traits but the degree of explanation of the relationships was limited, 20–28%. The recovery of survival between S₂ and S₃ might be attributed to random effects with only three S₃ families. It was also discussed that purging of lethal alleles might have taken place during the first generation of selfing leaving some detriments that would cause strong inbreeding depression for survival in later generations of selfing. The inbreeding depression in growth traits was attributed to mildly deleterious mutations. The family effect was significant (p<0.05) for all four traits.

Crosses between B. pendula trees originating from selfing were studied by Wang et al. (1996). Some of the families are provenance hybrids (Fig. 2-20), which complicates the interpretation since the photoperiodic response of the families, most likely, varies. Besides, the different types of crosses are represented by 1-12 families, which allows for random effects to disturb the interpretation of the results. Another complicating factor was that the degree of inbreeding varied among the parents included in these crosses.

However, it was observed that there was a relationship between the degree of inbreeding of the parents and stem volume (Fig. 2-20) with a good fit to the exponential relationship. As seen from Fig. 2-21 the mean of the 12 crosses southern x southern Finland shows a great superiority over the mean stem volume of the four reference crosses from the same region. Thus, this result suggests a true heterosis effect following crosses between inbred trees. It is likely that part of the superiority of the Finnish x non-domestic origin can be attributed to south-north transfer effects. The poor growth of the two Finnish north x south crosses may also be attributed to early growth cessation of these families. As expected the within-family variation estimated by the coefficient of variance decreased with increasing inbreeding coefficient from 48% for the reference material to 22% for F = 0.75. Similarly the between
family coefficient of variation dropped from 24% at $F_i = 0$ to 7% at $F_i = 0.625$. According to expectation the variation within the crosses should decrease and the variation between families should increase. One explanation for the deviation from these expectations was attributed to heterogeneous origin of the parents included in the crosses. It was concluded that exploitation of heterosis via crosses between selfed trees could be an option in birch breeding. However, the low level of germination of selfed seeds, estimated at 1.6%, is a strong constraint to this approach.

Wang (1996) tested the variation of twelve polymorphic isozyme loci in full-sibs and inbred material of *B. pendula* up to third generation of selfing. $S_i$ Stand progenies were used as control material and the non-inbred full-sibs were growing in three progeny trials. Five of the families were classified as fast growing and four as slow-growing. The percentage of polymorphic loci was highest in the stand offspring, 55.5% and the mean for the 10 full-sibs was 44.6. There was one outlier among the full-sibs with a percentage of polymorphic loci of 13.3. If this family is excluded the full-sib mean is 48.1%. Every step of inbreeding caused a drop of the percentage of polymorphic loci, especially in the two $S_i$ families this percentage was low amounting to 6.7% (Fig. 2-22). Parallel with the drop in polymorphic loci there was a drop in observed and expected heterozygosity (Fig. 2-23).

**Figure 2-21.** Stem volume superiority or inferiority of *B. pendula* crosses compared to the mean stem volume of 4 $S \times S$ full-sib families at age 9 in a Finnish progeny trial at latitude 60.7°N. All families were inbred x inbred with varying origin; $N$, $C$, and $S$ stand for northern, central and southern Finland, Est = Estonia, Lat = Latvia, De = Germany, Au = Austria. The number of families of the various types is indicated. Wang et al. 1996.

**Figure 2-22.** The relationship between inbreeding coefficient and percentage of polymorphic loci in *B. pendula* material growing in southern Finland. The non-inbred material consists of 10 full-sib families. Wang 1996.

**Figure 2-23.** The relationships between mean observed and expected heterozygosity of *B. pendula* families and inbreeding coefficient. Wang 1996.
The three clones of type II were aneuploid, with the tetraploid parent reached the height of the stand ploid parent. As seen from Fig. 2-25 none of the entries (II), or as clones from open-pollination (III) of the tetraploid (I), or in clones from one of the full-sib families of the entries share one tetraploid parent, either in full-sib triploids perform better than diploid B. pendula. The main objective of this investigation was to study if Five families originated from diploid x tetraploid parents. Naujoks (1998). The clones originated from four families.

No significant difference of the genetic variation in the selected material as compared to the unbred material. The higher observed heterozygosity than expected heterozygosity in this investigation suggests that survival is better in heterozygotes than in homozygotes.

2.5 Miscellaneous

Seven full-sib families, 10 clones, and one stand offspring of B. pendula were studied at age 2 and 4 by Schneck and Naujoks (1998). The clones originated from four families. Five families originated from diploid x tetraploid parents. The main objective of this investigation was to study if triploids perform better than diploid B. pendula. Eleven of the entries share one tetraploid parent, either in full-sib families (I), or in clones from one of the full-sib families (II), or as clones from open-pollination (III) of the tetraploid parent. As seen from Fig. 2-25 none of the entries with the tetraploid parent reached the height of the stand offspring. The three clones of type II were aneuploid, which might explain their poor performance. The open-pollinated clones (type III) were diploid. The ploidy level of the full-sibs is a mean value for several families. All of them did not have S1-S3 offspring, which might distort the relationship.

The two conclusions by the authors from this investigation were:

No significant difference of the genetic variation in the selected material as compared to the unbred material. The higher observed heterozygosity than expected heterozygosity in this investigation suggests that survival is better in heterozygotes than in homozygotes.

Ewald et al. (1993) compared the offspring from a tetraploid birch with diploid offspring with respect to leaf morphology, plant height, base diameter, stomata width and length. The latter varied much more than in the diploid progenies. The distribution of plant heights in the tetraploid offspring had its maximum at a higher level than the offspring from the diploid mother tree. It was concluded that the majority of the offspring from the tetraploid parent with high probability was triploid.

Laitinen et al. (2000 and 2002a) studied the variation in phenolic compounds among 30 trees from one population of B. pendula at age 20. It was stated that the chemical profile of individual trees suggest that the phenolic composition is genetically regulated. Four chemotypes of trees were distinguished. However, there was variation of individual compounds during the growth period as well as between years (Laitinen et al. 2002a), which complicates grouping of chemotypes or renders grouping doubtful. The ANOVA of the low-molecular-weight phenolics in Laitinen et al. (2002) revealed significant differences for all tested compounds.
2.6 Conclusions

Significant difference for growth traits, and frost hardiness were noted in several cases. Variation in bud flushing was more limited than variation in growth cessation. A strong correlation between growth cessation and stem volume was noted. Some physiological traits were strongly correlated with stem volume. In one case there was a strong correlation between density and breast height diameter, which rarely occurs in other species. It was shown that bud flushing was dependent on storage temperature, duration of storage, temperature during forcing, and on the genetic constitution of the material. Mapping of the birch genome has started but the map is still rather unsaturated.

The purple phenotype of birch was attributed to a dominant allele, which in homozygote condition causes death of the carrier.

Species hybridization between *B. nana* and *B. pubescens* are rather frequent in Iceland. Inbred material up to the third generation exists in Finland. Although the material was not ideal for estimation of the relationship between inbreeding depression and the coefficient of inbreeding a good fit to a linear relationship was observed. The percentage of polymorphic loci dropped from about 40% to approximately 7% in the third generation of inbreeding. Growth data from crosses between inbred lines suggested that heterosis might be an option for birch breeding. However, the extremely poor seed set in the inbred material is a great constraint to such a breeding effort.

Triploid *B. pendula* did not grow well, which means that ploidy breeding is not a valid option for this species.
3.1 Growth, growth rhythm, and quality

Thirty B. pendula clones originating from southern Sweden growing in a field trial at latitude 56.95°N were assessed with respect to growth, exterior, and interior quality traits at age 11 (Stener and Hedenberg 2003). The trees in this trial originate from tissue culture propagation. The traits included are shown in Fig. 3-1, in which the broad-sense heritabilities are presented. The fibre traits emanate from 8-9 annual rings. They were high for growth, the three fibre traits, and number of branches. It should be mentioned that the genotypic coefficient of variation was around 5% for height, density, and the fibre traits. Especially the estimate for height was regarded as low compared with results from other studies. In Table 3-1 I have tried to summarize the main findings as regards the relationships between and within types of trait. As expected the correlations between growth traits were all strong and significant. As seen from this table growth and fibre traits were not correlated, which facilitates simultaneous breeding for both types of trait. Contrary to this, the growth traits were negatively correlated with density.

Some of the correlation coefficients in the group of exterior traits were far above 1.0 which might be attributed to low precision of additive variance components for some of the traits. The relationships between exterior quality traits and density or fibre traits were all weak. The correlation between fibre width and fibre coarseness was strong and significant.

There were minor losses in density, <1%, when selecting for height or dry matter (Fig. 3-2) while considerable losses were noted for stem volume and dry matter production when selecting for density. The young age of the trees and just one test locality caused the authors to warn for far-reaching conclusions from this study. However, the genetic parameters for stem volume and density were in line with many other studies.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Growth 4</th>
<th>Exterior quality 5</th>
<th>Density 1</th>
<th>Fibre traits 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>Mostly strong and positive; 5 of 6 significant</td>
<td>Varying, more than half negative; only 1 of 25 was significant</td>
<td>All 5 negative; 1 being significant</td>
<td>All 12 weak and non-significant</td>
</tr>
<tr>
<td>Exterior quality</td>
<td></td>
<td>All 10 positive 5 of 10 strong; 3 significant</td>
<td>All five &lt; 0.40 and non-significant</td>
<td>14 weak, 1 moderate, 0.41, and significant</td>
</tr>
<tr>
<td>Density</td>
<td></td>
<td></td>
<td>All 3 weak</td>
<td></td>
</tr>
<tr>
<td>Fibre traits</td>
<td></td>
<td></td>
<td></td>
<td>1 strong and significant; 2 weak</td>
</tr>
</tbody>
</table>

Figure 3-1. Broad sense heritabilities for nine traits in a B. pendula clone trial. ApD = apical dominance, BrTh = branch thickness, BrA = branch angle, and BRN = branch number, Dens = density, FiL = fiber length, FiW = fiber width, Fco = fiber coarseness. Stener and Hedenberg 2003.

Figure 3-2. The genetic gain after selection with different weights put on height (H), dry matter (DM), and density (D) based on data from a clone trial with B. Pendula in southern Sweden. Stener and Hedenberg 2003.

Table 3-1. A summary of the genotypic correlation coefficients between five types of trait and the number of traits within each category in a B. pendula clone trial. Stener and Hedenberg 2003.
Figure 3-3. The mean coefficients of genetic (CV_G) and additive variance (CV_A) for height and breast height diameter (DBH) in two series of clone trials and two series of progeny trials with B. pendula. All trials were established in southern Sweden in the latitudinal range 56-60°N. Stener and Jansson 2005.

Two series of clone trials and two series of progeny trials with open-pollinated families were analysed with respect to survival, growth, and quality traits by Stener and Jansson (2005). All trials were planted in southern Sweden between latitudes 56 and 60°N. Each series of clone trial contained three trials and each progeny trial series contained two trials. In each series 39 clones were included either as vegetatively propagated or as open-pollinated offspring. The clone trials included material that was micropropagated with varying size at plantation. To overcome this problem, initial plant height at planting was used as a covariate in the analysis. Except for two trials the survival was >80% and as a consequence of this the broad-sense heritability was mostly low for survival. As seen from Fig. 3-3 the mean genotypic

![Genetic coefficients of variation; CV_G and CV_A](image1)

Figure 3-4. The range of broad sense heritabilities for five quality traits in two series of clone trials with B. pendula in southern Sweden in the latitudinal range 56-60°N. Str = straightness, ApD = apical dominance, BrT = branch thickness, BrA = branch angle, and BRN = branch number. Stener and Jansson 2005.

and additive coefficients of variation for height were in most cases relatively low, less than 10% while the corresponding for breast height diameter were all above 10%. The heritabilities varied more between trials within series. Thus, the narrow-sense heritability for height in series 3 was estimated at 0.08 in one trial and at 0.54 in the other trial. The corresponding range for breast height diameter in these two progeny trials was 0.11-0.59. These differences could not be attributed to large differences in growth between the trials within this series since the mean height in the trial with low heritability was 5.6 meters and 6.6 meters in the other trial. Nor did the DBH differ much, 47 and 56 mm, respectively.

Except for apical dominance (ApD), a fairly wide range of heritabilities was noted for the quality traits in the clone trials (Fig. 3-4). Except for one clone trial, heritabilities for branch thickness (BrT) were above 0.25. As seen from this figure the heritability mean values were fairly large. The genotype x environment interaction was modest or absent for most traits as revealed by the pair-wise correlation coefficients (Fig. 3-5). The low correlation coefficients for height in series 2 might be attributed to a large difference in mean heights of the three trials 4.7, 7.4, and 8.0 meters, respectively. There was no assessment of DBH from the poor-growing trial, which explains the difference between the correlation coefficients for tree height and DBH in this series. A possible transfer effect was tested in regression analyses with all R² estimates <0.17. The pair-wise genotypic correlations between growth traits were all strong and significant in both clone trial series (Table 3-2). Similarly, the correlations within the straightness/apical dominance group and branch angle/branch number groups were strong in trial series 1 while this was not the case in trial series 2. Significant correlations occurred in a seemingly random way among the other types of trait. It should be noted that none of the three significant correlations between growth traits and

![Genetic correlation coefficients r_G](image2)

Figure 3-5. The genetic correlation coefficients, r_G, between the same trait over trials within a series in two series of B. pendula clone trials in southern Sweden in the latitudinal range 56-60°N. Height and breast height diameter, DBH, at age 10, Str = straightness, ApD = apical dominance, BrT = branch thickness, BrA = branch angle, and BRN = branch number. Stener and Jansson 2005.
branch angle/branch number were strong.
The gain following selection of the 10% tallest clones for establishment of a seed orchard was estimated at 10% for height and 18% for diameter. The effect of early selection on tree height in trials 1A, 1B, and 2B were estimated by the below equation:

\[ \frac{CR}{RX} = H_Y \cdot r_G / H_X, \]

in which

- \( CR \) = indirect selection of trait Y
- \( RX \) = direct selection of the target trait X
- \( H_Y \) and \( H_X \) = the square root of the of \( H^2 \) for the selected trait Y and the target trait X, respectively
- \( r_G \) = genotypic correlations between traits X and Y.

As seen from Fig. 3-6 the relative gain was considerably higher from selection at age 6 than at age 4, which was explained by the stronger age–age correlations between ages 6 and 10 than between ages 4 and 10. The mean of the former was 0.94 while the mean for the 4–10 correlation was 0.67. Since the gain per year was the same following selection at age 4 and age 6 it was recommended that selection should be carried out at age 6.

There was no relationship between growth and origin of the selected trees. Since the trees selected may not be representative for the populations they were selected in, a relationship between geographic location and growth is not expected.

In conclusion the results are promising for breeding of \( B. \) pendula in southern Sweden. Based on the genetic parameters substantial gains of growth may be obtained with limited impact of genotype x environment interaction.

In their study of the relationship between growth and resistance against several herbivores the tree heights at age 11 for eight \( B. \) pendula clones were presented (Tikkanen et al. 2003). The variation in tree height among the eight clones was strongly significant (Fig. 3-7). The trial was located at latitude 61.80°N in southeast Finland.

**Figure 3-6.** The relative gain and gain per year in tree height at age 10 from selection at age 4-6 (A4-A6) and in three clone trials (1 – 3) with \( B. \) pendula in southern Sweden; latitudinal range 56.95 – 58.77°N. Stener and Jansson 2005.

**Figure 3-7.** The tree height at age 11 of eight Finnish \( B. \) pendula clones growing in a trial at 61.80°N in southeast Finland. Tikkanen et al 2003.

**Table 3-2.** A summary of genotypic correlation coefficients between 5 types of trait and the number of significant correlations within each category in a \( B. \) pendula clone trial. Abbreviations as in Fig. 3-4. Stener and Jansson 2005.
Thirty *B. pendula* clones originating from offspring of 10 trees were studied with respect to root weights and root/shoot ratios by Lavender et al. (1993). The root weight after 122 days cultivation in sand varied in the range 3.0–6.0 grams; the range for the root/shoot ratio was 0.3–0.5. Based on the performance of these 30 clones, four representing the range of variation, were selected for a detailed study of root response to two levels of nitrogen, 73 mg N per ml and 7.3 mg N per ml. The traits studied were:

- Root weight
- Root length
- Root/shoot ratio
- Woody root mass, roots that have developed secondary tissues
- White root length, non-woody roots with a diameter > 1mm
- Specific root length white root
- Fine root length, *i.e.* root diameters < 1 mm

In the preliminary study root dry weight was fairly strongly correlated with fine root length ($R^2=0.68$) and white root length ($R^2=0.68$).

It was stated that the two treatments had few differential effects on the root system. However, the root weight was 2–3 times lower in the Low-N treatment than in the High-N treatment. The root length was also 2–3 times lower in the Low-N treatment (Fig. 3-8). These reductions in the Low-N treatment are somewhat surprising since it is expected that the limited amount of nitrogen in the substrate should lead to increased root growth. It was reported that there were clone x treatment interactions for:

- Fine and wood root weights
- Specific root length for white roots
- Proportion of the root system as woody roots

One way to get a visual impression of a clone x treatment interaction is to calculate deviation from the mean with standard deviation as unit. I did this for root weight (Fig. 3-9), which suggests that there is an interaction for this trait. Clone 1 contributes least to the interaction. It was concluded that there may be possibilities to select for root development.

Two *B. pendula* clones with contrasting root characteristics according to a screening of 30 clones (Lavender et al. 1993) were selected for studies of root formation at two levels of nitrogen and phosphorus by Mackie-Dawson et al. (1998). The above-ground parts of the two clones did not differ much. The phosphorus treatment was complemented with inoculation of the mycorrhiza forming *Tel-ephora* sp. fungus. The nitrogen treatment lasted for 14 weeks and the phosphorus treatment lasted for 24 weeks. As seen from Fig. 3-10 clone 1 had a lower proportion of fine roots than clone 2 accompanied with a higher proportion of white roots. In Fig. 3-11 I have illustrated the specific root length (SPR = fine root length divided by fine root dry weight) and length of fine roots. There was a significant difference between the two clones with respect to fine root lengths in both treatments, $p < 0.01$. The High-N treatment did not cause any change of SPR in the two clones while the length of the fine roots was doubled in

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**Figure 3-8.** Root lengths in four *B. pendula* clones cultivated for 9 months under high, 73 µg nitrogen per ml, and low-N, 10% of this amount of nitrogen. Lavender et al. 1993.

**Figure 3-9.** The deviations from the mean values for root weight of four clones expressed in units of the standard deviation. High-N = 73µg nitrogen per mL, Low-N = 7.3µg per ml solution. Four clones of *B. pendula* were included in this study. Lavender et al. 1993.

**Figure 3-10.** The proportion of of fine roots, woody roots, and white fine roots in two clones of *B. pendula*. Mackie-Dawson et al. 1998.
this treatment. In spite of the differences in root growth the total dry weight of the two clones did not differ much within each of the two N-treatments.

As seen from Fig. 3-12 there was a drop in fine root length in clone 2 after mycorrhiza association while there was a sharp increase in clone 1. The strong drop in shoot dry weight and length of fine roots in clone 1 in the High-P treatment was attributed to a drain to the Telephora fungus. (In Table 3 of the Mackie-Dawson paper all treatments are shown as Low-P, which must be a printing mistake.) However, it was stated in the paper that there was no significant effect of added inoculum on root growth in genotype 2. Besides what is presented in Fig. 3-12, there was a stronger reduction of SPR of clone 1 at High-P+mycorrhiza than in clone 2. The proportion of fine roots in the High-P+mycorrhiza treatment was higher in clone 2 than in clone 1. In the three other treatments this proportion was almost identical. Except for the Low-P treatment the above-ground growth is reflected in the length of fine roots. It is evident that the plants have to allocate more resources to roots when the availability of nutrients is limited.

It may be concluded that mycorrhiza presence resulted in a different response in the two clones.

A physiological-genetic investigation including four B. pendula clones were reported by Aspelmeier and Leuschner (2004). Two clones originated from Austria, one clone from northern Germany, and finally one clone from southern Sweden were included in the study. The focus of the investigation was more on physiology than genetics. Half of the material was exposed to drought treatment, during which the soil water volume content was kept at 1-3% for 12 weeks during the second growth period and 14 weeks during the third growth period. The following traits showed significant clone and treatment effects:

- Stomatal conductance
- Intercellular CO₂ concentration
- Photosynthetic water-use efficiency
- Chlorophyll-based photosynthetic capacity
- Foliar carbon isotope composition, δ¹³C

In addition the two following traits showed significant clone effects:

- Saturating CO₂ concentration, CO₂ sat
- Bulk modulus of tissue elasticity, ε₅₅₂₀

Carboxylation efficiency, leaf area-based photosynthetic capacity, and leaf area-based net-photosynthesis did not show any significant clone effect. The clone x treatment interaction was non-significant for all traits studied. Graphics were used to illustrate the development of some of the traits. I have preferred to illustrate water use efficiency, WUE, at the end of the treatments during growth periods 2 and 3 in Fig. 3-13. The strong treatment effect is clearly seen in this figure. There is a fairly good agreement in performance in the control treatment while the WUE varied more between years in the drought treatment.
The photosynthetic capacity varied to a large extent between years within the two treatments (Fig. 3-14). The significant effect of clone is clearly visible for this trait. The phenotypic plasticity (= the maximum difference in performance between treatments) of WUE was said to be larger for the AU2 and DE clones than for the other two clones, which may be true within treatments but not for all four observations (Fig. 3-13). The authors noted that several of the physiological traits suggested that the DE and SE clones were better adapted to drought conditions than the two Austrian clones. The latter originated from a locality with a precipitation double as large as the localities in Germany and Sweden. Therefore, the results may reflect adaptation to drier conditions of the German and Swedish clones.

A study of acclimation was carried out by comparison of two genotypes, wild-type and an ABA-deficient genotype, as regards building up of frost tolerance, ABA content and some LEA (late embryogenesis abundant) proteins (Rinne et al. 1998). Both B. pubescens genotypes originated from northern Finland, latitude 65.08°N. In the water stress treatment the soil water content was reduced to 70% of the well watered treatment.

Three weeks of water stress or ABA treatment did not improve frost tolerance much in any of the two genotypes while both genotypes responded strongly on long night treatment for 3 weeks (Fig. 3-15). Both water stress and long night treatments for 7 weeks caused a strong increase of frost tolerance in the wild-type genotype whereas 7 weeks of water stress did not cause any increase of frost tolerance in the ABA-deficient genotype. It should be noted that frost tolerance of the wild-type plants after 7 weeks of water stress may be stronger than -13.5°C since it was the lowest temperature used for testing frost tolerance of the wild-type plants in this treatment. The water content of buds dropped in the wild-type during the long night and water stress treatments while it did not change in the ABA-deficient genotype. It is evident that there was almost no increase of ABA in the ABA-deficient genotype under outdoor conditions. Whether the peak of ABA in the wild-type was of short duration or not is not possible to evaluate since assessment intervals were three weeks. Both three weeks before and after August 9 the ABA content was much lower and did not differ much from the starting value. There was an accumulation of Group 2 and Group 4 LEA proteins in the wild-type following long night treatment and under natural autumn outdoor conditions.

It was concluded that ABA, Group 2 LEA proteins, and osmoregulation were involved in the long night-induced acclimation. It was also stated that the ability to swiftly

**Figure 3-14.** The photosynthetic capacity in 4 B. pendula clones originating from Austria (AU) northern Germany (DE), and southern Sweden (SE). Light green and dark green stand for controls in 1999 and 2000, respectively. Aspelemeier and Leuschner 2004.

**Figure 3-15.** Frost tolerance estimated as LT₅₀ of two B. pubescens genotypes for control (0) long night treatment (LN), water stress (WS), and ABA application. Blue columns refer to an ABA deficient genotype; green is the wild-type. The figures indicate the number of weeks of each treatment. Rinne et al. 1998.

**Figure 3-16.** Maximum increase of ABA following 16h night length treatment, water stress, and under outdoor conditions of two B. pubescens genotypes; wild-type ABA-deficient. Rinne et al. 1998.
respond to long nights under high-latitude conditions is
of adaptive value. It is rather the avoidance of becoming
exposed to early frosts than the absolute level of frost tol-
erance during winter that is critical for fitness.

Bud flushing of *B. pendula* clones was followed during
four or six years at two test localities in south eastern Fin-
land (latitude 61.80°N) by Rousi and Pusenius (2005). One
of the trials was located on an abandoned field poor in nu-
trients while the other trial was on a fertile field but the
establishment took place two years later. Growth termina-
tion (= 95% of the final increment reached) was assessed
during one or two years. Four of the eight clones followed
over six years were also followed at the other test locality
approximately 1 km away from the first locality. However,
only three clones were common according to the identi-
fication numbers in Figs. 4-5 in the paper, which I have
interpreted as a mislabelling.

The individual analyses of bud flushing in each trial re-
vealed significant effects of clone and clone x year inter-
action in both of them. It was concluded that bud flush-
ing was mainly related to heat sum and the most likely
threshold temperatures were in the range -1°C- +2°C. The
joint analysis of bud flushing data in the two trials with
the four common clones revealed significant clone and
clone x year interaction while clone x trial and the three-
way interaction clone x year x trial were non-significant.
I have preferred to illustrate the temperature sums for
reaching of 90% bud flushing in the two trials for the years
1993-1996 (Fig. 3-17). It should be noted that there was a
two-year difference in age between the two trials, which
might have some impact on the heat sum requirement for
bud flushing. It is obvious that the year effect is strong as
revealed in the ANOVA. Similarly, it may be seen that the
classic x year interaction is stronger than the clone x trial
interaction. Growth termination was not assessed more
than twice in the nutrient poor trial and once in the other
trial. I have illustrated the Julian days to growth ces-
sation in Fig. 3-18. There is a clear effect of year on growth ces-
sation. It was noted that the maximum clone differences
in bud flushing were 8 and 9 days in 1993 and 1992 while
the corresponding differences for growth cessation were
49 and 30 days. There was a significant clone difference in
duration of the growth period in both trials. Several rela-
tionships between phenology traits and increment were
calculated. The degree of explanation was not high in any
of the relationships. The strongest $R^2$ was noted for in-
crement 1993 in the infertile trial, estimated at 0.55.

It may be concluded that this paper with many instructive
illustrations is an important contribution for understand-
ing of growth and its components. The obtained results
were discussed from an evolutionary perspective and
the impact of obtained results was related to their conse-
quences for herbivores.
3.2 Chemical composition and resistance

From the same parental population as used by Laitinen et al. (2004; page 62) 14 micropropagated clones were studied with respect to secondary metabolites in shoots (Laitinen et al. 2005). The clones were planted at four test localities in the latitudinal range 61.60-61.78°N in Finland. There were significant differences among clones for all compounds but for some of them, such as the triterpenoid groups of compounds, the variation within a tree was larger than among clones. This group of compound showed the largest difference between parental trees and their micropropagated progeny. Significant genotype x test locality interaction was noted for all individual terpenoids as well as for total terpenoids. So called Finlay-Wilkinson diagrams were illustrated for two compounds, chlorogenic acid and papyriferic acid based on their performance at the four test localities. The latter compound was a good example of clone x site interaction.

The material used by Laitinen et al. 2000 and 2002b (cf. above) was also used for a study of variation in litter decomposition among 19 clones (Silfver et al 2007). In August before leaf abscission, one branch per plant was enclosed in a mesh bag and litter samples were collected after leaf fall in November. A bioassay with humus collected from a birch stand was carried out and the loss of weight in the litter was recorded after assays lasting for 3 or 6 months.

There was a significant difference in litter loss among the 19 clones for both assessments. From an evolutionary perspective the importance of the genetic differences in litter decomposition was questioned by the authors. In a stand there is a mixture of genotypes and litter is mixed which means that individual differences may not play a significant role in natural selection. Lignin concentration was also assessed without finding any significant genetic variation.

The relationships between litter loss at both times and insect resistance, growth rate, soluble proteins, specific leaf area, nitrogen %, and lignin % were estimated by Pearson correlations. In most cases there were no significant relationships. This might be attributed to absence of significant differences for some of the traits listed above. The only two significant relationships are shown in Fig. 3-19. As indicated the degree of explanation was not large for nitrogen percentage. It might be questioned if all of these relationships are based on hypotheses of causal relationships with litter decomposition.

3.3 Genetically modified material

One wild-type *B. pendula* line (R) and one transgenic line (R3.1) with delayed senescence originating from the wild-type clone were used in a physiological, biochemical, and molecular study of leaf senescence (Sillanpää et al. 2005). Plants were cultivated under controlled conditions in a greenhouse for 70 days. Assessments were carried out at five occasions between day 23 and 70. When possible, two types of leaf were included in the assessments, so called life span leaves (LSL) and young fully developed (YFD) leaves. There was a tenfold difference in height growth between the two lines in favour of the R clone. The leaves of the R3.1 line were small and curly.
The maximum efficiency of photosystem II was estimated by the \( F_{v}/F_{m} \) ratio, \( F_{v} \) is the variable fluorescence and \( F_{m} \) is maximum fluorescence in the dark-adapted state of the leaves. In the LSL-leaves of the R clone this ratio dropped between days 44 and 57 while it was constant between days 34 and 70 in the R3.1 LSL-leaves. There was not much variation in the non-photochemical quenching (NPQ) in the three types of leaves tested, R-LSL, R-YFD, and R3.1-LSL leaves. The NPQ was almost constant throughout the test period. A difference in the size of plastoglobuli between the R and R3.1 leaves was noted in electron microscope. The amount and total activity of Rubisco declined from day 34 in both types of leaf in the R-line while it remained constant in the R3.1-line leaves.

Six genes were used for an analysis of changes in gene expression through the growth period. I have tried to summarise the main results from this part of the Sillanpää et al. paper (Table 3-3). As seen from this table, there are some cases with mRNA levels that show a specific performance. It should be stressed that the R3.1-leaves had a later decline of Rubisco mRNA.

The photosynthetic activity in the YFD leaves was maintained until day 70 and continued even if the height growth was terminated. The size of the chloroplasts and starch grains in the palisade parenchyma cells of the R3.1-YFD leaves were approximately twice as large as in the R-YFD leaves.

In conclusion, it is not surprising that the R3.1 plants with a mean height of approximately 3 cm have another metabolism than the R plants with a mean height close to 50 cm. In addition there was a steady but extremely poor growth of the R3.1 clone until termination of the experiment.
In an earlier study Valjakka et al. (1999) had followed the gene activity during seedling development over seven weeks in greenhouse. The main objective of this study was to improve the knowledge on genetics of leaf senescence. Total soluble protein, Rubisco, and chlorophyll concentration were assessed with a start when the seedlings had 11 leaves and continued to day 47 from onset of sampling. These substances are indicators of senescence. Instructive graphic illustrations were presented. There was a tendency that the amounts of the three substances increased during the early sampling occasions, which was followed by a decline at later samplings. The sharpest decline was noted for the oldest leaves, i.e., leaves that were fully developed at the first sampling occasion. Expression of four senescence-related genes was assessed at the same sampling days. Also for these mRNAs instructive graphics were shown. The mRNA of the 1-aminocyclopropane-1-carboxylic acid (BP-ACO) increased in the young leaves up to sampling day 11. In the oldest leaves the level sank but from a high level. The youngest leaves had high levels at all samplings. The mRNA of the ribonuclease-like pathogenesis-related protein 10 (Ypr) in the young leaves followed the pattern of BP-ACO. The youngest leaves showed a slight increase of Ypr mRNA from a low level while this mRNA peaked at first sampling and then dropped continuously to a low level. The Mpt11 mRNA (mitochondrial phosphate translocator protein) increased sharply between sampling days 11 and 27 for all but the youngest leaves, in which the increase was less pronounced. The LAP (leucine amino peptidase) mRNA showed a fairly similar pattern as Mpt11. The RubiscoSmRNA declined between sampling days 11 and 27 in leaves of all ages. In the young leaves there was a sharp increase of this mRNA between onset of sampling and day 5. It was concluded that age and shading by later formed upper leaves had a strong effect on down-regulation of photosynthesis. It was evident that photosynthesis gradually moved to younger leaves during this 7-week period. It was shown that the expression of four senescence-related genes probably had an effect on onset of senescence.

The sugar beet chitinase IV gene with the enhanced CaMV promoter was transferred to a wild-type B. pendula clone by Pasonen et al. (2008). Fifteen transgenic lines were selected and planted at age 1.5 years in a field trial close to Helsinki in southern Finland. Growth, phenology, plant condition, and infection by Phytophia were studied up to an age of five years. Twisted growth of the stem and deviating branching pattern was used to classify loss of apical dominance. Tree condition was visually recorded in four classes, dead, partly dead, many brown leaves, few brown leaves, and green leaves only.

In Table 3-4 I have summarised the results as regards significant differences among transgenic lines and differences between the control and transgenic lines. The compilation in Table 3-4 shows that significant differences occurred for most of the traits. Frost damage constituted an exception but this absence might be attributed to very low frequency of frost damage. The lines were grouped in four classes, dead, partly dead, many brown leaves, few brown leaves, and green leaves only.

In Table 3-4 I have summarised the results as regards significant differences among transgenic lines and differences between the control and transgenic lines. The compilation in Table 3-4 shows that significant differences occurred for most of the traits. Frost damage constituted an exception but this absence might be attributed to very low frequency of frost damage. The lines were grouped in high, intermediate, and low level of sugar beet chitinase IV mRNA. The three lines with significantly better height growth than the control originated from each of the three groups. Similarly, the four lines growing significantly less than the control represented all three groups. The lines that differed significantly from the control for the traits bud flushing and autumn colours were later than the control.

The observed variation in Phytophia infestation was attributed to plant size since there was a strong correlation between these two traits. It was hypothesized that sugar beet chitinase IV transcript accumulation might explain the variation among the 15 transgenic lines but as the authors expressed it only little support was found for that. The amount of red colour of the leaves constituted an exception since it was the only...
trait that deviated from the general pattern of no relationship with transcript accumulation. The observed variation among lines was attributed to position effect of the transferred genes. Thus, dependent on the location within the genome the effect on the recorded traits varied. If this is the case gene transfer may lead to desired effects as well as undesired effects in the transgenic recipient plant.

Sutela et al. (2009) studied phenolic compounds and lignin characteristics in three transgenic B. pendula lines after inoculation with *Paxillus involutus*. The transgene used was the caffeate/5-hydroxyferulate O-methyltransferase (COMT) from *Populus tremuloides*. The inoculation took place during co-cultivation between the host plants and the fungus for eight weeks. No difference in the quality or quantities of phenolic compounds between transgenic and control plants was noted. The interaction between *P. involutus* and host plants was not affected by the presence of the PtCOMT gene.

3.4 Miscellaneous

Six *B. pendula* street trees with a wide range of phenotypes were selected for a study of a possible variation under the specific conditions along roads (Fostad and Pedersen 1998). The progenies were cultivated on peat and a soil from the roadside. The seedlings growing on peat showed a much better growth than the seedlings growing on roadside soil. One of the open-pollinated progenies grew much less than the other five progenies both in the control soil and roadside soil but the growth reduction in roadside soil did not differ among the six progenies, varying in the range 32-38%.

Three *B. pendula* clones were tested for their suitability as street trees at two localities in Arkansas (Gu et al. 2007). The *B. pendula* Trost’s dwarf had no surviving trees after four years. The clone *laciniata* had 13 surviving trees out of 14 at one test locality and none of the six trees survived at the other test locality. The third clone had a survival of 83 and 17% surviving trees at the two localities. At the test locality with good survival the two *B. pendula* clones ranked 6-7 with respect to growth among the 17 surviving genotypes. The long nights during the summer in Arkansas would intuitively result in very short growth periods for the *B. pendula* clones from much higher latitudes. This is expected to result in a poorer growth than the five meters attained after four growing seasons.

Two *B. pendula* cell cultures, one embryonic and one non-embryonic, were exposed to two culture media lacking sucrose or ammonium or nitrate in suspension cultures (Nuutila and Kauppinen 1992). There were sufficient amounts of ammonium, nitrate, and sugars during the first weeks of culture in the embryonic cell line while the non-embryonic cell line suffered from starvation. Thus, starvation should be used to enrichment of embryonic cell lines in mixes with non-embryonic cell lines.

3.5 Conclusions

Some papers in this chapter aim at better understanding of different processes leading to phenotypic variation rather than estimates of genetic parameters. Clonal differences for growth and quality traits were noted but the genotypic coefficient of variance was usually rather small, < 10%. Genetic correlations between types of trait such as growth, density, exterior quality, and fibre traits were in the majority of cases non-significant. Selection priority for density would lead to considerable loss in growth. In one series of clone trials the broad sense heritabilities varied much. This variation was hard to explain from growth data. This series indicated that clone x test locality was of minor importance. A considerable gain might be obtained from selection.

As observed in progeny testing, there was a larger variation in growth cessation than in bud flushing. Root growth was strongly affected by nitrogen supply in short-term tests. The root weight at 73 μgN/litre was 2-3 times lower than in the 7.3 μgN/litre treatment. Clone x N-treatment interactions occurred for several root traits. Several physiological traits related to photosynthesis showed clonal differences.

Abscisic acid, ABA, was studied in an ABA deficient and one wild type clone after long night treatment and ABA application. Long night treatment improved frost tolerance in both clones while drought stress for 7 weeks did not improve frost tolerance in the ABA deficient clone. It was concluded that ABA was involved in the long night acclimation towards frost tolerance.

Litter loss of clones was positively correlated with soluble protein but the degree of explanation was relatively modest, R² = 0.51.

Over time relative mRNA levels for genes related to photosynthesis, cell maintenance, antioxidants, and defence were studied in one transgenic line with late senescence and in two types of leaves, young fully developed and life span leaves. Instructive graphic illustrations were presented. Sharp increase of the antioxidant lipogenase was noted in the senescence line between days 57-70. In the wild type there was a sharp increase of the mRNA of cysteine proteinase (cell maintenance) and a pathogenesis related protein between days 44-70.

Differences among transgenic lines with the sugar beet chitinase IV gene with the CaMV promoter were studied with respect to growth, growth rhythm, and *Phytobia* resistance. Significant differences among lines were found for the majority of traits. For most traits differences could not be attributed to the level of chitinase accumulation in the lines, which varied considerably.
Herbivory

4.1 Population level

At age 10 moose browsing in a *B. pendula* provenance trial at latitude 60.65°N in Finland, containing 28 populations and one full-sib family from the latitudinal range 53.84 – 67.74°N, was recorded by Viherä-Aarnio and Heikkilä (2006). The longitudinal range was 4.75°E – 38°E. Originally the trial was fenced but the fence was broken at age 5. After that moose browsing was frequent in the trial, which offered an excellent opportunity to analyse the relationships between injuries and tree height or latitudinal origin. At age 5 the maximum was rather flat with a better fit to the second order regression curve than at age 10. Instructive graphic illustrations were presented for the dependence of population latitudinal origin on the below three ways of estimating damage:

- Percentage of browsed trees, PBT
- Percentage of trees with stem breakage, PTSB
- Percentage of repeatedly browsed trees, PRBT

A joint analysis of covariance was given for these three injuries and for number of browsed branches per browsed tree. For all three injuries there were clear relationships between damage and latitudinal origin. For the percentage of browsed trees the relationship was strongly significant (p <0.001). I have illustrated the dependence on latitudinal origin on two of the injuries in Fig. 4-1. For all three injuries the trend is similar with the most affected trees in the southern origins, south of latitude 59. The highest percentage of browsed trees was noted for a Swedish population from latitude 55.72°N. There was a strong relationship between tree height at age 5 and percentage browsed trees at age 10. The reason for relating the injuries to the height at age 5 is obvious since it was the height at start of browsing. The strong relationship between tree height at age 10 and latitude showed a maximum close to the latitude of the trial site but the dispersal along the curve was large. In passing it may be mentioned that there was no relationship between tree height at age 10 and injuries.

It was pointed out that palatability is highest during active growth when deterring substances in leaf and branches may be at a minimum. It was stated that moose browsing mainly occur during the period June-September when moose feeds from green leaves and young shoots. This was given as one explanation for high browsing of southern populations with their late growth cessation. Part of the populations from northern latitudes have already leaf colouring and or even leaf fall in August-September.

One experiment with a focus on species differences in tolerance against hare browsing was reported by Rouhi et al. (1996). A similar design as in Rousi et al. (1997) was used. The palatability index of six Russian *B. pendula* populations differed significantly but did not show any relationship with longitude; the range in longitude being 55.00–78.00°E. Similarly there was a significant difference in palatability among four Finnish *B. pendula* families, palatability index range was 17–52.

4.2 Within-population level

4.2.1 Mammals

Rousi (1991a) recorded the number of resin droplets and hairs, the latter in a 4-degree scale, in families or populations of *B. pendula*. Genetic entry is here used as a common term for families and populations. Hare feeding experiments were carried out as multiple-choice experiments with caged hares. Each experiment was run for three nights. In one experiment the effect of fertilization on hare feeding was carried out. One-year seedlings or eight-year twigs were used in the experiments. Twelve experiments were carried out. The paper contains a large number of instructive tables and graphic illustrations. Therefore, I will only in condensed form present the major results from this comprehensive investigation.

There was a large and strongly significant variation in resin droplets among families in all 12 experiments. Younger seedlings had higher number of resin droplets than older material.

There were significant relationships between height and number of resin droplets in two out of twelve experiments.

For hare browsing there was significant variation among entries in 11 of the 12 experiments.

There were significant, negative relationships between number of resin droplets and hare browsing in 11 of the 12 experiments.

![Figure 4-1. The percentages of browsed trees and trees with stem breakage in a B. pendula provenance trial at 60.65°N in southern Finland. The recordings were done at age 10. The number of the populations from different latitude classes is indicated. Viherä-Aarnio and Heikkilä 2006.](image-url)
Hares preferred twigs with low number of resin droplets when twigs with high or low number of droplets within individual families were offered for feeding. There were significant difference in height among families at age one and at age 8. Tall seedlings were less affected by hare browsing. A good relationship between entries at age 1 and 8 as regards hare browsing was noted. Fertilization resulted in tall seedlings with a large number of resin droplets but its effects were not fully clear. 

*B. platyphylla* seedlings had higher number of resin droplets than any of the *B. pendula* entries and these seedlings were less affected by hare browsing. It was suggested that the differences observed could be attributed to genetic differences among the entries. The carbon-nitrogen hypothesis says that carbon-based secondary metabolites are accumulated under conditions when all carbon cannot be used for growth. In the present investigation fertilization resulted in good growth and high number of resin droplets, which is opposite to the carbon-nitrogen hypothesis. The experiment with twigs from the same family with high or low number of resin droplets indicated that the hares are very specific in their feeding search. Based on the data in Fig. 1 in the Rousi paper I plotted hare browsing of one-year old seedlings after three nights against the number of resin droplets. Such a relationship is based on mean performance of the entries. The relationship was not strong (not shown, \( R^2=0.21 \)) nor did I find strong relationships of the individual experiments with one-year seedlings. The carbon-nitrogen hypothesis was the best estimate of moose preference since all parts of shrubs and trees are not edible. It was also suggested that moose tried to avoid or minimize their consumption of birch resin. The resin droplets contain papyriferic acid, which is a digestion inhibitor. There was some agreement of the feeding preferences between hare, moose, and voles of the clones tested.

Ten moose feeding stations were established along a forest edge with nine clones represented by 4 plants each (Jia et al. 1997). Growth and growth components were assessed. The weight and density of resin droplets on the current annual shoots were also assessed. The following five moose-related variables were recorded:

- Bite diameter
- Number of bites per tree
- Biomass removed per bite
- Biomass removed per tree
- Relative consumption = biomass removed/biomass available

The clones differed significantly for all five moose-related traits (\( p<0.01-\text{p}<0.001 \)). Similarly there were significant differences among the clones for the majority of growth and "resin" traits. Total amount of phenol and acid detergent fibre varied significantly among the clones. One of the objectives with recording of the large number of traits was to see if there were any relationship between moose-related traits and the other types of trait (cf Table 4-1). It was concluded that the relative consumption was the best estimate of moose preference since all parts of shrubs and trees are not edible. It was also suggested that moose tried to avoid or minimize their consumption of birch resin. The resin droplets contain papyriferic acid, which is a digestion inhibitor. There was some agreement of the feeding preferences between hare, moose, and voles of the clones tested.

<table>
<thead>
<tr>
<th>Relative consumption</th>
<th>Biomass removed/tree</th>
<th>Biomass removed/bite</th>
<th>Number of bites/tree</th>
<th>Bite diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth traits</td>
<td>1 of 9</td>
<td>4 of 9</td>
<td>1 of 9</td>
<td>3 of 9</td>
</tr>
<tr>
<td>Resin traits</td>
<td>2 of 2</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Phenol and ADF</td>
<td>1 of 2</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>ns</td>
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</tr>
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</table>

*Table 4-1.* The significances of Pearson correlations between moose related traits and growth, resin, phenol, and nitrogen. Green boxes indicate significant positive relationships and the light red box indicates significant negative relationships. ADF = acid detergent fibre. Jia et al. 1997.
There was a significant difference in height among the clones but not for basal diameter or stem volume. There was a significant difference in palatability with a large variation among the clones (Fig. 4-2). It was noted that the most eaten clone was the first preference of the hares. There were negative relationships between palatability and flavonoid aglycones and total triterpenoids, $r=0.72$ and 0.70, respectively for linear relationships. However, the graphic illustrations suggest that the relationships rather are exponential than linear. In spite of the within-clone variation it was stated that there was a strong genetic basis for the chemical compounds as well as for mountain hare palatability.

The study by Laitinen et al. (2002b) was a contribution to testing the CNB-hypothesis (carbon-nutrient balance, cf above). Hare palatability was studied on five $B. pendula$ clones at age 8 from two field trials, one on mineral soil and the other on peat, both located close to latitude 61.8$^\circ$N in Finland. As homogenous as possible 40 cm long twigs were cut and offered in bundles to five caged hares; one hare per cage. A palatability index, equal to the mean loss of biomass in percent, was calculated. The experiment lasted for 15 nights and a mean palatability index was calculated for the 15 nights. The cage experiment was complemented with recording of feeding in 7 field stations of the same clones. Bundles were placed on boards in the field experiment and a palatability index based on loss of twig length in relation to initial length was calculated. The results were related to a study of palatability at age 1 of the same clones.

There was a significant difference among the clones for tree height, DBH, biomass and twig diameter in each of the treatments. As revealed from Fig. 4-3 one clone contributed strongly to the clone x treatment interaction for biomass. To test the agreement between the estimates of palatability under different treatments I have calculated the strength of the relationships in Fig. 4-4. This figure reveals that only the field peat palatability differed from the others. Considering that only five pairs of comparisons exist the relationships are unexpectedly strong. To test the relationship between growth and palatability I related the palatability in the four treatments to the growth under corresponding soils conditions. Only in one case, the degree of explanation for the relationship between palatability and growth exceeded 50%; field peat palatability – biomass on peat soil. Thus, the strongest relationship was noted for the palatability that deviated most from the three others. Only ranks of palatability of the recordings at ages one and eight were presented and rank changes occurred.

The frequency of resin droplets that are supposed to be deterrent to herbivores was also studied. Also in this case the clones differed significantly. Significant relationships between frequency of resin droplets and palatability index were noted for all four treatments. However, these relationships were based on data from individual trees. The relationships did not exceed 50% of explanation in any case.

In conclusion this study did not give any strong support to the CNB-hypothesis presented above.
Growth at age 1 of four *B. pendula* full-sib families in six treatments were reported by Rousi et al. (1993). Fertilization and no fertilization were the major treatment. Within each of these treatments three shading regimes were used 0, 30, and 60% shading. A multiple-choice experiment with caged mountain hares (*Lepus timidus*) was carried out during three nights in February. A palatability index was estimated as the biomass loss in percent of the initial biomass. Ten caged voles (*Microtus agrestis*) were offered two birch families each (two-choice-experiment). The debarked area after 12 hours was recorded.

There were significant family, family x fertilization, and family x fertilization x shading effects both for shoot and root biomass. In addition, the family x shading interaction was significant for root biomass. In absence of any data on shoot or root biomass I have selected to present the root/shoot ratios for the two families with the largest contrast in this trait in the unfertilized and non-shaded treatment (control, left column) in Fig. 4-5. The family with the highest root/shoot ratios was a Finnish x Siberian family. It was speculated that the reason for the performance of this family might be attributed to adaptation to other climatic and edaphic conditions of the Siberian parent.

Resin droplet numbers decreased after fertilization and shading. This decrease was not related to plant size. With one exception the palatability indices from the hare feeding experiment increased in the shaded treatments (Fig. 4-6). Of the four families included it was only the Finnish x Siberian family that showed an increase of palatability after fertilization. There were significant and negative relationships between plant growth traits (shoot biomass, root biomass, and total biomass) and the palatability index in each of the fertilization and non-fertilization treatments.

It was stated that *vole feeding had a tendency to decrease with shading*, which was true only for one of the Finnish families but the decrease was non-significant (Fig. 4-7). The Finnish-Siberian hybrid family had a drastic increase in the shading treatment from its low palatability estimate in the non-shaded treatment. Fertilization increased the palatability to voles.

Even with such a limited number of families, there were clear genetic differences and different responses to growth modifying treatments. The latter had an impact on the palatability, and in different ways to the two types of herbivores, hares and voles.

In an experiment mainly focusing on growth rhythm after sowing at several occasions a spontaneous attack of voles on the seedlings was analysed (Rousi 1988). In Fig. 4-8 I have illustrated the plant heights and the damage during the first growth period according to my understanding of the graphic data presented by Rousi. It is evident that the Finnish x Russian hybrid Punkaharju x Novosibirsk, 61.80 x 54.17°N had the lowest frequency of damage and grew well. The low number of entries does not allow any general conclusions but the good performance of the Finnish-Russian hybrid calls for replication of the study with a larger number of hybrids.

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**Figure 4-5.** The root/shoot ratios in two *B. pendula* families in six treatments with or without fertilization and three levels of shading 0, 30, or 60%. Rousi et al. 1993.

**Figure 4-6.** The palatability index in two *B. pendula* families in six treatments with fertilization (green surface) or without fertilization (white surface) and three levels of shading 0, 30, or 60%. Red columns refer to a Finnish x Siberian family and blue columns refer to a Finnish x Finnish family. Rousi et al. 1993.

**Figure 4-7.** Mean removed bark area of four *B. pendula* families tested in 2-choice experiments with vole feeding. The hybrid family is a cross between one Finnish and one Siberian clone. Rousi et al. 1993.
4.2.2 Mammals and insects

A detailed study of genetic variation in insect and mammal herbivory among *B. pendula* clones and some seed offspring were reported by Rouxi et al. (1997). Very sophisticated experimental techniques were used both in field and under controlled condition. It is beyond the scope of this summary to explain all details of the experimental design. It suffices to state that the design was scientifically solid. A palatability index, percentage of loss of weight of the stuff offered to the herbivore was used as measure of preference by the herbivore. There was a large variation in the palatability index for hare browsing and an agreement between the two years when the study was carried out. Two clones of *B. pubescens* were among the three clones with highest palatability index. Vole damage was recorded in three field trials with different levels of average damage, 10, 25, and 50% of the seedlings damaged. Only in the field trial with 25% damage, significant differences among clones were noted. Similarly, pooled data from all three trials resulted in significant clonal differences. In the so called two-choise experiments in cages the basal parts of two dormant plants of two clones were offered to one vole in each cage. One clone was used as a standard in these tests comprising 15 clones.

No significant differences among clones were noted in these tests. Weevil feeding experiments revealed significant differences among clones in palatability against *Phyllobius argentatus*, *Ph. maculicornis*, *Ph. oblongus*, *Ph. piri*, and *Polydrosus mollis*.

One objective of this study was to estimate if the palatability of clones ranked in the same way for the different types of herbivore. The ranking of the eight clones is illustrated in Fig. 4-9. There is some agreement of ranking with two strong deviations, clones 1 and 4. It is evident that clones 5 and 6 were attractive to all four herbivores.

In conclusion there are clonal differences in palatability but a complete generality of palatability does not exist.

The above study was complemented with recordings of attacks by various types of herbivores, browsers, defoliators, phloem feeders, a leaf miner, and a cambium miner (Tikkkanen et al. 2003). The occurrence of nine insect species was recorded in the eight clones in a field trial. There were significant clone differences in susceptibility against all insect species. No general tolerance against all insects was observed. No strong relationships between tree growth and insect attacks, $r < 0.50$, were found.

Twenty open-pollinated families were exposed to insects and voles in a controlled way by Puseni et al. (2002). The occurrence of resin droplets after cutting of plants was recorded.

A significant difference among OP-families was noted for vole attacks. However, the proportion of attacked plants was low and varied in the range 4 – 6%. None of the interaction effects was significant. Voles preferred tall plants and they clipped them at an approximate height of 10 cm. The effects of OP-family and fertilization were significant for plant height and resin droplet density while all interaction effects were non-significant. As expected fertilization caused a better growth of the seedlings but a lower resin droplet density in most families (Fig. 4-10).
There was a significant relationship between insect and vole preference but the degree of explanation by the relationship was less than 25%. Similarly, there was a significant correlation between seedling height and droplet density but the degree of explanation was only 25%. Plants previously attacked by insects were not preferentially selected by the voles. It was speculated that vole attacks on tall plants might lead to reduction of growth of the coming generation. However, the low proportion of attacked seedlings and recovery of some of the attacked seedling would probably not lead to any large change of the genetic setup of the population.

Prittinen et al. (2003b) studied the effect of insect or vole feeding on plant survival and plant height in open-pollinated families of *B. pendula* from a stand at latitude 61.78°N in Finland. Half of the material was fertilized and the other half was unfertilized. The insect-free treatment was accomplished by spraying with pyrethrin and the effect of vole feeding was done by keeping one vole within each fenced plot. Significance for mortality was noted for the below effects:

- **OP-family**
- **OP-family x insect**
- **Insect**
- **Fertilization**

All other pure effects and interaction effects were non-significant. One of the OP-families deviated somewhat from the rest of families with approximately 48% mortality (Fig. 4-11). The mortality was approximately 70% higher in the fertilized than in the unfertilized treatment. Part of the mortality in the fertilized plots was attributed to self-thinning among the vigorously growing seedlings. The mortality was twice as large in the insect exposed treatment as in the pyrethrin sprayed plots. Noteworthy was the absence of any effect on mortality of exposure to voles. One reason for this was that the plants recovered from vole damage by resprouting. Vole damage was mainly during the rest period while insect feeding took place during the growth period. There was a fairly strong relationship between mortality in the insect removal and insect exposed treatments.

There were negative relationships between plant height and mortality, independent of treatment as is illustrated in Fig. 4-12. The strongest relationships were noted for the fertilization treatments. The authors stated that there was a trade-off between insect tolerance and plant growth such that the proportion of insect tolerance decreased after insect herbivory.

**Figure 4-11.** The mortality in open-pollinated families of *B. pendula* in a trial in southern Finland at latitude 61.78°N. Prittinen et al. 2003b.

**Figure 4-12.** The correlation coefficients between plant mortality and plant height in open-pollinated families of *B. pendula* in a trial in southern Finland at latitude 61.78°N including four treatments. Prittinen et al. 2003b.

**Figure 4-13.** Total dry matter at age 3 of 15 *B. pendula* clones in four treatments; control, fertilization, defoliation, and fertilization + defoliation. Red label means that the clone originates from a cross. Mutikainen et al. 2000.
4.2.3 Insects

Mutikainen et al. (2000) studied tree growth and feeding of the autumn moth, *Epirrita autumnata*, in a clone trial of *B. pendula* with 15 clones at ages 2-3. Artificial defoliation was carried out during end of June at ages 2 and 3. Earlier studies had indicated that defoliation may increase foliar resistance against herbivores. The bioassays were carried out on two undamaged leaves of each tree. One treatment with fertilization was also carried out. As many as 34 secondary chemical compounds were analysed in 10 of the 15 clones and their concentrations were related to plant growth and *Epirrita autumnata* growth rate since some of the compounds may increase tolerance against this moth.

Strongly significant clone effects were noted for height and total dry weight both years. All two-way interactions clone x treatment as well as the three-way interaction clone x defoliation x fertilization were non-significant. The strong treatment effect of fertilization is seen from Fig. 4-13. The clone effect was significant for relative growth rate of *E. autumnata* at age 3 but not at age 2 (Fig. 4-14). The clone variance component for age 3 assessments amounted to 13%. None of the interaction effects were significant. The clone effects for resin droplets and pupal mass were significant. The interactions were significant for resin droplets but not for pupal mass. Although fertilization was regarded as a fixed effect an estimate of a variance component of 94% was presented.

Several relationships between various compounds and relative growth rate or pupal mass of *Epirrita autumnata* were estimated using clone mean values. (These relationships were erroneously referred to as genetic correlations.) Of a total number of 72 relationships only five were significant. One of the strongest relationships is shown in Fig. 4-15. It should be noted that the separate relationships for fertilized and non-fertilized materials resulted in weaker relationships, $R^2 = 0.37$ and 0.73, respectively. The authors concluded that these and previous results suggest that a single hypothesis on environmental effects is not comprehensive enough to explain the phenotypic variation in plant resistance traits even within a single species. It is unlikely that one specific chemical compound should bring total resistance against insects in such a long-generation time species as *B. pendula*. The many generations of insects during one birch generation would most likely favour insects that can overcome the toxicity of one particular compound and thus become virulent.

![Figure 4-14. Relative growth rate of *Epirrita autumnata* in four treatments; control, fertilization, defoliation, and fertilization + defoliation, of 15 *B. pendula* clones. The red labels indicate full-sib families. Leaves from age 3 were used. Mutikainen et al. 2000.](image1)

![Figure 4-15. The relationship between clonal means for condensed tannins and pupal mass in 10 *B. pendula* clones. Blue refers to fertilized treatment and red to non-fertilized treatment. Units were read from Fig. 7B in Mutikainen et al. 2000.](image2)
The same experiment and plant material was used in a study (Mutikainen et al. 2002) of the cost for herbivore tolerance/resistance. The cost was estimated by relationships between clonal mean values for growth traits on one hand and insect resistance, vole and hare resistance, or chemical compounds. Three out 27 relationships were significant in the non-fertilized treatment while 5 out of 27 relationships were significant for the fertilized treatment.

The negative relationship between relative height growth in the fertilized treatment and DHPPG (3,4' dihydroxypropriophenone 3-glucoside) was strongest of all relationships, \( r = -0.78 \) (Fig. 4-16). Contrary to this, the same relationship in the non-fertilized treatment was estimated at 0.22. The lack of strong relationships in many cases was attributed to the young material in the present study. It was speculated that juvenile plants with high resistance may lose in biomass production over time and strong relationships may occur at a higher age.

There was no significant relationship between resistance against hares or voles and insect resistance. This was attributed to difference in feeding of these two types of herbivores. The former feed from leaves while the latter feed from shoots.

Genetic variation in insect damage and growth in 20 open-pollinated \( B. \) pendula families was studied in a Finnish field trial at latitude 61.68°N by Prittinen et al. (2003a). Four treatments were used:

- No fertilization + exposure to insects
- No fertilization + removal of insects
- Fertilization + exposure to insects
- Fertilization + removal of insects

The removal of insects was done by spraying the seedlings with a pyrethrine insecticide once per week. The insect damage was scored in classes as average damage per leaf and proportion of leaves damaged. The scores were multiplied to obtain a damage index at the two occasions of damage recording in July and September.

At both recordings there was a strongly significant difference for insect damage among the open-pollinated families. The damage index was approximately three times higher in September than in July. The interaction OP-family x fertilization was non-significant. There was a significant difference in height among the OP-families. Fertilization resulted in a significantly increased growth in spite of the low difference in mean values, 25.4 and 27.6 cm, respectively. As regards growth it was stated that the families did not respond differently to insect treatment. However, this was contradicted by the significant plant height x insect treatment interaction. There was a significant and positive relationship between insect damage and growth in the fertilized treatments. However, only 35% of the variation was attributed to this relationship.

It was concluded that trees in natural populations differ in their tolerance against herbivory. The findings were discussed in an evolution context and it was stated that small differences in herbivory may have profound effects on the genetic constitution.

Prittinen et al. (2006) presented another study based on the same material (my assumption). One specific objective of this investigation was to estimate the genetic effect of herbivory. This was done by comparing random and selective mortality by use of the so called Simpson's reciprocal index, SRI. This index was calculated for the entire population and for the canopy population. The latter was defined as the 25% tallest trees in each subplot.

The SRI decreased from its initial value of 20 in all treatments and the drop was especially pronounced in the combination insect exposure + fertilization. It was found that SRI was increased significantly in the canopy layer in the vole herbivory treatment while insect exposure and fertilization did not affect SRI. It was pointed out that the insect herbivory in this investigation only caused minor damage during the two years the study lasted. It was stressed that herbivory fluctuate over years, which thus
indirectly might be a source for keeping high genetic variability in birch populations.

The miner *Phytobia betulae* causes brown discoloration, which reduces the price of the wood. Occurrence of this miner was recorded at ages 1-6 in three *B. pendula* field trials in southern Finland; two at latitude 61.80° and the third at 60.35°N (Ylioja et al. 2000). A convenient way to assess the infestation of this miner is to count the pith flecks at the base of the trunk. One objective of the investigation was to relate the infestation to the growth of the trees.

A total number of 370 trees were examined for infestation of *Phytobia betulae*, of them only 27 trees were free from infestation. One example of the relationship between growth and infestation is given in Fig. 4-17 for trial II. As seen from this figure there was a strong relationship between tree height and number of pith flecks. Therefore, adjustments for growth differences were done in the analysis of possible clone differences for this trait. Figure 4-18 reveals that the adjusted number of pith flecks is much higher in the southern trial (III) than in the two other trials. A separate ANOVA in the three trials revealed significant clone effects for number of flecks. There was a fairly parallel development in the individual clones. In spite of this, the clone x trial interaction was significant. Figure 4-19 reveals that the tree height was fairly similar in Trials I and II while the growth was substantially better in Trial III. It is likely that the insect females select large shoots for their oviposition. Since large shoots are to be found on good growing trees the relationship between growth and infestation is easily understood. This relationship is a great constraint to breeding for good growth and infestation free trees. The authors stated that only eight clones were included in their study. Therefore, it cannot be ruled out that genotypes with good growth and absence of *Phytobia betulae* may exist.

Figure 4-17. The relationship between tree height and number of pith flecks caused by the tree miner *Phytobia betulae* in eight *B. pendula* clones grown in trial II at latitude 61.80°N in south-eastern Finland. Ylioja et al. 2000.

Figure 4-18. The number of pith flecks (adjusted for differences in basal area) caused by *Phytobia betulae* in eight *B. pendula* clones growing in three Finnish clone trials; I and II at latitude 61.80° and III at 60.35°N in Finland. Ylioja et al. 2000.

Figure 4-19. Tree height in eight *B. pendula* clones growing in three Finnish clone trials; I and II at latitude 61.80° and III at 60.35°N in Finland. Ylioja et al. 2000.

to assess the infestation of this miner is to count the pith flecks at the base of the trunk. One objective of the investigation was to relate the infestation to the growth of the trees. A total number of 370 trees were examined for infestation of *Phytobia betulae*, of them only 27 trees were free from infestation. One example of the relationship between growth and infestation is given in Fig. 4-17 for trial II. As seen from this figure there was a strong relationship between tree height and number of pith flecks. Therefore, adjustments for growth differences were done in the analysis of possible clone differences for this trait. Figure 4-18 reveals that the adjusted number of pith flecks is much higher in the southern trial (III) than in the two other trials. A separate ANOVA in the three trials revealed significant clone effects for number of flecks. There was a fairly parallel development in the individual clones. In spite of this, the clone x trial interaction was significant. Figure 4-19 reveals that the tree height was fairly similar in Trials I and II while the growth was substantially better in Trial III. It is likely that the insect females select large shoots for their oviposition. Since large shoots are to be found on good growing trees the relationship between growth and infestation is easily understood. This relationship is a great constraint to breeding for good growth and infestation free trees. The authors stated that only eight clones were included in their study. Therefore, it cannot be ruled out that genotypes with good growth and absence of *Phytobia betulae* may exist.
Twenty-two *B. pendula* trees were selected in a stand at latitude 61.80°N in southern Finland and vegetatively propagated for a study of tolerance against herbivore insects (Silfver et al. 2009). Clone trials were established at two localities in southern Finland, latitudes 61.60 and 61.78°N. Half of the plants were treated with a pyrethrin insecticide. This treatment was coined insect removal and the other half of the plants was untreated and designated as insect exposure treatment. The tolerance against insects was estimated by counting the number of undamaged leaves of each plant during 30 seconds. Observations were carried out during two years, 2003 and 2004. There was a significant genotypic difference in insect tolerance. There were also significant effects of year. In Figs. 4-20 and 4-21 I have plotted the insect resistance between years at each of the test localities. There were poor agreements between years in tolerance at both test localities. In spite of this it was reported that the genotype x year interaction was non-significant (p = 0.66). Nor was there any significant genotype x test locality interaction (p = 0.80) or three-way interaction; genotype x year x test locality (p = 0.64).

The authors reported a strong relationship between growth responses to insect feeding. However, for the test locality at 61.60°N I tried to verify this by plotting height increment in both treatments, insect exposure and insect removal, against relative insect tolerance (Fig. 4-22). As seen from this figure the fit to the two curves is extremely poor. Also at the other test locality the corresponding relationships were weak. The ANCOVA run indicated no significant genetic difference for height while the effects of test locality, genotype x test locality, and treatment were significant. Based on the graphic presentations in the paper it is surprising that the plant height increment did not differ significantly among the 22 clones.

In conclusion there was a significant genetic difference in insect tolerance. This should allow the population to respond to increased insect pressure. Whether or not the difference in insect tolerance influenced the tree growth is unclear.

Herbivory-related traits were studied in 30 randomly selected parents, their open-pollinated families and full-sib families of *Betula pubescens* from latitude 69.75°N by Haviola et al. (2006). The seedlings were cultivated at latitude 61.60°N in Finland. Twelve phenolics, five carbohydrates, and 16 protein-bound amino acids were included in the study. Besides the compounds, the heritabilities of seed weight, germination, and seedling height were estimated.

Twenty percent of the individual and pooled phenolic compounds had a heritability significantly different from zero. Only one of the carbohydrates and one of the amino acids had significant estimates of the heritability. One disturbing factor was the difference in the estimates based on female and male parents. It was stated that the estimates...
of heritabilities based on parent-offspring relationships, full-sib, or half-sib families gave equal results. Each family was represented by five seedlings at most, which means that the precision of the estimates is low. This might result in fewer significant effects of compounds than there really are. I assume that a study with a larger number of seedlings per family would have been too laborious to carry out. It was concluded that *B. pubescens* would be able to respond to changing selection pressure as regards the phenolic compounds. Based on the limited percentage of significant differences this conclusion might be somewhat too brave.

A factorial experiment with fertilization, partial defoliation, and a combination of these two treatments were carried out by Keinänen et al. (1999) to study:

- if chemical responses of birch leaves vary among phenological stages
- the chemical composition of remaining leaves after defoliation
- and the response of chemical compounds to fertilization
- possible genetic differences of the points above

Significant clone effects were noted for many phenolic compounds (number of significant interactions among all 2-way and 3-way interactions 4/21), cinnamoylquinic acids(1/12), flavonol glycosides (10/48), and flavone aglycones (11/21). Except for the last group of compounds the interactions clone x treatment were in many cases of less importance than the clone effect. No significant 3-way interactions were noted for any of the compound groups. Most of the compounds studied are believed to be of significance for defence against insect herbivory.

### 4.2.4 Insects + fungi

Ten *B. pendula* clones were inoculated by *Melampsoridium betulinum* to test the effect on the herbivore *Epirrita autumnata* (Saikonen et al. 2001). Relative growth rate and pupal mass was assessed.

The inoculation caused an increase of the infection level from a few per cent to 6-25% in the inoculated trees. There was no pronounced difference between rust inoculated and non-inoculated as regards *E. autumnata* relative growth rate or pupal mass (cf Fig. 4-23). There were significant differences among clones for both traits.

A study of the relationship between six insect species and three fungus genera was reported by Ahlholm et al. (2002). Open-pollinated progenies from *B. pubescens* trees in northernmost Fennoscandia were planted at two test localities, 90 and 270 masl, at latitude 69.75°N in Finland. The abundance of the two most common endophytic genera, *Fusicladium* and *Melanconium*, was recorded after growing them from surface-sterilized leaf discs to a growth medium. The study covered three growth periods of mature trees. The relative growth rate (RGR) of the herbivores was estimated as:

$$\text{RGR} = \ln(\text{biom}_2) - \ln(\text{biom}_1),$$

in which biom and biom are the initial and final biomass of larva, respectively. For one of the insects *Epirrita autumnata* the pupal biomass was also estimated. For *Dendroaspis betulae* density of leaf rolls was recorded. Recordings of insect performance were not carried out for all six insect species every year.

A total number of 74 family mean correlations (erroneously referred to as genetic correlations) between herbivore traits and occurrence of fungi were estimated. Separate correlations for the high elevation and the low elevation trials and for each year were estimated. Six of the seven significant correlations occurred with fungus occurrence in the high elevation trial. Four of the significant correlations were negative. The degree of explanation of the significant correlations varied between 46 and 72%.

In conclusion this investigation does not give much support for a general resistance against different kinds of herbivores or fungi. Rather, different traits in the host trees are responsible for host tolerance against other organisms, as was stated by the authors.
4.2.4 Fungal resistance


Inoculation of leaf discs from six *B. pendula* and two *B. pubescens* clones with urediniospores of *Melampsoridium betulinum* was carried out by Poteri (1992). The spores were obtained from trees of the two species in stands. The discs were examined for number of sporulating pustules 5-12 days after inoculation. Three experiments with each type of inoculum were carried out. The frequency of sporulating pustules varied much between the experiments. To standardise a comparison between the experiments I have calculated the deviation from the experimental mean of the eight clones included in this study. As seen from Fig. 4-24 the two *B. pubescens* clones were affected to a limited extent by the isolate from *B. pendula*. The response to inoculation was slower in *B. pubescens* than in *B. pendula*. One of the *B. pendula* clones had values above the mean in all three experiments. The pattern of response to the *B. pubescens* isolate was different. There was no longer superiority of the two *B. pubescens* clones, although one of the *B. pubescens* clones performed rather well. The strong sensitivity of one of the *B. pendula* clones in the first experiment against both inoculates is puzzling. The illustrations in Figs 4-24 and 4-25 suggest that there was a strong clone x experiment interaction. This interaction was not estimated or discussed. The seemingly large clone x experiment interaction for many clones complicates the interpretation of the results obtained.

Poteri and Rousi (1996) studied the variation among seven *B. pendula* clones at age 2 in resistance against *Melampsoridium betulinum* in a leaf-disc bioassay, a field experiment, and a fertilization experiment with three treatments in pots. The two highest fertilization levels were 5 and 10 times higher than the lowest level. The number of postules per disc nine days after inoculation showed a large difference among the clones (Fig 4-26). The score of diseased area after inoculation in field varied considerably too. In the fertilization experiment both clone and clone x treatment interaction were strongly significant for clonal
The interaction is clearly seen in Fig. 4-27, which also shows that the clonal disease index did not differ much at the two highest levels of fertilization. There was a fairly strong relationship between the leaf assay results and the results in field, \( r = 0.71 \). There were strong relationships between leaf assay data and results from the two highest levels of fertilization.

Poteri et al. (1997) used two isolates of *Melampsoridium betulinum*, one from *B. pendula* and the other from *B. pubescens*, for inoculation of *B. pendula* and two other non-domestic *Betula* species. Leaf disc assays with material collected indoors and outdoors were started on August 19. The number of postules per disc was recorded 8-12 days after the inoculation. Significant differences among species, between environments (indoors outdoors), and origin of the inoculum were noted. The resistance of the *P. pubescens* entry against *B. pendula* *Melampsoridium betulinum* inoculum is noteworthy. The low susceptibility of the *B. resinifera* x *B. pendula* hybrid is also worth mentioning.

The same trials were included in a study of occurrence of endophytic fungi and water content of the leaves (Elamo et al. 1999). The recordings were carried out during three years and were mainly focused on *Fusicladum* and *Melanconium* sp.

I have selected a representative sample of the results of the assessments during 1994-1996 in the two trials in Fig. 4-28. There was a significant OP-family variation in number of *Fusicladum* colonies per cm\(^2\) but no significant difference for family x trial or family x year interaction in spite of the differences shown in Fig. 4-28. All family mean correlations between observations different years within each trial were non-significant; all \( r \)-estimates were < 0.60.

None of the effects, family, family x year, family x trial, were significant for *Melanconium* infection. Five of the six family mean correlations between observations different years were strongly significant for *Melanconium* infection. The relationships between infection and leaf water content and *Melanconium* was strongly significant for years 1994 and 1995 in the Low-altitude trial. However, the low incidence of *Melanconium* infection in these trials renders any estimates of genetic parameters low reliability.
Establishment of two field trials at latitude 69.75°N at 90°.

Strong relationship between the percentage of spontaneous infection or artificial inoculations with Melampsoridium betulinum in two field trials at latitude 69.75°N and altitudes 90 and 270 masl. High-A stands for the B. pubescens trial at 270 masl and Low-A stands for the trial at 90 masl. The artificial inoculations were carried out on material collected in the two trials. The spontaneous infections were studied at ages 20-22. Elamo et al. 2000.

Open-pollinated families from ten B. pubescens trees originating from latitudes 69.75-69.94°N were used for establishment of two field trials at latitude 69.75°N at 90 (Low-A) and 270 masl (High-A), respectively (Elamo et al. 2000). Naturally occurring birch rust was assessed at the end of the growth period when maximum infection was expected. Inoculation of leaf discs were carried out four weeks earlier with urediniospores of Melampsorium betulinum.

The two northernmost OP-families, which originated from the highest altitudes, had highest incidence of rust. The coefficients of additive variance (Fig. 4-29) were fairly high, 13-32. These estimates were based on whole tree data. The corresponding heritabilities after spontaneous infection in field varied in the range 0.44-1.0. The standard errors were large but the reason for this was not discussed. Instead the reason for the low heritabilities (!) in the present study was considered. Considering the low percentage of infection at age 21 in the High-A trial the CVs was unexpectedly high. Another unexpected observation was the similar size of the CVs for artificial inoculations of the two materials. The range of mean values was much larger for the High-A material than from the Low-A material, approximately 2.9 uredinia per cm² versus 1.3 in the Low-A material. This would normally have led to a higher CVs for the High-A material. It would have been useful to estimate the family by trial interaction. One possible way to get information on this is to study the relationships between the results from the two trials and the inoculation experiment. The results from three of the experiments were plotted against the percentage of infected trees in High-A trial. Mean values from the three years of observation were used for the spontaneous infections in Fig. 4-30. As seen from this figure there was a fairly strong relationship between the percentage of spontaneous

Figure 4-29. The coefficient of additive variance for spontaneous infection or artificial inoculations with Melampsoridium betulinum in two field trials at latitude 69.75°N and altitudes 90 and 270 masl. High-A stands for the B. pubescens trial at 270 masl and Low-A stands for the trial at 90 masl. The artificial inoculations were carried out on material collected in the two trials. The spontaneous infections were studied at ages 20-22. Elamo et al. 2000.

Figure 4-30. The relationships between spontaneous infection % at a High-A trial at 290 masl, and spontaneous infection % in a Low-A trial at 90 masl; filled red squares

No of uredinia/cm² after artificial inoculation of leaves from the Low-A trial; open red squares

No of uredinia/cm² after artificial inoculation of leaves from the High-A trial; blue squares. Elamo et al. 2000.

Artificial inoculation with Melampsoridium betulinum of ten B. pendula clones from the latitudinal range 60.75-63.30°N in Finland resulted in strongly significant differences among the clones (Poteri et al. 2001). The approximate range of % coverage of uredinia was 5-25. There was no relationship between natural infection and artificial inoculation. It should be noted that four of the ten clones had spontaneous infections below 1%, which erroneously may contribute to significant clone x treatment interaction. The relationship between leaf age and
infection was tested by analyses of ten consecutive leaves from the branch apex. With one exception there were negative relationships both after inoculation and spontaneous infection. Seven of the Spearman rank correlations were significant. Strangely enough, two of the clones with spontaneous infection percentages <1% showed significant relationships between leaf age and spontaneous infection percentage. Generally, the oldest and youngest leaves had lower percentages of infection than leaves of intermediary age.

4.3 Transgenes

Studies in other species have shown that chitinase genes confer increased tolerance against pathogens. Presence of chitinase is assumed to break down chitin in fungal cell walls.

The purpose of an investigation by Pappinen et al. (2002) was to test if this holds true for the Pyrenopizizia betulicola fungus attacking B. pendula. The sugar beet chitinase IV gene with the enhanced CaMV 35S promoter was introduced into explants of B. pendula. Thirteen randomly selected lines among the 52 lines obtained were selected for inoculation with Pyrenopizizia betulicola to study if the introduced gene increases resistance to this fungus. All leaves of the plants were assessed with respect to disease symptoms in three classes: 0 = healthy leaf, 1 = 1-30% of the leaf area affected, 2 = > 30% of leaf area affected. A disease index was used in which the most affected class was given a higher weight than class 1. The transgenic plants grew normally without any morphological abnormalities or growth retardation. The number of copies of the transferred gene varied between 2 and 4 among the lines. Four groups of transgenic lines were identified following the analysis of total RNA from leaves. There was a continuous variation in disease index from 0.015 to 0.38. A significant relationship between chitinase mRNA and disease index was noted. A pairwise comparison of the disease indices and chitinase mRNA levels revealed that there were no differences at low chitinase mRNA levels between the control plants and transgenic plants. Contrary to this, the differences against the control were strongly significant for lines with high chitinase mRNA levels. The improved tolerance indicates that chitinase is produced and thus not only an increased level of chitinase mRNA. In conclusion incorporation of the sugar beet chitinase IV gene confers increased tolerance against P. betulicola in some transferred lines but with a large variation in tolerance in different transferred lines. This is disturbing for any application if breeding for tolerance against P. betulicola would be carried out.

The sugar beet chitinase IV gene was transferred by Agrobacterium to B. pendula in tissue culture (Pasonen et al. 2004). Fifteen lines were selected and transferred to greenhouse after in vitro propagation. After 18 months the plants were planted in a field trial close to Helsinki.

Three leaves were examined for leaf spot disease caused by Pyrenopizizia betulicola and birch rust disease caused by Melampsoridium betulinum. The traits used are shown in Table 4-2. The lines that were transgenic in greenhouse remained transgenic after three years in field.

Leaf spot disease. At age 2 in field, 14 of the lines differed significantly from the control for general disease score but without any improvement of the tolerance against this disease. As seen from Table 4-2 there was no consistency as regards the differences among the cell lines between the two ages in field. Contrary to expectation some of the transgenic lines were less tolerant against the leaf spot disease. The expression of the chitinase gene varied and the lines were classified as high, intermediate, and low. At age 3 in field, the number of disease spots varied significantly among the three classes of expression. At age 4 in field, the percentage area of disease spots and general disease score varied significantly among the groups.

Birch rust disease. The attacks of birch rust were more severe during the first years in field than during the last year. Also for this trait there was not full consistency between years. At ages 3 and 4 in field there was a large variation among the lines for number of rust postules, 5-58. In spite of this, the variation was non-significant. Four of the lines had significantly fewer postules than the control at age 3 while one line differed significantly from the control at age 4. At age 3 the latter line had the third highest number of postules. Generally, the intermediate class of expression had the lowest values for all three traits at all three ages while the low expression lines had the severest attacks. Five of the nine cases were significant.

One problem to sort out is the inconsistency between years for the various parameters. It is evident that the weather conditions vary between years. This is one possible explanation for the results obtained. It is certainly hard to evaluate the impact of many of the environmental factors that may influence the susceptibility against fungi.
Eight of these transgenic \textit{B. pendula} lines were used in a study of the impact of the chitinase gene on the ectomycorrhiza fungus \textit{Paxillus involutus} on its ability to colonize transformed plants (Pasonen et al. 2005). Twelve transgenic seedlings from each line were inoculated with \textit{P. involutus} and six seedlings were used as controls. The seedlings were grown for four weeks under controlled conditions; 6h night and 22/15°C. Number of root tips, mycorrhiza, shoot weight, and root weights were assessed. The expression of the chitinase gene in root tips was determined. Two of the eight lines did not show any expression of the chitinase gene and the six other lines were classified with respect to expression of the gene as low, intermediate, or high.

The degree of expression did not cause any difference in the morphological structure of mycorrhiza. Nor was there any difference between control and transgenic plants for this trait. The results from the statistical evaluation of differences among lines are listed below (2nd column) together with differences between lines of different expression (3rd column):

<table>
<thead>
<tr>
<th>% mycorrhizal root tips</th>
<th>ns</th>
<th>ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of root tips</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Root fresh weight</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Shoot fresh weight</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>Root/shoot ratio</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Total fresh weight</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

As seen from this compilation only half of the traits showed significant differences. It is worth mentioning that the transgenic lines had lower shoot weights than the control. The intermediate and high expression lines had a lower percentage of root tips with mycorrhiza but the difference was not significant.

In conclusion, the results do not indicate that the chitinase gene injuriously influence the ability for the normal mycorrhiza fungus \textit{P. involutus} to colonize transgenic seedlings.

Leaf litter decomposition from eight chitinase lines of \textit{B. pendula} was reported by Vauramo et al. (2006). The transgenic lines were grown in greenhouse for 18 months and then transferred to a field trial. At age 3 in field 10 or 15 leaves per tree were collected in July before any senescence could be seen. The 10 leaf samples were used for determination of the C/N ratio while 15 leaves were used for determination of the soil flora and fauna. This procedure was repeated in September when yellowing had started. Equal parts of the leaves were allowed to decay in field for 8 or 11 months in litter bags. Nematodes were counted alive under binocular microscope within two days after collection of the litter bags in the field trial. The identification of nematodes to trophic groups took place after fixing in ethanol. Determination of ergosterol was carried out to estimate biomass of fungi. A substrate induced respiration assay was used on the July sample of green leaves with 11 months decomposition to estimate the microbial response to the addition of glucose. Before adding glucose the basal respiration activity was measured during 36 hours.

The results from the statistical evaluation of the variation among all entries are compiled below:

<table>
<thead>
<tr>
<th></th>
<th>Leaf mass loss</th>
<th>C/N ratio</th>
<th>Ergosterol content</th>
<th>Basal respiration</th>
<th>Substrate induced respiration</th>
<th>Number of nematodes, 8 or 11 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ns</td>
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</table>

As seen from this compilation the presence of the chitinase gene caused a significant difference among the entries only once. A pairwise comparison between chitinase lines and their control as regards C/N ratio did not reveal any significant difference for this comparison. The results suggest that the decomposition of leaf litter was marginally affected by the presence of the chitinase gene.

Fifteen sugar beet chitinase IV transgenic lines and their corresponding non-transgenic line were planted in a trial in southern Finland together with seven wild-type \textit{B. pendula} clones and monitored with respect to insect occurrence and damage (Vihervuori et al. 2008). During the fourth season monitoring took place early, late, and during the middle of the season.

The expression of the chitinase transformed lines varied. Several insect orders were observed in the trial but the density was totally dominated by aphids. No significant differences were noted among the chitinase expression lines as regards the number of insect orders observed. Similarly, there was no difference between transformed lines and the control group. The insect density was lowest during the late season recording. Significant differences among the transgenic lines were noted for all three observation dates. Generally, the density was higher in transgenic lines than in the control clones. The damage of individual cell lines is illustrated in Fig. 4-31, which shows variation in percentage damage but no relationship with degree of chitinase expression. It was concluded that \textit{no clear harmful effects of the transgenic chitinase gene on insect population could be detected}.

The effect of the sugar beet chitinase IV gene on the intensity of ectomycorrhizal colonization and fungal species composition was studied in 15 transgenic lines and control lines (Pasonen et al. 2009). The 15 transgenic lines and 8 non-transgenic clones were cultivated for 18 months in greenhouse and planted in a field trial at latitude 60.22°N in Finland. The percentage of ectomycorrhizal root tips was assessed and identified with respect to ectomycorrhiza species after three years in field.

The expression of the chitinase IV varied much among the 15 lines. The percentage of colonization approached 100 both in the transgenic and control lines. In spite of this, seven lines had a significantly lower number of my-
corrhiza root tips than the control. There was a significant difference as regards the fungus species composition between the wild-type and the transgenic lines. Among the control clones there was no detectable difference. The cluster analysis revealed two groups of plants as regards fungus composition, the transgenic lines and their control formed one group while the other seven control clones formed another group. Some of the fungi were more frequent in transgenic lines than in the controls. It was disclosed that the genetic structure was more important for the fungus composition than being transgenic or not.

ethylene may have a role in the development of resistance against herbivores. The dominant \textit{ert1-1} allele prevents the binding of ethylene to the mutated receptor leading to ethylene insensitivity. The effect of one or two copies of the \textit{ert1-1} mutant allele on \textit{Epirrita autumnata} feeding on \textit{B. pendula} leaves was studied by Alatalo et al. (2008). Two clones were used; labelled J and V, with different phenology. The non-transformed J-clone has a later bud flushing than the non-transformed V-clone. Three leaves were offered to larvae to feed on for three days. Leaf area consumed, larval biomass, and pupal biomass were assessed. From Fig. 4-32 it is seen that the larval biomass and leaf area fed by the larvae in the J clone declined with increasing number of the \textit{ert1-1} allele. The \textit{ert1-1} allele in the V-clone did not change feeding or larval biomass much. It is known that phenology and water content of leaves are good predictors of insect attacks. Therefore, a separate ANOVA was run with phenology and water content as covariates in the analysis. The picture becomes totally different for the J-clone with an increase of larval biomass and leaf area consumed with increasing number of \textit{ert1-1} alleles (Fig. 4-33). The clone effect was significant for both traits and both ways of running the ANOVAs. However, the copy number of the transgene was significant only for final larval mass. Similarly, the interaction clone x copy number for larval biomass was significant in both ways of running the ANOVA. Survival of the larvae of \textit{Epirrita autumnata} by the eclosion date differed significantly dependent on the copy number of the transgene while there was no significant clone effect on survival.

Figure 4-32. The larval biomass in mg of \textit{Epirrita autumnata} and leaf area (LA) x 10 cm$^2$ consumed by \textit{E. autumnata} larvae on leaves of two \textit{B.pendula} clones and their transformed \textit{ert1-1} mutant from \textit{Arabidopsis thaliana}. Index 1 refers to one \textit{ert1-1} allele and 2 refers to two \textit{ert1-1} alleles. Alatalo et al. 2008.

Figure 4-33. The same as Fig Ala-1 but the estimates are obtained after ANOVA with water content and phenology as covariates. Alatalo et al. 2008.
There was a marked effect of the transgene copy number on the number of long shoots in the J-clone from approximately 12 to 45 long shoots. The corresponding increase in the V-clone was less pronounced. Contrary to the long shoots, there was limited effect of transgene copy number on the frequency of short shoots. Also phenology in the J-clone was affected with the earliest flushing of plants with two copies of the \textit{ert1-1} allele. It was concluded that the genetic constitution seems to be influential on the effect of the mutant \textit{ert1-1} allele both on birch growth performance and the possibilities for \textit{Epirrita autumnata} to feed on birch leaves. Pleiotropy and the location of the \textit{ert1-1} allele in the genome were suggested as explanations for the different response of the two clones.

\subsection*{4.4 Phenotypic variation}

Helander et al. (1998) recorded the occurrence of \textit{Melampsoridium betulinum} on \textit{Betula pubescens} ssp. \textit{czerepanovii} in northern Finland, latitude 69.75°N during four years. Fifteen groups of three trees were selected within an area of 2,500 m². Each group had one tree with low occurrence of the fungus and one tree with high incidence of rust. Finally, the third tree should take an intermediate position. There were no shifts in ranking over years as regards rust infection in the three incidence classes. This suggests a genetic variation in susceptibility against \textit{M. betulinum}.

The annual occurrence of larval tunnels of \textit{Phytobia betulae} was recorded in 25 and 30 trees in two stands with mixtures of \textit{B. pendula} and \textit{B. pubescens} in southern Finland at latitude 61.80°N (Ylioja et al. 1999). The two stands were 47 and 65 years old, which gave opportunities to follow the development of this herbivore over long time series.

In both stands and species there was a relatively flat maximum of \textit{Phytobia} per tree at ages 20-30. As a consequence of this, stand age explained 58 and 32% of the variation in \textit{Phytobia} abundance. The variation among trees was strongly significant in both species. Within years there was a strong relationship between tree growth and occurrence of \textit{Phytobia}. Similarly, a large population of \textit{Phytobia} one year resulted in a reduction of the \textit{Phytobia} population next year.

\section*{4.5 Conclusions}

Thanks to a broken fence of a provenance trial, variation among populations in moose feeding could be studied. There was a clear latitudinal effect on the feeding with the southern populations most affected by moose damage. The earlier growth cessation of the northern populations might be the reason for less moose feeding and as consequence of this a variation among populations. There was a strong relationship between tree height before the fence was broken and moose feeding damage.

Significant differences among genetic entries were noted for vole, hare and moose damage. Growth traits were in most cases not significantly correlated with the various types of moose damage. Resin droplets on birch plants and several chemical compounds have been assumed to play a role in birch plant defence against herbivores. Similarly, the effect on animal browsing of fertilization, soil, and shading were studied. Significant differences in resin droplets among open-pollinated families were noted. Strong relationships between the hare palatability in mineral soil in cages and in mineral soil in field were noted. Mostly there was a negative relationship between palatability and growth traits. In studies comprising several types of herbivores or individual herbivores within type, such as different insects, no general feature of the host plant seems to confer general tolerance against herbivores.

Experiments testing the effect on \textit{Epirrita autumnata} of different treatments were carried out. Significant differences among clones were noted for growth rate of \textit{E. autumnata} larvae. Condensed tannins correlated strongly with \textit{E. autumnata} pupal mass.

Pit flecks caused by \textit{Phytobia betulae} were studied in three trials with strongly varying number of such flecks, 20, 35, and 80 respectively. Significant clone x test locality interaction was noted. The number of pith flecks was highest in the trial with best growth; tree height 1.5-2 times higher than in the two other trials.

The chitinase IV gene resulted increased incertolerance against \textit{Pyrenopizza betulicola} in some transgenic lines. Significant differences in tolerance against \textit{P. betulicola} and \textit{Melampsoridium betulinum} were observed. However, inconsistency in tolerance between years occurred.

The chitinase IV gene did not have any significant effect on mycorrhiza association with transgenic plants. Based on data from one field trial it was stated that \textit{clear harmful effects of the transgenic chitinase gene on insect population could be detected.}

The genetic constitution seemed to influence the effect of the mutant \textit{ert1-1} allele both on birch growth performance and the possibilities for \textit{Epirrita autumnata} to feed on birch leaves.
5.1 Heavy metal tolerance

Seeds from five birch trees (3 from metal contaminated heaps and 2 from mixed forests) were collected and grown on nutrient agar medium for eight weeks (Brown and Wilkins 1985). After that, cloning took place and the cuttings were exposed to various levels of zinc and the effect on growth was recorded after growth under controlled conditions for another eight weeks. It was stated that a discrimination of the two birch species, *B. pendula* and *B. pubescens*, was impossible at the juvenile stage of the plants. The percentage growth under zinc treatment compared to control was estimated and coined as tolerance index.

The progenies from trees growing on contaminated spoil heaps had a higher tolerance index for stem length than the progenies from ordinary sites (Fig. 5-1). Similarly, the dry weights were higher in the progenies from contaminated soil. The response to different levels of zinc in the culture media were presented for progenies from four trees, two from contaminated heaps and two from ordinary sites (Fig. 5-2). There was a sharper drop of the tolerance index in the plants originating from ordinary birch sites than in the progenies from the lead/zinc contaminated sites and tolerance index was zero at the highest concentration of zinc. It was noted that increasing concentrations of calcium in the growth media increased the tolerance index for zinc when tested in an 1.5 mMol zinc solution. It was found that the tolerant plants had lower contents of zinc than the sensitive plants. It was speculated that the progenies from the contaminated heaps probably originated from fertilizations with pollen from surrounding stands on non-contaminated soil. In spite of this, significant differences in zinc tolerance between progenies originating from contaminated spoil heaps and progenies from ordinary birch sites were noted.

![Figure 5-1. The tolerance indices of five birch progenies from non-contaminated and lead or lead/zinc contaminated sites after growth for eight weeks on 1.5 mMol zink. Tolerance index was defined as the percentage growth in the zinc treatment compared to control. Brown and Wilkins 1985.](image1)

![Figure 5-2. The relationship between tolerance index and zink treatment in progenies from four trees, two from contaminated (red and brown) and two from ordinary birch sites (green and blue). Squares with the same colour originate from the same parent tree. Brown and Wilkins 1985.](image2)
Zinc and copper tolerance in micropropagated emblings of 8 *B. pendula* and 2 *B. pubescens* clones were studied in hydroponic cultures by Utriainen et al. (1997). The clones originated from one locality in Great Britain and 2 localities in Finland. The 4 British clones originated from a mine spoil heap and 3 *B. pendula* and 2 *B. pubescens* clones from one locality close to a metal smelter in southern Finland. Finally one Finnish *B. pendula* clone had unknown origin but must be regarded as non-exposed to heavy metal pollution.

It was hard to follow which clones were used in which experiment. Therefore I have tried to extract the information in Table 5-1. As seen from this Table there were significant clone x treatment interactions for the dose-response curves. Differences between clones were stated in the text but statistical evidences for that was not presented. This does not mean that I suspect that there were no clonal effects, but just that hard facts were not given. The 2-way ANOVAs run by the authors ought to have allowed estimates of clonal effects.

In conclusion the pollutants did not cause much change to earlier thawing close to the smelters. Heavy metal treatment resulted in a delayed bud flushing while the seedling original position in the gradients had no impact on flushing in the control material. Since there were no signs of changed phenology of any of the untreated materials it was suggested that observed difference in flushing in native forest could be attributed to earlier thawing close to the smelters.

In a series of papers a group at Turku University has tackled problems related to pollutions from metal smelters in the Kola region of Russia (Eränen 2008, Eränen and Kozlov 2008, 2009, Eränen et al. 2009). The focus of these papers is more on ecology and plant establishment than on genetics.

It was noted that flushing of *B. pubescens* takes place earlier close to metal smelters in the Kola Peninsula. Therefore, Kozlov et al. (2007) studied flushing along three gradients from heavy metal smelters in the Kola Peninsula. Flushing was studied in native forests of *B. pubescens var. czerepanovii*, on twigs brought indoors, and on seedlings grown in greenhouse from one of the smelters. One half of the seedlings was exposed to heavy metal treatment. Sites and trees within sites contributed equally much to the variation in flushing. In one of the gradients there was a significant difference in flushing between sites while there was significant differences among trees within all three gradients. The correlation between the observations during two years was weak.

Heavy metal treatment resulted in a delayed bud flushing while the seedling original position in the gradients had no impact on flushing in the control material. Since there were no signs of changed phenology of any of the untreated materials it was suggested that observed difference in flushing in native forest could be attributed to earlier thawing close to the smelters.

In conclusion the pollutants did not cause much change in phenology.

Eränen (2008) collected *B. pubescens* seeds from 12 sites in six gradients in the Kola Peninsula in north-western Russia. Three different types of gradients were selected, pollution, altitudinal, and sea-shore gradients. Each population was represented by seeds from five trees. Two weeks after planting of the seedlings heavy metal (HM) treatment was started with NiSO₄ and CuSO₄ in concentrations such that the amounts of Ni and Cu should be 170 mg Ni and 80 mg Cu per kilo substrate. This should be

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**Table 5-1. The number of clones from different types of soil that were included in studies of copper and zinc tolerance of birch emblings in hydroponic cultures. Utriainen et al. 1997.**

<table>
<thead>
<tr>
<th>Response curves Cu</th>
<th>B. pendula exposed GB</th>
<th>B. pendula exposed FIN</th>
<th>B. pubescens exposed FIN</th>
<th>B. pendula non-exposed FIN</th>
<th>Significant effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td>Clone x treatment ***</td>
</tr>
<tr>
<td>Response curves Zn</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Clone x treatment ***</td>
</tr>
<tr>
<td>50% root growth inhibition Cu</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
<td>Clone differences noted</td>
</tr>
<tr>
<td>50% root growth inhibition Zn</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>Clone differences noted</td>
</tr>
</tbody>
</table>

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approximately one third of the concentrations in the polluted, barren land close to the smelter industries. Besides growth traits, the ratio of variable to maximum fluorescence under the artificial light treatment ($F_v/F_m$) was assessed together with the time ($T_1/T_2$) for reaching 50% of $F_m$.

The survival was extremely low in the HM treatment, 11.2%, but was fairly good in the control, 96.8%. These figures differ considerably from the final mortality of the material originating from the so called pollution gradient, in which the survival of both types of origin, stress and non-stress, did not exceed 20%. The population effect was non-significant for plant height, leaf length, $F_v/F_m$, and $T_1/T_2$ while the family effect was significant for all these traits at the 5% level.

The seedlings originating from polluted sites were significantly taller than the seedlings from non-polluted sites in the HM treatment. The opposite was the case for the control. The differences on growth between control seedlings and plants from sites with the two other stress factors, seashore and high altitude, were less pronounced. This investigation also suggests that there was an adaptation to the heavy metal polluted conditions during the 60-70 years of exposure. This was built on the results presented in Fig. 5-3, in which it is shown that the seedlings from the stress grew better than the control seedlings in the heavy metal treatment. In the other treatment the non-stress seedlings outperformed the seedlings of stress origin. As far as I can see from the paper it was not shown that the origin x treatment interaction was significant. Besides, I have assumed that the height growth, that was shown in the paper, is the final at the end of the experiment. Of the original 144 seedlings from the pollution gradient in the heavy metal treatment approximately 25 seedlings were alive at the end of the experiment. This figure is based on the mortalities reported above. This means that the lengthy discussion in this paper on a possible adaptation to heavy metal tolerance in $B. pubescens$ was based on a rather limited material and probably not well founded.

In another study by Eränen and Kozlov (2009) seeds of $B. pubescens$ were collected in a similar way as before, i.e. one pollution gradient, one sea-shore gradient, and one elevation gradient. In each gradient one low-stress population and one high-stress population was selected. Each population was represented by five trees. Three treatments were used; seedlings were planted individually, in pairs, or in groups of eight seedlings in field at low-stress and high-stress sites of their own gradient. The pairs contained one seedling from high-stress and one from low-stress origin. The groups contained equal numbers of seedlings from low-stress and high-stress. Planting distance was approximately 10 cm. Survival, plant height, length of largest leaves, and chlorophyll fluorescence were assessed.

Both for the high-stress and low-stress origins there was 100% survival at the high-stress locality in the pollution gradient (Fig. 5-4; based on Fig. 3 in Eränen and Kozlov 2009). This is just amazing for such harsh conditions. Both low- and high-stress origins at the high-stress locality in the sea-shore gradient had low survival close to 20%. The difference in survival at the high-stress site in the sea-shore gradient was significant. In no other case the origin had a significant impact on survival. This suggests that there is limited variation for survival in the materials tested.
There was only a minor difference in height growth between the two origins tested at high-stress sites (Fig. 5-5) while the seedlings of low-stress origins grew better than the seedlings from high-stress origins at the low-stress locality. Such a performance leads to significance for the interaction test site x stress conditions at origin. The female effect was non-significant for all traits analysed. It should also be noted that the authors reported other height growth data. Thus, the high-stress origin seedlings from the pollution gradient grew taller in the high-stress test site in this gradient than in the low-stress site of this gradient, 13.6 cm versus 8.3 cm. This difference, which was strongly significant, constitutes a contrast to the results for the pooled material in Fig. 5-5. It is regrettable that no information of the performance of individual open-pollinated families was presented.

Ten _B. pubescens_ open-pollinated progenies from two localities at latitudes 66.47° (low altitude) and 67.52°N (high altitude) were included in a study of the effect of sheltering on stand establishment (Eränen and Kozlov 2008). Two pairs of test localities were included; in each pair one locality was designated as high stress-locality and the other as low stress-locality. The seedlings were planted within 20 cm of a host tree and in the open space at a minimum distance of two meters at two gradients. The plant survival, growth, length of two longest leaves, and chlorophyll fluorescence were assessed. The focus of the paper was on afforestation under extremely stressful conditions.

There was a positive effect of sheltering, especially at the high-stress-localities. Although there were ten open-pollinated families from two different localities the genetic information was limited to three p-values in one table. Only the difference in leaf length was significant, p=0.04, while plant height and time to 50% of the maximum fluorescence of chlorophyll were non-significant.

There was a positive effect of sheltering, especially at the high-stress-localities. Although there were ten open-pollinated families from two different localities in three northern Russia localities. The plants were planted at high-stress and low-stress conditions in field. Eränen and Kozlov 2009.

Seeds were collected from 5 _B. pubescens_ trees at each of 18 localities in northern Russia (latitudinal range 66.48-69.48°N) with varying site conditions (Eränen et al. 2009). Some of the localities suffered from heavy metal pollution; part of them had an open exposition and others were sheltered. Seedlings were exposed to drought and heavy metal contaminated soil in growth chambers. Heavy metal stands for nickel and copper sulphates at the concentration of 10 and 5 mg per litre. Besides growth, the ratio of variable to maximum fluorescence under the artificial light treatment (\(F_v/F_m\)) was assessed together with the time (\(T_1/T_2\)) for reaching 50% of \(F_m\). Seedlings were also planted at two contrasting test sites in each of two localities in northern Norway. One of the test localities was sheltered and the other exposed to strong winds. Growth was recorded at the end of the second and fourth growth period. Besides damage, leaf area, leaf dry weight, and leaf fluctuating asymmetry were assessed.

In the growth chamber experiment the family effect was significant for height, number of long shoots, shoot length, leaf length, and \(F_v/F_m\), but not for \(T_1/T_2\), while the family x treatment interaction was significant for the three last mentioned traits.

Only two traits showed a significant family effect in the field trials, No of long shoots and \(T_1/T_2\). The interaction family x test locality was significant for seedling height only.

According to the authors tall seedlings and high \(F_v/F_m\) ratios are indications of higher fitness than their counterparts. It was stated that We found evidence in the phytotron data, with polluted origin seedlings showing less stress in HM treatment than the control origin seedlings (Fig. 1c). To
test this statement I related the values in the heavy metal treatment and drought treatment to the origins performances in the control treatment. The reduction of growth in the heavy metal treatment did not differ between the two types of origin (Fig. 5-6). As seen from this figure, the differences in reduction of the Fv/Fm ratio were marginal in the heavy metal treatment. The plants from the polluted origins had a somewhat higher tolerance against drought than the non-polluted origins.

A similar way of illustration of the importance of the origin on three traits in the field trials is presented in Fig. 5-7. Also in the field trials there are only marginal differences between the two types of origin.

It is regrettable that only significances of various effects were given without any estimates of heritability or coefficients of additive variance. Such estimates would give information on the potential for adaptation.

5.2 Ozone alone and in combination with other agents

A large number of papers treating ozone tolerance in families or clones of B. pendula have been presented by Finnish scientists. The focus of these papers was mainly on the effect of elevated ozone. In some cases combined effects of ozone and other agents such as CO2 and frost exposure are presented. In most cases significances of genetic differences are not reported while the significance of the interaction genetic entry x treatment is presented in some cases. Thus, these investigations do not give any genetic parameters that reveal breeding potentials. However, it is praiseworthy that physiological investigations are carried out with well defined genetic material. In Table 5-3 (pages 92-96) I have compiled these investigations and I will not comment all these papers. It should be noted that data from the same experiment in some cases were reported in more than one paper.


Figure 5-7. The seedling height and Fv/Fm (variable to maximum fluorescence) of B. pubescens populations originating from heavy metal-polluted sites and non-polluted sites cultivated in growth chamber on ordinary substrate and Ni- and Cu-polluted substrate. Eränen et al. 2009.

Figure 5-8. The percentage change of growth, leaf area, and root dry weight after fumigation of five B. pendula clones with 150 nL ozone/L air for 24 days. Significant differences are indicated. Pääkkönen et al. 1993.
Occurrence of large necrotic flecks, established chlorosis, and yellowing of leaf veins was classified as severe damage. The mean value for severe damage in the control and the treated plants did not differ much mainly attributed to the high percentage of damage in clone D (green squares in Fig. 5-9). The results suggest that there is a strong clone x treatment interaction. However, the low percentage of severe damage calls for cautious interpretation of the results.

Effects of ozone on leaf anatomy and ultrastructure were studied by Pääkkönen et al. (1995). Samples were collected on July 6, August 3, and August 14 for anatomical examination. Cross-sections of leaves were photographed in microscope and measured on pictures magnified 373 x. I have illustrated the variation in leaf thickness at the end of the growth period in the control and the ozone treatment (Fig. 5-10). The clone x treatment interaction was strongly significant. Based on the standard errors presented by Pääkkönen et al. (1995) I assume that the clones differed significantly in both treatments. Strongly significant interactions were reported for spongy mesophyll and for palisades. Only at one occasion (August 3) there was a significant interaction for length of chloroplasts while interactions for number of plastoglobuli in chloroplasts were significant at two sampling dates (August 3 and 14). In spite of the significant treatment effects in three of the clones no interactions were noted for size of plastoglobuli.

Lavola et al (1994) reported that the total amount of sucrose, fructose, glucose, and raffinose in leaves increased after 8 weeks of ozone fumigation of three B. pendula clones (Fig. 5-11). Only some of the compounds reacted to the limited increase of ozone. Based on this experiment it was concluded that the use of secondary compounds as an ozone stress indicator in birch is not possible.

Pääkkönen et al (1997b) reported on ozone sensitivity variation among 39 B. pendula and 6 B. pubescens clones after ozone fumigation during two growth periods under field conditions. Twelve B. pendula and 2 B. pubescens clones were studied in more detail as regards anatomical features. An ozone tolerance index was based on four traits, visible damage, foliage area, autumn yellowing of leaves, and dry weight of shoots. Except for dry weight, assessments from both growth periods were included in the index. Each trait was scored in four classes. From Fig. 5-12 it can be seen that the six B. pubescens clones had high values of ozone tolerance while the variation was much larger among the 40 B. pendula clones. One of the B. pubescens clones reached the maximum score of 28. Detailed information on the data for individual clones was not given except for the scoring into four classes but the statistical analyses revealed significant clone and clone x treatment effects for visible damage, number of leaves,
mean leaf size, foliage area, shoot dry weight, and stomatal density. There was a positive relationship between ozone tolerance index and increase of stomatal density at both ages (Fig. 5-13).

At age 2, plants belonging to one sensitive and one ozone-tolerant *B. pendula* clone were fumigated for 43 days in growth chambers (Pääkkönen et al. 1998a). The dose given was 100nL per litre of air. Four treatments were used in this investigation; control, drought stress, elevated ozone fumigation, and a combination of drought and ozone stress. The relative growth rates (RGR) of individual parts of the plants was estimated as 

\[
\frac{(\ln \text{ final dry weight}) - (\ln \text{ initial dry weight})}{\text{number of weeks}}.
\]

In Fig. 5-14 I have illustrated the results as regards RGR of roots, which was the only case where the two clones differed in RGR. In all four treatments the sensitive clone had a higher RGR than the tolerant clone. The reduction of RGR of the sensitive clone was pronounced in the two treatments with drought.

The transcript levels of two stress proteins PR10 and PAL were reported for two occasions June 17 and June 19. PR proteins are defence-related enzymes induced by pathogens, PAL is the key enzyme regulating the phenylpropanoid pathway. In Table 5-2 I have summarized the results as regards intensity of expression of the PR10 protein genes in leaves from the first flushing. In two cases the expression of the genes differed in spite of the limited difference in time for flushing between samplings. There may be a difference between the clones in their response to drought but not to elevated ozone.

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The transcript levels of two stress proteins PR10 and PAL were reported for two occasions June 17 and June 19. PR proteins are defence-related enzymes induced by pathogens, PAL is the key enzyme regulating the phenylpropanoid pathway. In Table 5-2 I have summarized the results as regards intensity of expression of the PR10 protein genes in leaves from the first flushing. In two cases the expression of the genes differed in spite of the limited difference in time for flushing between samplings. There may be a difference between the clones in their response to drought but not to elevated ozone.

At age 2, plants belonging to one sensitive and one ozone-tolerant *B. pendula* clone were fumigated for 43 days in growth chambers (Pääkkönen et al. 1998a). The dose given was 100nL per litre of air. Four treatments were used in this investigation; control, drought stress, elevated ozone fumigation, and a combination of drought and ozone stress. The relative growth rates (RGR) of individual parts of the plants was estimated as 

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Oksanen and Rousi (2001) exposed open-pollinated *B. platyphylla* and *B. pendula* families and full-sib *pendula × pendula*, *pendula × platyphylla* and *pendula × resinifera* families to an elevated level of ozone. In the paper there is conflicting information on origins between Materials and Methods and Table 2. I will rely on the information from Material and Methods. The open air exposure of ozone was aimed at 1.3 times the ambient level and the treatment took place during the second and third growth periods.

The above ground dry weights in the different types of crosses are illustrated in Fig. 5-15, which shows that four of the interspecific hybrids have higher dry weights than the other entries at the end of the third growth period. Full-sib family No. 3 between two Finnish *B. pendula* plus-trees had a high dry weight at the end of the second growth period in the control. However, its superiority at age 2 did not remain during the third growth period.

Unfortunately no statistical test of differences among the families was presented. Looking at the data presented in Fig. 5-15 it is likely that the differences among families are significant for dry weight at both ages and both treatments.

In four cases of 18, stem height was significantly higher in the ozone treatment than in the control treatment. The same proportion of significant differences was noted for leaf dry matter but in this case ozone treatment resulted in a decrease of dry matter. A significant reduction of stem dry matter was noted in five cases. The effect on root dry weight of ozone treatment was most pronounced at age 3 resulting in five significant effects at this age (Fig. 5-16). The mean percentage of leaf damage for ages 2 and 3 varied in the range 3-30%. Entries 1, 3, and 4 showed all more than 25% damaged leaves while 2, 8, and 9 had lower than 5% damage. It is noteworthy that the above ground dry weight ratios (ozone/control) of progenies 2, 8, and 9 at

**Figure 5-15.** Above ground dry weights at ages 2 and 3 in controls and ozone treatment in genetic entries of three different types. Interspecific refers to crosses between *B. pendula* and *B. platyphylla* (5 and 6) or *B. resinifera* (7, 8, and 9). Origin 1 is *B. platyphylla*. Oksanen and Rousi 2001.

**Figure 5-16.** The ratio ozone treatment/control for root dry weight at ages 2 and 3 in nine birch genetic entries (cf the legend to Fig. 5-15). Ozone treatment was aimed at 1.3 times the ambient ozone in field conditions. Significances are indicated. Oksanen and Rousi 2001.

**Figure 5-17.** The ratio ozone treatment/control for foliar potassium at age 2 in nine birch genetic entries (cf the legend to Fig. 5-15). Ozone treatment was aimed at 1.3 times the ambient ozone in field conditions. Oksanen and Rousi 2001.
age 3 all were above 100. Only entry No. 9 showed such a high ratio for root dry weight. A comparison of Figs. 5-15 and 5-16 reveals that roots are more affected by the ozone treatment at age 3 than at age 2 while above-ground dry weight is most affected at age 2. The authors remarked that potassium has a key role in plant metabolism and that an ozone induced reduction of potassium might lead to disturbances in the metabolism (Fig. 5-17). There was no relationship between leaf potassium content and response to ozone treatment. It was expected that the differences in response to ozone treatment will be more pronounced at tree maturity. Such a statement is according to my opinion premature.

A short-term experiment in growth chambers with fumigation with ozone and freeze testing of six open-pollinated families of *B. pendula* was carried out by Prozherina et al. (2003). During the first 34 days May temperatures were simulated, followed by June temperatures until the end of the experiment 17 days later. The ozone fumigation took place with 65 ppb, which was approximately 30 % higher than ambient ozone. Prozherina et al. 2003.

and significant variation in flushing among the OP-families both in the control and the ozone treated material. Ozone caused a strongly significant delay of flushing and an OP-family x ozone treatment interaction is likely based on the data presented in Fig. 5-18. High percentages of damaged leaves were noted in the material exposed to elevated ozone (Fig. 5-19). Strangely enough, the combined treatment of ozone and freezing resulted in lower percentages of damage. It was suggested that there is a wide range of interaction between freezing and ozone treatment among OP-families. There was a moderate negative relationship between flushing in the control material and visible ozone damage ($R^2=0.59$).

The family effect was significant for number of leaves, leaf area, and leaf dry matter. Since these traits have a common component it is not surprising that significance occurs for all of them when one of them is significant. The other dry matter components, root, shoot, and wood, did not show any significant family effect. Leaf dry matter was the only growth component that showed significant ozone x family and freeze testing x family effects. As is evident from Table 5-3 (page 94) significant family effects were noted for many of the leaf and chloroplast structure traits. Many family x ozone, family x freezing, and family x ozone x freezing effects were also significant. It is worth mentioning that the size of the chloroplast in the parenchyma amounted to approximately 60% of the area in the control material.
As is evident from Table 5-3 (page 95) Yamaji et al. (2003) studied a huge number of traits after exposure to elevated ozone at ages 2-3 of 17 B. pendula clones. Unfortunately, clone differences were only reported for chemical compounds. Except for luteolin derivative all other compounds showed strongly significant differences. For a majority of traits the clone x treatment effect was significant. As seen from Fig. 5-20 the percentage of visible damage varied strongly among the 17 clones and it might be expected that this variation is significant. The relationships between leaf damage and 9 phenolic acids and phenolic-glycosides, 10 flavonoid-glycosides, and 7 flavonoids were estimated by Pearson correlations. The numbers of significant relationships in the three types of compound were 9, 7, and 4, respectively. However, the degree of explanation was low in all cases; all coefficients ($r$) were below 0.60.

Fumigation with ozone, carbon dioxide, and a mix of them was carried out for three growth periods in a 7-year old field trial with one ozone sensitive and one ozone tolerant clone (Riikonen et al. 2004). For both gases a doubling of the ambient concentration was aimed at. I found it worthwhile to illustrate the effect on stem volume increment from such an old trial exposed for three growth periods (end of May until late September or early October, Fig. 5-21). The clone classified as ozone tolerant in earlier tests grew less than the clone classified as ozone sensitive. It was stressed that a classification of ozone tolerance in young plants may not be valid for adult trees. It was speculated that $O_3$ sensitivity may be partly explained by higher respiratory costs and lower photosynthetic rates and stomatal conductance in mature trees compared to saplings (Oksanen 2003).

Six open-pollinated B. pendula families from each of two Polish populations were exposed to elevated ozone and UV-B irradiation individually or in combination during the first growth period (Pliura et al. 2008). The two populations originated from two contrasting environmental

![Figure 5-20. Percentage of visible damage following exposure to elevated ozone during growth periods 2-3 in 17 B. pendula clones. Yamaji et al. 2003.](image1)

![Figure 5-21. Stem volume increment during treatments for 3 years in two B. pendula clones, one sensitive and the other tolerant against ozone. OC = open air control, CC = chamber control, EC = elevated CO$_2$, EO = elevated ozone, EC + EO = elevated CO$_2$ and O$_3$. Riikonen et al. 2004.](image2)

![Figure 5-22. Lamina width in two B pendula families, the tallest and shortest in the control, in 9 treatments with ozone and UV individually or in combination. UV3 and UV9 stand for 3 and 9 J/m$^2$ and minute; O$_3$-120 and O$_3$-360 stand for 120 and 360 mg O$_3$/m$^3$. UV3 and O$_3$-120 were pretreatments for one week directly followed by the strong treatments. Pliura et al. 2008.](image3)
conditions, one from continental climatic conditions and the other from maritime conditions with lower ambient ozone and UV-radiation. In the first experiment the seedlings were exposed to an increased duration of UV-B irradiation, 3J/m² and minute during a 5-day period. One month later exposure to ozone took place at 80 and 240 μg/m³. The second experiment was factorial with initially weak treatments (referred to as preadaptation) with ozone (120 μg/m³) or UV (3J/m² and minute) followed by stronger treatments; UV = 9J/m² and minute and/or ozone 360 μg/m³ (cf Fig. 5-22).

The population effects for plant height and lamina width were non-significant while the family effects were significant (Table 5-3, page 96). Any adaptation to higher levels of pollutants had not taken place in the population originating from continental climate. The percentage family variance components for plant height and lamina width directly after treatment were estimated at 26 and 21, but they dropped to 10 and 8 two weeks after the treatment. These variance components estimate ¼ of the total additive variance which means that the estimate of 26% was beyond the allowed limit. Maternal effects were suggested as a reason for this. The homogeneous conditions in the growth chamber may be another contributing factor to high estimates of the variance components. Also in the second experiment the family variance components for height and lamina width were extremely high, 16 – 31% while the component for leaf damage was low, 1.6%. This low estimate for damage might be attributed to a large interaction family x treatment component, 12.5%. I have selected to illustrate the lamina width in all treatments for the tallest and shortest families in the control treatment in Fig. 5-22. This figure reveals that the difference between these two extreme families remains in most of the treatments leading to a limited impact of family by treatment interaction. However, it was noted that the genetic differences among families increased after exposure to the higher doses of UV-B and ozone. Concerning ozone effects, this investigation is the only one so far, in which genetic parameters were estimated.

Net photosynthesis, stomatal conductance, pigments, and nutrient elements were reported after freeze testing and ozone fumigation over 62 days of six open-pollinated families by Oksanen et al (2005a).

The variation in net photosynthesis among the six families 10-12 days after freeze testing (2 days at -2°C) is illustrated in Fig. 5-23. In spite of the family variation shown in this figure the family effect was non-significant. A few days before the freeze testing there was a significant family effect for net photosynthesis. Comparing the net photosynthesis for the freeze testing and control treatments in Fig. 5-23 it is somewhat surprising that the effect of freezing on net photosynthesis was significant while the family effect was non-significant. Contrary to net photosynthesis, the family effect was strong for stomatal conductance (Fig. 5-24). As in most other papers the main focus was on the effect of ozone and freezing exposures. Freezing caused a marked drop in net photosynthesis that was attributed to significant losses of photosynthetic pigments, dilatation, and distortion of thylakoid membranes, and to a reduced size of chloroplasts (Prozherina et al. 2003). It is surprising that such a temperature as -2°C should have so drastic effects on the metabolism. Visible, exterior damage usually requires lower temperatures than a few degrees below zero.
Four open-pollinated families of *B. pendula* were selected for an experiment with exposure to ozone by Silfver et al. (2008). Two of the families were known to be tolerant to ozone and fast-growing; the other two families were less tolerant against ozone with one slow-growing and one fast growing. During the third growth period one control and three treatments were applied:

- Elevated ozone, 1.5 times ambient ozone
- Freeze testing one month after bud flushing to -3.2 and -4.7°C
- Combined ozone and freeze testing

Several growth and physiology traits were recorded. As seen from Fig. 5-25 there was a drastic drop in net photosynthesis in the two freezing treatments immediately after the exposure to low temperatures. Three weeks was a too short time for full recovery.

Kontunen-Seppola et al. (2010) focused on gene expression in two *B. pendula* clones during the end of the growth period after exposure to elevated ozone, carbon dioxide, individually, and in combination. These agents were given in amounts twice as high as the ambient conditions. There was a significant difference between the two clones as regards leaf abscission (Fig. 5-26). It is clearly seen that elevated ozone caused an advancement of leaf abscission. The study showed that there is a linkage between ozone tolerance and possibilities to change the metabolism.

### Miscellaneous

*B. pendula* pollen was collected yearly from various contaminated localities with different degrees of radioactive irradiation after the Chernobyl nuclear plant accident for studies of DNA repair (Boubriac et al. 2008). Unscheduled DNA synthesis (UDS) in the haploid pollen was used as criteria for repair.

Initially UDS did not occur but at the end of the study in 1998 UDS had recovered in pollen from the localities with combined γ- and β-radiation but not in sites with combined α-, β-, and γ-radiation. It was concluded that adaptation to chronic γ- and β-irradiation had taken place.

Mitotic abnormalities in root meristem cells from five localities close to Voronezh in Russia were analysed by Kalaev and Karpova (2003). Four of the localities were highly polluted by industries, the fifth served as control. Seeds were collected from four phenotypically normal *B. pendula* trees in each locality. The percentage of abnormalities was higher in the polluted material than in the control material. There was no information on any variation among the trees from each locality.

### 5.3 Conclusions

A large number of studies were carried out. In the majority of studies the focus was on physiological rather than genetic effects.

Open-pollinated families from leaf and zink contaminated soil had higher tolerance against zink than OP-families from non-contaminated soil and a slightly better growth. In some experiments there were minor differences in growth between the two types of origin, both in field and laboratory. Family differences were noted. Delayed flushing was observed after heavy metal treatment. A broad span of percentage of survival was noted in transplantation and laboratory tests.

There are numerous studies on the effect of ozone fumigation on birch with the main focus on physiology. In one experiment root growth but not above-ground growth was negatively and strongly affected by ozone fumigation. However, in other experiments this was not the case.
Drought stress alone or in combination with ozone resulted in a drastic reduction of root growth in most cases. Following ozone treatment the expression of genes regulating the stress protein PR10 was observed both in a tolerant and a sensitive clone. A wider range in ozone tolerance was noted in *B. pendula* than in *B. pubescens*. The latter had higher scores of ozone tolerance. Genetic differences in above ground and root growth were noted in investigations, in which several entries were studied.

Ozone treatment provoked delay of bud flushing and a G x E interaction for this trait. Strangely the combined freeze testing and ozone fumigation resulted in a reduction of the percentage of damage. Two populations from different climatic conditions did not differ in growth after UV- and ozone treatment, individually or in combination. The family effect was significant for plant height and lamina width with high family variance components.
Table 5-3. Investigations on the effect of ozone on growth and physiology. In several papers the number of traits assessed is tremendous large owing to repeated assessments during the season as well as during more than one season. It should be noted that significant genetic entry or genetic x interaction effects for all assessed traits are in many cases not presented. The terminology of genetic entries is sometimes confusing but I hope that I have understood whether there were n clones and not several clones from n half-sib families that were studied.

<table>
<thead>
<tr>
<th>Author</th>
<th>Materials and methods</th>
<th>Traits studied</th>
<th>Proven significant effect of entry</th>
<th>Proven effect of the interaction entry x treatment</th>
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<tbody>
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<td>Pääkkönen et al. 1993&lt;br&gt;Fig. 5-8&lt;br&gt;Fig. 5-9</td>
<td>5 clones fumigation in field and growth chamber</td>
<td>Height growth, Biomass, Visible damage, Stomatal density, Diffusive resistance to water vapour</td>
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<tr>
<td>Lavola et al 1994&lt;br&gt;Fig. 5-11</td>
<td>3 clones fumigation for 8 weeks with 1.2 x ambient ozone</td>
<td>5 sugar traits leaves, 5 sugar traits stems, 19 glucosides, flavonoids, triterpenoids, and proanthocyanidins</td>
<td>2 of 5 *, 2 of 5 *, 13 of 19 *</td>
<td>1 of 5 *, All 5 ns, 6 of 19 *</td>
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<tr>
<td>Pääkkönen et al. 1995&lt;br&gt;Fig 5-10</td>
<td>5 clones; the same material as Pääkkönen et al. 1993 Sampling at three occasions</td>
<td>Leaf thickness, Palisade thickness, Spongy thickness, Length of chloroplasts, No plastoglobuli, Size of plastoglobuli</td>
<td>*** at 2 occasions, *** at 1 of 2 occasions, *** at 2 occasions, * at 1 of 3 occasions, * at 2 of 3 occasions, Ns at 3 occasions</td>
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<tr>
<td>Pääkkönen et al.1996</td>
<td>2 clones&lt;br&gt;Field study</td>
<td>Height, Foliage area, Net photosynthesis, Transpiration rate, Water use efficiency, 12 traits related to chloroplasts</td>
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<tr>
<td>Tuomainen et al. 1996</td>
<td>2 clone&lt;br&gt;(1 hour exposure to ozone)</td>
<td>Cell viability, Activity of detoxifying enzymes, Gene encoding of PAL</td>
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<td>Pääkkönen et al.1997a</td>
<td>2 ozone sensitive clones and 2 clones with intermediate sensitivity were planted at 3 localities and studied for 2 years</td>
<td>5 growth traits, Damage, 2 stomata traits, 18 chloroplast related traits</td>
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<td>Authors</td>
<td>Year</td>
<td>Experiment Description</td>
<td>Traits</td>
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<td>Pääkkönen et al. 1997b</td>
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<td>40 B. pendula and 6 B. pubescens clones were followed in field for 2 years</td>
<td>4 growth traits</td>
<td>Visible damage, stomatal density, 3 ultrastructural traits (12 + 2 clones), 5 chloroplast related traits (12 + 2 clones)</td>
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<td>Pääkkönen et al. 1998a</td>
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<td>2 clones and 4 treatments in growth chamber: control, drought stress, elevated ozone, and a combination of these 2 agents. Treatments lasted for 43 days</td>
<td>10 growth related traits</td>
<td>Visible damage, stomatal conductance was measured at 3 occasions</td>
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<td>2 clones 3 watering regimes in growth chamber and field</td>
<td>Height, 3 leaf traits, visible injuries, stomata density and conductance, 6 photosynthesis and transpiration related traits, ultrastructural traits</td>
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<td>Oksanen and Saleem 1999</td>
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<td>2 clones exp 1; 10 clones exp 2 long-term experiment</td>
<td>9 growth traits</td>
<td>5 nutrient elements, 7 phenolic compounds, 6 photosynthesis and transpiration related traits, 6 anatomical characteristics, carry-over effects</td>
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<td>Oksanen and Holopainen 2001</td>
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<td>2 clones exposed to different profiles of ozone in growth chamber</td>
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<td>Oksanen and Rousi 2001</td>
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<td>10 growth related traits</td>
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<td>Saleem et al 2001</td>
<td>2 clones 1.5-1.6 x ambient ozone for 3 growth periods</td>
<td>5 growth traits</td>
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<td>3 photosynthesis and transpiration related traits</td>
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<td>Oksanen et al 2003a</td>
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<td>with 6-year old field-grown trees of one clone</td>
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<td>13 out of 16 leaf and chloroplast structure traits *</td>
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<td>Stomata density * at 3 of 4 occasions B, Ca, Fe, K, Mg, P, and Zn *</td>
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<td>Vahala et al. 2003</td>
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<td>Significant Differences</td>
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<td>Yamaji et al. 2003</td>
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<td>12 growth traits, Visible damage, Chlorophyll, Carotenoid, Proteins, Nutrient Elements, Gas exchange traits, Phenolic Acid, Phenolic Acid Glycosides, Flavonoid Glycosides, Flavonoids</td>
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<td>Repo et al. 2004</td>
<td>2 clones over 2 growth periods 4 electrical impedance spectroscopy parameters of leaves</td>
<td>Leaf thickness</td>
<td>5 of 8 *</td>
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<td></td>
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<td>1 of 8 *</td>
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<tr>
<td>Riikonen et al. 2004</td>
<td>2 clones studied over 3 growth periods</td>
<td>A large number of growth traits</td>
<td>Leaf abscission * Seed weight *** % Shoot, branch, leaf of total biomass * Leaf area ratio &amp; mean leaf size *</td>
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<tr>
<td>Kasurinen et al. 2005</td>
<td>2 clones studied over 3 growth periods 8 short root morphotypes,</td>
<td>Degree of colonization</td>
<td>Light brown/orange mycorrhiza *</td>
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<tr>
<td>Oksanen et al. 2005b</td>
<td>2 clones exposure to ozone and carbon dioxide alone and in combination</td>
<td>35 variables of leaf structure, Nutrients and cell wall Chemistry of green leaves</td>
<td>Significant differences: for 5 of 14 leaf related traits For 5 of 11 nutrient elements * For 4 of 6 cell wall chemistry related traits *</td>
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<td></td>
<td></td>
<td>Not reported</td>
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<tr>
<td>Oksanen et al. 2005a</td>
<td>6 OP-families ozone and spring frost exposure in growth chambers</td>
<td>Net photosynthesis and photosynthesis related substances</td>
<td>10 of 19 photosynthesis traits and nutrient elements * 4 of 19 traits showed significant OP-family x ozone treatment</td>
<td></td>
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<tr>
<td>Fig. 5-23, Fig. 5-24</td>
<td></td>
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<tr>
<td>Riikonen et al 2005</td>
<td>2 clones CO2 and ozone + combinations of them over 3 years</td>
<td>Photosynthesis characteristics</td>
<td>net photo-synthesis ** Stomatal conductionance *** water use efficiency ***</td>
<td></td>
</tr>
<tr>
<td>&quot;Rubisco traits&quot; 3 of 6 *</td>
<td></td>
<td></td>
<td>Rubisco traits year x clone x ozone: 1 of 6 *</td>
<td></td>
</tr>
<tr>
<td>Kontunen-Soppela et al. 2007</td>
<td>2 clones 7 growth periods ages 2-8 elevated ozone</td>
<td>Growth and its components 339 low molecular weight compounds (including phenolics, polar and lipophilic compounds, and pigments)</td>
<td>Growth * Substantial growth reduction in ozone Significant differences for many of these compounds Most lipophilic and polar compounds * Polar phenolics *</td>
<td></td>
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<tr>
<td>Oksanen et al. 2007</td>
<td>Review paper</td>
<td>2 clones</td>
<td>(+)-catechin Chlorogenic acid</td>
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<tr>
<td>Ossipov et al. 2008</td>
<td>2 clones, elevated ozone for 7 years</td>
<td>331 compounds</td>
<td>Cluster and principle component analyses revealed differences</td>
<td></td>
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<tr>
<td>Pliura et al 2008</td>
<td>2 populations, each with 6 open-pollinated families Short term ozone and UV treatments individually and in combination</td>
<td>Experiment 1: Plant height Lamina width Leaf damage Experiment 2: Plant height Lamina width Leaf damage</td>
<td>Experiment 1: Population ns Family * Population ns Family * Population ns</td>
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<tr>
<td>Ossipov et al. 2008</td>
<td>331 compounds Cluster and principle component analyses revealed differences</td>
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<tr>
<td>Pliura et al 2008</td>
<td>2 populations, each with 6 open-pollinated families Short term ozone and UV treatments individually and in combination</td>
<td>Experiment 1: Plant height Lamina width Leaf damage Experiment 2: Plant height Lamina width Leaf damage</td>
<td>Experiment 1: Population ns Family * Population ns Family * Population ns</td>
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</tr>
<tr>
<td>Silfver et al. 2008</td>
<td>4 OP-families spring frost 9 growth traits 10 photosynthetic characteristics Bud flushing Ozone injuries</td>
<td>Growth 7 of 7 * Photosynthesis related traits 6 of 10 * Bud flushing *</td>
<td>No significant interactions for any of the traits</td>
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<td>Silfver et al. 2008</td>
<td>Fig. 5-25 Fig. 2-12</td>
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<tr>
<td>Baliuckienë 2009</td>
<td>2 populations from Poland Factorial experiments with elevated UV-B and O3 of juvenile plants</td>
<td>Plant height, leaf lamina, and leaf damage</td>
<td>Height and lamina width both at population and family level</td>
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<tr>
<td>Baliuckienë 2009</td>
<td>Leaf damage large interaction component</td>
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<tr>
<td>Kontunen-Soppela 2010</td>
<td>2 clones exposed to elevated ozone and carbon dioxide individually, and in combination for 2 years</td>
<td>Gene expression after CO2 and ozone exposure Photosynthesis related traits Leaf abscission</td>
<td>Different transcriptional responses Leaf abscission * Chlorophyll *</td>
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<td>Kontunen-Soppela 2010</td>
<td>Fig. 5-26</td>
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Breeding

6.1 General

The Finnish birch breeding programs have attracted much international interest. As a consequence of this, several papers have treated these programs (Viherä-Aarnio 1991 and 1994, Viherä-Aarnio and Velling 2001b, Koski and Rousi 2005). The following description refers mainly to the last paper.

In Finland a large-scale tree breeding of *B. pendula* started in 1961 and began with selection of superior stands. Good raw material for mechanical processing of the trunks was the focus in birch breeding. By the end of the seventies there were 60 approved stands with a total area of 135 hectares. In 1988 a zonation was developed for *B. pendula* in southern and central Finland. Each zone should have 600-700 plus trees. No less than 1,880 plus trees were selected based on good growth, stem quality, *i.e.* straight stems, circular trunks with good natural pruning. The variation in herbivory among progenies called for inclusion of trees less attractive to herbivores in the breeding population. Crosses between plus trees were carried out, mainly as single-pair matings. Both within-population and between-population crosses were carried out. Also open-pollinated progenies were included in progeny trials. The earliest progeny trials date back to 1965. The first evaluation took place at age ten when the trees had reached a height of ten meters. The genetic variation occurred mainly within stands while the among-stand variation was negligible. Rather impressive gains from the plus tree selection were noted with a superiority of the best family over trial mean being 20-30%. These results were obtained in trials located on suitable sites and with good maintenance. However, all trials were not of this kind; approximately one third of the 656 progeny trials had to be abandoned owing to various disturbances. The great superiority of certain families called for establishment of biclonal seed orchards in polythene houses. The first age-age correlations of stem volume were strong, $R^2=0.83$. This means that early evaluation may result in substantial gains.

Two different mating designs were used, one being disconnected half-diallel mating and the other according to reciprocal recurrent selection program. Around 350 hectares of progeny trials existed in the early nineties. It was reported that attempts were made for polyploidy breeding, inbred lines, and species hybridization. Chromosome doubling resulted in aneuploids or polyploids with slow growth and aberrant habitus. Since birches suffer from strong self-incompatibility, offspring from selfing was rare and when it occurred individuals were poorly growing. This means that copying the maize breeding with its crossings of inbred lines was not a realistic option for birch.

In conclusion birches have some advantages from a breeding point of view. Especially, the rapid generation turnover time is attractive to breeders. Vegetative propagation via sprouting and coppicing allows for rapid multiplication of valuable genotypes. The first results from the progeny trials suggest considerable potential for birch breeding. However, pest and diseases constitute constraints to breeding although most pests and diseases do not reduce growth substantially and become serious at decline of growth at ages 45-60 years. Cutting of birch stands with improved material can be done at age 40 with approximately 400m$^3$/ha.

Rousi (1991b) gave a presentation entitled *Breeding birches for resistance to mammalian herbivores*. However, this paper did not discuss breeding in any depth but it treated the comprehensive studies on variation in tolerance against voles and hares among genetic entries that Rousi and coworkers had carried out. He suggested that species hybridisation ought to be carried out to combine good growth with tolerance against mammalian herbivores. Rau (1988) reported on the performance of families, populations, species, and species hybrids in German trials. I preferred to treat the Rau paper in this chapter since it is a strong applied focus in the paper, and especially focused on material for problematic sites. Thus, there are no estimates of genetic parameters.
Three series of trials were studied:
1. *B. pubescens* progenies in one field trial, age 20
2. Species and provenances in four trials, age 6 (Also presented by Rau 1991)
3. Birch hybrids in four field trials, age 5 (Also presented by Rau 1991)

In all cases, the performance of a category of progeny was compared with commercial seed lots. Fig. 6-1 shows that the commercial seed lots had the poorest production in the first series of trials. The good performance of the two selfed progenies in the *B. pubescens* progeny trial is surprising. Firstly it is expected that few seeds are developed after selfing owing to self-incompatibility and secondly a strong inbreeding depression is expected in such a species as *B. pubescens*. It is extremely hard to avoid pollen contamination in artificial crossings in such a wind-pollinated species as *B. pubescence*. Therefore, there might be illegitimate seedlings in the selfed progenies. However, this topic was not discussed.

The four trials established for point 2 suffered all from various calamities leading to poor survival in several cases, which was particularly expressed for *B. alleghaniensis* populations. Generally, the *B. pendula* populations performed best. The mean values for height in *B. pendula* and *B. pubescens* were highest of all populations and they were almost identical. The plant survival was slightly higher in *B. pendula* than in *B. pubescens* and much better than in the exotic birch species.

Seven of the entries in series 3 exceeded the growth of commercial seed lots with 10%. It was a heterogeneous group consisting of two open-pollinated *B. pubescens* progenies, two *B. pubescens* crosses, two hybrids with *B. pubescens* × *B. papyrifera*, and one *B. pubescens* × *B. alleghaniensis* hybrid. It was evident that the site conditions of the four trials in this series were not suitable for *B. pendula*.

It was concluded that there was a large variation among *B. pubescens* progenies and this variation ought to be capitalized in future breeding. Damage by foraging mammals was higher in the exotic species than in the domestic ones.

In a general presentation of tree breeding in Britain Burlcy (2004) reported that a birch group was founded in 1997 for breeding to enable genetically based recommen-

dations for planting of *B. pendula* materials under British conditions. Four provenance trials with 25 populations and two indoor seed orchards were established. One important focus of the breeding is to improve the stem quality.

The state of the art of *B. pendula* breeding in Scotland was presented by Hubert et al. (2010). Four seed tree stands were identified and two clonal seed orchards were established in polythene houses during 2004-2008. The selection of plus trees was guided by knowledge of provenance performances.

Werner (1991) summarised the Swedish birch breeding. It was started already in 1940. However, the interest of birch wood declined and no real breeding took place until the 1980ies. Four breeding zones for *B. pendula* were identified, three for northern Sweden north of latitude 60°N and one zone south of this latitude. Plus tree selection was carried out in the limited number of progeny trials that existed. To reach the 200 plus trees per breeding zone selection had to be carried out in stands. Good growth and timber quality were targeted in this selection. Four breeding populations were created for the southern breeding zone; one northern and one southern, plus one population from southern Finland, and one from the Baltic states. For northern Sweden mainly open-pollinated offspring will be used for establishment of progeny trials while in southern Sweden micropropagated plants will be used for testing of selected trees and backward selection of the best parents. Clonal testing of 20-25 trees per family will be carried out to enable forward selection. This will be carried out at age 5-10, which ought to be satisfactory based on the observed strong juvenile-mature correlations. During the late 1980ies it was not decided which type of seed orchard that should be used. A series of bi-clonal seed orchards were mentioned as one possibility to exploit non-additive genetic effects. This breeding program is currently under implementation (Rosvall personal comm.).

6.2 Flowering in *B. pendula*

The use of transgenic plants and trees may not be permitted owing to the risk for spreading of undesired traits from such plants. Therefore, the possibilities to introduce female and male sterility to transgenic plants are of great significance.

Micropropagation of seedlings selected for early flowering in Germany was carried out by Lemmetyinen et al. (1998):
- to identify growth condition that stimulate flowering to study the possibilities for transformation of these clones
- to elucidate the functioning of the CaMV 35S promoter in different lines

The twelve earliest flowering seedlings in the German material were selected; of them ten could be micropro-
pagated and be transformed. Ten explants per clone were
grown under flowering stimulation conditions in plastic
boxes with elevated CO₂.
The first signs of flowering were noted 3-5 months after
rooting. One plant of the earliest clone produced flow-
er at a height of 6 cm. In the control the first flowering
was noted after 9-10 months. Transformation by aid of
Agrobacterium with CaMV-35S-GUS INT was carried out
in two early flowering clones. Three and five transgenic
lines were obtained from these clones. Two transgenic
lines from a control plant were also produced. Most of
the lines had several copies of the transferred gene and
were inserted at different loci. There was a large variation
in expression of the 35S-GUS in leaves from 1.6 to 5,000
times stronger than in the control plants. Further, in vitro
cultivation reduced the level of expression to 0.3-7.0% of
the original expression.
Two genes, BpMADS1 and BpMADS6 are expressed from
an early stage in development of inflorescences to seed de-
velopment (Lemmetyinen et al. 2004a). However, it was
concluded that it seems as these genes do not have any es-
sential role in the timing of flowering.
Transfer of the construct BpMADS1::BARNESE result-
ed in 81 kanamycin resistant lines (Lemmetyinen et al.
2004b) into two early flowering clones. Of the 81 lines, 36
performed like the wild-type while seven did not produce
any flowers and initially they showed no deviating veget-
etive development (Fig. 6-2). The last 38 lines did not
flower but showed vegetative defects. Even the promising
seven lines showed some side effects after the induction of
flowering. It was concluded that this study was the first, in
which prevention of flowering in a tree species was demon-
strated. Since there were some side effects it was sug-
gested that the expression of BARNES was too strong so
that there were effects also on the vegetative parts. There-
fore, one should aim at a somewhat lower expression of
BARNESE but still large enough to prevent flowering. In
conclusion, it is of significance that female and male ste-
tility can be transferred in birch.
Lännenpää et al. (2005) studied the prevention of flower
initiation in an early flowering B. pendula clone by use
of the construct BpFULL::BARNES. In all, 21 transgenic
lines were obtained. The results are summarized in Fig.
6-3, which shows that nine lines performed as the wild-
type with normal growth and inflorescence formation. In
four lines the vegetative growth was severely disturbed
and the plants were dwarfish. In additional four lines, the
development was initially good. However, after some time
the apical parts of the plants became bushy. Three of the
remaining lines had a slightly lower growth, 90-97% of
the wild-type, and no formation of male inflorescences.
No male inflorescences were formed in the final line that
had a strongly reduced growth 70% of the wild-type. Most
of the non-flowering lines contained one construct of
BpFULL1::BARNES.
Three of the non-flowering lines were cultivated for a
longer time including a winter to see the effect of the con-
struct on female flowering. Flowering did not occur in
any of the lines.
The effect on flowering of two constructs, the sense construct of BpMADS4 and the antisense construct BpMADSS4as, were studied by Elo et al. (2007). In an earlier study Elo et al. (2001) had identified two more genes involved in formation of female and male inflorescence in B. pendula, BpMADS3 and BpMADSS. Moreover, they seemed to have a role early during the transition from vegetative to flowering phase.

The cDNA of BpMADS4 in full length was inserted in normal and in reverse orientation into the DNA of the early flowering clone BpM2. Two normally flowering clones were used as controls in the experiments. In four of the lines with the sense construct the time to inflorescences did not differ from the time in the control and only male flowering was noted. The two lines with extremely early flowering had both female and male inflorescences. The time between the first and the last appearance of inflorescences was limited in the 23 lines of line 2 and no variation in the three plants in line 1. The plant heights in these lines varied between 2 and 4 cm. Line 1 did not grow well and died after some time. All lines showed abnormal growth pattern. In addition to these constructs, transfers were carried out to normal plants as well. The time to flowering in two lines was 86 and 138 days after rooting at plant heights 6-9 cm. The control plants in this case were kept for more than a year and no flowering occurred. The construct was reported to be most strongly expressed in the apical meristem before the appearance of any floral meristem. During vegetative growth the expression of the BpMADS4 gene is weakly expressed. Therefore, a high level of expression is needed for flower initiation or that other genes are required for initiation.

Ten lines with the antisense construct of the BpMADS cDNA were obtained. The time to first flowering was longer in five of the lines and it varied more in the antisense construct lines than in the control. Moreover, in nine of the ten lines no flowering at all was noted in some of the plants.

The practical consequences of these results are:
- A possibility to reduce the generation time in the breeding population
- A possibility to induce male sterility, which is of significance if GMOs should be used.

6.3 Seed production and pollen competition

Holm (1994a) tested the hypothesis that increased pollen density would lead to increased pollen competition. In vitro germination of pollen from three B. pubescens trees growing at latitude 64°N was tested with droplets having a variable number of pollen grains.

In the low range of pollen density, < 1000 pollen grains per droplet, there was an increase of germination with increased pollen density. At high pollen densities there was no further increase of the germinability. Pollen tube growth after artificial pollination of two trees revealed an initial difference in pollen tube growth between the two trees but this difference was reduced over time. With increasing number of pollen tubes per style the mean length of pollen tubes either decreased or remained relatively constant. Contrary to this, there was an increase in the length of the longest pollen tube with increasing number of pollen tubes per style.

Seed quality was recorded during six years in ten B. pendula, 13 B. pubescens and 2 B. tortuosa populations by Holm (1994b). The populations of the two former species covered a latitudinal range of three degrees and an elevation range of 45-520 masl. The focus of the paper was on the relationship between altitudinal origin and seed characteristics. There is some confounding between latitude and altitude since populations from low altitudes always originated from low latitudes.

I have tried to extract the most important results in Table 6-1. As seen from this table there is not total agreement between the two species. Thus, the percentage of germinable seeds decreased sharply with altitude in B. pendula and altitude since populations from low altitudes always originated from low latitudes.

Table 6-1. The relationship between altitude and several seed characteristics in B. pendula and B. pubescens populations. Holm 1994b.

<table>
<thead>
<tr>
<th></th>
<th>B. pendula</th>
<th>B. pubescens</th>
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<tbody>
<tr>
<td>% germinable seeds</td>
<td>Decreased significantly with altitude all 6 years</td>
<td>Decrease 3 years and increase 3 years</td>
</tr>
<tr>
<td>Seed germination rate</td>
<td>Increase with altitude</td>
<td>Slight increase with altitude</td>
</tr>
<tr>
<td>Seed weight</td>
<td>No clear trend</td>
<td>Increase with altitude</td>
</tr>
<tr>
<td>proportion of viable and filled seeds that failed to germinate</td>
<td>Significant decrease with increasing altitude</td>
<td>Significant decrease with increasing altitude</td>
</tr>
<tr>
<td>Empty seeds</td>
<td>No relationship</td>
<td>No relationship</td>
</tr>
<tr>
<td>Gall midge infected seeds</td>
<td>Increase with altitude with 2 exceptions</td>
<td>Low level of infection and no trend</td>
</tr>
<tr>
<td>Other types of damage; small undeveloped seeds and high incidence of Sclerotina betulae in two populations</td>
<td>No relationship</td>
<td>Significant negative relationship with altitude</td>
</tr>
<tr>
<td>Relative seed production</td>
<td>No relationship</td>
<td>No relationship</td>
</tr>
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Pollination and seed quality in two coastal and two mountainous populations of each of the species B. pendula and B. pubescens from northern Sweden. Recordings took place three years. Holm 1994c.

The proportion of germinable seeds in the two high altitude populations (700 and 730 masl) of B. tortuosa was low while the seed weight was relatively high. Seed germination rate in one of the populations was much higher than in B. pubescens populations. In five populations of B. pendula and B. pubescens the relationships between seed germinability and temperature sums the current year or the previous year were estimated. In both species, significant relationships with temperature sum of the previous year were noted for four of the five populations. No significant relationship was found with the temperature of the current year.

The sapling density in an area of 314 m² around a tree was estimated at 1.25 per m² for B. pendula, 16.47 for B. pubescens, and 0.72 for B. tortuosa. According to the author, the results of decreasing reproduction against the distribution limit suggest that the two species are in equilibrium with the prevailing climate. It is evident that clinal variation prevailed for many of the traits studied in this investigation.

Pollination and seed quality in two coastal and two mountainous populations of B. pendula and B. pubescens in northern Sweden were studied by Holm (1994c). The mountainous populations originate from latitudes 66.27-66.40°N and 450-480 masl while the coastal populations originate from latitudes 63.88-64.20°N and 17-220 masl. Six traits were studied, of them the seed germination is of greatest significance.

The number of cases with seed germination in three classes, <5%, 5-20%, and >20%, is shown in Fig. 6-4. The germination was tested during three years. In 1988 the germination was <5% in all populations. The poor germination of seeds from the two mountainous B. pendula populations is apparent. One of the B. pubescens mountainous populations had the highest germination percentage in this species, >50%. Supplemental pollinations were carried out in two years with B. pendula and one year with B. pubescens. Similarly there was a higher infestation of the gall midges (Semidobia) in B. pendula.

The sapling density in an area of 314 m² around a tree was estimated at 1.25 per m² for B. pendula, 16.47 for B. pubescens, and 0.72 for B. tortuosa. According to the author, the results of decreasing reproduction against the distribution limit suggest that the two species are in equilibrium with the prevailing climate. It is evident that clinal variation prevailed for many of the traits studied in this investigation.

Supplemental pollination with coastal pollen of a mountainous population increased the percentage of germinable seeds. For both species supplemental pollination had no significant effect on germinable seed % in the coastal populations. Seed germinability was significantly correlated with frequency of pollinated flowers in B. pubescens. Even if the effects of supplemental pollinations were significant in some cases, the effects were never strong. One B. tortuosa population was included in the study and its values for the six traits studied were lower than in the B. pubescens populations.

In conclusion, in the mountain area there was a larger effect of supplemental pollination in B. pubescens than in B. pendula. This suggests that there might have been less pollen available in B. pubescens than in B. pendula. The latter species was heavily affected by gall midge attacks.

In vitro pollen germination of individual B. pendula clones was compared with the germination in 2-clone mixes (Pasonen and Käpylä 1998).

The percentage deviations from the clonal means of the 2-clone mixes were positive in 7 of the 8 cases tested (Fig. 6-5). For two of the clones involved in this test, the individual percentages of germination differed according to Fig. 1 in the Pasonen and Käpylä paper. In one case, clone V 5066, the percentages were 53 and 85, and for clone V 505 the corresponding percentages were 50 and 71. Probably, these differences are due to more than one germination test of these clones. It was also shown that pollen density affected the germination percentage strongly and strongest for the pollen originating from outdoor collection, which showed a steep increase with increasing pollen density. Addition of pollen extracts from dead pollen changed this relationship, even to a reduction of germination with increasing pollen density. The effect of the positive deviation of germination in the 2-clone mixes was discussed from an evolutionary point of view. It might be advantageous for the female partner and negative for the male partner. Such an interpretation was regarded as improbable.
Pollen tube growth rates and siring success of six male parents was investigated after pollination with mixes of two clones on female *B. pendula* clones in a greenhouse (Pasonen et al. 1999). The siring success was estimated by aid of isozyme markers in six females after pollination with five pollen mixes. All pollen mixes had one male clone, E1970, with a unique isozyme genotype, which allows for a proper identification of its parentage in the progeny. As a complement to the pollination experiment, pollen tube growth of the six male clones after 12 hours of germination was assessed in vitro on 11 female clones and *in vivo*.

In Fig. 6-6 I have illustrated the mean siring success of the six male clones. In 20 of the 29 cases tested (one pollination failed) there was a significant difference in success rate. Clone E 9427 was consistently above 50% in all cases. There were significant differences in pollen tube growth on all females as well as in the in vitro test. There was a relationship between pollen tube growth and siring success (*r* = 0.63). The pollen tube growth on the stigma of 11 females showed a mixed pattern. In some females there was a limited variation in pollen tube growth while it was much differentiated in other cases. Therefore, I preferred to test the relationship including the two females with the largest difference in pollen tube growth. As seen from Fig. 6-7 the relationships were in these cases much stronger. It should be added that the success rates were rather consistent and independent of the female partner in the cross. This means that pollen competition probably exist in *B. pendula*.

The pollen tube growth of six *B. pendula* males on two female clones was studied in a polythene house and in a clone archive. Significant differences in the two environments are indicated. Pasonen et al. 2000.

The pollen tube growth of six *B. pendula* males on two females in a clone archive and in a polythene house. Each male has a specific colour. The diamonds and squares represent the two females. Significant differences in the two environments are indicated. Pasonen et al. 2000.

![Figure 6-6](image1.png)

*Figure 6-6. The mean success rate of male clones in mixes of two clones, in which the B. pendula clone E 1970 was used as a standard. Pasonen et al. 1999.*

![Figure 6-7](image2.png)

*Figure 6-7. The relationship between mean siring success rate of B. pendula male clones in mixes of two clones in crosses with females V 591 and V 5835. Pasonen et al. 1999.*

![Figure 6-8](image3.png)

*Figure 6-8. The pollen tube growth of six B. pendula males on two females in a clone archive and in a polythene house. Pasonen et al. 2000.*
0.001) and also a significant difference among the males (p < 0.05).

The evolutionary significance of variation in pollen tube growth and the parent x environment interaction in pollen tube growth was discussed. With such an interaction the seed crop produced may vary genetically dependent on the weather conditions during the flowering phase. This may have impacts on evolution as well as on breeding. A final answer related to this matter will only be obtained after studies of the performance of progenies.

Pasonen et al. (2002) reported on a similar study carried out in a greenhouse and in an open-air clone archive. The temperature conditions varied strongly between the two localities. Thus the maximum temperature in the greenhouse was around 30°C while the corresponding in the archive had maximum temperatures around 15°C. Seven B. pendula female and male clones were included in the study. Besides individual pollinations, two-clone pollen mixes with clone E 1970 as standard in the mixes were carried out. The pollen tube growth after individual pollinations was recorded at 12 hours after germination.

In Table 6-2 the significances of the different effects on pollen tube growth and siring success are presented. Almost all effects were significant for siring success while several effects were non-significant for pollen tube growth. The pollen tube growth was strongly affected by the pollination site with a much larger growth in the greenhouse than outdoors. The pollen tube growth was rather consistent among females, which means that a male with good growth on the stigma of one female generally grew well on stigmas of other females. There was a weak negative and significant relationship between pollen tube growth in greenhouse and in outdoor conditions, r = -0.39.

The percentage of siring success was larger in the outdoor clone archive than indoors. There was a strong relationship between the siring success in the two environments, r = 0.80. In spite of this, significant interactions of female x pollination site and male x pollination site were noted (Table 6-2). All correlations between pollen tube length and siring success for individual females in greenhouse were positive but non-significant. Contrary to this, most of the same type of correlations in the outdoor archive was negative and only once significant. When the data was pooled for all females the correlations became significant, r = 0.65 in greenhouse and r = -0.54 in the clone archive.

In conclusion the pollination site conditions strongly influence the pollen tube growth and siring success. The pollen tube growth rate under controlled conditions is a fairly good predictor of siring success, which is not the case for the variable outdoor conditions. There was a marked difference in the success rate of the labelled clone in the two environments. In Fig. 6-9 I have given the approximate percentages of success rate based on the graphic illustrations from Fig. 2 in the Pasonen et al. (2002) paper. The labelled clone was evidently less competitive under outdoor conditions and it was stated that the environmental conditions influence the mating pattern. As a consequence of this, the genetic constitution of seed crops from seed orchard will vary dependent on ambient conditions, which results in a broadening of the variation. This is not the case for seed orchards only, but will be the case in nature as well. A broader genetic raw material in nature means greater possibilities for adaptation via natural selection.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Pollen tube growth</th>
<th>Siring success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Male</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Female x male</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Pollination site</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Female x pollination site</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Male x pollination site</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>Female x male x pollination site</td>
<td>***</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 6-2. The significances of different effects on pollen tube growth and siring success in pollinations with mixes of two B. pendula pollen donors. Pasonen et al. 2002.
Table 6-3. The significances of various genetic effects on four seed and pollen traits in an 8 x 7 factorial mating of B. pendula clones. Pietarinen and Pasonen 2004.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Female</th>
<th>Male</th>
<th>Female x male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen tube length according to in vivo testing</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Seed biomass</td>
<td>***</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Seeds/inflorescence</td>
<td>***</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>No. aborted seeds</td>
<td>***</td>
<td>*</td>
<td>ns</td>
</tr>
</tbody>
</table>

Pietarinen and Pasonen (2004) tested two hypotheses:
1. Pollen donors with large pollen grains have faster growing pollen tubes; the larger pollen grains would have more resources and in this way stimulate good growth of pollen tubes.

and

2. Maternal plants produce more seeds when inflorescences have been pollinated with pollen from pollen donors with fast pollen tube growth.

Seven males and eight females were selected in an indoor seed orchard with B. pendula clones (one clone served both as female and male). All 8 x 7 pollinations had one male clone as pollen donor. The number of female flowers per inflorescence was counted. Pol len grain size and pollen tube growth after 24 hours in in vitro cultures was determined. The in vivo pollen tube growth was assessed after 12 hours for each of the 8 x 7 samples, excluding selfing of one clone. Seeds were harvested three months after crossing and 100-grain seed weight was determined. Total weight of the seeds and number of seeds per inflorescence were also determined.

A strongly significant difference in pollen grain size was noted but there was no correlation between grain size and length of pollen tube from the in vitro pollen germination test. Since the material was growing indoors under fairly uniform conditions environmental variation was ruled out as an explanation for the variation in pollen grain size. The correlations between pollen tube growth in vivo on one hand and seed biomass, seed number per inflorescence, and number of aborted seeds on the other hand were all non-significant. Similarly, the correlations between pollen grain size and the same three traits mentioned above were non-significant. I have summarized the results from the ANOVA for four of the traits studied in Table 6-3, which shows strongly significant female effects for the four traits, seed biomass, number of seeds per inflorescence, pollen tube length, and number of aborted seeds. At least the two former traits ought to be strongly regulated by the female itself. There was consistency among the male clones as regards pollen tube growth in different females. Generally, good growth in one female was noted but there was no correlation between grain size and length of pollen tube from the in vivo pollen germination test. Since the material was growing indoors under fairly uniform conditions environmental variation was ruled out as an explanation for the variation in pollen grain size. The correlations between pollen tube growth in vivo on one hand and seed biomass, seed number per inflorescence, and number of aborted seeds on the other hand were all non-significant. Similarly, the correlations between pollen grain size and the same three traits mentioned above were non-significant. I have summarized the results from the ANOVA for four of the traits studied in Table 6-3, which shows strongly significant female effects for the four traits, seed biomass, number of seeds per inflorescence, pollen tube length, and number of aborted seeds. At least the two former traits ought to be strongly regulated by the female itself. There was consistency among the male clones as regards pollen tube growth in different females. Generally, good growth in one female resulted in good growth in the other females. The total seed biomass could to 52% be attributed to average number of seeds per inflorescence.

In conclusion, the two hypothesis presented above could not be verified in this investigation.

Pasonen et al. (2001) studied the impact of females and males on seed and seedling performance with particular emphasis on the relationship between pollen tube growth and other traits. Six B. pendula males and nine females were included in this investigation.

As seen from Figs. 6-10 and 6-11 there were fairly strong relationships between pollen tube length and the three traits illustrated in these figures: 100-grain weight, filled seed percentage, and plant height after cultivation for 85 days. It should be noted that the pollen tube growth in one outlier female had an extremely poor pollen tube growth, which influences two of the relationships strongly (Fig. 6-10 and 6-11).

Negative relationships between 100-grain weight and seedling height were noted for eight of the nine females. However, none of them was significant. Another contributing factor to the absence of significance was the limited variation in 100-grain weight, 19-27 mg. For three females there was a significant and positive relationship between pollen tube length and percentage filled seeds, r varying in the range 0.84-0.94. The ANOVAs run for number of seeds per inflorescence, percentage of germinable seeds, and filled seeds showed significant female effects while the male effects were non-significant. It is evident and expected that these traits are regulated by the parent plant and not by the newly formed embryo. Neither female nor male effects were found for 100-grain weight. Female, male, and female x male interaction were all significant for plant height at 85 days after germination. The number of aborted seeds differed significantly among the three females tested. No relationship...
between aborted seeds and pollen tube length was noted. It was concluded that there was no strong evidence for the theory (hypothesis; my remark) of pollen-tube growth rate as an indicator of progeny quality is provided by this study. I do not understand the background for such a hypothesis and reasons for such a hypothesis were not elaborated in the text.

6.4 Plant type and seed production

Four types of plants, seedlings, micropropagated plants, grafted plants, and cutting-propagated plants, were compared morphologically and with respect to juvenility (Brand and Lineberger 1992). The ortets were 15 years old and growing in a seed orchard in Pennsylvania. Two *B. papyrifera* clones were included in the study together with one *B. pubescens* x *B. papyrifera* hybrid clone.

In all three clones there was 100% of flowering at eight months in the cutting-propagated mature material while there was no flowering in seedlings and tissue-cultured materials. Only in the hybrid clone there was flowering in the grafted plants with the percentage being 73. At eight months there was no difference in stem diameter or leaf length between the four types of plants of the hybrid clone. The internode length of cuttings and grafted material was larger than for the two other types of plant. A logical conclusion of the results was that reversion from mature to juvenile characteristics, facilitated by in vitro culture, appears to occur in birch.

Which type of material should be used for seed orchards - seedlings, grafts, or micropropagated trees? This question was addressed by Vihervää-Aarnio and Ryynänen (1994 and 1995), who studied growth and flowering during four years in a polythene house. Ten clones were selected and grafts, micropropagated plants, and seedlings were produced from each of the ten clones. Topping of the plants was carried out at age 2, which resulted in levelling of the branching in the different plant types.

At age 2 only the grafts were flowering. The two following years there was an abundant flowering while it dropped at age 5. The seed production was almost ten times larger at age 4 than at age 3. Therefore, a direct calculation of a mean value will be misleading. Therefore, I calculated the percentage contribution of each plant type for the individual clones in the grand total seed crop (Fig. 6-12). In seven clones the seed production was highest in the seedlings while it was lowest in the grafts of all ten clones. For ages 3-4 the difference between plant types was strongly significant while there was no significant difference at age 5 when the flowering was very poor. It was noted that birch flowering in natural stands also was poor this year. The difference between clones was strongly significant at age 4 but not at ages 3 and 5. Only at age 3 the clone x plant type interaction was significant. This was attributed to varying speed of maturation or degree of rejuvenation among genotypes.

Figure 6-11. The relationship between pollen tube length and 100-seed weight, percentage filled seeds, and plant height in 9 *B. pendula* females. Height was measured 85 days after germination. Pasonen et al. 2001.

Figure 6-12. The mean percentage contribution of seeds at ages 3 and 4 for seedlings, micropropagated plants, and grafts from the same genotype. Ten *B. pendula* genotypes were studied in a polythene house located at latitude 61.82°N and 90 masl in southern Finland. Vihervää-Aarnio and Ryynänen 1994.
The growth of the seedlings and micropropagated trees was much better than the growth of the grafts (Fig. 6-13). The more vigorous growth resulted in more abundant flowering than in the grafts even if the flowering started earlier in grafts than in seedlings or micropropagated trees. It should be noted that the standard deviation was large, especially for male catkins per branch in the seedlings. These two investigations show that micropropagated material can advantageously be used in *B. pendula* indoor seed orchards to obtain satisfactory seed crops.

The possibilities for large-scale micropropagation of birch call for studies of possible pitfalls for commercial application of this technique. To this end a study of more than 29,000 trees of micropropagated and seed-born trees were carried out by Viherä-Aarnio and Velling (2001a). Three series of field trials were assessed at ages 6-7 with respect to survival, tree height, vole and moose browsing as well as stem lesions. The authors correctly pointed out that comparisons should be done with closely related entries. Series 1443 fulfilled this requirement in a good way. Since there sometimes was a large variation of mean values among trials within a series of trials I have related the performances of the micropropagated to the seed-born progenies in each trial of a series and then calculated a mean value for each series (Fig. 6-13). It should be noted that damage by voles and moose was extremely low in some trials. For those cases with one affected seedling and two affected micropropagated plants such a calculation would exaggerate differences and was therefore not carried out. The percentage survival in two series varied in the approximate range 50-90. In the third series there was one clone with 25% survival, which differed from the other clones and seedlings, which occupied the same range as in the two other series. There were strongly significant differences for survival and height between entries in all three series of trials while the entry x test site was significant in two series. Fig 6-14 reveals that the differences between clones and seedlings is limited for survival and growth while there are differences between clones and seedlings for lesions and vole browsing in some cases. The vole affected trees was low in series 1336 and in two of the three trials in series 1444. The third trial showed a strongly significant difference for vole damage between the two types of material with less damage in the clones. The percentage of stem lesions varied in the range 10-70 with the largest variation among trials in series 1336 and the variation among sites within the three series was significant.

The authors presented the performance of individual clone/seedling pairs for trial series 1443, which had the best comparison material. The number of significant differences between clones and seedlings was low as seen from Fig. 6-15. This important investigation for application of large scale multiplication via micropropagation does not suggest that there should be any drawbacks of using this technique in breeding or production populations. However, it should be remembered that the trials are still young and the advice by the authors to carefully select and test the material to be used in applied forestry is justified.

### 6.5. Conclusions

Three types of papers were presented in this chapter:
- Breeding programs,
- Flowering with a focus on siring success and male sterility,
- Type of material to be used in seed orchards and in the production population.

Since birch breeding has been more important in Finland than in other countries it is not surprising that several reports from Finland treated breeding in this country. Large-scale breeding started 1961 in Finland. In 1988 a
zonation of the country was carried out. Each zone should have 600-700 plus trees. Crosses between plus trees were carried out, mainly as single pair matings. Impressive gains from the plus tree selection over trial means were reported, 20-30%. Age-age correlations were strong, which suggests that early selections could be done. It was also noted that pests and diseases occur but they become severe at decline of growth at an age of 45-60 years.

Based on various types of trials in Germany it was concluded that there was a large variation among *B. pubescens* progenies and this variation ought to be capitalized in future breeding. Damage by foraging mammals was higher in the exotic species than in the domestic ones.

A breeding program was established in United Kingdom in 1997. Two clonal seed orchards were established during the years 2004-2008.

Birch breeding started already during the fourties in Sweden but was abandoned for several decades. During the 1980ies birch breeding was again taken up. Four breeding zones for *B. pendula* were identified, three for northern Sweden north of latitude 60°N and one zone south of this latitude. Four different breeding populations were established for southern Sweden; one northern and one southern plus two populations from southern Finland and the Baltic countries. Each zone should have 200 plus trees.

Prevention of flowering was studied after transfer of two genes *BpMADS1* and *BpMADS6*. It was concluded that this study was the first, in which prevention of flowering in a tree species was demonstrated. Some side effects were noted, which was attributed to too strong expression of the construct.

The cDNA of *BpMADS4* in full length was inserted in normal and in reverse orientation into the DNA of the early flowering clone BpM2. Flowering in two transgenic lines took place after 86 and 138 days when the plants were 6-9 cm tall. It was shown that the generation time might be reduced considerably. The antisense construct of the *BPMADS* gene might have induced male sterility.

In the low range of pollen density, < 1000 pollen grains per droplet, there was an increase of *in vitro* germination with increased pollen density. With increasing number of pollen tubes per style, the mean length of pollen tubes either decreased or remained relatively constant.

During a 6-year period the percentage of germinable seeds decreased significantly with altitude in *B. pendula* while this was the case during three years for *B. pubescens*. A significant relationship between seed germinability and temperature sum of the previous year was noted. At high altitude supplemental pollination had a larger effect in *B. pubescens* than in *B. pendula*.

The percentage deviations of pollen germination in vitro from the clonal means in 2-clone mixes were positive in 7 of the 8 cases tested.

In 20 of the 29 cases tested with respect to siring success there was a significant difference in success rate. There were also significant differences in pollen tube growth on all six females.

Pollen growth of several clones outdoors and indoors in a polythene house differed. Some of the matings contributed to genotype x environment interaction. It was concluded that the pollination site conditions strongly influenced the siring success.

The below two hypotheses were tested with no support for any of them:

1. Pollen donors with large pollen grains have faster growing pollen tubes; the larger pollen grains would have more resources and in this way stimulate good growth of pollen tubes.

2. Maternal plants produce more seeds when inflorescences have been pollinated with pollen from pollen donors with fast pollen tube growth

Pollen tube growth did not seem to have any effect on progeny quality.

All studies related to type of regeneration material showed that there are no disadvantages to rely on micropropagated material for seed orchards or for the production population. One of the studies contained an impressive number of trees, 29,000.
Martin et al. (2008) used RAPDs to estimate variation within and between six isolated populations of *B. pendula* subspecies *fontqueri* in Spain. Some of the populations were small with less than ten trees. In addition, two *B. pubescens* populations were included. Samples were taken from 94 *B. pendula* trees and 10 trees from each of the two *B. pubescens* populations.

Eighty-three RAPD markers were found in *B. pendula* and 81 in *B. pubescens*. Eleven of the *B. pendula* markers were monomorphic while 31 markers were shared by all *B. pendula* populations. Twenty of the *B. pubescens* markers were monomorphic. The cluster analysis showed two distinct groups one containing the Sierra Nevada population and the second group the other five populations. The variation among populations was estimated at 64.2%.

The pairwise differences were in all cases high and ranged between 0.59 and 0.71. As seen from Fig. 7-1 there is no strong relationship between geographic and genetic distance. The low number of trees (2-4) tested in some populations will result in random variation. It was pointed out that there are natural barriers that may prevent gene flow among these populations, which should explain the large differentiation.

Since the main objective of this investigation was to obtain genetic information that could guide gene conservation, one section of the paper was devoted to this topic. Unfortunately, no geographic data were presented for the sampling localities. The obtained differentiation among the populations strongly suggests that each of them should be included in a gene conservation program for *B. pendula*. Since three of the populations contained 20 or less trees the efficient population sizes of these populations are so small that adaptation to the site conditions at their localities may not take place owing to too strong genetic drift. If the environmental conditions are the same or almost the same at these three localities, an exchange program between these three populations would be a sensible measure to guarantee adaptation and long-time survival of the populations. This is supported by the suggestion in the paper that these populations should be treated as a group with a common management program. As in all cases with gene resource populations in nature measures should be taken to promote regeneration of the gene resource populations.

![Figure 7-1. The relationship between geographic distance and genetic distance, \( F_{ST} \), based on RAPDS in six disjunct Spanish populations of *B. pendula*. Martin et al. 2008](image-url)
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