Henna Pihlajaniemi

SUCCESS OF MICRO-PROPAGATED WOODY LANDSCAPE PLANTS UNDER NORTHERN GROWING CONDITIONS AND CHANGING ENVIRONMENT
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Abstract

Plant registration and selection work aimed at identifying the best genotypes for northern landscaping has been carried out in Finland since the 1980’s. In the University of Oulu Botanical Gardens, micropropagation methods have been developed for several woody plant taxa registered during the POHKAS (Northern Hardy Plants) project. Micropropagation is an effective method to conserve valuable genetic characteristics and to produce plantlets from woody species with limited mother stock material and in a limited time period.

In this study the long-term field phenology and success of 19 micropropagated shrub and tree taxa was followed in plant selection experiments. Experiments were conducted at four northern field sites presenting different climatic conditions. Of the phenological monitoring parameters, the onset of foliation and flowering in the field revealed a strong relation to spring time temperature, being obviously latest to occur in northernmost site. The gradient between southern and northern sites for autumn phenology was not so obvious. However, between the different genotypes, the greatest differences were observed in the timing of autumn colouration and defoliation. Winter hardiness also showed clear differences between genotypes. Of the success parameters, it was most decisive as winter hardy genotypes had a higher occurrence of flowers and ornamental appearance, for example in *Rosa majalis* ‘Tornedal’. Some of these hardy genotypes with known characteristics were introduced to northern tourism areas to create examples of sustainable landscaping. Further, a list of potential plants for different northern sites was compiled.

Special forms with both scientific and ornamental value are occasionally found in wild species. One example of this is the red-leaved form of a pubescent birch, *Betula pubescens* f. *rubra*, which was studied in the plant selection experiments, and was used as a model tree to evaluate the role of anthocyanins in northern plants in a case study of northern birches. In the case study, the red-leaved pubescent birch showed some differences in flavonoid responses and growth rate in comparison to *Betula pubescens* and *Betula pubescens* ssp. *czerepanovii*. Phenology of the *B. p. f. rubra* was corresponding to that of the *B. pubescens*.

For cultivated woody plants the most important selection criteria for the northern areas are the suitability to local climate i.e. timing of phenological events and winter hardiness. Foliar anthocyanins seem to increase adaptation to northern growing conditions with high light intensity and low temperature.

Keywords: anthocyanins, landscaping, micropropagation, northern area, phenology, plant selection, winter hardiness, woody plant
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All the original papers were written with the support of co-authors. The plant selection experiments, original papers one, two and three, were established in the Northern Hardy Plants project before the author was involved in the study. The author participated on the field surveys, designed the experiment and data sorting for the original papers, and wrote and revised the original manuscripts. The fourth study was planned by Satu Huttunen and the author. The author was responsible for the data collection, microscopy and chemical analyses, and wrote the original manuscript. The fifth study was planned by the LABPLANT work group in which the author worked as a researcher. The author wrote and revised the original manuscript.

Oulu, November 2009

Henna Pihlajaniemi
List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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9
Introduction

The number of native woody plant species in Fennoscandia is small in comparison to the amount of native woody plant taxa occurring in other geographical areas - Eastern Europe, Eastern Asia and North America - in the boreal zone (Silander et al. 2000, Alanko 2001, Hämet-Ahti 2008). Also, the selection of hardy woody ornamentals for northern landscape gardening is considered limited, even though the cultivated woody plant selection has been enriched with foreign species and cultivars over centuries (Parvela 1930, Kerkkonen 1959, Alanko 2005). More problematic from the landscaping point of view, are the quality and origin of plant material commercially available (Tarvainen et al. 1993, Joy et al. 1994, Lagerström & Eriksson 1996, Juhanova et al. 1998, Raisio & Alanko 2008, Öberg 2009). It is generally accepted that the selection of cultivated plants, in the case of foreign taxa, should be enriched with species coming from climatically corresponding areas (Hämet-Ahti et al. 1992, Alanko 2005). The Finnish botanical gardens and Northern arboretum committee have made plant collection expeditions for example to West Siberia, Japan, China, South Korea and North America (Siuruainen et al. 2004, Koponen & Koponen 2008). The selection of cultivated forest trees in Finland has mainly been extended with different provenances of a Picea abies (L.) H. Karst., Pinus sylvestris L. and Larix sibirica Ledeb. (Silander et al. 2000, Hagman & Raisio 2008). In Sweden Pinus contorta Douglas ex Loudon has been exploited in forestry since the 1970’s (Engelmark et al. 2001).

Botanical gardens are scientific gardens that maintain living plant collections. They are specified by the facts that the collections are well documented, and that they serve botanical research and teaching in Universities (Piirainen & Schulman 2008, Primack & Miller-Rushing 2009). Botanical gardens nowadays have an important role in sustaining biodiversity, acting as gene banks both for valuable, old plant cultivars and endangered wild plant species (Bramwell & Kiehn 2000, Pautasso & Parmentier 2007, Piirainen & Schulman 2008). A further important role is related to studies on how climate change affects plant behaviour (Primack & Miller-Rushing 2009). In this thesis the plant register and collections in the University of Oulu’s Botanical Gardens in Finland play a critical role. Oulu Botanical Gardens (65°N, 25°E) has a gene bank register of hardy northern woody plant species that today includes some 300 plant taxa (Siuruainen et al. 2004). Oulu Botanical Gardens is also involved with acquisition and experimental cultivation of woody plants of different provenances, in order to test their
suitability for ornamental purposes in northern Finland (Siuruainen et al. 2004, Monem 2007).

Oulu Botanical Gardens is one of the world’s northernmost scientific gardens with about 4000 species or 5000 taxa growing in the outdoor garden (Alanko et al. 2004a, Monem 2007). The Tromsø Arctic Alpine Botanic Garden in Norway is located in the highest latitude 69°N of all the botanical gardens in the world (Torbjørn et al. 1997, Salmia 2004, Monem 2007). Its collection of arctic and alpine plants includes species from all over the northern hemisphere (Monem 2007). Lystigardur Akureyrar Public Park and Botanical Garden in Iceland (65°N) has about 7000 alien and about 430 native plant taxa growing in the garden (Index Seminum 2008). The Kirovsk Polar-Alpine Botanical garden located in Russia has the largest collection of woody plants, 763 taxa in 1998, north of the Arctic Circle (Kazakov 1998). The Georgeson Botanical Garden in Alaska, Fairbanks (64°N) is also one of the world’s northernmost botanical gardens (BGCI).

1.1 Selection of hardy plant material

Northern growing conditions for plants are considered harsh due high annual fluctuation in light and temperature, a cool and short growing season and nutrient poor soils. As the native plants have adapted to these growing conditions, the cultivation of introduced perennial material is challenging (Heide 1983, Laine et al. 2007, Wielgolaski & Karlsen 2007). The aim of plant selection in brief is to identify superior plant genotypes for cultivation (Webster 1988, Collicut & Davidson 1992). This work usually includes comparative field experiments where the performance of promising plant genotypes, clones, provenances or accessions is observed over several years under different climatic or hardiness zones (Webster 1988, Tigerstedt et al. 1998). In the selection experiments conducted in northern areas, most attention is paid to winter hardiness, disease and pest resistance, and ornamental value (Brander 1982, Juhanaja et al. 2001, Carlson-Nilsson 2002, Juhanaja 2007). Some experiments test propagation ability and recommendations may at final stage be based on this (Bengtsson & Jansson 1992, Juhanaja et al. 2001). Experiments can also include more specific measurements of plant cold hardiness e.g. artificial freezing tests (Suojala & Linden 1997). Further, selection experiments are needed for identification and naming of new plant cultivars, and in defining special characteristics of individual genotypes (Brander 1982, Webster 1988, Widrl echner 1990, Bengtsson & Jansson 1992, Juhanaja et al. 2001).
Selection experiments or plant trials have been established in several countries throughout the northern hemisphere. For example the NC-7 plant selection trials for evaluation of woody ornamentals in north central United States began in 1954, and the project has continued over decades (Widrlechner 1990). Canadians are famous especially for their shrub rose selection and breeding work (Ogilvie & Arnold 1992, Joy et al. 1994). In Europe plant selection projects have been conducted for example in Britain, Denmark, Netherlands, Norway and Sweden (Brander 1982, Webster 1988, Bengtsson & Jansson 1992, Sæbø et al. 2003, Carlson-Nilsson 2008, Öberg 2009).

In Finland, the extensive work aiming to improve the selection, quality and production of ornamental plants began in the 1980’s when several projects were launched. Of these KESKAS (Acquisition of hardy true-to-type parent plants, and culture of park and landscape plants in Finland) research concentrated to Central and Southern Finland (Alanko & Tegel 1989, Juhanoja 1992, Juhanoja et al. 2001). In northern Finland, the first project Pohjoiskalottitutkimus (Arctic region of the Nordic countries research) began in 1984 in co-operation with Sweden and Norway (Hellsten 1991, Pasanen et al. 1991). At the end of 1980’s it was followed by projects that aimed to improve the nursery garden material and nursery business in the north, and to develop micropropagation methods for hardy woody plants (Hellsten 1991, Laine et al. 2007). The potential plant material found during these projects was collectively registered in to POHKAS (Northern Hardy Plants) register maintained by the Oulu Botanical Gardens and the term “POHKAS plants” was established (Süruruainen et al. 2004, Laine et al. 2007). Of these registered genotypes the most promising were micropropagated and transferred to the selection experiments in order to determine their field performance in northern sites (Väinölä et al. 1995, Pihlajaniemi et al. 2003, Pihlajaniemi 2004). The design of the POHKAS plant selection experiments and the monitoring parameters used were as uniform as possible with the ones in the KESKAS clone selection (clone i.e. study plants were propagated from cuttings) experiments (Tarvainen et al. 1993, Juhanoja et al. 2001). The POHKAS register is located in the archives of the Oulu Botanical Gardens with limited access. It includes data about the origin, quality and propagation of the registered plants, and also digitalized photographs (Süruruainen 2009, personal communication).
1.2 Phenology and plant selection

In the northern areas, the photoperiod (i.e. day length) in combination with temperature, affects the annual growth rhythm and survival of woody plants. In spring the temperature has a significant effect on the onset of budburst and foliation, as the growth cessation and start of dormancy in autumn is strongly affected by a shortening photoperiod (Chunyang et al. 2003, Pudas et al. 2008). Plant phenology is a study in which the timing of seasonal periodic events such as budburst and flowering are observed (Rathcke & Lacey 1985, Lappalainen 1996, Cleland et al. 2007). Phenological records together with climatic data provide valuable information about the annual life cycles of plants and how the plants are adapted to the local climate (Lappalainen 1996). Knowledge of phenology is important in agriculture and forestry, and is used for example in the annual crop production estimations and in pollen warnings for allergy sufferers (Caprio et al. 1970, Lappalainen 1992, Beaubien & Hall-Beyer 2003, Meier et al. 2009). In northern plant selection experiments, phenology offers important method to understand why some plant genotypes are more successful than others, and it is used with other factors in identification of the most suitable genotypes for particular sites (Juhanoja et al. 2001, Pihlajaniemi et al. 2003, Pihlajaniemi 2004). Phenological knowledge is also used in the selection of seed sources of introduced plant material (Caprio et al. 1970).

One predicted effect of the global climate change is increase of the mean temperature that would have particular significance in the northern areas of the Northern Hemisphere (Karl & Trenberth 2003, IPCC 2007, Wielgolaski & Karlsen 2007). This may positively impact upon the cultivation possibilities of some desired plant taxa in northern areas, whereas the growth of plants adapted to the local combination of light and temperature can be disrupted, and species with limited adaptability may be lost (Beaubien & Hall-Beyer 2003, Wielgolaski & Karlsen 2007). Long-term phenological time series can be used to evaluate and predict future scenarios for the effect of climate change on both cultivated and native perennial plants (Beaubien & Hall-Beyer 2003, Aono & Kazui 2008, Meier et al. 2009).

1.3 Reproduction of hardy woody plants

In order to develop propagation methods, and to clone and test selected northern woody plants a micropropagation laboratory was established at the Oulu
Botanical Gardens (Laine 1990, Hellsten 1991). The operation in the micropropagation laboratory was started in 1990 with the aid of a grant from the Finnish Ministry of Agriculture and Forestry. The idea was that laboratory could partly apply the results from plant physiological and ecological research done at the Department of Biology, and vice versa support the research done at the department (Laine 1990). One example of this was the development of micropropagation method for the red-leaved pubescent birch, *Betula pubescens* f. *rubra* T. Ulvinen, and applying the method for commercial production (Kauppi & Ulvinen 1989). With the aid of the Oulu Botanical Gardens, micropropagation laboratories were also established in the northern horticultural colleges in Rovaniemi and Kempele (Hellsten 1991, Pasanen et al. 1991). The purpose of these laboratories was the commercial mass production of hardy plants for local nurseries (Hellsten 1991, Väinölä 1995, Siuruainen et al. 2004).

Micropropagation is vegetative plant propagation *in vitro* through aseptic cell, tissue or organ culture in which regeneration occurs via organogenesis or somatic embryogenesis. Today it is used worldwide by the plant propagation industry, with the majority of propagated species being ornamental plants (Haapala 2004, Winkelmann et al. 2006, Read 2007). Besides mass propagation, it is applied in production of genetically modified plant material, in plant breeding, in cryopreservation of valuable plant genotypes and in production of *in vitro*-derived plant secondary metabolites (Qiaochun et al. 2005, Häggman et al. 2007, Read 2007). In comparison to traditional plant propagation methods, micropropagation has several benefits. These include rapid and efficient multiplication of desirable plant genotypes, even from very small plant parts, and production of disease free plant material (Haapala & Niskanen 1992, Haapala 2004, Winkelmann et al. 2006, Read 2007). For some plant species difficult to propagate or with limitation of stock plant material, micropropagation may be the only method to obtain new plantlets. An additional benefit is that it can be performed all year around, even during the long winter months in Finland (Laine et al. 2007, Pihlajaniemi et al. 2008). Disadvantages can also be listed and these include, for example, the expensive costs of establishing the micropropagation laboratory and in production the labour costs of the laboratory personnel (Haapala & Niskanen 1992, Winkelmann et al. 2006). Problems are also related to transferring the plantlets from *in vitro* to *ex vitro* conditions (Winkelmann et al. 2006, Fuentes et al. 2007).
1.4 Anthocyanins in leaves of northern plants

Anthocyanins are water-soluble pigments derived from the flavonoid pathway with a colour range of red, purple, pink and blue (Lee & Gould 2002, Timmins et al. 2002). They are common pigments in flowers and fruits facilitating pollination and seed dispersal (Manetas et al. 2003). In leaves anthocyanin pigments are commonly masked by chlorophyll, though in several plant species visible concentrations of anthocyanins are accumulated transiently in juvenile and/or senescing leaves (Hoch et al. 2001, Feild et al. 2001, Manetas et al. 2003, Hughes et al. 2007). Some species, especially ornamental cultivars, exhibit permanently red leaves due to high anthocyanin concentrations (Manetas et al. 2003, Schmitzer et al. 2009). Anthocyanin accumulation in leaves has been noticed after stressful conditions like excess radiation, cold temperature and nutrient deficiency (e.g. Chalker-Scott 1999, Mendez et al. 1999, Timmins et al. 2002, Manetas et al. 2003).

In northern Fennoscandia, anthocyanins occur in the autumn foliage of deciduous trees like Prunus padus L., Sorbus aucuparia L. and Populus tremula L. (Keskitalo et al. 2005, Taulavuori 2006, Lev-Yadun & Holopainen 2009). In northern birches anthocyanins are apparent in the senescing red leaves of Betula nana L. and occasionally in its hybrids (Sulkinoja et al. 1981, Anamthawat-Jónsson & Thórsson 2003). A single specimen of pubescent birch producing high levels of anthocyanins was found in the early 1970’s from northern Finland (Mäkirinta & Similä 1980). The tree form, Betula pubescens Ehrh. f. rubra Ulvinen f. nova, was named and described by A. Kauppi and T. Ulvinen from the University of Oulu in 1989 (Kauppi & Ulvinen 1989). Betula pubescens f. rubra is an eye-catching tree due to its unusual colouration. In spring the bud scales are red, after bud burst the new leaves are delicate green, but red colour soon starts to spread from the leaf margins and leaves turn dark red (Kauppi & Ulvinen 1989). This colouration is evident throughout the growing season, and in autumn, leaves turn brilliant red as the chlorophyll in the leaves disintegrate. Since the tree form was cloned by micropropagation, it has become an important ornamental tree in northern landscaping (Pihlajaniemi et al. 2008). The tree form has also both great genetical value and scientific significance (Kauppi & Ulvinen 1989).
1.5  Aims of the study

The northern area, here the northern part of Fennoscandia above latitude 64°N up to the forest line including mid and northern boreal vegetation zones, is among the world’s northernmost areas where horticulture and landscaping is practised. While the hardy plant registering, selection and breeding work is still strongly concentrated on southern parts of Fennoscandia, the results can only partially be applied in northern parts of the area. Further, the more intensive land use in the form of increasing tourism sets special demands for the landscaping plants and sustainable plant use in the north.

Those plants valuable to northern landscaping may occur as special forms of known species, as in the case of *Betula pubescens* f. *rubra*. Despite the scientific value of this special tree form, it has not been actively studied. The functional role of anthocyanins in leaves has been studied mainly on tropical and temperate zone species.

The present work examines the data from long-term plant selection experiments with micropropagated woody plant material (I, II, III), and special characteristics of well thriving plant material (IV). Further this work introduces hardy plant material with known characteristics to northern landscaping purposes (V). The main objectives of the study are:

1. to evaluate the long-term field phenology and success of micropropagated woody plants in different northern climatic conditions (I, II, III)
2. to test and define the characteristics of successful and unsuccessful plant genotypes, as well as to identify the most valuable plant genotypes and their cultivation possibilities in the north (I, II, III)
3. to evaluate the possibility to reproduce woody plant material for cultivation in northern areas (I, II, III, IV, V)
4. to evaluate the role of foliar anthocyanins and other protective features under changing conditions in a case study with *Betula pubescens* f. *rubra* as a specific example (IV)
5. to introduce hardy plants for sustainable landscaping and restoration in northern tourism areas and give examples of plant use in specific sites (V)
2 Material and methods


2.1 Plant selection experiments (I, II, III)

2.1.1 Plant material and study sites

At the beginning of the 1990s, 19 promising plant genotypes were micropropagated from POHKAS registered mother plants in order to determine their special characteristics and to identify the best genotypes for northern landscaping in field experiments (Haapala & Niskanen 1992, Väinölä *et al.* 1995). Micropropagation was carried out both in the Oulu Botanical Gardens and in the Lapland Vocational College, Department of Natural Resources and Environment in Rovaniemi (Pihlahiemi *et al.* 2003). The genotypes represent woody ornamentals from *Alnus*, *Betula*, *Populus*, *Prunus*, *Rosa*, *Syringa* and *Viburnum* genera (Table 1).

The micropropagated seedlings propagated in spring 1990 were transferred to the field in autumn 1991, and material propagated in spring 1991 was transferred to the field in autumn 1992. At the time of planting the height of the trees varied between 30 and 70 centimetres (cm), of the roses between 15 and 40 cm, of the lilacs between 40 and 80 cm, and of the snowball viburnum ‘Pohjan Neito’ genotypes between 15 and 80 cm. The heights of *Syringa vulgaris* ‘Alba’ and *Viburnum lantana* genotypes were approximately 10 cm when transplanted.
Table 1. Genotypes* in the plant selection experiments (I, II, III). Their registration numbers, provenances and characteristic features. Study site is given in the last column.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Registration no</th>
<th>Provenance1</th>
<th>Characteristic</th>
<th>Study site2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Syringa villosa</em> Vahl 'Hirvas'</td>
<td>329</td>
<td>Rovaniemi (66°35'N)</td>
<td>light pink inflorescence turns white</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Syringa vulgaris</em> L. 'Alba'</td>
<td>84</td>
<td>Tornio (65°50'N)</td>
<td>white inflorescence</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Syringa × josiflexa</em> Preston ex Pringle (S. josikaea × <em>S. reflexa</em>) 'Veera'</td>
<td>327</td>
<td>Rovaniemi</td>
<td>upright growth habit, flaccid purple inflorescence</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Syringa × henryi</em> C.K. Schneider (S. josikaea × <em>S. villosa</em>) 'Paulus'</td>
<td>297</td>
<td>Rovaniemi</td>
<td>dense growth habit, dense pink inflorescence</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Viburnum lantana</em> L.</td>
<td>87</td>
<td>Tornio</td>
<td>upright and dense growth habit</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Viburnum opulus</em> L. 'Pohjan Neito'</td>
<td>89</td>
<td>Kempele (65°20'N)</td>
<td>sterile white flowers in snowball-like inflorescence</td>
<td>Na, -, -, -</td>
</tr>
<tr>
<td><em>Viburnum opulus</em> L. 'Pohjan Neito'</td>
<td>293</td>
<td>Tervola (66°05'N)</td>
<td>luxuriant growth habit, sterile white flowers in snowball-like inflorescence</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Viburnum opulus</em> L. 'Pohjan Neito'</td>
<td>91</td>
<td>Tornio</td>
<td>sterile white flowers in snowball-like inflorescence</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Rosa majalis</em> J. Herrmann 'Tornedal'</td>
<td>1022</td>
<td>Rovaniemi</td>
<td>double pink flowers</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Rosa majalis</em> J. Herrmann 'Tornedal'</td>
<td>50</td>
<td>Tornio</td>
<td>double pink flowers</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td>*Rosa 'Poppius' (Hybrid Spinosissima Group)</td>
<td>1035</td>
<td>Rovaniemi</td>
<td>fragrant, semi-double pink flowers</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td>*Rosa 'Sipi' (Hybrid Rugosa Group)</td>
<td>1028</td>
<td>Rovaniemi</td>
<td>branches almost thornless, rose-red flowers</td>
<td>Na, -, Ruuk, Sot</td>
</tr>
<tr>
<td>*Rosa 'Splendens' (R. 'Frankfurt', Hybrid Gallica Group)</td>
<td>44</td>
<td>Tornio</td>
<td>simple scarlet flowers</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Rosa × spaethiana</em> Graebner (R. palustris × <em>R. rugosa</em>, Hybrid Rugosa Group)</td>
<td>290</td>
<td>Rovaniemi</td>
<td>robust growth habit, simple pink flowers</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td>Genotype</td>
<td>Registration no</td>
<td>Provenance(^1)</td>
<td>Characteristic</td>
<td>Study site(^2)</td>
</tr>
<tr>
<td>--------------------------------</td>
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<td>--------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>Paper III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alnus incana</em> (L.) Moench f. laciniata Loudon</td>
<td>2</td>
<td>Oulu (65°01'N)</td>
<td>densely-lobed leaves</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Betula pendula</em> Roth f. crispa (Reichenb.) Hämet-Ahti</td>
<td>9</td>
<td>Pietarsaari (63°40'N)</td>
<td>densely-lobed leaves</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Betula pubescens</em> Ehrh. f. rubra Ulvinen</td>
<td>12</td>
<td>Yli-Kilminki (64°55'N)</td>
<td>purple red-leaved</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Populus tremula</em> L. 'Erecta'</td>
<td>257</td>
<td>Oulu</td>
<td>columnar growth habit</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
<tr>
<td><em>Prunus maackii</em> Rupr.</td>
<td>38</td>
<td>Kilminki (64°55'N)</td>
<td>shiny and peeling red-brown bark</td>
<td>Na, Ro, Ruuk, Sot</td>
</tr>
</tbody>
</table>


\(^1\) In the table the coordinates are presented only once for the same location

\(^2\) Study sites: Na=Naruska, Roi=Rovaniemi, Ruuk=Ruukki and Sot=Sotkamo
Twelve (n = 12) replicates, individual plants, of each genotype were planted at four different study sites (Fig. 1). The northernmost site, Naruska experimental field, has acid peat soil, which was improved by adding sand and lime (Tarvainen et al. 1993). The site is characterized by a cold and continental climate, night frosts are common during the growing season (Tarvainen et al. 1993). The other sites used - Agricultural Research Centre of Finland’s regional research stations in Rovaniemi, Ruukki and Sotkamo - have a mineral soil. Ruukki and Sotkamo sites are located in the same latitude (64°N), but their local climate differs as Ruukki is located in the northern Ostrobothnian lowlands in vicinity of the Baltic Sea and Sotkamo is in eastern Finland, in the Kainuu esker region with a more continental climate. When the site at Rovaniemi was established, it was chosen at the basis that it represented most harsh conditions within the cultivation zone (Uusitalo M 2009, personal communication). The duration of the growing season and effective temperature sum along the north – south gradient of these sites increases (Fig. 2). Greatest daylength variation occurred between the sites at the time of the summer solstice (Fig. 3). Finland is divided into eight climatic zones in relation to the cultivation of fruit trees and woody ornamentals (Solantie 1988). Naruska site is located in the VII, Rovaniemi in VI, Sotkamo in IV/V and Ruukki in V zones.
Fig. 1. (A) Study sites (1-4) in the plant selection experiments (I, II, III). In the map Oulu Botanical Gardens and the demonstration areas established in the LABPLANT subproject (V) are also shown. (B) Photograph taken from the Sotkamo site summer 1998.

1. NARUSKA 67°N, 29°E, 213 m a.s.l.
2. ROVANIEMI 66°N, 26°E, 103 m a.s.l.
3. RUUKKI 64°N, 25°E, 45 m a.s.l.
4. SOTKAMO 64°N, 29°E, 150 m a.s.l.
Fig. 2. A) Duration of the growing season, and B) the effective temperature sum in the study sites during the plant selection experiments. Growing season began when the daily mean temperature exceeded 5°C on five days in succession and the ground was at least half snow free. It ended when daily mean temperature dropped continuously below 5°C. Effective temperature sum was calculated by summing the daily mean temperatures of the days on which the temperature exceeded 5°C from the beginning of the growing season. The presented data from Rovaniemi, Ruukki and Sotkamo were obtained from the regional research stations. In the case of Naruska, data were obtained from the Finnish Meteorological Institute from the nearest meteorological station located 45 km SW of the study site.
Fig. 3. Annual variation in the day lengths at the study sites. Day lengths were calculated from the sunrise and sunset data obtained from the Almanac Office at University of Helsinki. The Naruska data were from measuring point located 45 km SW and Ruukki data were from measuring point located app. 30 km W of the study site.

2.1.2 Experimental design and study parameters

In the field, replicates were planted in rows in random order with 1.5x2 m between shrubs and 2x3 m between trees. They were marked with metallic labels, on which number code for the genotype, replicate number and plantation year was engraved. Trees and shrubs were planted to form separate groups in the field. A covering sheet (Mypex) was placed on the ground for weed control, and the interlinear walkways were sown with grass. Plants were fertilised with Kemira NPK 10:7:14 during the first three years. Dead and broken branches were cut off every spring, and weeds were removed mechanically.

The monitoring parameters used in the field experiment are presented in Table 2. Height growth of the plants was also measured in spring and autumn, but not systematically every year. A qualitative description of autumn colouration was performed occasionally. Observations in the field were made approximately twice a week by the local field staff, and results were written into observational tables. The recorded data were saved at each study site and also sent to the Oulu Botanical Gardens. The data presented in the original papers I, II and III was
recorded between the years 1993 and 1999. In data processing the monitoring parameters were divided into two categories; phenology (date, continuous scale) and success (five-point grade, ordinal scale). Depending on the scale, genotype-specific mean or median values were computed separately for every study site. The data were analyzed by means of one-way ANOVA or with non-parametric Kruskal-Wallis test in the SPSS (version 13.1; SPSS Inc., Chicago, IL, USA) statistical package with genotype as grouping factor. Comparisons were made within plant groups; roses, lilacs, viburnums and trees, and separately for each study site.

The design is currently available in Naruska site. Gene banks for the genotypes exist in the living plant collections of the Oulu Botanical Gardens and Lapland Vocational College, and in the arboretum of the Agricultural Research Centre of Finland’s regional research station in Rovaniemi. Some genotypes are also maintained in micropropagation at the Oulu Botanical Gardens.
<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Scale explanation</th>
</tr>
</thead>
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<tr>
<td>April–June</td>
<td></td>
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</tr>
<tr>
<td>Onset of foliation</td>
<td>date</td>
<td>when 10% of bud scales are open and buds are green</td>
</tr>
<tr>
<td>Full foliation</td>
<td>date</td>
<td>when over 90% of buds are open</td>
</tr>
<tr>
<td>Winter hardiness</td>
<td>1-5</td>
<td>1:dead, 2:dead to ground level, 3:dead to snow level, 4:shoot tips dead, 5:tip buds dead or no damage</td>
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<tr>
<td>May–July</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset of flowering</td>
<td>date</td>
<td>first 10% of flower buds flushed</td>
</tr>
<tr>
<td>End of flowering</td>
<td>date</td>
<td>last 10% of flower buds flushed, 90% of flowers wilted</td>
</tr>
<tr>
<td>Occurrence of flowers during full flowering</td>
<td>1-5</td>
<td>1:non, 2:few, single (1-10), 3:moderate (11-30), 4:rather abundant (31-50), 5:abundant (over 50)</td>
</tr>
<tr>
<td>July</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ornamental appearance</td>
<td>1-5</td>
<td>1:ugly, 2:rather ugly, 3:moderate, 4:rather beautiful, 5:beautiful</td>
</tr>
<tr>
<td>Occurrence of leaf diseases</td>
<td>1-5</td>
<td>1:over 90% injury, 2:70% injury, 3:50% injury, 4:30% injury, 5:no significant injury</td>
</tr>
<tr>
<td>Occurrence of leaf pests</td>
<td>1-5</td>
<td>1:over 90% injury, 2:70% injury, 3:50% injury, 4:30% injury, 5:no significant injury</td>
</tr>
<tr>
<td>Occurrence of shoot diseases</td>
<td>1-5</td>
<td>1:over 90% injury, 2:70% injury, 3:50% injury, 4:30% injury, 5:no significant injury</td>
</tr>
<tr>
<td>Occurrence of shoot pests</td>
<td>1-5</td>
<td>1:over 90% injury, 2:70% injury, 3:50% injury, 4:30% injury, 5:no significant injury</td>
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<tr>
<td>July–August</td>
<td></td>
<td></td>
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<tr>
<td>Occurrence of fruits</td>
<td>1-5</td>
<td>1:non, 2:few, single (1-10), 3:moderate (11-30), 4:rather abundant (31-50), 5:abundant (over 50)</td>
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<tr>
<td>August–October</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset of autumn colouration</td>
<td>date</td>
<td>first 10% of leaves autumn coloured</td>
</tr>
<tr>
<td>End of autumn colouration</td>
<td>date</td>
<td>last 10% of leaves autumn coloured</td>
</tr>
<tr>
<td>Onset of leaf defoliation</td>
<td>date</td>
<td>first 10% of leaves shed</td>
</tr>
<tr>
<td>End of defoliation</td>
<td>date</td>
<td>last 10% of leaves shed</td>
</tr>
</tbody>
</table>
2.2 Foliar anthocyanins - A case study of northern birches (IV)

2.2.1 Garden trees in situ

Phenology - bud burst, flowering, leaf development and beginning of leaf senescence - was recorded in micropropagated *Betula pubescens f. rubra* (n = 6) and naturally occurring *Betula pubescens* Ehrh. (n = 3) trees over the growing seasons 2001 and 2002 (IV). The trees were growing at the margin of a park like area and scarce forest stand near Oulu Botanical Gardens (Fig. 1). Leaf samples were collected in liquid nitrogen at seven different collection dates during both growing seasons (IV). Total anthocyanin content was measured (Shimadzu Bio Spec-1601 spectrophotometer) from leaf discs extracted in acid methanol (Mancinelli *et al.* 1988) and chlorophyll concentration was measured (Shimadzu UV-1700 PharmaSpec) from leaves homogenized in 80% acetone (Arnon 1949). Climatic data were obtained from nearby automatic weather station (Milos 200 DM21). Individual anthocyanins in *Betula pubescens f. rubra* leaf samples were identified by solid phase high performance liquid chromatography (HPLC-DAD Shimadzu LC-10 Avp, Phenomex Inertsil 5 ODS 2 column) with methods by Fuleki & Francis (1968), Huopalahti *et al.* (2000) and Terahara *et al.* (2000). Localisation of anthocyanins was studied from fresh leaf strips in 10% sucrose solution using a light microscope (Nikon Optihot-2). No statistical analyses were performed.

2.2.2 Enhanced UV-B experiment

Micropropagated seedlings of *Betula pubescens f. rubra*, *Betula pubescens* (provenance 65°N, 25°E) and *Betula pubescens* ssp. czerepanovii (N.I. Orlova) Hämet-Ahti (provenance 68°N, 25°E) were placed in an enhanced UV-B radiation experiment, simulating a 20% ozone depletion over the growing seasons 2003 and 2004 (IV). The experiment was arranged in the experimental field of the University of Oulu with 14 lamp banks half of which were covered with polyester filters (absorption below 315 nm) for the control treatment, and half with cellulose acetate filters (transmission down to 290 nm) for the enhanced UV-B treatment (Laakso *et al.* 2001). Radiation levels (100% capacity between 10 am - 4 pm each day) were measured with a spectroradiometer (model 754, Optronics, Orlando) between 250 nm – 400 nm. The radiation levels measured in 2003 were higher than those in 2004. Leaf samples were collected for pigment analyses and
scanning electron microscopy in June and August 2003 and in July and August 2004. Electron microscopy samples included newly developed leaves from the tip of the branches and lower, fully developed leaves. Height of the stems and radial diameter of the base of the stems was measured at the beginning of June and at the end of August in both years.

Total anthocyanin content was calculated as cyanidin-3-glucoside equivalents from leaves homogenized in acid methanol with a method by Taulavuori et al. (2004). Total concentrations of soluble UV-B absorbing compounds were measured (Beckman DU-64 spectrophotometer) at 320 nm, 300 nm and 280 nm from leaf discs homogenised in acid methanol (Mirecki & Teramura 1984). Leaf surface waxes and trichomes were observed with a field emission scanning electron microscope (FESEM, JSM 6300F) from adaxial sides of leaf discs sputtered with a gold-palladium layer (80:20, 45 nm, Polaron E5100). The distribution of crystalline wax deposits was evaluated using the classification by Vanhatalo et al. (2001) in which class I represented now or few wax sculptures (0–10%) and class V abundant wax sculptures (70–100%). The amount of non-glandular trichomes was evaluated as: absent, sparse, fairly abundant or very abundant (Valkama et al. 2003). The amount of glandular trichomes (number/mm²) was calculated from the micrographs.

The data, except abundance of non glandular trichomes, was tested with T-test and one-way ANOVA, or in the case that assumptions of the parametric tests were not met, with Mann-Whitney U-test and with Kruskal-Wallis Test. All tests were performed using the SPSS (version 16.0; SPSS Inc., Chicago, IL, USA) statistical package.

### 2.3 Plant material for northern landscaping and restoration, LABPLANT subproject (V)

Hardy plants were introduced in tourism areas in Finnish Lapland between the years 2004–2007 in the LABPLANT (Production of Plant Material for Landscape Planning, Greening and Restoration) subproject. Aim was to transfer the information about propagation methods and cultivation possibilities of the registered hardy plants and other potential plant material in the collections and databases at Oulu Botanical Gardens and Lapland Vocational College in to practise. LABPLANT was one of the four subprojects of the EU LIFE Environment project Tourist Destinations as Landscape Laboratories – Tools for Sustainable Tourism (LANDSCAPE LAB) (Jokimäki & Kaisanlahti-Jokimäki
and concentrated on selecting, listing and producing hardy plants for landscaping and restoration in northern tourism areas (Laine et al. 2007, Pihlajaniemi et al. 2008).

Hardy plants were selected based on the results from previous projects and plant collections and databases in the Oulu Botanical Gardens and Lapland Vocational College (Laine et al. 2007, I, II, III, V). Besides deciduous trees and shrubs, conifers, herbaceous and dwarf shrub species were selected and included in the plant list, but they are not discussed in this thesis. The subproject emphasised especially on wild plants, native to Finland. A second list including information about the suitability of these plants to different sites was made based on the expertise of the participating institutes (Laine et al. 2007, V). Over 100 taxa, including 35 deciduous tree and shrub taxa, were propagated at the Oulu Botanical Gardens and Lapland Vocational College between the years 2004 and 2006, and transplanted to demonstration areas in years 2006 and 2007 (Fig. 1). Woody taxa, for example *Betula nana*, *Betula pubescens* ssp. *czerepanovii*, *Rosa majalis* ‘Tornedal’ and *Sorbus aucuparia*, were mainly propagated by micropropagation. Some willow taxa, for example *Salix lanata* L. and *Salix myrsinites* L., were propagated from cuttings. The six demonstration areas represented (i) urban and rather densely built tourist village, (ii) transition zone between urban and natural landscape, and (iii) natural landscape from the Pallas-Yllästunturi National Park, and Levi and Ylläs tourism areas (75°N, 25°E).
3 Results

Recorded climatic data during the plant selection experiments (I, II, III) showed both annual variation between the study sites and also interannual variation between the study years. During the experiments duration of the growing season and effective thermal sum were every year the lowest in the Naruska site. On the average, the growing season was longest in Sotkamo and the thermal sum highest in Ruukki. During the cold winter, year 1994, at Naruska, Rovaniemi and Sotkamo sites minimum temperature dropped down to -40°C temperature.

3.1 Plant selection experiments

The micropropagated woody plant genotypes were viable under northern field conditions (I, II, III). Most of the studied 19 woody plant genotypes grew well in Ruukki and Sotkamo, however some differences in the field performance within genotype were observed. In the northernmost Naruska site, Viburnum lantana, Syringa vulgaris ‘Alba’ and all the tree genotypes except Betula pubescens f. rubra died after the first or second winter in the field. The selection of successful plants included ‘Tornedal’, ‘Sipi’ and ‘Poppius’ genotypes from the roses studied (II), Syringa × henryi ‘Paulus’ from the large shrubs (I) and Betula pubescens f. rubra from the trees (III). In Rovaniemi, the selection of potential genotypes was higher including both Viburnum opulus ‘Pohjan Neito’, Syringa × josiflexa ‘Veera’ and Alnus incana f. laciniata genotypes (I, III). None of the 12 replicate plants of Prunus maackii were alive in the third growing season in Rovaniemi and most of the Betula pendula f. crispa genotypes also died during the experiment (III).

3.1.1 Phenology

Onset of foliation was latest in the northernmost site - starting at the beginning of June - and buds opened within a few days (Fig. 4). At the other sites, the onset of foliation and the foliation period (days) showed more variation, though on average onset of foliation was in Rovaniemi at the end of May and in Ruukki and Sotkamo around mid-May (I-III, Fig. 4). Of the roses, ‘Tornedal’ genotypes were the first and ‘Splendens’ the last to start foliation (II). Within other groups - viburnums, lilacs and trees - early or late genotypes were not identified, as the foliation period varied inconsistently (I, III). Most genotypes began foliation
between one to ten days earlier in the western Ruukki than in the eastern Sotkamo site, both located at 64°N, (I, II, III).

Shrub genotypes started to flower during their second growing season in the field. Of the trees, *Betula pubescens* f. *rubra* flowered during the last two years in the experiment, and *Prunus maackii*, flowered in eastern Sotkamo for the first time in the summer of 1996 (III). The same phenology trend that was seen between the sites in the onset of foliation was also seen in the onset flowering (Fig. 4). With all the shrubs, the onset of flowering was latest at the northernmost site and earliest at the Ruukki site. However, no clear trend for a longer flowering period at the more favourable sites was noticed (I, II, Fig. 4). The onset of flowering for some shrubs at the Naruska site was so late as to severely limit their potential as ornamental plants in this area. For example with *Rosa* ‘Splendens’, *Rosa* ‘Poppius’ and *Viburnum opulus* ‘Pohjan Neito’ genotypes flowering started at the end of July, and with *Syringa × josiflexa* ‘Veera’ in August (I, II). Of the roses, ‘Tornedal’ and ‘Sipi’ genotypes had generally shorter flowering periods than ‘Splendens’ and ‘Poppius’ (II). Of the viburnums, ‘Pohjan Neito’ genotypes had longer flowering period than *Viburnum lantana* (I). The lilacs with whitish coloured inflorescence ‘Alba’ and ‘Hirvas’ flowered poorly in the two northernmost sites, lilacs with pink ‘Paulus’ and purple ‘Veera’ inflorescence had slightly longer flowering periods (I). The lengths of flowering periods varied significantly between roses at every site, between lilacs in Sotkamo and between viburnums in Ruukki and Sotkamo sites (I, II).

The onset of autumn colouration occurred for most genotypes at the end of August and at the beginning of September (Fig. 4). *Viburnum lantana*, *Syringa vulgaris* ‘Alba’, *Rosa* ‘Splendens’ and *Betula pendula* f. *crispa* genotypes were noticed to be the last to start leaf senescence (I, II, III). In the case of *Alnus incana* f. *laciniata*, no autumn colouration was observed (III). With all the shrub genotypes, the period for full autumn colour to change was longest in Ruukki (I, II, Fig. 4). The defoliation, in the case of lilacs, started after mid September, with ‘Alba’ being the last genotype (I). With viburnums, the onset of defoliation was in between late September and early October (I). The defoliation of roses varied from the end of September at the Rovaniemi site, to the beginning of the same month in Ruukki, with ‘Splendens’ being the last one to start defoliation (II). The onset of tree defoliation occurred in September, except *Betula pendula* f. *crispa* in Rovaniemi and Ruukki, and *Populus tremula* ‘Erecta’ in Rovaniemi, which began at the beginning of October (III).
The genotypes were leafless in mid or late October, with the exceptions of *Betula pubescens* f. *rubra* in Ruukki and Sotkamo at the end of September, and *Viburnum lantana* in Ruukki at the beginning of November (I, III). For most genotypes, full defoliation took over 20 days (I, II, III). Roses had long defoliation periods at Ruukki, with an average of 40 days (II, Fig. 4). Notably the full defoliation of *Betula pendula* f. *crispa* took 43 days in eastern Sotkamo site, but only 9 days in Ruukki (III). Occasionally with *Betula pendula* f. *crispa*, *Viburnum lantana* and *Syringa vulgaris* ‘Alba’, the development of autumn colouration or full defoliation was not complete on the last date of observation (I, III).

![Phenology profile](image)

**Fig. 4. Indicative phenology profiles of four genotypes in the different study sites.**

The weather statistics and correspondingly the timing of phenological events showed clear interannual variation between the years 1993 and 1999. The onset of foliation in the genotypes studied was in most cases several days later than the onset of thermal growing season (Table 3). The onset of autumn colouration varied yearly so that it occurred before or after the end of the thermal growing season. The data show that even though the growing season continued until late autumn the start of leaf senescence, on average, was not delayed (Table 3).
Table 3. Interannual variation in phenology. The onset of foliation and autumn colouration in the study sites during the survey period 1993–1999. The dates for the plant groups 'lilacs', 'viburnums' and 'roses' are average values (I, II). From group 'trees' single genotype *Betula pubescens* f. *rubra* is presented (III). The onset and end of thermal growing season is in the parentheses. For other details, see figure 2 legend.

<table>
<thead>
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<th>Year</th>
<th>Rosa Onset of foliation (Onset of thermal growing season in parentheses)</th>
<th>Syringa</th>
<th>Viburnum</th>
<th><em>Betula pubescens</em> f. <em>rubra</em></th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Rovaniemi</td>
<td>Ruukki</td>
<td>Sotkamo</td>
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- no data from the site
3.1.2 Success

Genotypes suffered most winter injury during their first or second winter in the field. At every site the hardiest genotypes were ‘Tornedal’ roses, *Syringa × henryi* ‘Paulus’ and *Betula pubescens* f. *rubra* with no or only small injuries (I, II, III). *Betula pendula* f. *crispa* was most susceptible to winter injury (III). *Rosa* ‘Splendens’, *Syringa vulgaris* ‘Alba’ and *Alnus incana* f. *laciniata* genotypes experienced also more damage than the genotypes generally (I, II, III). Still in most cases, winter hardiness was considered rather good or good, with median values 4 and 5 respectively (Fig. 5). Least winter damage was recorded in the Sotkamo site. Winter hardiness differed significantly between viburnums in Naruska, Ruukki and Sotkamo (I), between lilacs in Naruska, Rovaniemi and Ruukki (I), between roses in each site (II), and between trees in Rovaniemi and Ruukki (III). It was observed that the branches carrying the metal labels with codes for the genotypes died during winter.

For most genotypes, the ornamental appearance in July was evaluated as moderate or rather beautiful, median values 3 and 4 respectively. *Viburnum lantana* and *Syringa vulgaris* ‘Alba’ were rated ugly in Rovaniemi and *Betula pendula* f. *crispa* in Ruukki (I). The highest rating, beautiful, was given for *Betula pubescens* f. *rubra* at the northernmost site (Fig. 6), and for *Alnus incana* f. *laciniata*, *Rosa* ‘Poppius’ and *Rosa* ‘Sipi’ in eastern Sotkamo site. The ornamental appearance differed significantly between viburnums in Rovaniemi and Sotkamo (I), between lilacs and roses at every site (I, II), and between trees in Ruukki and Sotkamo (III).

Lilac and viburnum flowering was poor in the two northernmost sites, as the occurrence of flowers during full flowering was few or none (I) (Fig. 5). Further at the northern sites, flowering of roses was at best moderate, except for abundant flowering of *Rosa majalis* ‘Tornedal’ genotypes in Naruska (II) (Fig. 7). Occurrence of flowers in *Viburnum lantana* was poor or moderate, even at the more favourable sites (I). Of the *Viburnum opulus* ‘Pohjan Neito’ genotypes, genotype 293 had the lowest abundance of flowers (I). Occurrence of flowers in lilacs ‘Alba’ and ‘Hirvas’ was low, but moderate or rather abundant in ‘Veera’ and ‘Paulus’ (I). The abundance of flowers was higher in the rose genotypes grown in Ruukki than in Sotkamo site, both located in the same latitude (II). Of the trees the occurrence of flowers in *Prunus maackii* was scant (III). Occurrence of flowers between viburnums (I), lilacs (I) and roses (II) varied significantly at each study site, except for viburnums at the northernmost site. The occurrence of fruits
was evaluated in roses and in *Viburnum lantana*. *Rosa majalis* ‘Tornedal’ genotypes had no fruits at all (II). *Rosa* ‘Splendens’ had the highest number of fruits of the studied roses (II). Occurrence of fruits in *Viburnum lantana* was poor (I).

No serious plant infections or pest damage were observed during the experiment. Damage was observed more often in leaves than shoots. Of the sites studied, the highest frequency of damage was recorded in Rovaniemi, and the lowest in Naruska (I, II, III). Rose leaves were infected by mildew and rust fungi, *Rosa* ‘Poppius’ had the lowest resistance against diseases in the field experiment (II). Roses also suffered from herivory due to hares and aphids (II). Viburnums and lilacs had limited damage to leaves only in Rovaniemi due to undefined leaf diseases and pests (I). Of the tree genotypes studied, the leaves of *Betula pubescens* f. *rubra* had a small amount of rust fungi, and also suffered from minor damage due to leaf pests (III). *Populus tremula* ‘Erecta’ trunks were damaged by mountain hares and leaves were partly infected by fungal disease (III) (Fig. 8).

Autumn colouration was described as ‘beautiful’ - with at least partial change to red or multi-colour in *Betula pubescens* f. *rubra*, viburnums, roses, *Syringa × josiflexa* ‘Veera’ and *Syringa × henryi* ‘Paulus’ and *Populus tremula* ‘Erecta’ (I, II, III). The leaves of *Betula pendula* f. *crispa* and *Prunus maackii* were single-coloured yellow (III). At the continental sites, most genotypes were considered beautiful during their full autumn colouration. It is interesting to note that the genotypes which died during the first winters at Naruska, did not change colour and were still green when the first snow came.
Fig. 5. Snowball viburnum ‘Pohjan Neito’ genotype 91 in Naruska (smaller picture) and in Ruukki (larger picture). In Naruska the shoots over wintered under snow, while parts above snow level experienced winter injury every year. Photo: V. Saariniemi 23.7.1998. In Ruukki the same genotype had reached over 120 cm height and flowered abundantly. Photo: K. Sorvari 15.7.1998. In Naruska winter hardiness had median value 4 and in Ruukki 5.
Fig. 6. In the northernmost site *Betula pubescens f. rubra* grew densely. All twelve replicate plants of this genotype were growing well when the site was visited summer 2008. Photo: H. Pihlajaniemi 14.7.2008.

3.2 A case study of northern birches

3.2.1 Garden trees in situ

Bud burst in *Betula pubescens* f. *rubra* and *Betula pubescens* occurred in spring when the effective temperature sum reached 37 dd (IV). In *Betula pubescens* f. *rubra* leaves, the anthocyanin concentration rose during the growing season, with highest levels being found in June and August. Chlorophyll content showed small variation between the birches, while chlorophyll a/b ratio was higher in *Betula pubescens* leaves (IV). Anthocyanins were located in the upper and lower epidermis of *Betula pubescens* f. *rubra* leaves. The leaf epidermis was a mosaic formed by red and green pigmented cells, leaf veins were red and stomatal guard cells green (IV). HPLC analysis quantified four anthocyanins from *Betula pubescens* f. *rubra* leaves; cyanidin-3-arabinoside, cyanidin-3-glucoside, cyanidin-3-galactoside and delphinidin-derivate (IV).
3.2.2 Enhanced UV-B responses

The total foliar anthocyanin content of the micropropagated birch seedlings was not affected by enhanced UV-B radiation (IV). There was also no strong response in the concentration of soluble UV-B absorbing compounds (IV). Only *Betula pubescens* f. *rubra* had a higher concentration of UV-B absorbing compounds at 300 nm in the UV-B treatment than in the control, with the only statistically significant difference between treatments occurring in 2004 (IV). In *Betula pubescens* and *Betula pubescens* ssp. *czerepanovii* leaves the amount of UV-B absorbing compounds was higher in the 2003 samples, corresponding to higher radiation levels. No such response was seen in *Betula pubescens* f. *rubra* (IV). Of the taxa studied *Betula pubescens* ssp. *czerepanovii* had the highest amount of UV-B absorbing compounds in both years and treatments (IV).

No clear effect of the enhanced UV-B radiation on the abundance of leaf waxes was seen. The abundance varied more or less randomly between and within the different birch taxa in different treatments and between leaves of different developmental stages. The abundance of (short and long) non-glandular trichomes varied from sparse to very abundant with the birch taxa studied, but no difference between treatments was seen (IV). Interestingly, in 2004 there where more glandular trichomes on the leaves grown under control conditions, than on those under UV-B treatment for every birch taxa, but the result was not statistically significant (IV). Generally, the leaves of *Betula pubescens* ssp. *czerepanovii* had more glandular trichomes than the leaves of *Betula pubescens* f. *rubra* and *Betula pubescens* (IV).

The growth of micropropagated birch seedlings was not affected by enhanced UV-B radiation. By comparison to the green leaved taxa, *Betula pubescens* f. *rubra* showed significantly faster growth rate (IV).

3.3 Hardy plants for northern landscaping and restoration

The selection of potential plants for northern landscaping and restoration was rather large, including 70 deciduous native and foreign tree and shrub taxa from the genera *Amelanchier*, *Alnus*, *Aronia*, *Betula*, *Caragana*, *Cotoneaster*, *Crataegus*, *Dasiphora*, *Lonicera*, *Malus*, *Populus*, *Prunus*, *Ribes*, *Rosa*, *Salix*, *Spiraeae*, *Sorbaria*, *Sorbus*, *Syringa* and *Viburnum* (Laine et al. 2007, V). These plant taxa were evaluated as suitable for seven different site types in the northern tourism areas presented in Table 4.
To demonstrate plant use in these typical northern site types, six experimental areas were established. At the two demonstration areas near Pallastunturi Visitor Centre, representing natural landscape, only wild herbaceous and dwarf shrub species like *Vaccinium uliginosum* L. were planted in order to accelerate the natural succession (V, Laine *et al.* 2007). In the two demonstration areas located at the transition zone in Levi tourism area mainly wild, for example *Betula nana, Juniperus communis* L., *Salix myrsinites* and some ornamental taxa were planted (Laine *et al.* 2007). In the two demonstration areas located in densely built environment in Ylläs tourism area, several ornamental taxa, for example *Dasiphora fruticosa* (L.) Rydb. ‘Tervola’, *Rosa majalis* ‘Tornedal’ and *Spiraea betulifolia* Pallas together with some wild plant taxa, like *Salix glauca* L., were planted (Laine *et al.* 2007). The demonstration areas established during this study are pilot sites for future research. As the success of the transplanted plant material in the areas will be assessed after several years monitoring, the results are not presented in this thesis. Some of the propagated plants were also planted into the collections of the Oulu Botanical Gardens and Lapland Vocational College (V, Laine *et al.* 2007, Pihlajaniemi *et al.* 2008).
Table 4. Selection of potential deciduous tree and shrub taxa for landscaping and restoration purposes at different sites in the northern (tourism) areas (Laine et al. 2007, V). Names of cultivated plants are according to Räty & Alanko (2004) and native plants* according to Hämet-Ahti et al. (1998) and Hämet-Ahti et al. (2005). For restoration local provenance of native species are used.

<table>
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<tr>
<th>Plant taxa</th>
<th>1. Dry and open e.g. low herb meadow</th>
<th>2. Mesic and open e.g. tall herb meadow</th>
<th>3. Transition zone</th>
<th>4. Dry with scarce tree stand e.g. heath forest</th>
<th>5. Mesic with dense tree stand e.g. herb-rich forest</th>
<th>6. Wetland and shore</th>
<th>7. Peat land with scarce tree (pine) stand e.g. marsh</th>
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<td><em>Salix glauca</em> L.</td>
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<td><em>Salix hastata</em> L.</td>
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<td><em>Salix lanata</em> L.</td>
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<td><em>Salix lapponum</em> L.</td>
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<td><em>Salix myrsinifolia</em> Salisb. ssp. myrsinifolia*</td>
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<td><em>Salix myrsinifolia</em> Salisb. ssp. borealis (Fr.) Hyl.</td>
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<td>Plant taxa</td>
<td>1. Dry and open e.g. low herb meadow</td>
<td>2. Mesic and open e.g. tall herb meadow</td>
<td>3. Transition zone</td>
<td>4. Dry with scarce tree stand e.g. heath forest</td>
<td>5. Mesic with dense tree stand e.g. herb-rich forest</td>
<td>6. Wetland and shore</td>
<td>7. Peat land with scarce tree (pine) stand e.g. marsh</td>
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<td>Salix myrtilloides L. *</td>
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<td>Salix repens L. ssp. repens</td>
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<td>Salix repens L. ssp. repens var, argentea (Sm.) E.G. Camus &amp; A. Camus</td>
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<td>Salix reticulata L. *</td>
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<td>Sorbaria sorbifolia (L.) A.Braun</td>
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<td>Spiraea betulifolia Pallas</td>
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<td>Spiraea chamaedryfolia L.</td>
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<td>Spiraea chamaedryfolia L. var. ulmifolia (Scop.) Maxim.</td>
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<td>Spiraea japonica L.f. (callosa) ‘Odensala’</td>
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<td>Spiraea media Fr.Schmidt</td>
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<td>Spiraea salicifolia L.</td>
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<td>Spiraea × rosalba Dippel (×ba × latifolia, &quot;salicifolia&quot;)</td>
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<td>Syringa josikaea Jacq.f. ex Rchb.</td>
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<td>Syringa × henryi C.K.Schneid. (josikaea × villosa)</td>
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<td>Syringa × josiflexa Preston ex</td>
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<td>Prunus (josikaea × reflexa) ‘Veera’</td>
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<td>Viburnum opulus L. ‘Pohjan Neito’</td>
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1Transition zone is area located between managed and natural sites. In this marginal area the light and soil moisture conditions vary.
4 Discussion

When the whole Fennoscandia is considered, the northern areas of Finland represent an intermediate climate between highly oceanic sections of the Norwegian cost line and the continental section in eastern, Russian parts of Fennoscandia (Karlsen et al. 2007). Distinctive features of northern areas of Fennoscandia are the arctic light conditions (polar day and night), but milder thermal conditions due to the Gulf Stream in comparison to other geographical areas in the boreal zone (Kallio et al. 1985). Agriculture, for example extends in Fennoscandia up to the latitude 70°N, whereas in northern Canada 58°N is the limit (Kallio et al. 1985, Richards 2007).

4.1 Long-term phenology and success

In woody plants spring phenological events occur when integrated climatic conditions, mainly temperature are satisfactory (Rousi & Pusenius 2005, Aono & Kazui 2008, Lüttge & Hertel 2009). In the plant selection experiments the onset of foliation of all genotypes showed obvious relation to spring time temperature, being last in the northernmost site, where a sufficient thermal sum for bud opening accumulated at a later date (I, II, III). With the *Betula pubescens* f. *rubra* and *Betula pubescens* in the case study, the bud burst occurred when the temperature sum reached and exceeded 37 dd (IV). In an experiment conducted in southeaster Finland the satisfactory degree day temperature sum for bud burst in *Betula pendula* was between 31 and 33 dd (Rousi & Pusenius 2005).

In Naruska, the foliation periods were shortest of all the sites (I, II, III). Short springs in the northern regions force plants to act more synchronously in comparison to those in southern regions (Siljamo et al. 2008). The rhythm of flowering followed foliation (I, II), as the woody plants need to reach a certain cumulated temperature sum also before flowering and seed maturation (Rathcke & Lacey 1985, Fenner 1998). However, the length of flowering was not predicted by the northern or southern location of the site (I, II). For example, hot weather can shorten the flowering period in lilacs (Kolkka 2006).

The autumn phenology of the woody plant genotypes between years showed consistent pattern in a sense, that it was not prolonged by an unusually warm growing season (Table 3). In woody plants, the main trigger for the initiation of growth cessation and leaf senescence is the shortening photoperiod, but there is an obvious relationship between temperature (heat sum) and day length (Koski &
Sievänen 1985, Junttila 2007, Lüttge & Hertel 2009). The autumn phenological events considered in this study – colouration and senescence – were found to have largest differences between genotypes (I, II, III). However between sites no clear differences in timing of autumn phenology was seen (I, II, III).

Between July and August the difference in the day lengths between the sites in the selection experiment was within two hours (Fig. 3). When growing at high latitudes, woody plants need to initiate dormancy without such an obvious shortening photoperiod as those in more southern latitudes i.e. they react more sensitively (Junttila 2007). For example, Betula pendula and Picea abies seedlings of northern provenance stop their growth with shorter night length than those with southern provenance (Partanen 2004). Nevertheless, some woody species have autonomous growth rhythmicity, which means that they do not respond to photoperiod changes at all (Wareing 1956, Junttila 2007, Lüttge & Hertel 2009). With some Prunus species the response to photoperiod depends on temperature (Heide 2008). It is likely that the taxa studied here have more than one genetically and environmentally controlled stimulus for growth cessation and onset of dormancy.

Though the photoperiod and temperature are the significant determinants in the autumn phenology, other biotic and abiotic factors may accelerate or delay leaf autumn colouration and senescence. Blights in birch leaves can cause premature yellowing and defoliation (Huxley 1992, Lilja & Hantula 2008). The birch rust infection may explain the earlier defoliation of Betula pubescens f. rubra in Ruukki and Sotkamo by comparison to the other genotypes (I, II, III). Also the age and stage of development of a plant influences the timing of growth cessation (Partanen 2004).

From the success parameters in the field experiments, the winter hardiness was most decisive and it sets also prerequisites for the mid summer ornamental value and abundance of flowering (I, II, III). In northern areas, the limit for cultivation of a woody plant is based on winter hardiness i.e. in the capacity of the plants to withstand freezing cold temperatures during the long winter (Stushnoff et al. 1983, Laine et al. 2007). Winter hardiness means also resistance to other abiotic and biotic factors, besides cold temperatures, encountered during winter (Palonen 1999). Die back of branches after winter lead to slow growth due loss of photosynthesizing tissues and loss of flowering potential like was the case with Viburnum opulus ‘Pohjan Neito’ in northernmost site (I). Further, hardening which includes degradation of macromolecules and remobilization of important
substrates for storage in stems requires energy (Lüttge & Hertel 2009) and good growth is needed for the development of proper winter tolerance.

Albeit some observed damage, the genotypes in this study can be considered hardy against diseases or vice versa, no serious pathogens occurred in the experimental sites (I, II, III). In northern latitudes herbivory is more likely to cause damage to the types of plants studied in selection experiments than diseases. In future, damage due pathogens and herbivores are predicted to increase under warming climate because the amount of time available for growth and reproduction will increase (Roy et al. 2004). Beautiful autumn colouration and ripening fruits give additional value for northern landscaping plants in seasonal climate. The appearance of these is obviously related to climatic conditions at the sites (I, II, II).

In the selection experiments the difference in phenology and field success was clear between the sites in the northern vs. southern location, but not so obvious in the western vs. eastern location (I, II, III). In Finnish phenological research, latitude is a more important parameter than longitude, although the latter does cause variation in the phenological phases (Kubin et al. 2008). Traditional method obtaining the effective temperature sum in boreal climates does not make any of difference between the day and night temperatures nor take into account the soil frost (Solantie 2008). The difference in the effective temperature sum between northern and southern and also maritime and continental sites is not as informative as it should be (Solantie 2008). Of the study sites here, in Ruukki the thermal sum was higher, but in Sotkamo the growing season was longer. This difference was reflected in phenology in that the onsets of all events were earlier in Ruukki than in Sotkamo site (I, II, III).

### 4.2 Selection experiments as a method to identify hardy plants

Selection experiments under field conditions can be considered as the ultimate way to identify the plant genotypes that are most suitable for the local climate. They are time consuming, but long observation periods are needed as the growth of woody plants is slow and flowering in the field may take several years (Mac Carthaigh 2008). Also, within a long observation period, five to ten years, extreme climate conditions are more likely to occur. In this study, the phenology and success of the genotypes in the field were recorded systematically for six years which is considered a sufficient period to obtain reliable data (I, II, III, Beaubien & Hall-Beyer 2003).
The most important monitoring parameters in identifying hardy plants for northern areas are the suitability to local climate i.e. phenology, and winter hardiness (I, II, III). Unlike in the paper by Juhanoja et al. (1998), the differences in phenological events actually have practical value in this study. Resistance to plant diseases, and characteristics valuable in landscaping, like flower abundance, are also important parameters in plant selection (Brander 1982, Juhanoja et al. 2001, I, II, III). In this study, after phenology and winter hardiness, an important selection parameter for flowering shrubs, *Viburnum*, *Syringa* and *Rosa* is the flower abundance and for the trees, *Alnus*, *Betula*, *Populus* and *Prunus*, ornamental appearance (I, II, III). Generally the emphasis of the selection criteria depends on the type of environment in which the plants are to be used (Sæbø et al. 2003).

If the field conditions are mild or no clear differences between genotypes are obtained during selection experiments, a controlled freezing test can be used to study the rhythmicity of cold hardiness to identify hardy genotypes (Suojala & Linden 1997, Linden 2002). Also as the evaluation of disease resistance is based on natural infections, controlled infections with specific pathogens would provide important information about resistance of certain genotypes (Brander 1982, Carlson-Nilsson 2002). Information about cold hardiness would be especially informative in the future selection experiments of hardy plants for northern areas.

The data gained from the selection experiments represent both objective and subjective values (I, II, III, Table 2). Evaluation of ornamental appearance depends on personal opinion, measuring numerical characteristics is much more reliable (Brander 1982). This problem is difficult to avoid in future experiments, but careful planning of the parameters and instructions for the field staff diminishes errors. The classification of winter hardiness carried out in this study was important, although somewhat problematic (Table 2.). The classes would have needed further explanation or few additional classes. Evaluation of interannual variation in phenological data were difficult due to the amount of missing data (Table 3, I, II, III). In some years with warm springs, foliation had already started before the actual recording began. With some plants, like alder and aspen, flowering occurs before foliation, and it was not within the recording period (III). In future experiments the recording period should start earlier in the spring in order to obtain all phenological data from the sites. The number of recorded parameters should be evaluated in relation to their importance and available human resources at the experimental sites. Finally, there are different ways for statistical processing and separating between valuable and poor
genotypes, but plant selection should also be based on non-statistical parameters like growth habit, cultivation area and number of types in cultivation (Brander 1982, Juhanoja et al. 1998, Juhanoja et al. 2001, I, II, III).

As the microclimate and soil properties greatly affect the cultivated plant success it is sometimes difficult to apply the plant selection results to areas outside the test site (I, II). Also in the case of the phenological surveys, there is always uncertainty in extrapolation of the data from a single site to the surrounding region for the same reason (Siljamo et al. 2008). Nevertheless, selection experiments provide new information about the cultivation success of certain taxa like the *Alnus incana* f. *laciniata*, *Populus tremula* ‘Erecta’ and *Prunus maackii* in northern parts of Finland (III). In plant cultivation, the interaction between soil and climate is crucial, though today with advanced management practises the climate has a determinant role (Skjelvåg 1998). The cultivation success of ornamental plants can be improved by creating a favourable microclimate and improving soil conditions (I, II). This means that, for example, cultivation of snowball viburnums in the corresponding cultivation zone where the northernmost Naruska site with peat soil was located can be attempted on a sheltered site with west-south exposition in mesic and nutrient rich soil (I).

One risk related to plant selection experiments is that they may lead to a reduced gene pool (Webster 1988). Conversely, they may promote the use of old and valuable plant cultivars such as the old rose cultivars in this thesis (II, Tegel 2000). These types of plants are hardy, with low nutrient demand, standing and flourishing over many years for example in old parks and gardens with low maintenance. Loss of these old cultivars and provenances reduces the genetic variation in landscaping plants (Öberg 2009).

Due to hybridization, especially with roses and lilacs, the nomenclature and characteristics of cultivars and strains vary (e.g. Juhanoja et al. 1998, Ki-Joong & Jansen 1998). Besides the actual selection of the most suitable and valuable genotypes for a local climate, selection experiments are important in naming and defining the characteristics of the plants (e.g. Webster 1988, Bengtsson & Jansson 1992, Juhanoja et al. 1998). In the plant selection experiments here, the plants were further identified and names of the genotypes were revised from the ones given at first registration (I, II, III).
4.3 Reproduction of hardy woody plants

Micropropagation is a suitable propagation method for several woody taxa that thrive in northern growing conditions (I, II, III, V). The actual in vitro propagation techniques used for the plants in the selection experiments, LANDSCAPE LAB subproject and case study are not discussed here. However, data from the explant source, media, method and their improvements etc. are carefully documented at the Oulu Botanical Gardens micropropagation laboratory.

The advantages of micropropagation in this study were the effective production of plant material from limited propagation sources (I, II, III), rapid production of plant material with a limited time schedule (V), obtaining true to type material from each particular genotype (I, II, III) and production of own-rooted material from taxa such as *Populus tremula* ‘Erecta’ and roses that are commonly grafted to rootstocks in production (II, III, Curir et al. 1986, Räty 2008a).

At the beginning of 1990s in Finland micropropagation was considered as a new and promising method to produce woody plants efficiently throughout the year. New micropropagation laboratories were established and the method and its applications were studied (Haapala & Niskanen 1992). At the end of 1980s, micropropagation was applied in Finnish forestry to production of the economically most important deciduous tree *Betula pendula*, but was concluded in 1994 because it was not profitable in comparison to seed propagation (Viherä-Aarnio & Velling 2001, Häggman et al. 2007, Viherä-Aarnio & Velling 2008). In Finland micropropagation is used in production of disease free (elite) plants, in plant breeding, gene conservation and in the propagation of special tree and shrub forms like the *Betula pubescens* f. *rubra* (Aaltonen et al. 2006, Viherä-Aarnio & Velling 2008, III). Micropropagation is practical, and sometimes only propagation method, for species and cultivars with low propagation success by traditional methods or that are sterile. Micropropagation is also applicable to mother stock production, plant breeding and in rejuvening old propagation sources (Jesch & Plietzsch 2000, Giri et al. 2004, Haapala et al. 2004, Andreu & Marín 2005, Laine et al. 2007). According to Haapala and Niskanen (1992) micropropagation of northern woody plants is relatively easy. Whether or not a certain plant is sensible and cost efficient to propagate in vitro has to be considered case-specifically.

Several genotypes studied in the field experiments and also propagated for the LABPLANT subproject are preserved in culture in vitro in the micropropagation facilities at the Oulu Botanical Gardens (I, II, III, V). New
subcultures of the desired genotypes and species can be established for future purposes. One problem related to conservation of plants in micropropagation is that during subculturing the growth of many species is retarded and eventually in vitro culturing is not successful. It is necessary to establish new in vitro cultures at regular intervals in some species (Litwińczuk et al. 2005). Cryopreservation techniques can be applied in long-term preservation of valuable woody plant genotypes in vitro (e.g. Jokipiän et al. 2004).

### 4.4 Characteristics of successful and unsuccessful plant genotypes

Several genotypes used in this study represented taxa with long cultivation histories in Finland (I, II, III, Table 2). As the genotypes were propagated from well thriving mother plants, it was assumed that genotypes have good adaptation and acclimation potential to a northern environment (Väinölä et al. 1995, Pihlajaniemi 2004, I, II, III). In plants, adaptation is a long-term process in which the modifications that enable certain species to grow and survive in their environment are heritable. Acclimatization is shorter-term phenotypic adjustment to the local growing conditions as the morphology and physiology of the plant adjust to the growing conditions (Heide 1983, Pelkonen et al. 1990). The evaluation in the field experiments was obviously performed on the phenotypes of the woody plant genotypes.

Propagation sources for all the genotypes were northern, except the provenance of *Betula pendula f. crispa*, 63°N, which is below the northern range defined by this study (I, II, III, Table 2). In forestry, the origin of *Betula pendula* seed source should be in the range of 150 km or 150 degree days north or south from the original site, although in eastern and western axis the distance can be longer (Hämä-Ahti et al. 1992, Viherä-Aarnio & Velling 2008). This range is not so strictly defined with ornamental plants as the reasons for their cultivation are different from those for forest trees. It was not anticipated that the cut-leaved silver birch would perform better at the continental Sotkamo site than in Ruukki, as the mother plant grows in Pietarsaari seaside town on the west coast of the Gulf of Bothnia (III).

In boreal climates, proper timing of frost hardening in autumn and dehardening in spring is essential for exploiting the full growing season and minimizing frost injury (Lüttge & Hertel 2009). In this study the correct timing of autumn senescence seemed to be crucial for plant survival as the late genotypes...
like *Betula pendula f. crispa*, *Viburnum lantana* and *Syringa vulgaris* ‘Alba’ died at the northern sites (I, II, III). Generally, unsuccessful genotypes had late or no leaf senescence at all, and they experienced severe winter damage at the northern sites (I, II, III). Native woody plants respond to local photoperiod (Junttila 2007), to introduced plants this is challenging. At high latitudes above 66°N, here Rovaniemi and Naruska, woody plants need to stop growth and set buds without a distinct dark period (Lütte & Hertel 2009, Taulavuori et al. 2010). This was likely one reason for poor success of some genotypes in the field experiments (I, II, III).

Field success was affected greatly by the age and size of the plant genotype, as the mortality of the plant individuals was highest during the first years of experiment (I, II, III, Caprio et al. 1970). Both the *Viburnum lantana* and *Syringa vulgaris* ‘Alba’, which were classified only as modestly succeeding, were small sized in comparison to other genotypes when transplanted to field (I, II, III).

### 4.5 Plants from selection experiments

Lilacs are mostly cultivated in temperate zone, but they are important and traditional ornamental shrubs also in areas with boreal climate due to their hardiness and showy flowering (Kolkka 2006, Fiala 2008, Alanko & Lagerström 2009). Two lilac species are native to Europe, growing in the south-eastern Balkan mountain region (Fiala 2008). Of these *Syringa josikaea* has been used as a parent in the hybrids *Syringa × henryi* and *Syringa × josiflexa* (Fiala 2008) of which genotypes ‘Paulus’ and ‘Veera’ were studied in the field experiment (I). Both of these are valuable and beautiful lilacs, with ‘Paulus’ having higher potential for cultivation in northern sites due to better winter hardiness (I). Cultivar ‘Veera’ has also been evaluated in the KESKAS clone selection experiments with sites located in southern and central Finland (Juhanoja et al. 2001). It has been granted the FinE (Finnish Elite) trademark, and disease free and true-to-type plantlets are available from the Laukka Research and Elite Plant Station (Hokka et al. 2007, Juhanoja 2007). The other European species, *Syringa vulgaris*, was introduced to Finland as an ornamental plant early in the 18th century (Suominen 1986). Although the common lilac has a long cultivation history in Finland, even in the northern parts of the country, genotype ‘Alba’ was characterized in this study with slow growth and modest hardiness (I). The majority of lilac species occur in north-eastern Asia like *Syringa villosa* (Erhardt et al. 2000) from which genotype ‘Hirvas’ was studied (I). Though hardy, the
modest flowering during the field survey suggests that ‘Paulus’ has more value in
northern sites (I). Generally lilacs thrive best in areas with cold winters, but their
success declines when grown in areas with arctic influence (Fiala 2008), evidence
from this study supports these findings (I).

It is likely that all the studied ‘Pohjan Neito’ provenances represented the
same strain (and also same genotype?) though there was some variation in their
field performance (II). In Finland two types of the vegetatively propagated
snowball viburnum are identified. The one sold under the cultivar name ‘Roseum’
thrives in southern and central parts of Finland. The other type that thrives in
northern Finland up to the Rovaniemi region, like the provenances in this study,
has been given cultivar name ‘Pohjan Neito’ (Hämet-Ahti et al. 1992, Räty 2008b,
Alanko & Lagerström 2009, I). ‘Roseum’ is considered a foreign, and ‘Pohjan
Neito’ a domestic cultivar (Aaltonen et al. 2006). The success of snowball
viburnum is best on mesic and moderately shaded sites, and planting on this type
of site prevents damage due to the leaf contorting aphid to which the shrub is
susceptible (Eskilson 1994). The cultivated *Viburnum lantana* in Finland
originates from southern and central Europe, areas with a temperate climate
(Alanko & Lagerström 2009). The species prefers lime soils and grows both on
moderately shaded and open sites (Kollmann & Grubb 2002, Alanko &
Lagerström 2009). Success of the genotype was best at Ruukki site with mildest
conditions of the study sites (II).

Roses are naturally distributed throughout the northern hemisphere, with
most species growing in the mountain valleys of the temperate zone. Some
species have spread to the arctic regions of the Eurasia and North America
(Alanko et al. 2009). Six rose species grow wild in Finland, from these *Rosa
majalis* (syn. *Rosa cinnamomea*) occurs throughout the country in mesic forest
sites (Hämet-Ahti et al. 1992, Alanko et al. 2009). Of the foreign taxa, *rugosa* and
*spinosissima* roses have adapted well to the Finnish climate (Joy 2005) which was
further proven in the field experiment (II). The multiple flowered forms of *Rosa
majalis* have been cultivated in areas where they naturally occur, as they appear
to have a similar hardness to the wild cinnamon rose (Alanko et al. 2009). The
cultivar ‘Tornedal’ has been cultivated in northern Finland (Tornio river valley)
for over 300 years (Rautio 2009). The name of the rose implies that it occurred
and was taken into cultivation in northern Finland (Joy 2005). It is also possible
that the rose has been introduced from Central Europe (Hämet-Ahti 2009). The
two hardy and beautiful ‘Tornedal’ genotypes studied most likely represent the
same strain, showing only small differences in field performance (II). ‘Tornedal’
cultivar is in this study considered the most valuable shrub rose to northern landscaping (II). Wild *Rosa rugosa*, native to northern Japan, north-east China and Russian Far East, has been used is numerous rose hybrids to introduce resistance to frost, heat, drought and salt (Ueda *et al.* 2000, Bruun 2005). In selection experiments *Rosa ‘Sipi’* and *Rosa × spaethiana* genotypes represent roses with vigorous growth and good winter hardiness (II). *Rosa × spaethiana* is a common remnant in old parks and gardens as besides cultivation it has been used as a rootstock for sensitive rose cultivars (Sennikov 2004, Alanko *et al.* 2009). Cultivar ‘Sipi’ has been found and named in Finnish Lapland (Laine *et al.* 2007, Alanko *et al.* 2009). Spinosissima roses have been cultivated in Finland at least since the 19th century (Rautio 2009). *Rosa ‘Poppius’* genotype here is a hardy and graceful rose which value is especially in the beautiful and fragrant flowers (II). ‘Poppius’ is an old cultivar, it was named in Sweden in the mid 19th century (Joy 2005, Rautio 2009). Generally the *Rosa ‘Poppius’* strains in Finland appear to be uniform (Juhanaja *et al.* 2001, Alanko *et al.* 2009). *Rosa ‘Splendens’* is appreciated as being one of the traditional and valuable shrub roses in Finland (Joy 2005, Alanko *et al.* 2009). However, the genotype here has least potential from the studied roses for cultivation in northern areas (II). The botanical background of the cultivar ‘Splendens’ is unclear, Gallica group roses originate from *Rosa gallica* which grows wild for example in Central Europe (Alanko *et al.* 2009, Rautio 2009, II).

The *Betula pubescens f. rubra* occurs in northern Finland and it obviously has the same tolerance in relation to climate and soil as the common *Betula pubescens* (Kauppi & Ulvinen 1989, Hämet-Ahti *et al.* 1992, Pihlajaniemi *et al.* 2008, III). From the tree genotypes *Betula pubescens f. rubra* is most valuable and hardiest genotype for northern landscaping, though the showy appearance may restrict its use in rural sites (III). Other red-leaved cultivars of birches also exist (Santamour & Mc Ardle 1989), but they are not cultivated at least in northern region of Finland.

The columnar aspen ‘Erecta’ was originally introduced to Finland from southern Sweden and has been cultivated here for decades (Räty 2008a). According to the field performance, its success is uncertain in the Rovaniemi area and northwards (III). The background of the studied cutleaf tree genotypes is unclear. They were represented here as *f. crispa* and *f. laciniata* instead of ‘Crispa’ and ‘Laciniata’ (Haapala & Niskanen 1992) as cultivar names belong to one single clone of the forma (III, Hämet-Ahti *et al.* 1992). It is likely they represent foreign cultivars as the cutleaf forms of silver birch and grey alder are rare in
nature in Finland (Hämet-Ahti et al. 1992, Oskarsson & Nikkanen 2001). Of the studied cutleaf forms, *Alnus incana* f. *laciniata* has more value in northern landscaping (III). *Prunus maackii* is a temperate species favouring fresh and mesic sites native to northeast Asia, Manchuria (Hämet-Ahti et al. 1992, Erhardt et al. 2000). The species is known to be hardy in southern and central parts of Finland and according to this study also in the eastern Sotkamo site (Hämet-Ahti et al. 1992, III).

### 4.6 Northern birches – case study

Birches are emphasized in this study for the following reasons (IV). They are widespread deciduous species in boreal areas. They are the main landscape trees in northern urban and suburban areas growing both wild and as planted trees. In Finland silver birch is among the most popular street and park trees (Sæbø et al. 2003). Further northern birches, *Betula pendula*, *Betula pubescens* and *Betula pubescens* ssp. *czerepanovii*, have been exposed to several climate change-related experiments in which the responses to elevated ozone, carbon dioxide and UV-B radiation have been studied (e.g. Vanhatalo et al. 2001, Tegelberg 2002, Manninen et al. 2009). The UV-B responses of *Betula pubescens* f. *rubra* were studied here for the first time (V).

The foliar flavonoid responses in the UV-B experiment were subtle with every birch taxa (IV). In the case of UV-absorbing compounds the green-leaved birches, *Betula pubescens* and *Betula pubescens* ssp. *czerepanovii*, showed clear difference in the concentrations between the two years, as the weather conditions and UV-B radiation levels differed between the experiment years (IV). *Betula pubescens* f. *rubra* on the other hand showed the only significant difference between the treatments, as there was a higher concentration of UV-B absorbing compounds in the enhanced UV treatment at 300 nm in the second year of the experiment (IV). These results are somewhat contradictory in relation to UV-B exposure, but it is obvious that the flavonoid responses generally differ between the ‘normal’ green leaves versus anthocyanic leaves (IV). The most apparent response to enhanced UV-B radiation in northern birches seems to be the accumulation of flavonoids that filter UV-B radiation as the growth and biomass responses are less obvious (Lavola 1998, de la Rosa et al. 2001, Tegelberg et al. 2001, Julkunen-Tiitto et al. 2005). The UV-B tolerance and responses are known to vary between birch provenances and even individuals (Julkunen-Tiitto et al. 2005).
The anthocyanin concentrations of the three birch taxa studied did not show short term response to enhanced UV-B radiation (IV). Whether or not anthocyanins have an important role in UV-B protection in plant leaves has been discussed in several papers (e.g. Woodal & Stewart 1998, Mendez et al. 1999, Gould 2004, Manetas 2006). In this study no clear evidence from the UV-B protective role of anthocyanins was obtained (IV). Nevertheless, according to light microscopy of *Betula pubescens* f. *rubra* leaves, anthocyanins are located in the upper epidermis which is an ideal position to protect against UV-B (IV, Burger & Edwards 1996). For most plants previously studied anthocyanins are found in the vacuoles of the palisade mesophyll and or on the spongy mesophyll of leaves (Lee & Collins 2001, Gould et al. 2002).

As in the case of flavonoids, the epidermal waxes and trichomes showed no clear response to enhanced UV-B radiation in comparison with the control treatment (IV). Since leaf epidermis is the first cell layer to interact with the environment, the trichomes and waxes are important protectants that for example, reflect UV-B radiation (e.g. Karabourniotis et al. 1992, Hollósy 2002, Holmes & Kieller 2002). According to Valkama et al. (2004), the amount of leaf trichomes in northern birches is high in young leaves, but decreases during maturation due to growth dilution. These findings are also supported in this study (IV). UV-B exposition has been shown to increase the density of glandular trichomes in *Betula pendula* (Kostina et al. 2001).

*Betula pubescens* ssp. *czerepanovii* had the overall highest concentration of UV-B absorbing compounds and also glandular trichomes regardless of the treatments (IV). These are adaptations of the subspecies to harsh growing conditions at the northern tree line in Fennoscandia (Atkinson 1992, Turunen & Latola 2005). Naturally, the highest anthocyanin concentrations were found in *Betula pubescens* f. *rubra* with an increasing trend throughout the growing season (IV). *Betula pubescens* ssp. *czerepanovii* had a higher anthocyanin content than *Betula pubescens* (IV). The proanthocyanidin content has been studied in Finland’s four native birch species, of those *Betula nana* had the highest content of proanthocydins and *Betula pendula* the lowest (Karonen et al. 2006). The northern populations of mountain birch had a high proanthocyanidin content, but when grown in southern Finland the proanthocyanidin content was similar to pubescent birch (Karonen et al. 2006). Here, in the UV-B experiment all the birches had yellow autumn colouration, though mountain birch is known to exhibit orange foliage at northern latitudes (IV, Vaarama & Valanne 1973). A qualitative latitude-associated gradient in foliar flavonoids has been identified in
pubescent birch, indicating protection against photoprotective stress in northern latitudes (Stark et al. 2008). The high anthocyanin or proanthocyanidin content in northern species or plant populations is likely to be a biochemical adaptation to northern growing conditions with high light intensity and low temperature (Oberbauer & Starr 2002, Karonen et al. 2006, Lätti et al. 2008).

Woody plants with permanently red leaves such as Betula pubescens f. rubra rarely occur in nature, but are common as horticultural plants due to selection and breeding (Steyn et al. 2002). For the northern areas of Finland and other areas with similar climate apparently no corresponding hardy red leaved landscaping tree is available (III, V). The leaves of the tree form have determined redness i.e. the red colour appears and develops in leaves with the same pattern every year, though the intensity of colour and anthocyanin concentration varies between years (IV). Though genetically determined, environmental factors like solar radiation act as external signal which partly controls anthocyanin synthesis and accumulation (Chalker-Scott 1999, Neill 2002). For example with the ornamental cultivar Cotinus coggygria Scop. ‘Royal Purple’ UV-light between the 300 and 400 nm is crucial for accumulation of foliar anthocyanins (Oren-Shamir & Levi-Nissim 1997).

Permanent red foliage is considered disadvantageous to plant growth due to the shading effect of anthocyanins in leaves, seen here as reduced chlorophyll a/b ratio of Betula pubescens f. rubra in comparison to Betula pubescens (IV, Manetas et al. 2003, Hughes et al. 2007). For example the red-leaved silver birch, Betula pendula f. purpurea has a slower growth rate than the green-leaved silver birch (Hattemer et al. 1988). The spring phenology was also approximately one week later with red-leaved silver birch in comparison with green-leaved (Hattemer et al. 1988). Here the phenology and performance of Betula pubescens f. rubra was not noticed to be abnormal or poor in comparison with green-leaved tree taxa (III, IV). In the UV-B experiment, the red-leaved in vitro propagated birch seedlings had significantly higher height growth rate than the green-leaved (IV). This could partly be explained by the effect of external factors, genotype and explant source of the mother stock on the in vitro propagation (Haapala & Niskanen 1992, Chalupa 2002). Photosynthesis of Betula pubescens f. rubra has been measured in previous studies and it is about the same level or lower than in the green leaves of the common pubescent birch (Portaankorva 1995, Pihlajaniemi 2004). In the case of lower photosynthetic capacity, no difference in the growth rate was noticed (Portaankorva 1995). Little information is found on the specific anthocyanin sugars of the Betula species. The anthocyanins identified
from the *Betula pubescens* f. *rubra* leaves are among the six most commonly occurring anthocyanins in plants (Bohm 1998, Schmitzer *et al.* 2009). The main flavonoid and other phenolic compounds in *Betula pendula* and *Betula pubescens* are myricetin, quercetin and campherol derivates (Keinänen & Julkunen-Titto 1998; Keinänen *et al.* 1999; Valkama *et al.* 2003).

As transient redness is the most common form of anthocyanin appearance in leaves, it suggests that the role and effect of anthocyanins in plant leaves is related to leaf age and developmental stage (*e.g.* Manetas *et al.* 2003, Archetti *et al.* 2009). In many woody species growing in temperate, boreal or arctic latitudes anthocyanins in leaves are evident in the young emerging spring leaves and again in the senescing leaves. The role of anthocyanins in juvenile and senescing leaves is likely to have a similar role - that is photoprotection of leaves during the stressful periods under conditions of high irradiance and low temperature (Feild *et al.* 2001, Hoch *et al.* 2001, Oberbauer & Starr 2002, Hughes *et al.* 2007). Juvenile reddening was not studied here, nevertheless the reddish appearance of for example young *Betula pendula* leaves in northern latitudes is easily observed by anyone.

The autumn colouration was observed in genotypes in the plant selection experiments. Reddish foliage was expressed in several taxa including both successful and unsuccessful genotypes at northern sites (I, II, III). Though some experiments support the idea of protective role of anthocyanins during leaf senescence, divergent photoprotective mechanisms exist (Feild *et al.* 2001, Hoch *et al.* 2001, Archetti *et al.* 2009). For example, the photochemical efficiencies and nitrogen resorption performance of *Betula papyrifera* with yellow autumn colouration were comparable with the anthocyanin-producing species in an experiment by Hoch *et al.* (2003). Generally, red autumn colouration is more apparent in the tree species growing in temperate North America and East Asia than in the deciduous tree species in temperate parts of Northern Europe (Lev-Yadun & Holopainen 2009). However, when moved to higher latitudes anthocyanins start to dominate in autumn leaves also in northern parts of Europe (Oberbauer & Starr 2002, Lev-Yadun & Holopainen 2009).

In *Betula pubescens* f. *rubra* foliage, the anthocyanin content started to decline in August and later measurements were not made (IV). The measurements performed in the same area with *Sorbus aucuparia* showed that the amount of anthocyanins in autumn leaves did not increase but the red autumn foliage was due to disintegration of chlorophylls (Taulavuori 2006). However, production of anthocyanins in several other tree species during autumn senescence has been
shown (Keskitalo et al. 2005, Archetti et al. 2009, Lev-Yadun & Holopainen 2009). According to Archetti et al. (2009) the autumn anthocyanins are synthesized after half of the leaf chlorophyll has degraded. Keskitalo et al. (2005) have shown accumulation of anthocyanins in the senescing Populus tremula leaves as a response to cold and clear days.

As the woody plants are slowly growing and reproducing organism, and also their leaves shed every year a longer period of ultraviolet exposures under field conditions are needed to see the long-term responses (Julkunen-Titto et al. 2005). With foliar anthocyanins the accumulation responses, especially in relation to light quantity and quality and leaf age, need further research.

4.7 Introduction of hardy woody species for northern landscaping

The results from plant selection experiments (I, II, III), and also from previous projects and research activities at the Oulu Botanical Gardens and Lapland Vocational College were put in practise when the hardy plants were listed, propagated and transplanted to northern tourism areas (V, Laine et al. 2007). Nature-based tourism has been increasing in Finnish Lapland and the ecological and social sustainability of this activity has been the target of research in recent years (e.g. Jokimäki et al. 2007, Törn et al. 2007). Though in the LABPLANT subproject the tourism areas were focused, the results are applicable to northern areas in a larger context (V). The subproject offered the possibility to introduce ‘natural’ landscaping by combining wild and ornamental plants. Natural landscaping was created by selecting plants according to the wild species growing in the surrounding environment. The use of wild species is important not only from the biological point of view, but also to maintain the genuine characteristics of the landscape (Lagerström & Eriksson 1996). Also sustainability is achieved with the use of local plant material, as it is well adapted to the prevailing climatic and site conditions (Lagerström & Eriksson 1996, Laine et al. 2007, V).

Foreign taxa and provenances are commonly encountered in northern landscaping. This is partly for the reason that northern nurseries are few and native plants are produced in low numbers or not at all (V, Laine et al. 2007). Today there is a growing recognition of the use of native species in landscaping to create more sustainable and ecological landscaping (Spellerberg & Given 2008). The use of native plants for example in tourist resorts is important because they provide habitats for native bird and animal species (Spellerberg & Given 2008, V). When native plants are used in landscaping, especially in ecologically fragile
areas such as in the fell areas of Finnish Lapland, it has to be decided whether they are propagated only from local plant sources, and whether species representing rare or endangered ones should be used in landscaping. These questions are as yet unresolved at least in Finland. As the LABPLANT subproject created gene banks for many northern native species, propagation material for interested nurseries is available for future needs (V, Laine et al. 2007).

The native plants introduced for landscaping by the subproject are also suitable for restoration (Table 4). Restoration is a complex processes that can be implemented in various ways (Urbanska 1995, Hagen 2007, Laine et al. 2007). For this reason, it is not discussed in more detail in this thesis. Here, restoration was performed by transplanting native species of northern provenance in order to accelerate recovery of the disturbed site in Pallastunturi (V).

The selection of plant taxa for the different site types (Table 4) represents species native to northern areas, and cultivars and foreign taxa that are known to be hardy to northern climate because they have been traditionally cultivated here or their success has been observed in the field (I, II, III, V, Juhanjo et al. 2001, Laine et al. 2007). One example of a versatile and hardy plant group is the willows, *Salix* spp., from which several species are suitable both for landscaping and restoration (Table 4, Viherä-Aarnio 1988). As natural looking and versatile plant groups include, beside the trees and shrubs, also a field layer with dwarf shrubs, herbaceous perennials and grasses, larger selection of species is presented in the guidebook ‘Pohjoisen matkailuympäristön kestävät kasvit’ (Hardy plants of northern tourist destinations) by Laine et al. (2007).

LABPLANT demonstration areas are trial plantings in which the suitability of the selected plants is assessed to different planting targets. The introduction of new taxa must be given careful consideration and risk evaluation in the future (Haywood 2009). For example the longer and warmer autumns may lead to harmful invasions of species which formerly were not able to produce mature seeds under northern growing conditions. The demonstration areas established in the LABPLANT subproject are important in identification any future changes both in ornamental and wild plants.
5 Conclusions

This study shows that long-term field experiments are important in identifying plant genotypes adapted to local growing conditions, and that are potential landscaping plants for the area. The experiments for this thesis were carried out over a six year period. This was considered sufficient since unsuccessful genotypes were identified within the first few years and those successful plants had time to show all growth phases, such as occurrence of flowers. The most important monitoring parameters in identifying hardy plant material were proved to be phenology and winter hardiness. The key characteristic of successful plants was the proper timing of hardening in the autumn, assessed in this study by the onset of autumn coloration and defoliation.

Micropropagation is a suitable propagation method for several woody species that thrive under northern growing conditions. The foremost benefit is the fast and efficient propagation of desired genotypes from a limited mother plant stock. In the future, cryopreservation techniques offer the possibility to preserve the cultures of valuable hardy plants, like those observed in this study, in a long-term gene bank. Despite the obvious benefits of micropropagation, each case should be considered individually, taking into account cost, species and purpose.

Betula pubescens f. rubra was found to be a potential landscape plant for northern areas due to several good characteristics. However, because of the unique appearance its suitability to various environments has to be considered. At the moment it is also known that micropropagated trees produce fertile seeds. Betula pubescens f. rubra is a suitable study plant for research that examines the ecophysiological differences in red versus green leaves. To reveal more of the role of anthocyanins in northern plants, research should be performed in situ with the species naturally occurring at the site. The foliar and growth responses to enhanced UV-B radiation in Betula pubescens f. rubra, Betula pubescens and Betula pubescens ssp. czerepanovii are not easily detected in a short-term experiment as trees are such slow-growing organisms.

Hardy woody plants with known characters, like the genotypes observed in the plant selection experiments, are important in sustainable landscaping. Use of native species should be increased in northern landscaping and restoration, and not only in the tourism areas. Native vegetation should also be used as a guideline when selecting landscape plants in those areas in close connection with the surrounding nature. Further, in the case of both ornamental and wild plants, local
propagation sources and provenances should be favoured to promote sustainable land use.

This thesis can be considered as pioneering work in its representation of northern plant selection work in a scientific forum, for it has traditionally only been considered on a local or national level. Hopefully this work provides a valuable basis for the planning and execution of future selection experiments in northern areas. These are still necessary to broaden the selection of hardy woody landscaping plants. Plant collections, for example, in northern botanical gardens may serve as sources for new woody landscaping plants. Improvement in future selection experiments is needed to determine the length of annual observation period - especially as the warming climate is predicted to make the onset of growing season earlier - and in defining of the parameters so that they are informative, objective and reasonable to carry out. In future the changing climate may positively impact on the cultivation possibilities of foreign taxa. There is a risk, however, that the success of native plants is negatively affected and some introduced species may become invasive. All genotypes in this study were healthy, but the warming climate may promote spread of serious plant disease and insects to northern latitudes. The demonstration areas established in the LABPLANT subproject are important for identification of potential future alterations in the performance of both foreign and native plants.
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Henna Pihlajaniemi

SUCCESS OF MICRO-PROPAGATED WOODY PLANTS UNDER NORTHERN GROWING CONDITIONS AND CHANGING ENVIRONMENT