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Control of growth and frost hardening of silver birch container seedlings: growth retardants, short day treatment and summer planting

Jaana Luoranen

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Jaana Luoranen

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Auditorium XII of the University Main Building, Aleksanterinkatu 5, on 22 September, 2000, at 12 o´clock noon.

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Cover photos: Seven years old plantation of silver birch in Suonenjoki (photographed by Leo Tervo). Inset: Two months old silver birch contaner seedling for summer planting (photographed by Risto Rikala).

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Abstract

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The aim of this study was to find methods to control or avoid problems concerning growth and frost hardening of silver birch container seedlings in nurseries. The objective was to find method(s) by which it would be possible: 1) to decrease the risk of frost damage during autumn frosts, 2) to control height growth of seedlings in nurseries and 3) to improve field performance of birch container seedlings.

For characterizing the annual development of birch seedlings, the growth rhythm and frost hardening of seedlings was monitored during the first growing season in nursery conditions. After cessation of height growth, diameter growth continued for a few days, and accumulation of dry mass to shoot (stem and branches) and roots continued for about a month. The ability of roots to egress from peat plugs was high until mid-August. After that root growth decreased linearly toward autumn.

One purpose of this study was to seek appropriate assessment tests of the frost hardiness of birch seedlings for evaluting cultural practices and monitoring methods of frost hardening for operational use in nurseries. The tests compared were electrolyte-leakage, stem-browning and whole-seedling viability as assessed after the freezing exposure.

Frost hardening can be observed by using combined measurements of height growth and water content of seedling apices. The first stage of frost hardening starts when height growth ceases. During the first stage water content decreases until stabilization at the time when the second stage of hardening, i.e. rapid increase in frost hardiness, starts. The yellowing and abscission of leaves correlate to some extent with frost hardening, but the previous growing conditions affect this relationship. Frost hardening can also be determined with regular exposure to –10°C. Damage can be evaluated after two weeks incubation by stem-browning test.

Effects of growth retardants on height growth were studied using daminozide application sprayed once (on 28 June at concentrations of 1.0–6.0 g l^{-1}) and CCC (chlormequat chloride) application sprayed twice (on 29 June and 27 July at concentrations of 0.5–3.0 g l^{-1}). The most suitable applications for retardation of height growth, without negative effects on other morphological variables, were daminozide concentration of 4 g l^{-1} and CCC concentration of 2 g l^{-1}. Neither of these compounds, however, affected the frost hardening of seedlings.

In the first crop (seeds sown on May), three-week short-day (SD) treatment (16 hours night length) begun at the end of June stopped height growth only temporarily. Therefore, it did not affect the frost hardening of seedlings. In the second crop (seeds sown in mid-June), the three-week SD treatment started at the end of July stopped height growth during the treatment period. This treatment also hastened frost hardening of seedlings and during the first stage of hardening improved frost hardiness by 2–6°C, depending on the date. Neither early or late SD treatment affected diameter- or root growth. SD treatments had no influence on growth after planting, but SD-treated seedlings grown in small

containers were more susceptible to hare damage and stem lesions than untreated seedlings were.

For avoiding problems related to excessive height growth and costs of large birch seedlings, it was investigated the possibilities to expand planting window to summer by planting small leaf-bearing container-grown seedlings during their first growing season. When seedlings were planted in July or before mid-August, the following seasons' growth improved and survival was the same compared to seedlings planted at the traditional time in early September or the following May.

The studied methods for controlling growth and frost hardening (growth retardants, short-day treatment) did not fulfill the objectives or uncertain results were obtained. The most promising results were obtained with summer planting. Leaf-bearing seedlings with actively growing roots, which are planted into warm soil during the growing season, have rapid root egress and, thus, increased field-performance potential.

Keywords: annual cycle, *Betula pendula*, CCC, chlormequat chloride, daminozide, diameter, electrolyte leakage, frost hardiness, growth cessation, height, leaf abscission, leaf yellowing, root egress, root growth potential (RGP), shoot, stem browning, water content, whole-seedling viability

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Abstrakti

Luoranen, J. 2000. Control of growth and frost hardening of silver birch container seedlings: growth retardants, short-day treatment and summer planting. Abstrakti: Rauduskoivun paakkutaimien kasvun ja karaistumisen hallinta: kasvunsääteet, lyhytpäiväkäsittely ja kesäistutus. Metsäntutkimuslaitoksen tiedonantoja 777. 167 s + 5 liitettä.

Tutkimuksen tarkoituksena oli löytää menetelmiä rauduskoivun paakkutaimien kasvussa ja karaistumisessa taimitarhalla ilmenneiden ongelmien hallitsemiseen tai välttämiseen. Työssä tutkittiin menetelmiä, joiden avulla olisi mahdollista 1) vähentää hallavaurioriskiä syyshalloissa, 2) säädellä taimien pituuskasvua taimitarhalla ja 3) parantaa rauduskoivun paakkutaimien maastomenestymistä.

Taimien kehitysrytmin selvittämiseksi niiden kasvua ja karaistumista seurattiin ensimmäisen kasvukauden aikana taimitarhalla. Pituuskasvun päättymisen jälkeen läpimittakasvu jatkui muutamia päiviä. Sen sijaan verson (ranka ja oksat) ja juurten kuivamassa lisääntyi vielä noin kuukauden ajan. Istutettujen taimien kyky kasvattaa juuria paakusta ympäröivään maahan oli korkea elokuun puoliväliin saakka ja hidastui sitten lineaarisesti syksyn edetessä.

Työssä vertailtiin myös koivun taimien pakkaskestävyyden määritykseen soveltuvia testejä kasvatusmenetelmien ja taimitarhoille soveltuvien karaistumisen seurantamenetelmien arvioimiseksi. Vertailtavina menetelminä olivat pakkasaltistuksen jälkeen käytettävät elektrolyyttivuoto-, ranganruskettumis- ja taimien kasvatustestit.

Karaistumista voidaan seurata pituuskasvu- ja latvan vesipitoisuusmittauksilla. Karaistumisen ensimmäinen vaihe alkaa pituuskasvun päättyessä ja sen aikana vesipitoisuus alenee kunnes se tasoittuu toisen karaistumisvaiheen, nopean pakkaskestävyyden lisääntymisen, alkaessa. Lehtien kellastuminen ja variseminen korreloi jonkin verran karaistumisen kanssa, mutta aikaisemmat kasvuolosuhteet vaikuttavat näiden muuttujien suhteeseen. Karaistumista voidaan seurata myös altistamalla taimia säännöllisesti –10°C:een ja arvioimalla vauriot kahden viikon jälkeen ranganruskettumistestillä.

Kasvunsääteiden vaikutusta pituuskasvuun tutkittiin käsittelemällä taimet kerran (28.6.) daminozidilla (pitoisuudet 1,0–6,0 g l^{-1}) tai kahdesti (29.6. ja 27.7.) CCC:llä (klormekvaattikloridi) (0,5–3,0 g l^{-1}). Soveltuvimmat käsittelypitoisuudet taimien pituuskasvun hidastamiseen ilman negatiivisia vaikutuksia taimien muuhun rakenteeseen olivat daminozidilla 4 g l^{-1} ja CCC:llä 2 g l^{-1}. Kumpikaan yhdisteistä ei vaikuttanut taimien karaistumiseen.

Toukokuun kylvöerällä kolmen viikon lyhytpäivä- (LP) käsittely (16 tunnin yön pituus) kesäkuun lopussa aloitettuna lopetti koivun taimien pituuskasvun vain väliaikaisesti. Käsittely ei vaikuttanut taimien karaistumiseen. Kesäkuun puolivälin kylvöerällä kolmen viikon LP-käsittely heinäkuun lopussa aloitettuna lopetti pituuskasvun jo käsittelyn aikana. Tämä käsittely aikaisti myös taimien karaistumista ja lisäsi taimien pakkaskestävyyttä karaistumisen ensimmäisen vaiheen aikana 2–6 °C:lla riippuen määritysajankohdasta. Kumpikaan LP-käsittely ei vaikuttanut taimien läpimitan tai juurten kasvuun. LP-käsittelyt eivät myöskään vaikuttaneet istutuksen jälkeiseen kasvuun, mutta pienissä paakuissa kasvatetut, LP-käsitellyt taimet olivat alttiimpia jänistuhoille ja versolaikuille käsittelemättömiin taimiin verrattuna.

Koivun paakkutaimien liiallisen pituuskasvun ja korkeiden taimi- ja istutuskustannusten vähentämiseksi tutkittiin myös mahdollisuutta laajentaa istutus-

kautta kesään käyttäen pienempiä, lehdellisiä, ensimmäisen kasvukauden taimia. Kun taimet istutettiin heinäkuussa tai ennen elokuun puolta väliä, kasvu parani seuraavina kasvukausina ja elävyys oli sama kuin perinteisesti syyskuun alussa tai seuraavana keväänä istutetuilla taimilla.

Tutkituilla taimien kasvun- ja karaistumisen hallintamenetelmillä (kasvunsääteet, LP-käsittely) ei saavutettu toivottuja tuloksia tai menetelmät osoittautuivat epävarmoiksi. Lupaavimmat tulokset saavutettiin kesäistutuksessa. Lehdelliset, kasvukauden aikana lämpimään maahan istutetut taimet, joiden juurten kasvu on aktiivista, juurtuvat nopeasti nopeuttaen taimien alkukehitystä istutuksen jälkeen.

Avainsanat: *Betula pendula*, CCC, daminozidi, elektrolyyttivuototesti, juurten kasvupotentiaali (RGP), juurtuminen, kasvun päättyminen, kasvatustesti, klormekvaattikloridi, lehtien kellastuminen, lehtien variseminen, läpimitta, pakkaskestävyys, pituus, ranganruskettumistesti, verso, vesipitoisuus, vuosirytmi

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Preface

This study was part of the nursery and forest regeneration investigations carried out at Suonenjoki Research Station, Finnish Forest Research Insitute. Experiments on summer planting of silver birch container seedlings were made at the suggestion of Mr. Matti Suihkonen, Director of the Pohjois-Savo Forestry Center; these experiments were made in cooperation with Itä-Suomen Taimi Oy. The study was supported by the Finnish Forest Research Institute, the Graduate School of Forest Ecology, the European Agricultural Guidance and Guarantee Fund, the Finnish Cultural Fundation, Metsämiesten Säätiö, and the Niemi Fundation. These are all gratefully acknowledged. In addition, I am grateful for the excellent working facilities provided by Suonenjoki Research Station.

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Suonenjoki, July 2000 *Jaana Luoranen*

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List of symbols and abbreviations

Symbol	Explanation	Unit	Section
RGP	Root growth potential	g or mg	3.1, 4, 6
SV	Seedling vitality		6
FW	Fresh weight	mg	2
DW	Dry weight	mg	2
WC	Water content	%	2, 4
y_i	Height, diameter, dry mass or injuries in sigmoid function used in estimation of frost hardiness	cm, mm, g, %	2, 3, 5
$f(x)$	Estimated function		2, 3
ε	Error		2, 3
x_i	Age or temperature in the sigmoid function used to estimate growth curves or frost hardiness	days or °C	2, 3
m	Parameter defining the range between upper and lower asymptote of the sigmoid function when estimated the growth responses	cm, mm, g	2, 3, 4.2
c	Point of inflection of the sigmoid function	day, °C	2, 3, 4.2, 5
b	Slope of sigmoid function at inflection point relative to parameter m or a	day^{-1}, $°C^{-1}$	2, 3.2
l	Lower asymptote of the sigmoid function when estimated responses in diameter growth	mm	2, 3, 4.2
x_t	Age of transplantation	day	2
w	Statistical weight coefficient used to homogenize variances		2, 4
$\hat{f}$	Estimate of sigmoid function		4
v	Fixed value used to calculate weight w		2
x_p	Parameter expressing the proportion p from the maximal value of parameter m or a	day, °C	2, 4
h	Point of inflection of the sigmoid function when estimated the shedding of leaves	day	2, 3, 4
k	Slope of sigmoid function at inflection point relative to parameter h	day^{-1}	2
r	Change point defining the age when growth curve change to abscission curve	day	2, 3.1, 4.2
RB	Relative browning	mm/mm	3.2
L_b and L_t	Length of brown tissue and total length of cutting in browning test	mm	3.2
REL	Relative electrolyte leakage		3.2
e_1 and e_2	Electrolyte leakage after freezing and killing	$\mu S\ cm^{-1}$	3.2
ET_{50} and ET_{10}	Temperature causing 50% and 10 % electrolyte leakage	°C	3.2, 4.2
BT_{50} and BT_{10}	Temperature causing 50% and 10 % stem injuries	°C	3.2, 4.2
D	Proportion of damaged seedlings in whole-seedling viability test	%	3.2
L	Proportion of seedlings dead in whole-seedling viability test	%	3.2
DT_{50} and DT_{10}	Temperature causing damage of 50% and 10 % of seedlings	°C	3.2
LT_{50} and LT_{10}	Lethal temperature for 50 and 10 % of seedlings	°C	3.2
d	Parameter used to define the lower asymptote of the sigmoid function		3.2
a	Parameter defining the injury range between lower and upper asymptote in sigmoid function		3.2

1 Introduction

1.1 Growing and planting of silver birch container seedlings

In Finland, regeneration with planted silver birch seedlings (*Betula pendula* Roth) started in the late 1960's as a result of large-scale studies of birch breeding, seedling production and planting techniques by Dr Jyrki Raulo (results have been summarized in Raulo 1978, 1981). At present, 15–20 million birch seedlings are planted annually (Figure 1.1a), of which most are silver birch grown in containers (Västilä and Herrala-Ylinen 1999). In the early 1990s, the annual production of birch seedlings reached its maximum (28 mill. seedlings delivered for planting) (Västilä and Herrala-Ylinen 1999). Because of changes in the annual afforestation area (Figure 1.1b), the demand for seedlings fluctuates greatly, making it difficult to predict the annual need for seedlings.

At present, seeds of silver birch are sown in spring directly into containers or broadcast sown into peat-filled trays, and after a few weeks the germinants are transplanted to container cells. Seedlings are grown in greenhouses until they are moved outside in mid- or late June. Seedlings are then raised in outdoor growing areas until leaf abscission, when they are lifted and stored either outside or in frozen storage until the following spring. A schematic diagram of the present practices in the production of silver birch container seedlings in nurseries is presented in Figure 1.2.

Growing birch seedlings in containers requires a lot of space. During their first growing season, seedlings grow 50–100 cm tall, depending on sowing time and growing conditions. This means that, to give enough growing space for shoot and root growth, seedlings have to be raised in large containers. After the early growth phase, there is also a need to enlarge the distance between containers in order to increase aeration and reduce the risk of disease (Lilja et al. 1997).

So far, it has been difficult to control the growth and frost hardening of silver birch seedlings in nurseries. If weather conditions during the growing season favor growth, seedlings may grow too tall in relation to root plug volume. During a warm autumn, the frost hardening of seedlings may also be delayed. Compared to conifer seedlings, birch seedlings are difficult to protect against frost. When autumn frosts are predicted, conifer seedlings are covered with thin

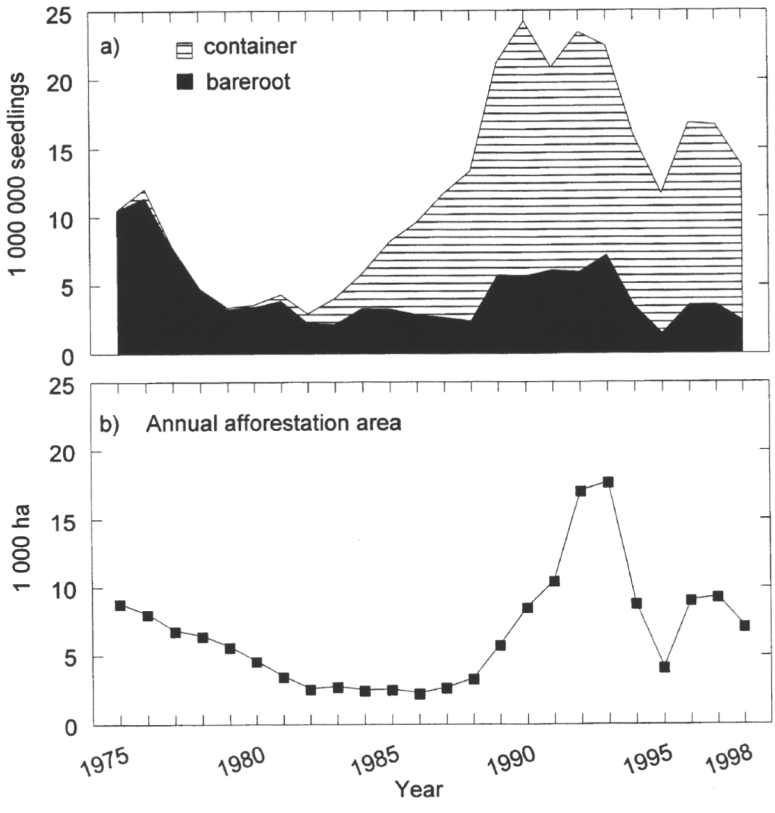


Figure 1.1 a) Annual production of silver birch container and bareroot seedlings (1 000 000 seedlings per year) in Finnish nurseries in 1966–1998 and **b)** total annual afforestation area (1000 ha per year) in Finland in 1970–1998. Figure based on numbers presented in Finnish Statistical Yearbooks of Forestry for the years 1976–1998 (Anonymous 1977–1989, Västilä and Herrala-Ylinen 1999).

cloth or are irrigated in order to prevent damage (Landis 1989). In birch, these methods are not usually used because the seedlings are tall and the areas extensive. The use of irrigation to protect seedlings against frost damage is based on the fact that the temperature of the plant will not fall below the freezing point during sprinkling as long as a change of state from water to ice is taking place (Rose and Haase 1996). The prerequisite for preventing damage to seedlings is the uniform film of water on the surface of seedlings. In birch, the uniform film of water may be difficult to form because of tall seedlings, large leaves and dense crops.

Frost means subzero temperatures at ground level. Hard frost, on the other hand, is defined as frost where the temperature is –0.1°C or lower 2 meters off the ground, which means the ground level temperature is lower than –3°C (Solantie 1987). In southern Finland (60–64°N) in regions with few lakes, hard frosts occur about every third year until the end of August. In the lake region, hard frost do not occur in August, but its probability increases to 25 % until mid-September (Solantie 1987). In late August and early September, the frost hardiness of seedlings may still be poor. Thus there is a need to develop methods to prevent frost damage of silver birch seedlings during autumn.

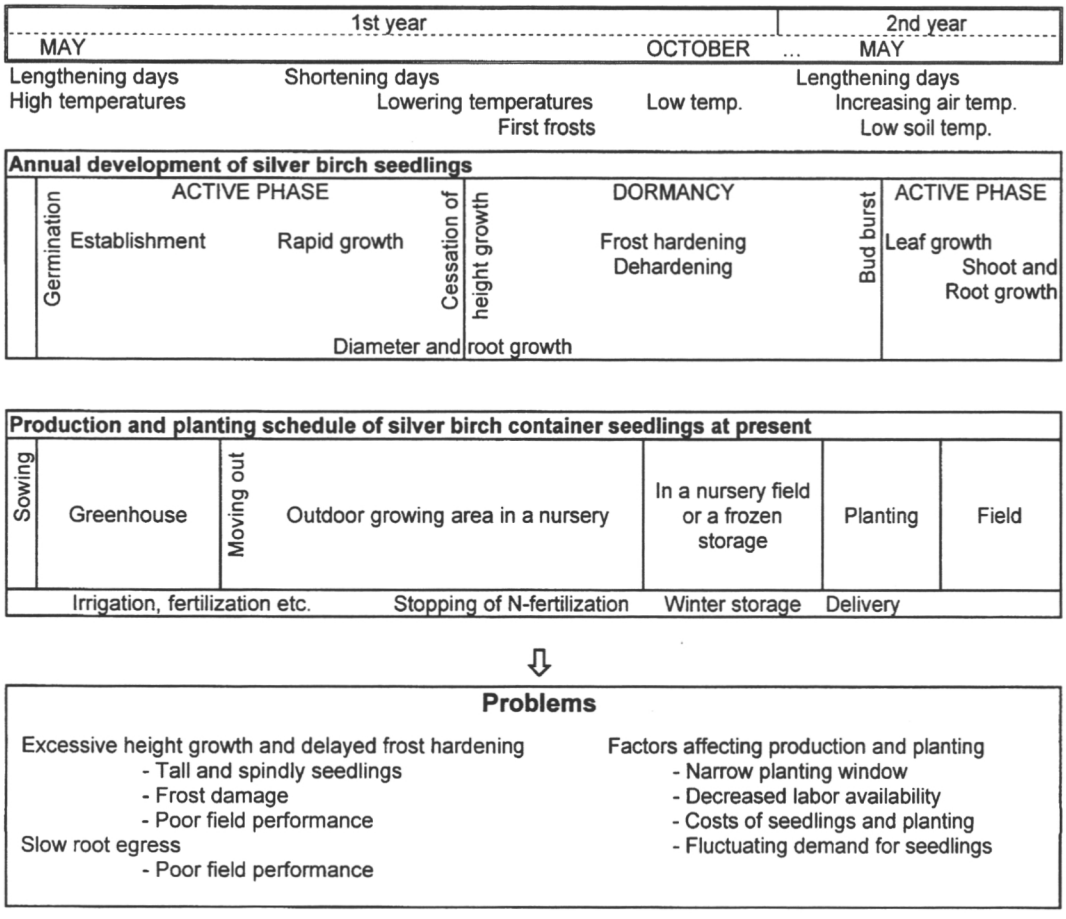


Figure 1.2 Schematic presentation of annual cycle, current production and planting schedule of silver birch container seedlings and their relation to seasonality of environmental factors. Problems concerning production, planting and field performance of birch container seedlings.

The need for methods to hasten frost hardening or at least to determine frost hardiness and frost damage was noticed in eastern Finland nurseries in 1993–1994. The growing season 1993 was cool (Helminen 1993), which delayed the frost hardening of seedlings (Kivivuori 1994b). On a few nights in late September 1993, minimum air temperatures were between –2 and –8°C, and at ground level the temperatures were as low as –10°C over large areas of central Finland (Helminen 1993). These exceptionally severe early autumn frosts caused extensive shoot damage to silver birch seedlings in nurseries in eastern Finland (Kivivuori 1994a,b). The resulting damage incurred losses of millions of Finnish marks for nurseries (Kivivuori 1994a,b). Problems and economic losses increased when some of the damaged seedlings were planted the following spring before the damage was discovered.

At present, it is recommended that silver birch seedlings be planted in May and, in addition, in southern Finland in September or in northern Finland in August (Vastamäki 1993). Most tree seedlings are planted during a few weeks in May and early June (Figure 1.2). During this short period much labor is needed for lifting, transporting and planting both in nurseries and on planting sites. However, the number of workers employed in silvicultural work decreased, for example, from 5000 persons in 1982 to 2300 persons in 1997 (Ylitalo 1998). In addition, it is predicted that in the near future there will be even fewer laborers to do this work. Thus, there is a need to enlarge the planting window, so that fewer laborers have time to plant all the seedlings. This means there is a need to investigate the possibility to plant seedlings in summer.

The field survival of silver birch container seedlings has been lower than that of bareroot seedlings (Parviainen et al. 1989, Hytönen 1995). Mortality as high as 40% of the planted seedlings may occur on afforestation sites during the first 5 years after planting (Hytönen 1995). At present, most of the planted birch seedlings are container grown (Figure 1.1a), and thus alternative methods for forestation using container seedlings to increase the survival of planted seedlings need to be investigated. It would be difficult to return to planting bareroot seedlings on a large scale because their production requires about a year longer than container seedlings. The longer time from sowing to planting bareroot seedlings makes it more difficult to forecast the number of seedlings required for planting. In addition, when bareroot seedlings are used, the total costs of forestation are higher than for container seedlings, because the seedlings are more expensive and the costs of planting are higher.

As described above and in Figure 1.2, there is a need to develop methods for growing seedlings that allow nursery managers to respond more rapidly and more effectively to changes in demand for seedlings. At the same time, nursery managers need better ways to control the height growth and frost hardening of birch container seedlings. Moreover, the field performance of container seedlings should be improved. There is also a need to enlarge the planting window so that 1) seedlings could be planted over a longer period during the growing season and 2) all silvicultural work required to ensure satisfactory regeneration on clear-cut areas could be made with less labor. Thus, methods should be found to reduce or avoid these problems.

1.2 Theoretical framework

1.2.1 The annual growth cycle and frost hardening

1.2.1.1 Active period

The growth and development of seedlings of woody plants are regulated by both internal and environmental factors. Genotype sets the limit for growth, i.e., growth potential; but environmental factors determine the amount of growth potential realized. Environmental factors are highly correlated in nurseries where many growth-limiting factors are kept at optimum levels. Major factors affecting growth and photosynthesis in nursery conditions are light, temperature, humidity, carbon dioxide, water and nutrients in the growing medium (Kozlowski and Pallardy 1997).

Landis et al. (1999) divided the annual growth cycle of container seedlings in nurseries into three development phases: establishment, rapid growth and hardening (Figure 1.2). Sowing of the seeds starts the establishment phase. It continues until the terminal shoot in the middle of the cotyledons begins to grow quickly, which starts the rapid-growth phase. This phase continues until the final height is achieved and the hardening phase starts. The effects of environmental factors and possibilities to affect them vary in each phase. In addition, the duration of each phase depends on the species, seed source, environment, and cultural practices (Landis et al. 1999).

At northern temperate and boreal latitudes, woody ecotypes and species are adapted to the local seasonal photoperiod and temperature conditions (Sakai and Weiser 1973). Due to seasonality, the active growth and dormancy phases are clearly separated (Sarvas 1972, 1974, Fuchigami et al. 1982). During the most intensive phase of growth, most woody species are not able to frost harden and are thus very sensitive to low temperatures (Larcher 1995).

Koski (1990) divided the active period into three phases with respect to response to photoperiod: during the first phase, shoots do not respond to photoperiod; in the second phase, height growth will stop in response to very long nights (>12 h); and in the third phase, shoots become gradually more responsive to night length. According to this hypothesis, in natural photoperiod conditions, the earlier the seedlings have been sown or the warmer the growing season, the sooner height growth should stop.

According to the photoperiod theory, height growth stops when the photoperiod shortens to less than the critical day length (or more correctly, the night is longer than the critical night length) (e.g. Vaartaja 1954, 1957, 1959, Dormling et al. 1968). On the other hand, annual changes in timing of cessation of height growth are explained by the interaction between photoperiod and temperature sum (Koski

and Selkäinaho 1982, Koski 1985, Koski and Sievänen 1985). The warmer the growing season the higher the accumulated temperature sum (>+5°C) and the shorter the night length that can induce cessation of growth (Koski and Selkäinaho 1982).

In addition to photoperiodicity, soil moisture, nutrients, and day and night temperatures affect cessation of growth. In growth chamber conditions, fluctuating day/night temperatures in downy birch (*B. pubescens* Ehrh.) and willow (*Salix pentandra* L.) seedlings (Junttila 1980) and high temperatures (+18–24°C) in Norway spruce (*Picea abies* (L.) Karst.) (Heide 1974a) hasten the response on long nights. On the other hand, warm nights may delay the cessation of growth in birch seedlings, which would normally be induced by long nights (Håbjørg 1972a, Downs and Bevington 1981). However, cool nights (<+4°C) may compensate for the influence of photoperiod and induce cessation of growth even under continuous light (Howell and Weiser 1970). However, temperatures may not greatly influence cessation of height growth under conditions of natural photoperiod (Håbjørg 1972a, Heide 1974a).

Both diameter growth of the shoot (Heide 1974a, 1977, Bjørnseth 1985) and root growth stop later than height growth of the shoot. In many conifers and certain hardwood species, under nonfrozen conditions, root growth may occur all year round (Ritchie and Dunlop 1980 and references therein). Photoperiod does not affect root growth (Heide 1977, Krasowski and Owens 1991, Hawkins, C. et al. 1996), but results concerning photoperiodic effects on cessation of diameter growth have been contradictory. According to Heide (1974a, 1977), photoperiod affects the activity of the cambium in Norway spruce; and the night length that will stop this activity is 2–6 hours longer than that which induces cessation of height growth. In the experiment of Håbjørg (1972a) both photoperiod and temperature affected the diameter growth of downy birch seedlings. Diameter growth may also be regulated by induction of bud dormancy (Ögren 1999a).

1.2.1.2 Frost hardening

Resistance to early autumn frost is closely related to timing of the cessation of height growth (Sakai and Weiser 1973); and in most species, cessation of growth is a prerequisite for frost hardening (Weiser 1970). Many environmental factors can temporarily cause growth to slow or even stop; but after the return of favorable conditions, growth usually continues (Weiser 1970). In some cases, the cessation of height growth induced by a stress factor might negatively affect the frost hardening later in the autumn. For example, when nights are long, low temperatures may prevent induction of frost hardening and reduce frost hardiness later in the fall (Fuchigami et al.

1971). According to Fuchigami et al. (1971), this is because the early stages of frost hardening are active processes, which are possible only above a certain threshold temperature. During the early part of dormancy (autumn dormancy or dormancy I, Sarvas 1972, 1974), called rest (Weiser 1970, Sarvas 1974, Powell 1987), plants have no growth competence. Due to the chilling temperatures of autumn and winter, growth competence increases and during the later stage of dormancy in early and mid-winter, which is called quiescence (Weiser 1970, Hänninen 1990), winter dormancy or dormancy II (Sarvas 1972, 1974), plants have full growth competence. During quiescence, however, bud burst does not take place before the buds are exposed for a prolonged period to high enough temperatures (e.g. temperatures >0°C) (Hänninen 1990).

Freezing injury to plants is caused by the formation of ice in their extracellular or intracellular spaces (Levitt 1980). During active growth, cells of woody plants are killed at the moment of ice formation; but in the frost hardy stage, they are able to tolerate extracellular ice formation in their tissues (Levitt 1980, Chen et al. 1995). Intracellular ice formation always kills the cells (Levitt 1980). In many deciduous species, at winter temperatures, woody tissues and buds survive by supercooling (Palta and Weiss 1993), i.e., the tissues are cooled to a temperature lower than the freezing point without freezing (Levitt 1980).

During frost hardening, the tissues and cells in a seedling change to tolerate low winter temperatures (Levitt 1980). Frost hardening is divided into two (or three) stages (Weiser 1970). The first hardening stage starts with cessation of height growth and is triggered by an increase in night length (Fuchigami et al. 1982). During this hardening stage, several physiological events occur (Weiser 1970). Seedlings accumulate organic compounds such as sugars, proteins, lipids and other cryoprotectants, which results in significant alterations in cellular metabolism (Gusta and Weiser 1972, Yelenosky 1978, Levitt 1980, Grossnickle 1992, Kacperska 1993, Ögren 1999b).

During frost hardening, the water content of the tissues decreases, which is associated with increased concentration of cell solutes and consequent freezing point depression (Pellett and White 1969, Burke et al. 1974, Chen et al. 1977, Gusta et al. 1979, Levitt 1980, Siminovitch 1982, Grossnickle 1992, Chen et al. 1995). This stress- (or photoperiod) induced modification in water relations in the hardening cells during the first stages of frost hardening is a prerequisite for the adjustment of plant growth and metabolism to low temperature (Kacperska 1993). The water content in cells is reduced by displacement of water in the cells during accumulation of sugars, increased water loss and decreased uptake of water (Levitt 1980).

The second stage of frost hardening begins within a few days after the first autumn frost and is characterized by a rapid increase in frost

hardiness (Van Huystee et al. 1967, Howell and Weiser 1970, Glerum 1973). In woody plants the most effective temperature for induction of this second stage of frost hardening is about –3°C (Kacpercka 1993). In this stage, the cell membranes and enzymes are modified in such a way that the cells become tolerant of the loss of water that occurs during formation of extracellular ice (Larcher 1995). The third stage of frost hardening, which leads to the highest level of frost hardiness, is achieved by uninterrupted exposure to temperatures of –30°C ... –50°C (Glerum 1973). Only very frost hardy species are able to attain this stage (Glerum 1973), and this type of frost hardiness can be lost very rapidly (Weiser 1970).

Frost hardiness of shoots is always greater than that of roots (Calmé et al. 1994, Colombo et al. 1995, Ryyppö 1998). In container-grown red oak (*Quercus rubra* L.), sugar maple (*Acer saccharum* Marsh.) and yellow birch (*Betula alleghaniensis* Britton) the roots harden more slowly in autumn and deharden faster in spring than the stems do (Calmé et al. 1994). McEvoy and McKay (1997) studied frost hardiness in several deciduous species and concluded that the frost hardiness of roots depends mainly on soil temperature; air temperature and precipitation have no direct influence. In conifer species the frost hardiness of roots is also increased by decreasing temperature and slightly affected by photoperiod (Bigras and D'Aoust 1992). According to Colombo et al. (1995), the frost hardiness of first-year black spruce (*Picea mariana* (Mill.) B.S.P) seedlings was greatest at the terminal bud and least at the root tip; and the differences in hardiness along the stem and roots were gradual.

Photoperiod and temperature are the major environmental factors that trigger and influence frost hardening of the shoot. Nutrients (Macey and Arnott 1986, Edwards 1989, Hawkins, B. et al. 1995, 1996) and soil moisture (Chen et al. 1977, Chen and Li 1978, Macey and Arnott 1986, DeHayes et al. 1989, Welling et al. 1997) have also been observed to modify frost hardiness. The effects of nutrients are well studied in conifer species. Unbalanced nutrient concentrations and fertilization continued too late in the growing season have usually been associated with poor frost hardiness (Aronsson 1980, Glerum 1985, Stimart et al. 1985). On the other hand, fertilization after bud formation may increase frost hardiness (Glerum 1985). In birch seedlings, high K concentrations in foliage have reflected low frost hardiness (Jozefek 1989). However, fertilization seems not to be a very effective method for controlling frost hardening of seedlings.

Although drought stress did not induce cessation of height growth in downy birch seedlings in long-day conditions, it increased the frost hardiness of buds after the one week of exposure (Welling et al. 1997). In seedlings of red osier dogwood (*Cornus stolinifera* Michx.), low temperature, short day or drought stress were capable of increasing frost hardiness, and the final level of hardiness was the sum of the

hardiness induced by the above-mentioned factors (Chen and Li 1978). As stress factors, nutrition and soil moisture may also disturb development of frost hardiness in shoots (Glerum 1985).

In conclusion, the most promising way to control frost hardening is to stop height growth by artificially increasing night length before the critical length, thereby inducing the first stage of frost hardening. Since decreasing temperature is the main factor promoting the later stages of frost hardening, this stage is less easily controlled in nursery conditions.

1.2.1.3 Response mechanisms to photoperiodic conditions

Responses to photoperiod are mainly sensed through the red/far-red light-absorbing phytochromes in the leaves (Thomas 1998). Several phytochromes sense the changes in the wavelength of light, but only phytochromes A and B have been assessed for their involvement in photoperiodism (Thomas 1998). During the light period, the red light (λ_{max} 660 nm) absorbing form of a phytochrome (Pr) changes to the far-red light (λ_{max} 730 nm) absorbing form (Pfr). During the dark period, the phytochrome reverse to the Pr-form (Chory 1997, Thomas and Vince-Prue 1997). When the duration of uninterrupted darkness is long enough, bud dormancy is induced (Thomas and Vince-Prue 1997).

The photoperiod signal is translocated from leaves to other plant parts in the phloem by plant growth regulators (also called plant hormones or plant growth substances) (Fuchigami et al. 1971, Timmis and Worral 1975, Larcher 1995). Phytochromes and plant growth regulators are associated with both cessation of growth and induction of frost hardening (Thomas and Vince-Prue 1997).

Previously it was thought that the reason for cessation of height growth and the onset of dormancy was the increase in the concentration of ABA in buds induced by short photoperiod (Vince-Prue 1985). Recent studies have showed that the alteration in ABA levels is not directly involved in the cessation of growth in short days (SDs) (Welling et al. 1997). Welling et al. (1997) have shown that drought stress and ABA treatment improved the frost hardiness of downy birch slightly, but could not compensate for SD-induced improvement of frost hardiness. Thus ABA could be a component of the endogenous system which controls dormancy.

At present, it is well established that gibberellins (GAs) play an important role in cessation of height growth. Application of GA can delay or prevent the induction of dormancy in several tree species even under SD-conditions (Junttila and Nilsen 1993). According to a hypothesis presented by Thomas and Vince-Prue (1997), the contents and/or composition of GAs in the buds change when tree seed-

lings are exposed to SDs. Consequently, the cessation of height growth and the induction of frost hardening can be regulated either by shortening the photoperiod or affecting the composition of GAs.

1.2.2 Alternatives for controlling growth and frost hardening

1.2.2.1 Growing methods

Several methods are used to induce the cessation of height growth and frost hardening in nurseries. When seedlings are grown, it is possible to manipulate photoperiod, water supply (drought stress), growth regulators, fertilization and temperature. For several woody species, the use of short-day (SD) treatment, drought stress, a combination of SD and drought stress, timing of fertilization and control of temperature has been widely studied in order to control height growth, cessation of height growth and frost hardening (Jinks 1994, Landis et al. 1999). The effects of treatments differ between species and between environmental conditions.

In birch species, as a method for nurseries, SD treatment has been studied by Kelly and Mecklenburg (1978) and by Luoranen and Rikala (1997) in container-grown silver birch and by Calmé et al. (1995) in container-grown yellow birch seedlings. According to Luoranen and Rikala (1997), the effectiveness of SD treatment to stop height growth and induce frost hardening of silver birch seedlings is dependent on the time treatment is started: if started too young, treatment caused only temporary cessation of height growth. In conifer seedlings, SD treatment has also been shown to hasten frost hardening by at least two weeks compared to seedlings grown under natural photoperiod (Aronsson 1975, Timmis and Worrall 1975, Christersson 1978, D'Aoust and Hubac 1986, Bigras and D'Aoust 1992, Folk et al. 1994, Hawkins, C. et al. 1996). SD treatment seems to be a promising method for controlling the development of birch seedlings in nurseries.

Plant growth retardants are organic compounds that retard cell division and cell elongation in shoot tissues and thus regulate plant height physiologically (Arteca 1996). The activity of most growth retardants is based on their ability to inhibit some step in GA biosynthesis. Which step is blocked depends on the chemical formula of the retardant (Sponsel 1995). Growth retardants that block GA biosynthesis are, for example, onium compounds (chlormequat chloride (CCC), mepiquate chloride, AMO-1618, phosphon D), pyrimidines (ancymidol, fluoprimidol) and triazoles (paclobutrazol, uniconazole, triapenthenol). Another widely used retardant is daminozide; its precise mechanism of action is unknown, although it might affect

gibberellin biosynthesis (Arteca 1996). In general, growth retardants are used for producing shorter, and often sturdier, seedlings (Arteca 1996).

Plant growth retardants have been used for several years to modify plant development, especially in agriculture, floriculture and horticulture. The effects of growth retardants have been studied in the production of forest tree seedlings such as container Norway spruce (Dunberg and Eliasson 1972), white spruce (*P. glauca* (Moench) Voss) (Weston et al. 1980, van den Driessche 1996), lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) (Weston et al. 1980, van den Driessche 1996) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (van den Driessche 1990, 1996) seedlings. Aphalo et al. (1997) studied the effects of growth retardants on the production of silver birch container seedlings.

The effects of drought stress on cessation of height growth and on frost hardening differ between species. The duration and the level of drought are also important. However, drought stress promotes cessation of height growth and frost hardening only in certain species. For example, it can hasten cessation of height growth in western red cedar (*Thuja plicata* Donn) (Grossnickle et al. 1988) but not in Douglas-fir (van den Driessche 1969, MacDonald and Owens 1988) or western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) seedlings (Grossnickle et al. 1988, Grossnickle et al. 1991a). Drought stress also reduces diameter growth (MacDonald and Owens 1988). In conifers, combined SD and drought stress usually gives worse results than one of these methods alone (Grossnickle et al. 1988). In red osier dogwood seedlings, however, their interaction is stronger than either one alone (Chen and Li 1978). When western hemlock seedlings are planted in spring, the combination of SD treatment and drought stress produces seedlings that perform well even in harsh field conditions (Grossnickle et al. 1991a); but in autumn the frost hardiness of western hemlock or Douglas-fir seedlings treated in this way may be reduced (Grossnickle et al. 1991a, MacDonald and Owens 1988).

According to the comparison of methods used to control height growth, induce cessation of height growth or induce frost hardening in several species, the most promising alternatives for silver birch container seedlings seem to be SD treatment and chemical growth retardants.

1.2.2.2 Monitoring of seedling quality in the nursery

High quality seedlings are usually defined as those that will survive well and grow vigorously after planting (Ritchie 1984). Testing of seedling quality is needed to avoid planting damaged stock, to convince costumers that seedlings are high quality, to ensure the correct

timing of nursery practices and to improve nursery practices (Dunsworth 1997).

The production of high quality seedlings demands knowledge of the timing of growth and the development of seedlings in the nursery. Ritchie (1984) categorized the characteristics of seedlings that reflect quality (i.e., performance potential) as either performance attributes or material attributes. Material attributes (seedling morphology, mineral nutrient status etc.) are rapidly measurable, either directly or indirectly, whereas performance attributes are physiological tests (root growth potential (RGP), frost hardiness) that evaluate the seedling's response to certain environmental variables (Ritchie 1984, Mexal and Landis 1990, Mattsson 1997). Puttonen (1997) classified seedling quality tests for the purposes of evaluating general applicability, readiness for lifting and prediction of planting success. He also rated these tests using the scoring criteria of Hawkins and Binder (1990) and concluded that morphological characteristics would be the best overall test method for grading seedling stock and predicting field performance.

Until now, performance attributes have not been necessary for measuring seedling quality routinely in Finnish nurseries. In some other countries with a large number of species, a range of site conditions, high costs of seedling establishment or history of plantation failures, operational testing of seedling physiology is used (Dunsworth 1997). Nowadays, the need for testing has also increased in Finland, as growing methods are changing and, more often, a nursery needs to grow several crops per season and produce stock from more seed origins. This means that there is a need to estimate the frost hardening of seedlings and thus ensure the correct timing of nursery practices (e.g., readiness for frozen storage).

Several methods have been used to evaluate whether seedlings are ready for cold or frozen storage or, in the case of "extended greenhouse culture", to determine the time for moving seedlings out of the greenhouse in autumn. In the Pacific Northwest of the United States frost hardening of conifer seedlings is determined by a whole-seedling viability test (Tanaka et al. 1997). In British Columbia, seedling storability is determined visually by scoring injuries after exposure to –18°C (Binder et al. 1996, Dunsworth 1997). In Ontario, the electrolyte leakage method has also been used after seedlings have been exposed to –15°C (Colombo 1997). The usefulness of chlorophyll fluorescence has also been studied (Binder et al. 1996). In evergreen conifer species, both electrolyte-leakage and chlorophyll-fluorescence methods seem to be more sensitive and quantitative than visual scoring. According to Binder et al. (1996), chlorophyll fluorescence is also suitable for deciduous species, if the seedlings have enough chlorophyll in their stems. In Sweden, several methods are used routinely. For example, frost hardening is monitored by deter-

mining the dry matter content (water content) in the apices of seedlings (without exposure to freezing), root growth potential or electrolyte leakage after exposure to freezing (Dunsworth 1997).

In Ontario, visual assessment, measurement of the chlorophyll fluorescence of the foliage and the RGP test are used in the spring to assess the physiological quality of nursery stock prior to planting (Sampson et al. 1997). In the United States, the RGP and seedling-viability tests are also used for minimizing the planting of poor quality stock (Tanaka et al. 1997).

Which methods are selected depends on the need for evaluation, the tree species and the resources available for assessment. It is possible to determine the time of growth cessation, and thus the triggering of frost hardening of shoot by regular measurement of height. In breeding studies, yellowing and abscission of leaves in silver birch seedlings is used to compare provenances (e.g. Velling 1979). However, to my knowledge, there are no studies in which the actual relationship between frost hardiness and visual scored development in leaf yellowing has been investigated. Blackburn and Brown (1988) observed that birch trees with some green leaves were not as frost tolerant as trees with yellow and/or abscised leaves. In that study, however, frost hardiness was assessed only once and the actual relationship between yellowing and abscission of leaves and frost hardiness was not determined.

During frost hardening, the water content in seedling tissues decreases. This information is utilized when frost hardening of seedlings has been monitored indirectly by regular determination of water content (dry matter content) in the apices of unfrozen seedlings (e.g. Rosvall-Åhnebrink 1977, Colombo 1990, Calmé et al. 1993). This method is commonly used in Swedish nurseries. When water content reaches a certain species-specific value, seedlings are considered to be frost hardened enough to withstand frozen storage or light frosts (Hultén 1980, Calmé et al. 1993, 1995). It has been proposed that the critical value would be, for example, 68–66 % (32–34 % as dry matter content) in Norway spruce, 69% (31 %) in Scots pine (*Pinus sylvestris* L.) (Hultén 1980), 60% (40%) in black spruce, white spruce and Jack pine (*P. banksiana*) (Calmé et al. 1993), and 55% (45%) in deciduous species (Calmé et al. 1995). The relationship between water content and frost hardiness is dependent on seedling origin and on previous growing conditions, as has been shown in Scots pine seedlings (Toivonen et al. 1991).

The electrolyte-leakage (conductivity, ion leakage) method is widely used, and the electrical-impedance spectroscopy used less, to describe freezing damage. Both methods are based on the principle that plasma membranes in tissues injured by freezing allow electrolytes to leak from the symplast into the apoplastic water (Ritchie 1991). In the electrical impedance method, changes in extracellular

resistance caused by freezing are measured (Glerum 1985, Repo 1994). This method is quite rapid (Ritchie 1991), but the results are affected by type of tissue and by some physical factors, such as tissue size (stem diameter) and temperature (Glerum 1985, Repo 1994).

1.2.3 Relation of root growth and root activity to planting windows

The field performance of planted seedlings is strongly affected by the seedling's ability to grow roots into the surrounding soil and to resume water and nutrient uptake (Kozlowski and Davis 1975). After planting, root growth is controlled by the growth phase, soil moisture, nutrition, light intensity and temperature (Lopushinsky and Max 1990, Noland et al. 1997). Photoperiod, on the other hand, does not directly affect root growth (Kelly and Mecklenburg 1978, Bigras and D'Aoust 1993); but the indirect effect of photoperiod on root growth is mediated by net daily carbon assimilation of a seedling.

The timing of root growth during the first growing season has been investigated mainly with conifer species. In northern Finland, the roots of container seedlings of Norway spruce, Scots pine and Siberian larch grow throughout the summer in which they germinate (Kinnunen and Lähde 1972). Time of germination also affects timing of root growth. Roots of Scots pine and Norway spruce seedlings sown in May stopped growing at the end of August, but roots of seedlings that germinated later continued to grow until mid-September (Valtanen et al. 1986). In the study of Mattsson (1986) the roots of Scots pine container seedlings that had germinated earlier grew more early in the summer than did the roots of seedlings that germinated later. In container-grown birch seedlings, the roots grow most in July, declining linearly towards autumn (Rikala 1996).

In some deciduous species, the roots can grow during bud dormancy (Farmer 1975, Webb 1976, Westergaard and Eriksen 1997). However, in birch species, carbohydrate reserves, those stored mainly in the roots, are used to produce leaves in spring; and thus new roots do not grow until the leaves are able to produce carbohydrates (Abod et al. 1991). In the spring of the second year, the root growth of silver birch seedlings begins slowly and speeds up just after the new leaves are fully expanded (Rikala 1996).

The temperature in the root zone strongly affects the growth and activity of the roots. Low soil temperatures decrease uptake of water (Lopushinsky and Kaufmann 1984, Örlander and Due 1986) and nutrients by roots (Bowen 1970, Karlsson and Nordell 1996). This is due to the slowed metabolism of roots and increased viscosity of water, when the temperature in the root zone is low (Ryyppö et al. 1998a). Water and nutrient uptake of old roots is also poorer than

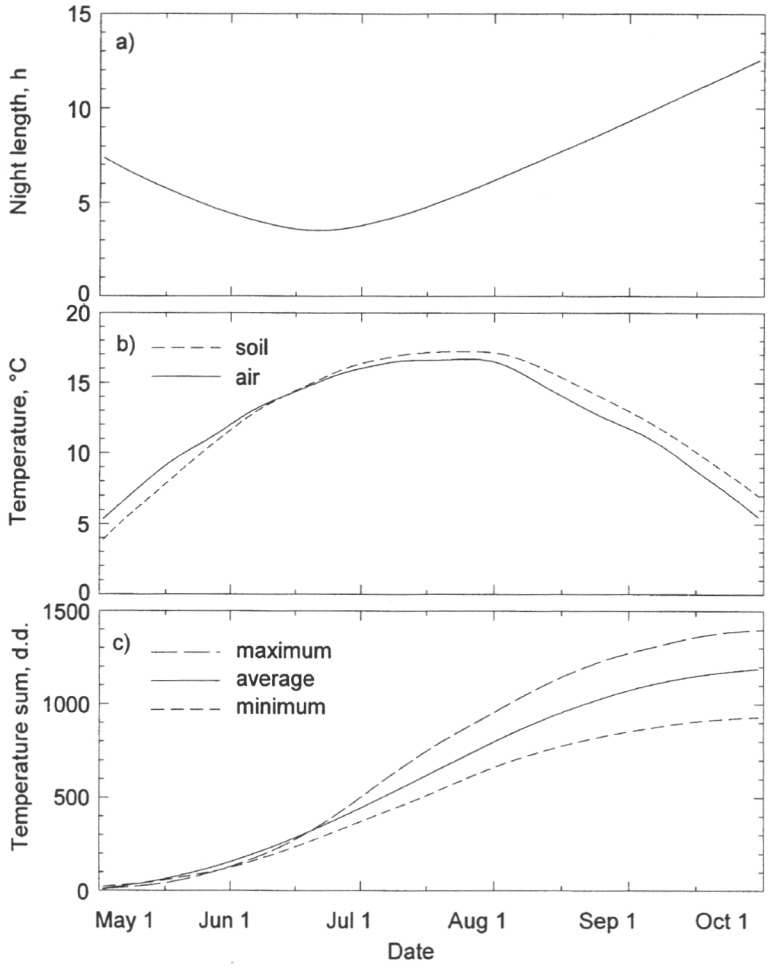


Figure 1.3 a) Changes in night length at the latitude of Suonenjoki, **b)** daily mean air (solid line) and soil (at a depth of 15 cm) (broken line) temperatures as mean values for 25 or 15 years, respectively, at Suonenjoki Research Station during the growing season. **c)** Accumulation of temperature sum (d.d.) during the coolest (1987) and warmest (1972) growing season and the average accumulation of temperature sum in 1972–1999 observed at Suonenjoki Research Station.

that of new, growing roots (Häussling et al. 1988, Ryyppö et al. 1994). In spring, this means that initiation and elongation of root tips are slow or are prevented completely (Lopushinsky and Kaufmann 1984, Vapaavuori et al. 1992, Ryyppö et al. 1994, 1998a, Landhäusser et al. 1996), which decreases the production of dry mass in the whole seedling (Lyr 1996). In summer, the soil temperature (Figure 1.3b) is more conducive to rapid rooting of planted birch seedlings, because the optimal temperature for root growth is about +15°C (Lyr 1996).

At present, most birch seedlings are planted in spring. The argument in favor of spring planting is that dormant seedlings are more resistant to stress (Heikinheimo 1941, Huuri 1974). This is true for bareroot seedlings, which run a great risk of exposing the roots to drying or mechanical damage (Heikinheimo 1941, Huuri 1974). Spring planted seedlings do not take up water efficiently. In deciduous species the roots cannot grow before bud burst (Abod et al. 1991, Rikala 1996) and the water and nutrient uptake capacity of the suberized, old roots is weak (Bowen 1970, Örlander and Due 1986,

Häussling et al. 1988, Karlsson and Nordell 1996, Ryyppö et al. 1994). In addition, after planting container seedlings have poor root-soil contact when all roots are in the peat plug. This also limits water uptake by seedlings (Burdett 1990).

In May and early June, when it is thought that there is enough water to ensure rapid rooting, soil temperatures are low but air temperatures are relatively high (Figure 1.3b). In these conditions, where water uptake by the roots is low but the water and nutrient needs of expanding leaves may be quite high (low air humidity and high evaporation), newly planted seedlings run a great risk of desiccation. The situation can be problematic for seedling establishment: seedlings have to assimilate carbon so that roots can grow, but photosynthesis depends on uptake of water and nutrients by roots (Burdett 1990).

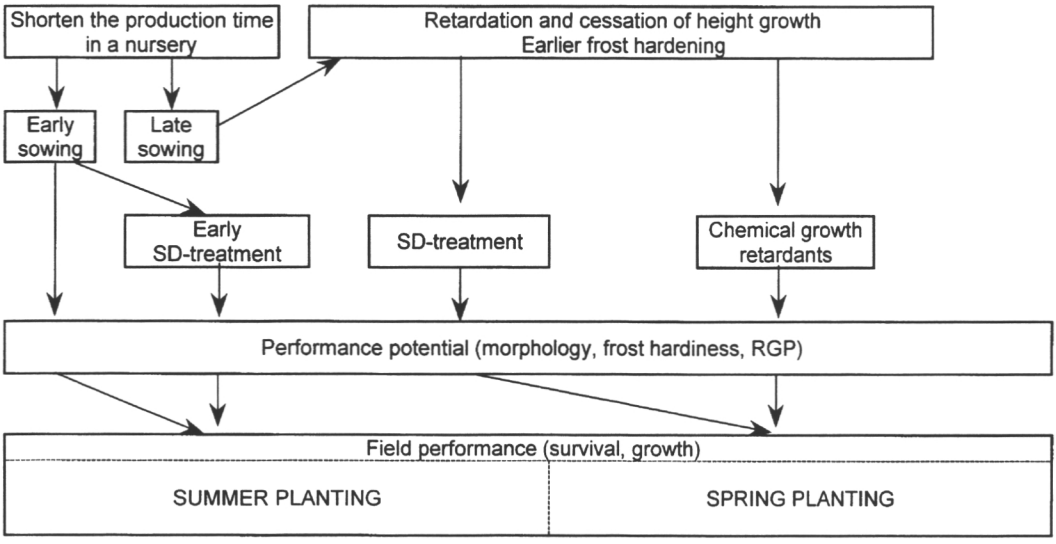
An alternative for solving the above-mentioned problems of slow rooting and increased risk for drought stress in spring is summer planting. In fact, one reason for raising container seedlings was to expand the planting season without increased risk of root damage due to drought (Leikola and Raulo 1973, Kinnunen et al. 1974). So far, container seedlings are mainly planted in spring. In summer, the potential for root growth is high and soil temperatures (Figure 1.3b) should favor both growth and water and nutrient uptake by the roots. Planting throughout the growing season has been studied in all important silvicultural tree species in Finland and Sweden (see Luoranen 1998 and references therein), but only a little in birch seedlings (Lähde and Raulo 1977, Raulo et al. 1994, Rikala 1996). In the late 1980's, however, silver birch container seedlings were summer-planted in some small-scale trials in central Finland with good results (A. Ruha and M. Suihkonen, personal communication).

After summer planting, growth in later years has increased when seedlings were planted at the time of most rapid root growth, the seedlings were relatively short (Kinnunen et al. 1974, Valtanen et al. 1986, Kinnunen 1989, Rikala 1996) and the soil temperature has been near the seasonal maximum (Rikala 1996). However, studies of summer planting are still scarce and not all factors affecting seedling production for summer planting and seedling establishment after planting have been evaluated.

Annual development of silver birch container seedlings (Chapter 3)

MORPHOLOGY	FROST HARDENING
Timing: height-, diameter-, root-growth Partitioning of dry mass	Progress of frost hardening Dormancy induction Methods for monitoring frost hardening

Methods for controlling development of seedlings (Chapter 4):



Enlargement of the planting window (Chapter 5)

Figure 1.4 Schematic presentation of methods which can be used to avoid or solve problems concerning production and planting of silver birch container seedlings. The numbers after the titles in the picture tell the numbers of chapters in which each subject is described.

1.3 Methods for controlling the development of silver birch container seedlings

The problems in the forestation chain of silver birch container seedlings are linked together. The cultural practices carried out in a nursery affect the field-performance potential of seedlings. On the other hand, if one wants to change the planting window, the cultural practices used to produce acceptable seedlings in a nursery must be altered accordingly. Currently, however, our understanding of the annual growth cycle of silver birch seedlings is inadequate. When the growth cycle is better understood, it will be possible to try to find methods either to regulate the growth cycle or to avoid the problems. Figure 1.4 presents alternative ways to solve problems concerning the production and planting of silver birch container seedlings.

One possibile way to solve problems of excessive height growth, delayed height growth cessation and delayed frost hardening is to avoid them. Monitoring of seedling development helps to direct nursery practices to control growth. In this way it may also be possible to predict the risk of frost damage and keep seedling quality high.

Another way to avoid these problems in a nursery is to shorten the production time in the nursery, i.e., to plant seedlings earlier or sow later. In this way it may also be possible to change the practices for production and planting of seedlings so that nurseries can respond more quickly and flexibly to fluctuations in demand for seedlings, enlarge the planting window and possibly decrease the costs of reforestation. However, there may exist other problems when these practices are changed: 1) how well unlignified or only partially lignified seedlings tolerate mechanical stress during lifting, transporting and planting; 2) how summer-planted seedlings frost harden; and 3) whether there is a risk of delayed frost hardening in seedlings sown later in summer.

It is also possible to control height growth of birch seedlings by retarding their growth during rapid growth phase or by inducing earlier cessation of height growth. Growth can be retarded both by timing and changing nursery practices, such as irrigation and fertilization. On the other hand, the growth of seedlings can be inhibited by reducing cell division and enlargement by applying chemical growth retardants. Another possible method is to induce cessation of height growth. An advantage of this method could be that with the same method it is also possible to hasten the frost hardening of seedlings. These methods might also be needed to increase stress tolerance of seedlings if duration of nursery growing is shortened.

1.4 Aims of the study

The objective of this study was to evaluate possibilities to change the forestation chain of silver birch container seedlings so that it would be possible to respond more quickly and flexibly to fluctuations in the demand for silver birch seedlings and to guarantee the field performance of seedlings. The objective was to find methods to control seedling development during the first growing season so that it would be possible:

I. to decrease the risk of frost damage to silver birch seedlings in nurseries during the first autumn frosts,
II. to control the height growth of seedlings without negative influences on root and diameter growth or field performance, and
III. to maintain or increase the field performance of seedlings compared to container seedlings grown and planted according to present practices.

To achieve these objectives

1. timing of height (Sections 3.1 and 4) and diameter growth, dry mass partitioning (Sections 3.1 and 4.2) and frost hardening (Sections 3.2 and 4.2) of silver birch container seedlings were charactrized during the first growing season;
2. methods of monitoring frost hardening in nurseries were compared to find suitable methods for operational use in nurseries. To do this the frost hardiness (Sections 3.2, 4.2 and 5) of birch shoots was assessed, water content was determined and leaf yellowing and abscission were scored (Chapters 3–5);
3. applications of growth retardants (Section 4.1) and short-day treatment (Section 4.2) were studied as methods of regulating growth and frost hardening of birch seedlings;
4. the ability seedlings to grow roots into the surrounding soil (root egress) during the first year was monitored to determine the best planting windows and hence improve the ability of seedlings to survive and grow after planting (Sections 3.1 and 4.2);
5. the possibility of expanding the planting window of silver birch container seedlings by planting growing, leaf-bearing seedlings in summer was investigated (Sections 4.2 and 5).

More specific objectives will be presented in each part of the study.

2 Seedling material, study conditions and common methods

2.1 Introduction

This chapter presents methods used in several chapters of the study. If a method was used in only one part of the work, the method is described in that chapter.

2.2 Growing conditions

These studies were carried out during 1995–1999. The studies on seedling development (Chapter 3) and growing methods (Chapter 4) were done at Suonenjoki Research Station (62°39'N, 27°03'E, altitude 142 m asl); and in all those studies, seeds of silver birch from seed orchard 379 (M29-92-0001) were used. The seedling material used in the summer planting experiment is presented in Section 5.2.

Daily mean and minimum temperatures as well as daily precipitation at Suonenjoki Research Station for each year are presented in Figure 2.1. In each crop, accumulative temperature sums (threshold value +5 °C) were calculated from the time of sowing using mean daily temperature data collected by thermographs (Lambrecht) placed at seedling level.

2.3 Morphology and nutrient analysis

Height growth of the stem was monitored weekly by measuring the same randomly selected seedlings in each treatment. Height (H) was measured (accuracy 1 mm) from the surface of peat (retained as a root collar) to the tip of the shoot apex. Diameter (D) (accuracy 0.01 mm) was measured 2 cm above the peat surface. Sturdiness (S) of seedlings (mm m^{-1}) was calculated as

$$S = \frac{D * 1000}{H} \qquad (2.1)$$

For monitoring dry mass partitioning, shoots (stems and branches), leaves and roots were separated, the roots washed, and all three sections dried for 24 h at 105°C before they were weighed (accuracy 1 mg). Samples to be used for nutrient analysis were dried for 48 h at

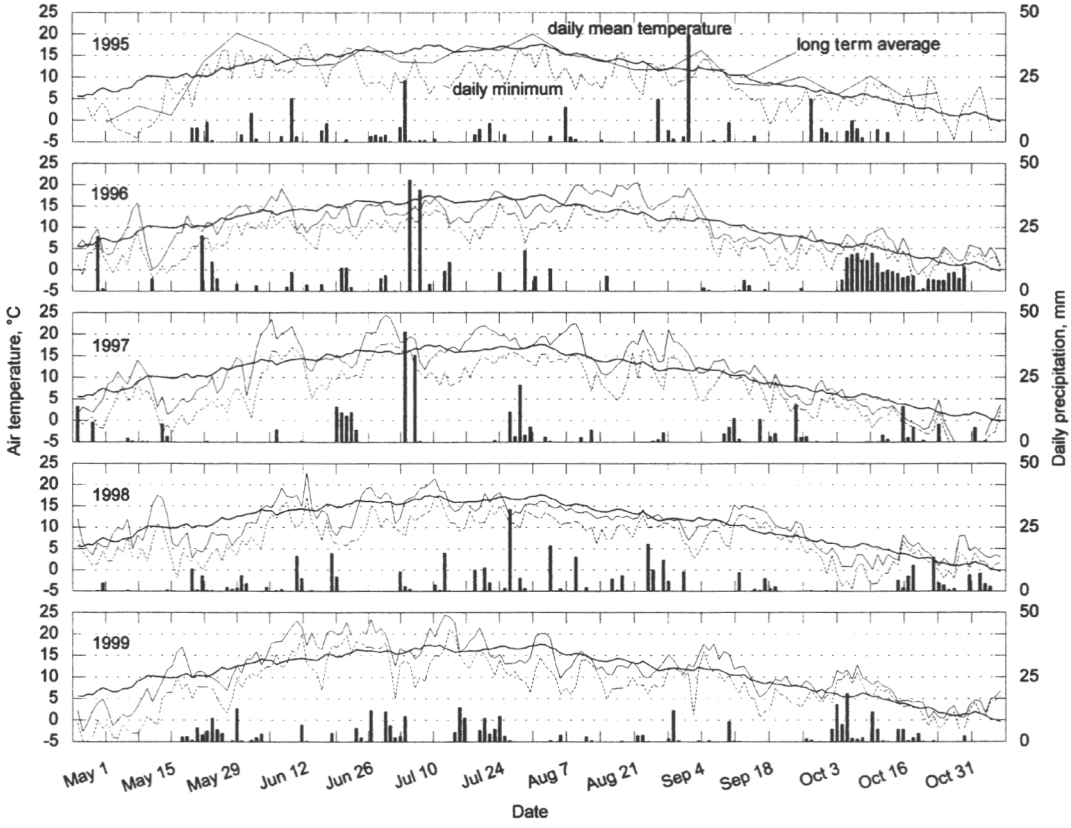


Figure 2.1 Daily mean (thin solid line) and minimum (dotted line) air temperatures (°C) and precipitation (bars) (mm) at Suonenjoki Research Station from 1 May to 31 October in 1995, 1996, 1997, 1998 and 1999. Air temperatures were compared to the 27-year average air temperatures (thick solid line) recorded at Suonenjoki Research Station.

60°C. Nitrogen concentration of samples was determined with a LECO CHN-600 analyzer (Leco Co, USA); and concentrations of P, Ca, K, Mg, Cu and B of samples were determined from dry-digested 2 M HCL samples (Halonen et al. 1983) using plasma emission spectrophotometric analysis (ICP, ARL 3800).

2.4 Water content

The procedures used for determination of water content were the same as those described by Rosvall-Åhnebrink (1977) and Colombo (1990) for conifer seedlings and by Calmé et al. (1995) for hardwood seedlings, but with slight modifications. Seedlings were watered the day before sampling. The uppermost 10 cm (Section 3.1) or 5 cm (Sections 4.1, 4.2) of stem from randomly selected seedlings were cut in the morning at 0730 h–0900 h. The cut apices were put into plastic bags and weighed (accuracy 1 mg) without leaves within two

hours from sampling (fresh weight, *FW*), then put into paper bags, dried in an oven for 24 h at 105°C and weighed again (dry weight, *DW*).

Here water relations are expressed as water content (*WC*) as described by Timmis and Fuchigami (1982) and Colombo (1990)

$$WC = \left(\frac{FW - DW}{FW} \right) * 100 \qquad (2.2)$$

2.5 Root growth potential and root-egress test

Root growth potential (RGP) is defined as the ability of a tree seedling to initiate and elongate new roots within a prescribed period of time in a standard environment that is optimized to promote root growth (Simpson and Ritchie 1997). Since RGP consists of the initiation, elongation and thickening of roots and can be measured quickly, in this study, RGP is expressed as the dry mass of roots grown out from the peat plug during the test period. When the effects of treatments were compared (Sections 4.1 and 4.2: the second crop), RGP was used as a conventional method of evaluating the vigor of seedlings in spring.

RGP was tested in frozen stored and thawed seedlings in spring. RGP was determined by planting seedlings in 0.75 l (the first crop in Section 4.2) or in 2.2 l (diameter at soil surface 17 cm, experiment in growth retardant in Section 4.1) plastic pots filled with sand. Seedlings were grown in a heated and lighted (day/night temperature: +20 – +22 °C/ +15°C, photon flux density (photosynthetically active radiation, PAR) 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from metal halide lamps (HQI-400W Power Star, Osram), photoperiod 18 h) greenhouse for four weeks. All seedlings were watered three times a week with tap water. At planting, the height and the diameter of the root collar were measured. After three weeks, all seedlings were harvested, and the height and diameter were measured again. Roots growing out of the peat plug into the sand ('new' roots) were cut and washed; roots in the peat plug ('old' roots) were washed, and the shoot was divided into stem and leaves. All plant parts (new and old roots, stems and leaves) were then dried in an oven for 24 h at 105°C and weighed (accuracy 1 mg) separately.

In an attempt to find suitable planting windows for silver birch seedlings grown in containers (Sections 3.1 and 4.2: the first crop), the ability of seedlings to root egress at different times of the year was evaluated by using a modificated RGP test (root-egress test). Seedlings were planted in 0.75 l (the first crop in Section 4.2) or in 2.2 l (diameter at soil surface 17 cm, growth rhythm experiment in

Section 3.1) plastic pots filled with sand and grown as in the RGP test in spring, except that the greenhouse was unheated and under natural photoperiod and light conditions. The seedlings were watered as necessary (at least twice a week) with tap water. Height, root collar diameter and dry mass of shoots, leaves and roots were measured as described above, except that roots in the peat plug ('old' roots) were excluded.

2.6 Statistical analysis

2.6.1 Statistical tests

The Bartlett test was used to determine the homogeneity of the variances and the Lillefors test to determine whether the data were normally distributed. When needed, log- or other transformation was used to obtain normal distributions before analysis of variance. When the differences between treatments in weekly measured height or water content were analyzed, an analysis of variance procedure for repeated measurements was used (Chapter 4). Differences between treatments or weeks were analyzed with Tukey's HSD test or with a t-test. If no transformation was found to transform the distributions to normal and to homogenize the variances, the effects of treatments were analyzed with non-parametric methods: Kruskal-Wallis one-way analysis if several treatments were compared, and Mann-Whitney U-test if only two treatments were compared. Results shown in the figures and tables are back-transformed. Possible linear correlation between variables was analyzed with Pearson's linear correlation analysis (Sections 3.1 and 3.2). Data were analyzed with Systat 5.05 statistical software (Systat Inc, USA) (Wilkinson et al. 1992).

2.6.2 Estimation of growth curves

Development of first-year seedlings was monitored in Sections 3.1 and 4.2. Height, diameter and dry mass of the shoot and roots of seedlings (later referred to as morphological attributes) increases with increasing age to a certain point sigmoidially (see Figures 3.1a,b and 4.7). For estimating growth responses in different crops, a simple four-parameter logistic model (Ratkowsky 1990) was fitted to the data.

The developmental function is

$$\begin{aligned} y_i &= f(x) + \varepsilon \\ &= \frac{m}{1 + e^{b(c - x_i)}} + l + \varepsilon \end{aligned} \qquad (2.3)$$

In the function, y_i is the value of a morphological attribute at the age x_i; and m, b, c and l are equation parameters. The upper and lower asymptotes of the function are determined by parameters m and l, respectively. Parameter l is the lower asymptote of function, the level of attribute at the beginning of development. Parameter m expresses the amount of growth between the lower and upper asymptotes. Thus, $m + 1$ indicates the upper asymptote, which tells the final value of an attribute in the end of growing season. Parameter c, the point of inflection, expresses the age of the maximum growth rate. Parameter b expresses the slope of the curve at the point of inflection, i.e., a maximum growth rate. This basic equation was used to estimate the development of diameter.

For the height and dry mass of shoot and roots the estimated equation was

$$y_i = \frac{m}{1+e^{b(c-x_i)}} - \frac{m}{1+e^{b(c-x_t)}} + \varepsilon \tag{2.4}$$

This equation was used due to the fact that these attributes were measured not from the time of sowing but after transplantation. It was assumed that at the age of transplantation (x_t) a value of an attribute is zero.

The variation in the measured attributes increased with time (see Figure 4.2). To homogenize the variances both sides of equation (2.3) were divided by the statistical weight (w)

$$w = \hat{f}(v - \hat{f}) \tag{2.5}$$

In the above equation, $\hat{f}$ is the estimate of $f(x_i)$ given to the first iteration and v is some fixed value (in the most of cases 10).

Cessation of growth was defined by estimating the age x_p. The height, diameter or dry mass at age x_p is the proportion p of the total amount of growth m (expressed as $f(x_p) = pm$). An inverse function of equation (2.3) is

$$x_p = -\frac{\ln\left(\frac{m}{f(x_p)} - 1\right)}{b} + c \tag{2.6}$$

x_p is the new parameter, and one of the original parameters has to be removed and expressed by x_p and other parameters to avoid overparametrization. The easiest way is to replace c or b. The slope b of equation (2.6) is

$$b = \frac{-\ln\left(\frac{m}{f(x_p)} - 1\right)}{x_p - c} \tag{2.7}$$

By substituting equation (2.7) into equation (2.3) and by using $f(x_p) = pm$, we get

$$y_i = \frac{m}{1+e^{\frac{-\ln\left(\frac{1}{p}-1\right)}{x_p-c}(c-x)}} + \varepsilon \tag{2.8}$$

Or replacing the inflection point c

$$c = x_p + \frac{\ln\left(\frac{m}{f(x_p)}-1\right)}{b} \tag{2.9}$$

and the equation for estimation is

$$y_i = \frac{m}{1+e^{b\left(x_p+\frac{\ln\left(\frac{1}{p}-1\right)}{b}-x\right)}} + \varepsilon \tag{2.10}$$

To estimate the growth cessation or the end of dry mass accumulation, equation (2.8) or (2.10) with a value of 0.95 for p was fitted.

Development of the dry mass of leaves consists of two parts: increase in dry mass and abscission of leaves. Both parts change with time to a certain point as a logistic function (see Figure 4.7c). Equation (2.4) can be used to express the increase in dry mass of leaves. Abscission of leaves is a decreasing sigmoidial curve with parameter h to the point of inflection and parameter k to the slope of the curve. The time when the growth curve changes to an abscission curve can be calculated using change point (Lappi 1993 and references therein). When $k < 0$, the equation for abscission of leaves is

$$\begin{aligned} y_i &= f_2(x) + \varepsilon \\ &= \frac{m_2}{1+e^{k(h-x_i)}} + \varepsilon \end{aligned} \tag{2.11}$$

In change point r, the growth curve changes to an abscission curve. At this point r,

$$f(x) = f_2(r) \tag{2.12}$$

or

$$\frac{m}{1+e^{b(c-r)}} - \frac{m}{1+e^{b(c-x_i)}} = \frac{m_2}{1+e^{k(h-r)}} \tag{2.13}$$

or

$$m_2 = \frac{m\left(1+e^{k(h-r)}\right)}{1+e^{b(c-r)}} - \frac{m\left(1+e^{k(h-r)}\right)}{1+e^{b(c-x_t)}} \tag{2.14}$$

Thus, the development of the dry mass of leaves from sowing to total abscission of leaves is

$$y_i = \begin{cases} \dfrac{m}{1+e^{b(c-x_i)}} - \dfrac{m}{1+e^{b(c-x_t)}} & , \text{ when } x < r \\ \dfrac{m_2}{1+e^{k(h-x_i)}} & , \text{ when } x \geq r \end{cases} \tag{2.15}$$

The end of dry mass partitioning into leaves was estimated by substituting equation (2.7) or (2.9) with a value of 0.95 for p into the first part of equation (2.15). In the same way, the time when all leaves were abscised was estimated by reparametrization of the second part of the equation (2.15) and using a value of 0.05 for p. The change point r was used to express the start of leaf abscission.

Equations were fitted by using nonlinear regression with Gauss-Newton of Systat 5.05-statistical software (Systat Inc, USA) (Wilkinsson et al. 1992). Equation (2.15) was estimated with the Simplex method of estimation. The Gauss-Newton method did not work as the derivatives of the response function with respect to change point r do not exist.

3 Development of first-year silver birch container seedlings

3.1 Shoot and root growth

3.1.1 Background and study objectives

In nurseries, knowledge of the timing of growth and partitioning of dry mass into different parts of seedlings during the growing season helps nursery managers to time and plan growing practices (sowing time, growing density – target height, outmoving time, fertilization, etc.). However, only Rikala (1996) has published information on growth of first-year silver birch seedlings grown in containers in a nursery. Timing of shoot but especially root growth is also important for choosing planting windows for silver birch seedlings. For seedling survival and rapid growth after planting, it is important that roots grow quickly from the peat plug into the surrounding soil (Kozlowski and Davis 1975).

The ability of a seedling to grow roots in an environment that is highly favorable for root growth has been measured by a test of root growth potential (RGP) (Mexal and Landis 1990, Ritchie and Tanaka 1990). RGP is a seedling performance attribute (Ritchie 1984). In general, the higher the RGP of a seedling the greater its chance of survival (Burdett 1987). RGP has been studied in deciduous bare-root seedlings before frozen storage or in spring after storage (e.g. Farmer 1979, Webb and von Althen 1980, Struve 1990). During the first growing season, the ability of the roots of silver birch seedlings to egress from the peat plug is highest in July and early August (Rikala 1996). In the first summer, root growth is also greater than with traditional spring planting (Rikala 1996). In the present study, the root egress (the dry mass of outgrown roots) was used as a parameter for measuring and comparing the root growth of seedlings at different times of the first growing year.

The objective of this part of the study was to investigate: 1) the time course of and relationship between height- and diameter growth and partitioning of dry mass to shoot, roots and leaves from transplanting to abscission of leaves in silver birch seedlings growing in a nursery and 2) ability of seedlings to grow roots out from the peat plug (root egress) at different times of the growing season.

3.1.2 Material and methods

In three crops, seeds of silver birch were sown on peat-filled germination trays in a greenhouse on 15 April (later this crop is referred to as APRIL96) and on 2 May (MAY96) 1996 and on 5 May 1997 (MAY97). Germinants were transplanted into Plantek 25 trays (25 cavities per tray, 380 cm^3 per cavity, 156 cavities per m^2, Lännen Plant Systems, Finland) filled with fertilized sphagnum peat (Vapo, XL, Finland) on 3 May (APRIL96) and on 23 May (MAY96) 1996 and on 29–30 May 1997 (MAY97). In each crop, the seedlings were irrigated according to normal nursery practice by maintaining the water content of the peat at 40–70 percent by volume during the growing season. In 1996, in both crops the seedlings were irrigated and fertilized after they had been moved to the same outdoor growing area according to the same regime; in late summer the water content in peat plugs was lower in APRIL96 than in MAY96. In the APRIL96, MAY96 and MAY97 crops, seedlings were fertilized with liquid fertilizer 6, 5 and 4 times, respectively. The total amounts of nutrients (including both base and liquid fertilizer) given were 144 mg N, 51 mg P and 162 mg K per seedling plus micronutrients for APRIL96, 132 mg N, 47 mg P, 150 mg K per seedling plus micronutrients for MAY96, and 87 mg N or 36 mg P and 106 mg K per seedling plus micronutrients for MAY97. Seedlings were grown in a greenhouse until they were moved to an outdoor growing area on 10 June or 20 June in 1996 and on 25 June in 1997. All seedlings were sprayed twice with Bayleton 25 (triadimefon 0.05 %) against birch rust (*Melampsoridium betulinum* (Pers.) Kleb.): on 12 July and 1 August in 1996 and on 29 July and 29 August in 1997. Seedlings were sprayed against fungi that cause stem lesions with Tilt 250 ES (propiconazole 0.5 l ha^{-1}) on 16 July 1996 and with Shirlan (fluazinam 0.4 l ha^{-1}) on 14 August 1996. The seedlings were sprayed against aphids with Ripcord (cypermethrin 0.05 %) on 28 July 1997.

Air temperatures at seedling level (about 10 cm from the ground) were monitored both years. The accumulation of the temperature sum (threshold value +5 °C) was calculated from the time of sowing. The climate in July 1996 was cool (14°C average temperature, 15-year mean daily temperature in Suonenjoki = 16°C) and rainy (monthly cumulative sum was 137 mm, 15-year mean monthly cumulative sum = 84 mm), but August was warmer (17°C average temperature, 15-year mean daily temperature 14°C) and precipitation was low (cumulative sum = 17 mm, 15-year mean = 60 mm) (Figure 2.1). The 1997 season was warmer than the 15-year average (average temperatures were 16, 19, 17°C, 15-year mean daily temperatures were 14.5, 16, 14°C in June, July and August, respectively) (Figure 2.1).

In each crop, there were 30 trays (25 seedlings in each) divided into 10 blocks, i.e., a total of 750 seedlings. In the crops of APRIL96

and MAY96, the height of the stems of the same two randomly selected seedlings per tray in all 30 trays were measured weekly from 2 weeks after transplanting to the middle of September. From 4 September to 9 October 1996, yellowing and abscission of leaves was recorded weekly.

In each crop, two seedlings per block, a total of 20 seedlings each week, were harvested from 15 May to 15 October, from 3 June to 14 October, and from 9 June to 13 October for APRIL96, MAY96 and MAY97 crops, respectively. Height and diameter were measured, and dry mass of the shoots (stem and branches), leaves and roots was determined. In 1996 and 1997 the roots were washed by different people, but during each year the person washing the roots was the same. Thus the results of different years are not totally comparable. Roots that grew into the ground often broke when the seedlings were lifted. Thus the results do not indicate the total dry mass of the roots. The aim of this study was to examine the amount of roots in seedlings for planting and it was not considered necessary to include broken roots in the samples. At the last harvesting date in October 1996, the shoot and root samples in each block were pooled for nutrient analysis. Nutrient concentrations in the stem and roots in both crops in 1996 are presented in Table 3.1. In 1997, water content in the uppermost 10 cm of all harvested MAY97 seedlings was determined from 11 August to 13 October.

For the root-egress test, 20 randomly selected seedlings per crop were planted weekly into 10 blocks from 26 June to 2 October and

Table 3.1 Nutrient concentrations of stems and roots in seedlings from crops sown in April or in May 1996. Nutrients were analyzed separately from stems and roots at the end of the first year (on 2 October or on 30 September 1996). Values are the means of five samples (drawn from a pooled sample of two seedlings) and the standard error of the means.

Nutrient	Stem		Roots	
	April	May	April	May
Nitrogen, g kg^{-1}	9.8 ± 0.36	9.0 ± 0.30	7.8 ± 2.24	8.0 ± 0.28
C/N	58 ± 1.93	71 ± 6.23	63 ± 2.00	68 ± 2.34
Phosphorous, g kg^{-1}	1.32 ± 0.05	0.99 ± 0.13	1.53 ± 0.09	1.53 ± 0.02
Potassium, g kg^{-1}	4.18 ± 0.09	3.18± 0.42	4.05± 0.20	4.11 ± 0.08
Calcium, g kg^{-1}	2.22 ± 0.21	1.46 ± 0.21	2.87± 0.19	3.11 ± 0.07
Magnesium, g kg^{-1}	0.89 ± 0.06	0.63 ± 0.08	1.13 ± 0.05	1.33 ± 0.03
Manganese, mg kg^{-1}	68.9 ± 5.3	56.5 ± 9.4	58.5 ± 5.0	64.9 ± 6.50
Copper, mg kg^{-1}	3.0 ± 0.2	3.5 ± 2.1	11.5 ± 0.4	11.0 ± 1.1
Zinc, mg kg^{-1}	77.8 ± 7.0	52.6 ± 8.4	57.2 ± 3.3	56.0 ± 2.88
Iron, mg kg^{-1}	28.2 ± 2.2	22.9 ± 3.3	263 ± 44	210 ± 36.3
Boron, mg kg^{-1}	8.5 ± 0.5	6.6 ± 1.0	7.1 ± 0.74	7.8 ± 0.19

Table 3.2 Estimated parameters of the growth curves for height (Equation 2.4), diameter (Equation 2.3) and dry mass of shoot and roots (Equation 2.4) and the parameters of the development curves for the dry mass of leaves (Equation 2.15) in seedlings sown on April 1996 (APRIL96), on May 1996 (MAY96) and on May 1997 (MAY97). The dates and accumulated temperature sums at the age of the maximum rate of growth (parameter c) and at the age when growth ceased ($x_{0.95}$) for each variable and dates and accumulated temperature sums at the age when leaves began to shed (r) and when all leaves were shed ($x_{0.05}$) for dry mass of leaves.

Parameters of estimated growth curves

Variable	Crop	m	b	c	$x_{0.95}$	l
Height	APRIL96	72	0.06	80	124	
	MAY96	67	0.07	78	120	
	MAY97	84	0.09	69	101	
Diameter	APRIL96	6.3	0.05	73	129	0.1
	MAY96	6.4	0.06	75	124	0.1
	MAY97	6.9	0.07	63	104	0.1
Shoot	APRIL96	5.0	0.08	107	145	
	MAY96	4.8	0.09	101	134	
	MAY97	5.4	0.09	92	124	
Roots	APRIL96	3.7	0.07	103	146	
	MAY96	3.5	0.08	98	134	
	MAY97	2.6	0.09	90	123	

Parameters of estimated development curves for dry mass of leaves

Variable	Crop	m	b	c	$x_{0.95}$	k	h	r	$x_{0.05}$
Leaves	APRIL96	2.4	0.09	75	107	–0.13	168	154	191
	MAY96	2.8	0.10	78	106	–0.23	154	140	167
	MAY97	2.2	0.17	63	81	–0.15	147	119	167

Dates and accumulated temperature sums at the estimated ages

		Max. growth rate c		Cessation of growth $x_{0.95}$		Beginning of shedding r		All leaves shed $x_{0.95}$	
Variable	Crop	date	d.d.	date	d.d.	date	d.d.	date	d.d.
Height	APRIL96	4 Jul	851	17 Aug	1288				
	MAY96	19 Jul	767	30 Aug	1212				
	MAY97	13 Jul	866	14 Aug	1306				
Diameter	APRIL96	27 Jun	790	22 Aug	1351				
	MAY96	15 Jul	741	2 Sep	1239				
	MAY97	7 Jul	878	17 Aug	1324				
Shoot	APRIL96	31 Jul	1133	7 Sep	1485				
	MAY96	10 Aug	987	12 Sep	1265				
	MAY97	5 Aug	1194	6 Sep	1536				
Roots	APRIL96	27 Jul	1094	8 Sep	1489				
	MAY96	7 Aug	949	12 Sep	1265				
	MAY97	3 Aug	1165	5 Sep	1527				
Leaves	APRIL96	29 Jun	810	31 Jul	1133	16 Sep	1493	23 Oct	1532
	MAY96	18 Jul	761	15 Aug	1046	18 Sep	1269	15 Oct	1306
	MAY97	7 Jul	801	25 Jul	1041	1 Sep	1500	19 Oct	1634

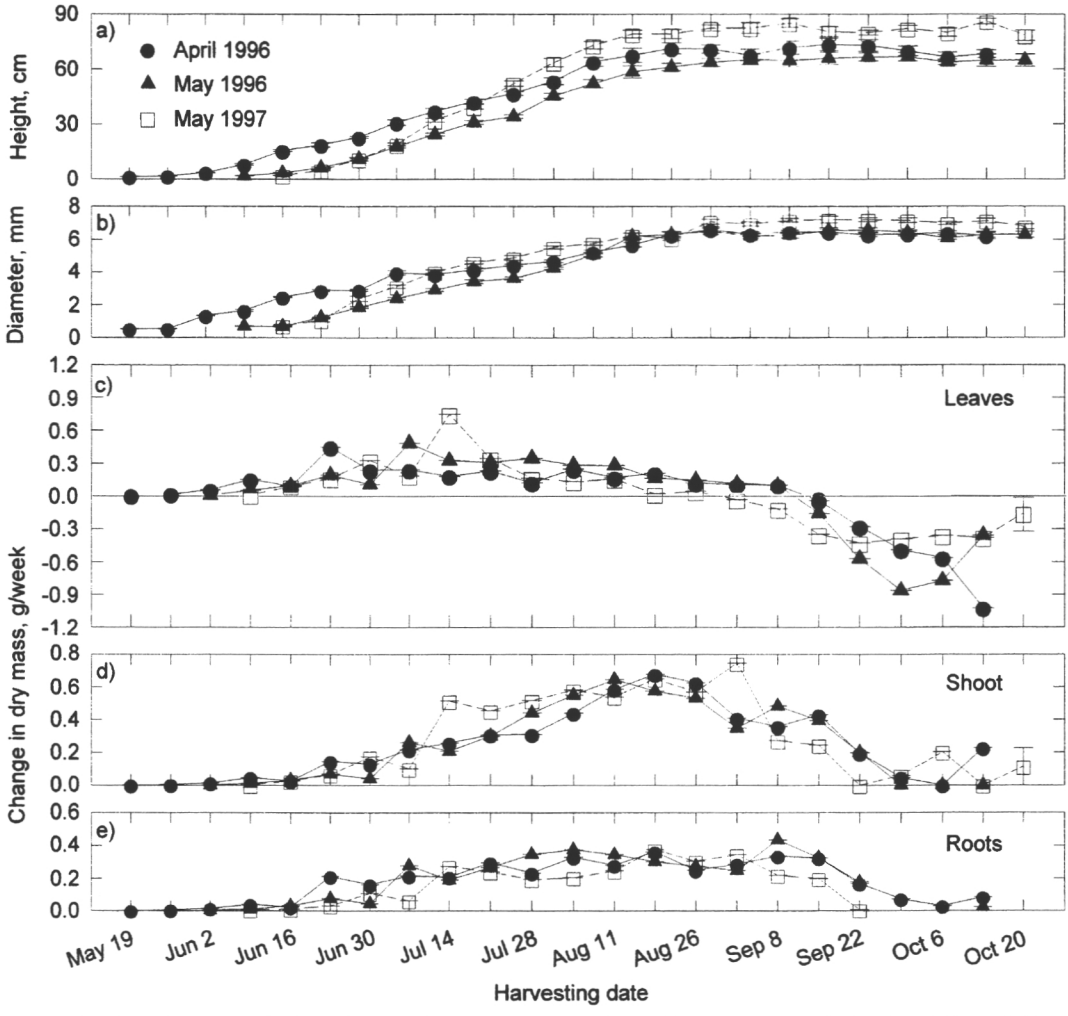


Figure 3.1 Development of dimensions and dry mass in birch seedlings sown on 15 April or 2 May 1996, or 5 May 1997. **a)** Height, **b)** diameter and **c)** changes in dry mass of leaves, **d)** shoot (including stem and branches) and **e)** roots of seedlings were measured weekly from transplanting to the middle of October 1996 or 1997. Each symbol indicates the mean of 10 blocks (mean of 2 seedlings per block) on each harvesting date.

from 1 July to 30 September 1996 for APRIL96 and MAY96, respectively. Seedlings planted in sand-filled plastic pots were kept in an unheated greenhouse under natural photoperiod and light conditions for three weeks. In the greenhouse, the daily mean temperature during the summer was about 21°C (the largest difference between minimum and maximum temperature was 12°C in the middle of August, but otherwise the difference was 1–5°C) and the RH 58% (48–65%). On 14 October, seedlings for the root-egress test the following spring were packed in plastic bags and stored at –4°C. From 17 May until they were planted in the test on 27 May, the seedlings were thawed and kept in dark (4 d at 5°C). After 3 weeks, the seedlings were

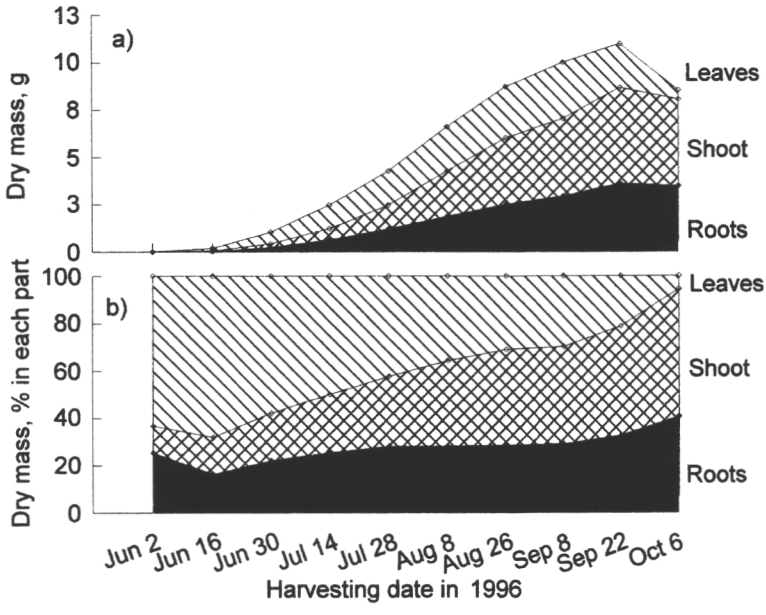


Figure 3.2 Dry mass partitioning to leaves, shoot (including stem and branches) and roots as **a)** dry mass (g) and **b)** percentage of total mass per birch seedling sown on 2 May during the 1996 growing season. Dry mass of 20 seedlings (2 seedlings in 10 blocks) was measured weekly from 2 June to 13 October 1996.

harvested and height, diameter, dry mass of shoot, leaves and new roots were assessed.

Statistical analysis. The growth curves were fitted as described in Section 2.5. In the root-egress tests, due to heterogenic variances, differences between crops in each week were analyzed with the Mann-Whitney U-test. In the root egress test of the APRIL96 seedlings, the weekly differences were tested by Kruskall-Wallis test and in the MAY96 seedlings by ANOVA and Tukey's HSD test after logarithmic transformation.

3.1.3 Results

During the growing season, height, diameter and dry mass of stem and roots increased logistically with time for all crops (Table 3.2, Figure 3.1a,b). During the first part of the season, seedlings allocated the most dry mass to the leaves and least to the stem (Figure 3.2). Dry mass of the leaves increased until the beginning of August. Diameter increased fairly linearly with increasing height of the stem (Figure 3.3a), and the dry mass of the roots increased with increasing dry mass of the shoot (Figure 3.3h). The relationship between dry mass (shoot and roots) and seedling dimensions (height and diameter) was divided into two parts (Figure 3.3b–e): 1) until cessation of height growth in the middle or end of August, dry mass increased fairly linearly as height and diameter increased (Figure 3.3b–e); 2) then the dry mass of the stem and roots increased for about a month until the decrease in dry mass of the leaves began in the be-

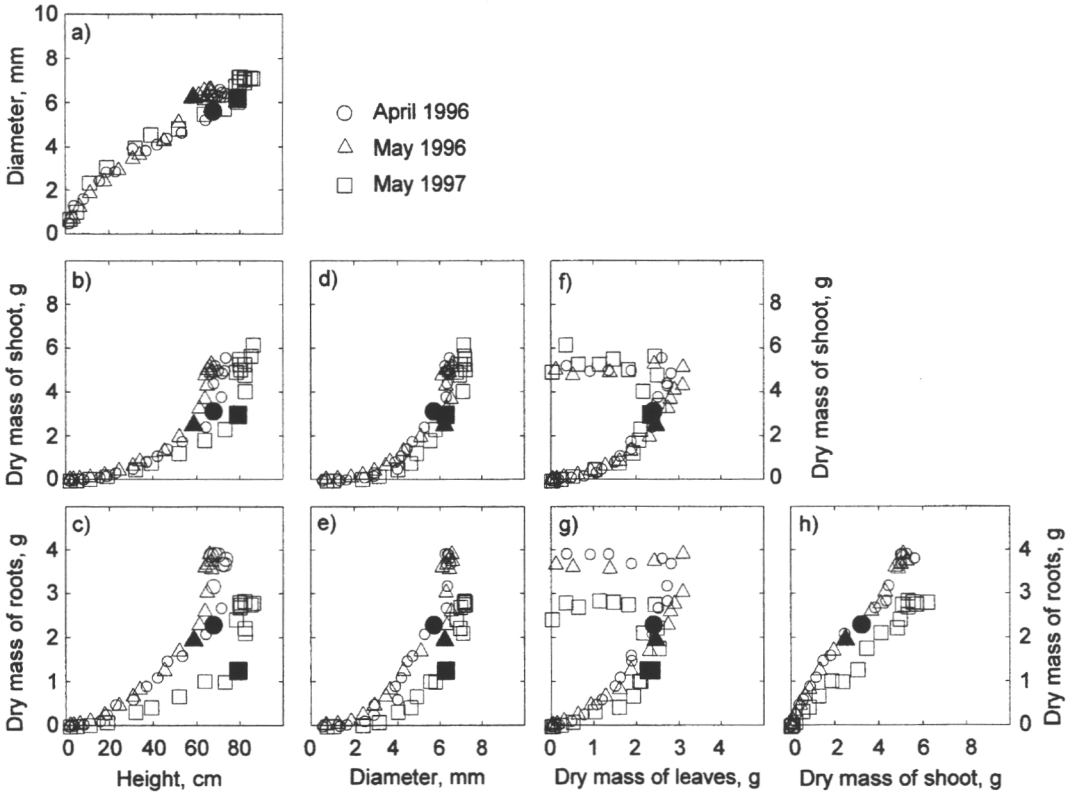


Figure 3.3 Correlations between seedling dimensions and dry mass of different plant parts assessed at different times during the growing season from crops sown on 15 April and 2 May 1996, and on 5 May 1997. The first harvesting dates are on the left and time moves on toward the right, except in **f)** and **g)** where time moves back to the left in autumn due to leaf senescence. To help indicate the timing of changes in relationships, mean values obtained in the middle of August (week 33, the average time for cessation of height growth) were drawn larger and with black symbols. Each symbol (for explanations of sowing times, see Figure 3.1) indicates the weekly mean of 20 seedlings (2 seedlings in 10 blocks) for each variable of each crop.

ginning or middle of September (Figures 3.1c and 3.3f,g). After that, leaf dry mass decreased due to leaf senescence (Figure 3.4) without a change in the dry mass of shoot and roots (Figure 3.3f,g). All leaves had dropped by the end of October (Figures 3.1c and 3.4).

In 1996, the height growth of seedlings sown in April and May stopped after the middle of August (Table 3.2). According to visual scoring, the leaves started to become yellow in the second week of September (Figure 3.4) and all leaves had been shed a month later. In 1997, the height growth of the MAY97 seedlings stopped on 14 August (Table 3.2). After cessation of height and diameter growth, water content (measured from the seedling apex) in the MAY97 seedlings decreased until leaf abscission began in the end of September (data shown only in Figure 6.4b).

At the end of the growing season, the APRIL96 and MAY96 seed-

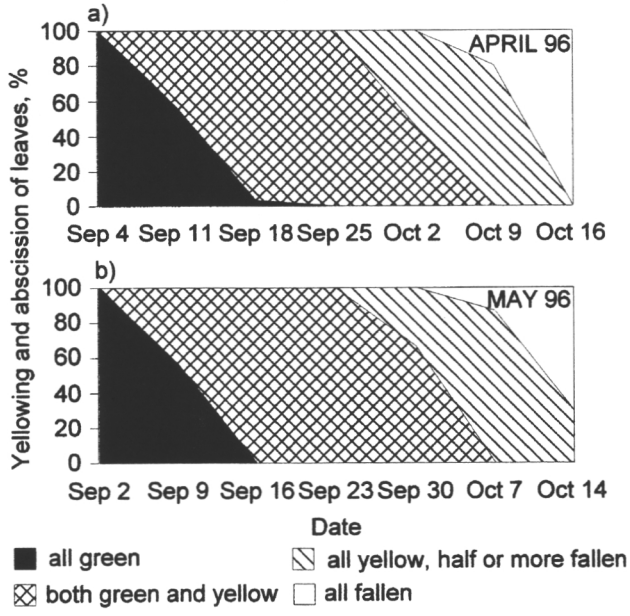


Figure 3.4 Yellowing and abscission of leaves (% of seedlings) in seedlings sown **a)** in April or **b)** in May 1996 and assessed weekly during autumn 1996. Leaf yellowing and abscission was recorded from 60 seedlings per crop according to the following classification: 1) all leaves green, 2) some leaves green, the rest yellow, 3) all leaves yellow, half or more fallen, 4) all leaves fallen.

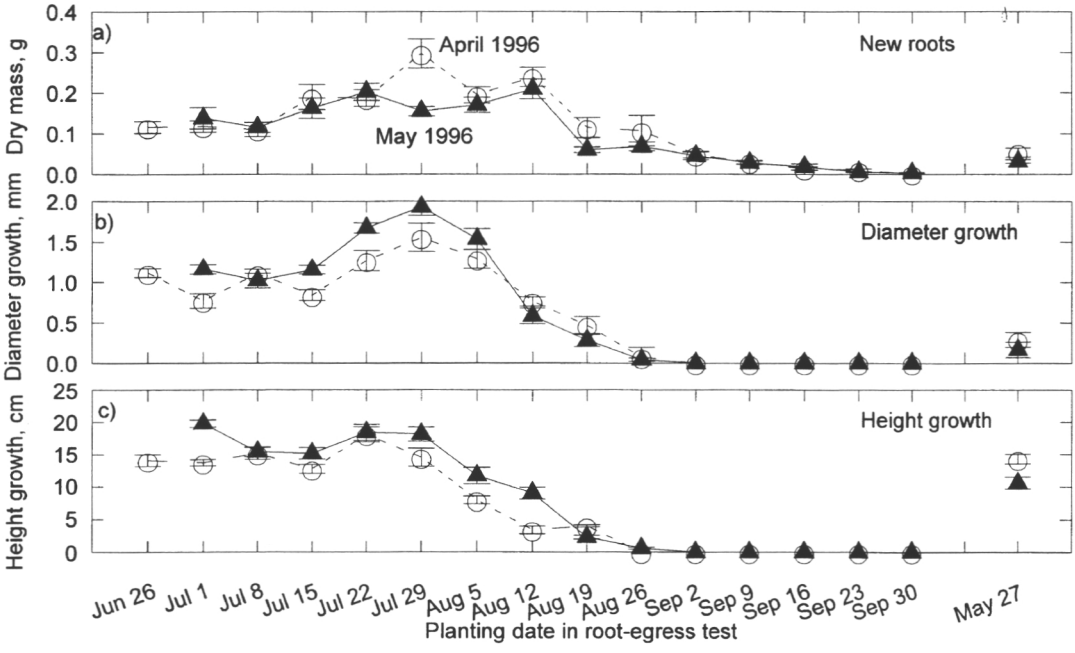


Figure 3.5 Growth of seedlings planted in root-egress test in summer 1996 and spring 1997. a) Dry mass of new (outgrown from peat plug) roots (g), b) growth in diameter (mm) and c) height (cm) of the stem of birch seedlings sown on 15 April or 2 May 1996 during the three-week test period. Bars indicate standard errors of the means for each day. (n = 10 block means, 2 seedlings per block).

lings were shorter than the MAY97 seedlings (Figure 3.1a), but there were no clear differences in diameter (Figure 3.1b), sturdiness or dry mass of the stem (Figure 3.3b, d) between crops. Root dry mass (Figure 3.3h) and root-to-shoot ratio in crops of 1996 were higher than in the MAY97 crop. During the growing season, the sturdiness and root-to-shoot ratio of the seedlings decreased until the beginning of July (data not shown).

In the root-egress test, the dry mass of new roots was large until the middle of August (Figure 3.5a). At the end of August, root growth had decreased but did not cease until the leaves dropped at the end of September. The decline in amount of new roots in late summer and early autumn was correlated to the cessation of height and diameter growth. The increments in height and diameter stopped at the same time at the end of August (Figure 3.5b, c). There were no differences between crops, except at the end of July when the APRIL96 seedlings had more root growth (Kurskal-Wallis $P<0.001$).

The following spring, root growth was the same (Kruskal-Wallis $P=0.112$) in both APRIL96 and MAY96 seedlings (Figure 3.5). The height at planting affected height growth, since seedlings in the APRIL96 crop that were taller at planting grew more in height ($P=0.005$). There were, however, no differences in diameter growth ($P=0.368$). Root and diameter growth in the spring test were comparable to growth in the test period that started in the middle of August, whereas height growth was comparable to the test period that started in the beginning of July (APRIL96) or August (MAY96) the previous year.

3.1.4 Discussion

3.1 4.1 Timing of growth and dry-mass partitioning

In the summer of 1997, more dry mass was allocated to shoots and less to roots compared to 1996 (Figure 3.3h). In 1996, sowing date did not affect the final height, diameter or dry mass of the stem and roots (Figure 3.1). The period of rapid growth started earlier in seedlings sown in April compared to seedlings sown two weeks later. However, at the end of the growing season there were no differences in seedlings in different crops. The weather conditions of the 1996 and 1997 growing seasons differed. July 1996 was cooler and rainy, August 1996 was warmer and dry; and both July and August 1997 were warmer and drier than the long-term average (Figure 2.1). It is probable that these differences in weather conditions also affected the height growth and dry-mass partitioning. Height and diameter growth of the APRIL96 seedlings slowed down in late July and early August (Figure 3.1a,b), possibly due to slight drought stress. Seedlings from both crops were irrigated and fertilized according to the

same regime. Taller APRIL96 seedlings were assumed to use more water due to the larger transpiring surface (not measured) compared to the MAY96 seedlings. Thus the APRIL96 seedlings may have suffered slight drought stress, which could have decreased their growth late in the season.

In 1997, in particular, considerably less dry mass was accumulated by roots than by shoots (Figure 3.3h). The dry mass of the roots determined in different years was not totally comparable, due to the fact that different persons washed the roots. It cannot, however, be proved how much of the effect was due to that; but because of the increased height growth, dry-mass allocation to the roots probably was reduced in the summer of 1997 compared to the summer of 1996. The optimal temperature for shoot growth is 21°C, but temperatures between 15 and 21°C do not affect root growth (Skre 1991b). Thus the shoot-to-root ratio usually increases with increasing air temperature.

During the early part of the season, most of the dry mass was allocated to leaves (Figure 3.2). Young growing leaves do not export assimilates to other tissues (Kozlowski 1991). Once leaves mature, they produce carbohydrates for growth of new leaves, stem and root tissues (Kozlowski and Pallardy 1997). In plants where height is increasing, roots are relatively weak sinks and tend to assimilate carbohydrates only from lower leaves or after the demand of the developing leaves is met (Dickson and Isebrands 1991).

In this study, the dry mass of the leaves increased until height growth ceased in the middle of August (Figure 3.1). After that, the dry mass of the leaves was unchanged until the beginning or middle of September, depending on the year (Table 3.2). Both short photoperiod and decreasing temperature in autumn are needed for yellowing and abscission of leaves (Thomas and Vince-Prue 1997). After growth ceases, leaves are important for proper frost hardening, since the early stages of frost hardening are dependent on growth regulators produced by the leaves and on other active metabolic processes (Fuchigami et al. 1971). Early and heavy frosts usually retard abscission of leaves by injuring the abscission zone, thereby impeding the normal processes (Kozlowski 1991). During this study, the first autumn frosts, which did not, however, damage the seedlings, were in the middle and end of September, in 1996 and 1997, respectively (see Figure 2.1).

In 1997 the seedlings were tall, mature leaves located lower on the stem were shed first, and the shedding started earlier than it had the year before (Figure 3.1c, Table 3.2). Shading, drought stress and other environmental stresses may also lead to senescence and to shedding of mature leaves, because lower leaves are not able to maintain a favorable carbon balance (Dickson and Isebrands 1991). Due to low levels of light, mature leaves are not capable of positive net photosynthesis and usually cannot use imported sugars (Dickson and Isebrands 1991).

The dry mass of the shoot and roots increased for more than a month after cessation of height and diameter growth (Figure 3.3b–e). During this period, the dry mass almost doubled. At the same time, however, growth of new roots decreased linearly toward autumn (Figure 3.5a). Actively growing shoots contain mostly water. However, as the shoots lignify and accumulate stored carbohydrates, the water content of the tissues decreases in association with frost hardening (Abod and Webster 1991b). The dry mass of the overwintering parts is increased by the active processes of leaf abscission (Addicott 1970) and transfer of nutrients and mobile compounds from leaves to stem and roots (Tamm 1951, Dickson and Nelson 1982, Chapin and Kedrowsky 1983, Abod and Webster 1991b, Nordell and Karlsson 1995). Abod and Webster (1991b) showed that for silver birch seedlings the roots are important sites for storage of assimilates. Accordingly, in the present study during leaf yellowing, the increment in dry mass in the roots was probably, at least partly, due to translocation of carbohydrates to winter storage. Dry mass may also increase due to absorption of nutrients. Deciduous, broadleaf species are able to absorb nutrients during autumn and winter, provided that the soil temperature is above 0°C (van den Driessche 1984). When all leaves had been shed, the dry mass of the stem and roots did not change (Figure 3.3f,g), thus, indicating a decrease in the metabolic activity of the seedlings (Weiser 1970).

3.1 4.2 Ability of birch-seedling roots to egress during summer

In general, the RGP test has been used to determine the ability of seedlings to grow roots in optimal radiation, photoperiod, temperature, moisture and nutrient conditions (Ritchie and Tanaka 1990). In an environment that is highly favorable for root growth, in a natural environment RGP usually overestimates root growth (Rikala and Puttonen 1988, Ritchie and Tanaka 1990, Folk and Grossnickle 1997). In this study, the objective of the modificated RGP test, the root-egress test, was, in addition to examining the timing of root growth, also to evaluate the seedlings' performance potential at different times of the summer and to compare that with the spring values. Since the experiment was done in a greenhouse, light intensity (not measured) was probably lower and temperatures higher than outside. Differences between day and night temperatures were minor compared to conditions in a nursery or in the field. In spite of a few differences in growing conditions, most of the results could be generalized to natural growing conditions.

Root growth of silver birch seedlings was strong until the middle of August (Figure 3.5a) and continued as long as the leaves were green. Similar results were shown earlier by Rikala (1996). When

yellowing and abscission of leaves was induced (Figure 3.4), growth of new roots slowed down and finally stopped. Root growth depends largely on current assimilates, which are produced mainly by mature leaves (Eliasson 1968, Burdett 1990). Here root growth was measured as dry mass of new roots grown out of the peat plug; thus it was not possible to say whether this was due to root initiation or root elongation. According to Lyr and Hoffmann (1967), both in conifer and deciduous trees uninterrupted root growth during winter might be possible at mild winter temperatures and in frost-free soils, but the growth rate and number of growing roots would decline markedly compared to the growing season.

In spring, the amount of root egress in the seedlings was lower than in summer (Figure 3.5a). Root growth needs carbohydrates produced by leaves. Storage carbohydrates are used mainly during bud flushing for growth of the leaves (Abod et al. 1991). In the early part of the growing season, expanding leaves use assimilates for their own growth and for growth of the upper leaves (Kozlowski and Pallardy 1997). This means that few carbohydrates are available for the roots, and more rapid root growth is possible only after some leaves mature (Lee and Hackett 1976, Abod and Webster 1991b, Abod et al. 1991).

3.1.5 Conclusion

After transplantation, silver birch container seedlings allocated most of their dry mass growth to leaves. Later, more and more of the dry mass was allocated to other parts of the plants. After cessation of height and diameter growth, the dry mass of the shoot and roots increased until leaf abscission. The results of root-egress tests indicated that the root growth of first-year birch container seedlings is strongest in July and early August. After height growth ceases, growth of the new roots declines, ending completely when the leaves are shed. In spring, root growth is not possible until new leaves are fully expanded; this means that root growth is weak compared to that of the previous summer.

3.2 Frost hardening and assessment of frost hardiness

3.2.1 Background and study objectives

There are several definitions of frost hardiness. Glerum (1985) mentioned, e.g, the lowest temperature below the freezing point to which a seedling can be exposed without being damaged, and the minimum temperature at which 50 percent of the seedlings are killed (expressed as lethal temperature 50 (LT_{50}). On the other hand, frost hardiness can be defined as concerning only a plant part, for example, stem, buds or roots.

Frost hardiness is widely expressed as LT_{50} (e.g. Bittenbender and Howell 1974, Sutinen et al. 1992, Lindén et al. 1996). However, the lowest temperature or the same kind of estimates are also used to express the frost hardniness of seedlings (Sakai et al. 1986, Lindstrom 1992, Anisko and Lindstrom 1995, DeHayes and Willams 1989). In addition, estimates of LT_{10} (Hansen 1992) or LT_{20} (Perkins et al. 1993), the temperature at which only 10 or 20 percent of seedlings or tissues are dead or damaged, are used. Ketchie et al. (1972) used the temperature range at which injuries appear (T_{10} and T_{90}). Odlum and Blake (1996) recommended the use of the temperature needed to induce an index of injury of 5 percent in the electrolyte-leakage test. Using the electrolyte-leakage method, Murray et al. (1989) expressed frost hardiness as the rate of electrolyte leakage. When the sigmoid function is used for calculation, frost hardiness is also described as the rate of freezing injury, or the point of inflection is used as a measure of maximum rate of freeze-thaw stress (Zhu and Liu 1987, Fry et al. 1991, von Fircks and Verwijst 1993).

According to Ritchie (1991), the LT_{50} value is reliable for testing intact seedlings and determines when the injuries develop completely during the incubation time. It might be useful to compare the growing methods or species using LT_{50} values; but considering the needs of nurserymen, a more suitable value might be, for instance, the lowest temperature at which the evaluated tissue survives.

Frost hardiness of woody plants has been assessed by several methods. In most tests the viability of a tissue or intact plant is assessed directly or indirectly after freezing. If frost hardiness is defined as the lowest temperature at which seedlings survive, the possibilities to use different methods to estimate this temperature have to be evaluated.

Stergios and Howell (1973) compared several viability tests in four species and concluded that, even though a method gives good results for one species in certain conditions, it is not certain that it will also work on other species or on the same species in other conditions.

However, according to them, in all species tested the most reliable tests were regrowth and tissue browning. The growth test evaluates growth after exposure of the plants to different temperatures. For species with no growth competence during the early part of dormancy (rest period), this test requires considerable time before regrowth occurs. Although there may be no visible symptoms of injury and viability may not be affected, invisible minor freezing injuries may decrease growth (Ritchie 1991). In the browning test, stems are visually scored as dead if the cambium and phloem are brown or yellow, and alive if they are 'apple green' (Ritchie 1991). In this test the labor needed is minimal and the incubation time is only 1–2 weeks (Stergios and Howell 1973, Glerum 1985).

The relative electrolyte-leakage (also called relative conductivity or relative ion leakage) method is based on the principle that plasma membranes in freeze-injured tissue allow increased leakage of electrolytes into the apoplastic water (Ritchie 1991). Since the work of Dexter et al. (1930, 1932), the electrolyte-leakage method has been widely used to assess the frost hardiness of different plant tissues. In woody plants this method has been used for both deciduous (e.g. Wilner 1960, 1961, Stregios and Howell 1973, Deans et al. 1995) and coniferous species (e.g. Aronsson and Eliasson 1970, Colombo et al. 1984). Dexter et al. (1930, 1932) measured only absolute electrolyte conductivity, but later Wilner (1960, 1961) showed that more reliable results could be obtained when the relative conductivity (ratio of conductivity after freezing to total conductivity after the sample is killed) is measured. Wilner measured the conductivity of frozen samples after 24 hours incubation time and then boiled the samples in water for two minutes to release total electrolytes. Recently, Deans et al. (1995) have questioned the previously used short incubation and killing times for assessing the frost hardiness of woody species, in particular, for assessing the stem of deciduous species. According to them, a reliable method for assessing freezing injury in small pieces of leafless stem tissues required 5–7 days after temperature exposure for electrolyte equilibration, followed by autoclaving for at least 90 min at 121°C and allowing an additional 24 h before conductivity measurement.

Exposure temperature, degree of damage and frost hardiness of the samples all affect the time response of electrolyte leakage (Zhang and Willison 1987): the rate of electrolyte leakage from more damaged samples caused by lower freezing temperatures is slower than that of less damaged samples exposed to less severe freezing temperatures. On the other hand, the hardier the tissue the slower the rate of electrolytes leakage out of the cells to deionized water. Thus, during the same, but too short, incubation time the measured electrolyte leakage of samples with different degrees of frost hardiness may differ, even though the damage is the same. This may cause problems

to estimate a proper frost-hardiness parameter.

Estimates of frost hardiness have been determined graphically from freezing response curves (Sutinen et al. 1992). By using statistical methods, it is possible to calculate the standard errors of the estimates and thus compare frost hardiness in different populations. The methods used are, for example, the Spearman-Kärber method (Bittenbender and Howell 1974), calculation of critical temperature with analysis of variances and interpolation (DeHayes and Williams 1989), logistic regression models (Zhu and Liu 1987, Anderson et al. 1988, Repo and Lappi 1989, Fry et al. 1991, Sulc et al. 1991, Hansen 1992, Odlum and Blake 1996, Lindén et al. 1996), the Richards function (von Fircks and Verwijst 1993, Lim et al. 1998) and the Gompertz function (Lim et al. 1998). Logistic models, the Richards function and the Gompertz function are sigmoid functions with a maximum of four parameters. Logistic models are symmetrical, and the others are asymmetrical to the point of inflection of the curve (Ratkowsky 1990). According to Ratkowsky (1990, pages 140–141) the Richards function, although widely used, is statistically poor at estimating the parameters, due to its strong non-linearity with respect to parameters. Logistic models have been widely used with good results (e.g. by Andersson et al. 1988, Repo and Lappi 1989, Odlum and Blake 1996, Repo et al. 1997, Ryyppö et al. 1998b) and were also used here.

The objective of this part of the study was to evaluate tests of electrolyte leakage, stem browning and viability of whole seedlings for assessing the frost hardiness of shoots of silver birch container seedlings in autumn. More specifically, the following objectives were included:

1) to compare the frost hardiness estimates given by the three methods; electrolyte-leakage and stem-browning tests were compared to the whole-seedling viability test, which was used as the method giving the most reliable results,
2) to evaluate the estimates for 10 and 50 percent lethal (damaging) temperature in practical nursery use,

and

3) to monitor the frost hardening of birch container seedlings raised according to normal nursery practices in Finland.

3.2.2 Material and methods

3.2.2.1 Freezing tests

Frost hardiness (see Section 3.1.2, crop sown in May 97) was tested five times in autumn: on 18–19 August, on 8–9 and 29–30 September, and on 13–14 and 27–28 October 1997. For each testing date, seven trays were randomly selected, so that 2 or 4 seedlings from each tray were exposed to each test temperature (a total of 20 (on the last date 10) seedlings per test temperature). Half of the seedlings were put on polystyrene trays, in which roots were protected by covering them with sawdust. The rest of the seedlings were cut into two parts: the water content was determined from the uppermost 10 cm (results shown only in Figures 6.4 and 6.5), the rest of the stems were exposed to freezing temperatures. In late October the air temperature decreased below 0°C, peat plugs for the survival test were frozen and it was impossible to lift seedlings from trays without damaging stems and roots. Thus, only samples for the electrolyte-leakage and stem-browning tests were exposed.

The samples were exposed to six test temperatures (presented in Table 3.3) in air-cooled chambers. Air temperature in the chambers was controlled by an external alcohol-circulating system [Lauda RUK90 Ultra-Kryomat together with a Lauda digital programmer R410 and PM351 (MGM Lauda, Germany)]. On the first three test dates, the temperatures in the freezers were lowered 3°C per hour, kept at the minimum for 3 to 11 hours depending on test temperature and test date, then raised 3°C per hour to +5°C. On the fourth and fifth dates, the temperatures in the freezers were lowered 3°C per hour until –15°C was reached, lowered 5°C per hour and kept at the minimum for 3 to 21 hours, raised 6°C per hour to –15°C, and finally 3°C per hour to +5°C. Air temperatures in the chambers and temperatures of the stem samples or stems of whole-exposed seedlings were recorded with thermocouples. On all testing dates the control temperature was +4°C.

Table 3.3 Exposure temperatures at each test date in autumn 1997.

	Exposure temperature, °C																	
Test date	+4	–2	–4	–6	–8	–10	–12	–14	–16	–18	–20	–22	…	–30	–32	–34	–36	…	–40
18 Aug	x	x	x	x	x	x					x								
8 Sep	x		x		x	x		x			x					x			
29 Sep	x		x		x	x		x			x						x		
13 Oct	x		x		x		x		x		x						x		
27 Oct	x			x		x		x			x			x					x

Frost hardiness was assessed by three methods: 1) visual scoring of damage on stem samples, 2) electrolyte-leakage, and 3) viability of whole seedlings.

Visual scoring of stem damage

After freezing, when the pieces were cut from the lower and upper parts of the stem for testing of electrolyte leakage, the rest of the stem samples were stuck onto wet floral foam for the stem-browning test. The cuttings for the browning test were then placed in a growth chamber [Weiss, type 10 Sp/5 DU-Pi, Lidenstruth, Germany; temperature: +20/15°C, 16 h photoperiod, photon flux density (PAR) 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from metal halide lamps (Powerstar HQI T-400/D, Osram)], where they were kept wet by adding water to plastic boxes with foam as needed and spraying the stems daily with tap water. According to the results of a preliminary study, 14 days in these conditions were necessary for injury to become visible. Thus, after 14 days each cutting was dissected and the length of the damaged stem measured. The stems were scored as damaged if the cambium and phloem were brown, and alive if they were still 'apple' green (Ritchie 1991). Stem damage was calculated as relative browning (*RB*)

$$RB = \frac{L_b}{L_t} \tag{3.1}$$

where L_b is the total length of the brown tissue and L_t is the total length of the sample.

Electrolyte leakage

After freezing, two 0.5 cm long pieces were cut from the lower and upper parts of the stem for testing of electrolyte leakage. The pieces were washed in deionized water and put into a test tube containing 18 ml of deionized water. On the first and second dates the tubes were shaken (110 revs min^{-1}) for 22 h at room temperature (+20–22°C) (Colombo et al. 1984) and on the other dates shaken for 120 h at +4°C (minimizing the microbial activity) in darkness (Deans et al. 1995). To minimize the risk for biased estimation of frost hardiness in samples with different degrees of hardiness, the incubation time for the last three testing dates used here was five days. It was assumed that in the first and second test the seedlings had such low hardiness and the temperatures used were so high that there was no risk of slow electrolyte leakage (see Zhang and Willison 1987) and the incubation time was 22 h, as suggested by Colombo et al. (1984).

After the incubation, the conductivity of the solution was measured (Radiometer CDM 83, water bath at 25°C ±1°C temperature, cell constant 1.008, μS cm^{-1}, without temperature compensation). According to the results of the preliminary experiment, to minimize overboiling, 15 ml of water was removed from the test tubes before the sample pieces were killed by autoclaving the tubes at 120°C for 60 minutes. The removed water was returned to the tubes and the total conductivity was measured after the 22-hour shaking period at room temperature in darkness. Relative electrolyte leakage (*REL*) was calculated as

$$REL = \frac{e_1}{e_2} \tag{3.2}$$

where e_1 is the result of the first conductivity measurement, which expresses the electrolyte leakage caused by freezing injuries and background leakage, and e_2 is the second conductivity measured after autoclave killing, which represents the total electrolyte leakage of the stem tissues.

Viability of whole seedlings

After exposure to freezing, seedlings on polystyrene trays were kept in a dark cold room at +5°C until the peat plugs had thawed (24–48 hours, depending on test temperature). Then the seedlings were placed in an unheated greenhouse, where they were irrigated as needed. On 14 October, the seedlings were removed from the trays, packed in plastic bags and stored at –2.5±0.5°C. From 22 January to 2 February, the seedlings were thawed in darkness (11 d at +2°C) and from 2 to 3 February at +17°C. On 3 February, the seedlings were put in Plantek 25 trays and transferred to a heated and lighted greenhouse (day/night temperature: +20 – +22 °C/ +15°C, photon flux density, PAR 150 μmol·m^{-2}·s^{-1} from metal halide lamps (HQI-400W Power Star, Osram), 18 h photoperiod) for 17 days. All seedlings were watered daily with tap water. On 20 February, seedlings were classified into four classes: 1) alive (undamaged), 2) alive, but smaller leaves than in class 1, 3) alive but stem apices dead and 4) dead. For statistical analysis, seedlings were reclassified as a) damaged (class 2–4) and undamaged, for calculations of proportions of damaged seedlings (*D*) (used to estimate damaging temperatures (*DT*), or b) dead (class 4) and alive (class 1–3), for calculations of proportions of dead seedlings (*L*), which was used to estimate lethal temperatures (*LT*).

3.2.2.2 Estimation of frost hardiness

Both in electrolyte-leakage and in stem-browning tests, the proportions of injuries [relative electrolyte leakage (*REL*) or browning (*RB*)] and in the test of viability of whole exposed seedlings, the damage (*D*) or mortality (*L*) of seedlings increased with decreasing temperature to a certain temperature as a logistic function (Figure 3.6). The same logistic function as has been used previously, e.g., by Andersson et al. (1988), Repo and Lappi (1989), Odlum and Blake (1996), and Repo et al. (1997), was used where

$$\begin{aligned} y_i &= f(x_i) + \varepsilon \\ &= \frac{a}{1 + e^{b(c - x_i)}} + d + \varepsilon \end{aligned} \tag{3.3}$$

where y_i is the measured injury (*REL*, *RB*, the proportion of injured or dead seedlings), x_i is exposure temperature (°C) and a, b, c and d are equation parameters. The inflection point c is the temperature (°C) at which the change of injuries (dy/dx) is maximal as temperature decreases (Zhu and Liu 1987). The upper and lower asymptotes of the function are determined by parameters a and d, respectively. Parameter d is the lower asymptote of function, the background level, e.g. indicating the *REL* from uninjured cells at non-damaging temperatures. Parameter a expresses the injury range between the lower and upper asymptotes. Thus, $a + d$ indicates the upper asymptote, which tells the *REL* value for the temperature where injuries are at maximum. The slope of the curve at the point of inflection is $ab/4$. Thus, parameter b [$(C°)^{-1}$] measures the slope at c relative to injury range a.

Parameters a and d were not estimated in tests of stem-browning and whole seedling viability. It was assumed that in the stem-browning test, living samples have no injuries ($d = 0$) and dead ones have brown phloem and cambium ($a + d = 1$). In the viability test all seedlings are assumed to be alive when the lower limit d was 0 and dead (damaged) when the upper $a + d$ was 1.

The variance of the distribution in the tests of stem browning and whole seedling viability (Figure 3.6f–r) is proportional to the variance of the binomial distribution ($p(1 - p)$). Thus the variance is greatest when $p = {}^1/_2$ and is 0 when $p = 0$ or $p = 1$. In nonlinear estimation, however, homogenous error variances are assumed. To homogenize these variances, both sides of the equation (3.3) were divided by the weight (w)

$$w = \sqrt{\hat{f}(1 - \hat{f})} + 0.01 \tag{3.4}$$

where $\hat{f}$ was the estimate of $f(x_i)$ given to the first iteration. To avoid dividing by zero, 0.01 was added to each value.

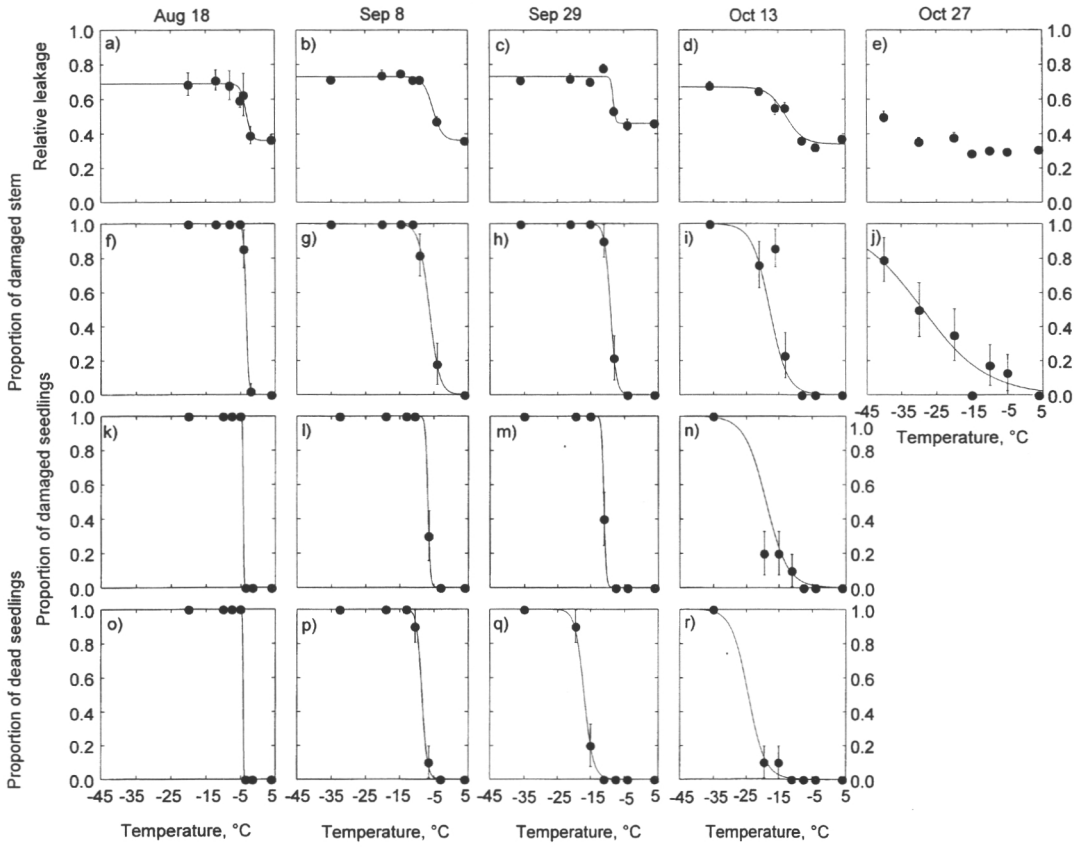


Figure 3.6 Results of freezing tests in autumn 1997. Methods for estimation of frost hardiness were **a–e)** electrolyte leakage, **f–j)** stem-browning test (relative proportion of damaged stem) and whole-seedling viability tests assessed either as proportions of **k–n)** damaged or **o–r)** dead silver birch seedlings. When whole seedlings were tested, the seedlings were classified as damaged when they were dead or their leaves were smaller than in living seedlings. For estimation of lethal temperature, seedlings with small leaves were scored as alive. The vertical bars indicate the standard error of the mean values for each method at each test temperature (n = 10 samples per temperature). Lines are the fitted sigmoid functions (estimated parameters are presented in Appendix I).

Injuries at temperature x_p are the proportion p of the range of electrolyte leakage (or damaged stem) (expressed as $f_p = pa + d$. The temperature x_p can be estimated as described earlier in the case of estimation of growth cessation (see section 2.6.2). The equations for estimations are

$$y_i = \frac{a}{1 + e^{\frac{-\ln\left(\frac{1}{p}-1\right)}{x_p - c} - (c - x_i)}} + d + \varepsilon \qquad (3.5)$$

or

$$y_i = \frac{a}{1+e^{b\left(x_p + \frac{\ln\left(\frac{1}{p}-1\right)}{b} - x_i\right)}} + d + \varepsilon \qquad (3.6)$$

Equations (3.3) and (3.5) or (3.6) with a value of 0.1 for p were fitted for data from each testing date and each method by using non-linear regression with the Quasi-Newton method. The logistic equation used is symmetrical (Ratkowsky 1990), and the estimated point of inflection c was used to express the temperature at which the *REL* is 50 percent of the observed range of *REL* (ET_{50}), half of the stem is brown (BT_{50}), and half of the seedlings are damaged (DT_{50}) or dead (LT_{50}) (Zhu and Liu 1987, Andersson et al. 1988, Repo and Lappi 1989, Fry et al. 1991, Von Fircks and Vervijst 1993, Odlum and Blake 1996). The estimated parameter x_p expresses 10 percent of the range of electrolyte leakage (ET_{10}), stem browning (BT_{10}), damaged seedlings (DT_{10}) or mortality (LT_{10}). Frost hardiness estimates given by electrolyte-leakage and stem-browning tests were compared to estimates assessed by whole-seedling viability; relationships between estimates were determined by fitting linear regressions.

3.2.3 Results

In early autumn (test on 18 August) the tissues were damaged at –4 to –5 °C, and the response curves were steep (Figure 3.6a, f, k, o). Later the slope of the curve (b) decreased with increasing frost hardiness (Figure 3.6). Frost hardening decreased the total amount of leaked electrolytes (Figure 3.7); and the differences between electrolyte leakage after freezing and killing decreased, making curve fitting more difficult or impossible (on the last date only BT could be estimated).

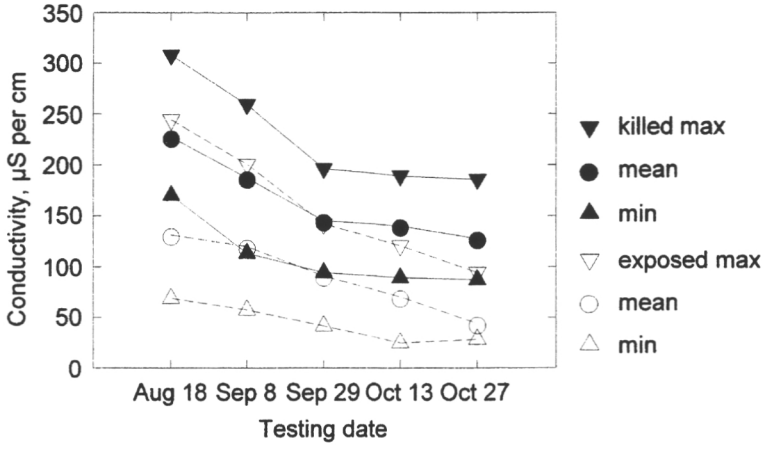


Figure 3.7 Changes in electrolyte leakage from stems of silver birch seedlings after freezing tests during autumn. Mean, maximum and minimum electrolyte leakage (measured as conductivity of the incubated solution) calculated from all measured conductivities after freezing (broken lines) and killing by autoclaving (solid line) on each testing date in autumn 1997.

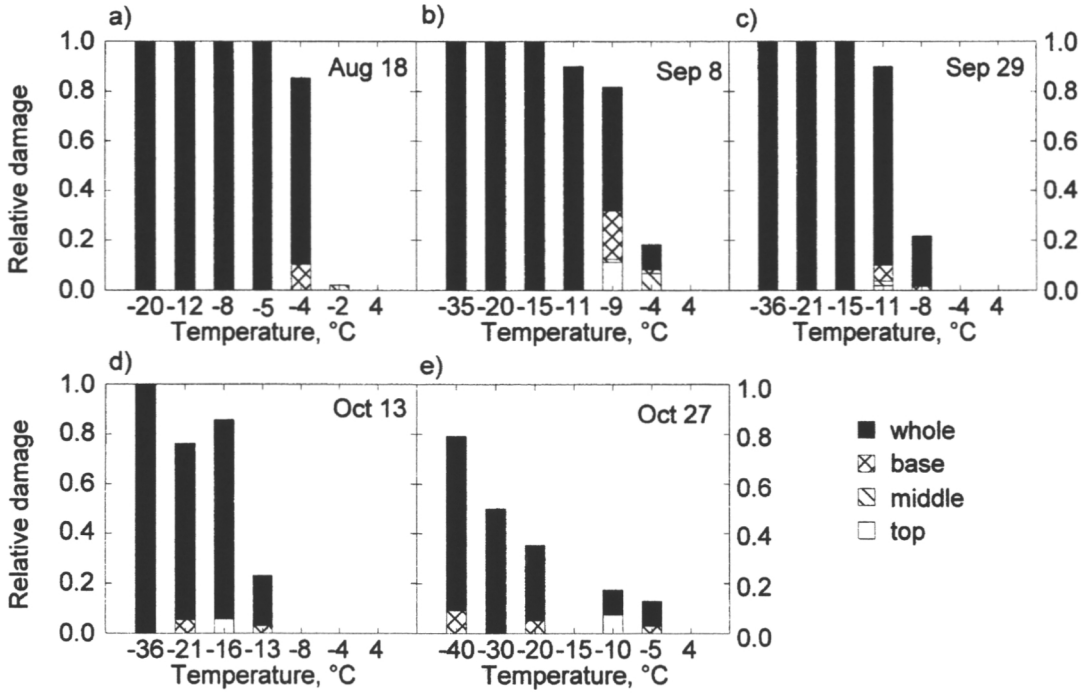


Figure 3.8 Position of damaged cambium and phloem in samples used in the stem-browning test on each testing date. Proportion of the length of the brown base, middle or top part of the total length of the sample at each test temperature is shown. The black part of the bar indicates that the cambium and phloem were damaged in the whole sample.

In the stem-browning test, the position of the injuries on the stem was also observed. In most cases the entire stem was either damaged or alive. However, when cuttings were only partially damaged, the lower part of the stem was damaged more often than the apices or the middle part of the stem (Figure 3.8).

Frost hardiness of silver birch seedlings increased slowly from the middle of August to the middle of September. After that, frost hardening became more rapid. The differences between the estimated BT_{50} and ET_{50} or BT_{10} and ET_{10} were minor (Appendix I). There were strong linear relationships between estimates, but BT_{50} and ET_{50} underestimated the viability of the stem compared to estimates of LT_{50} or DT_{50} (Figure 3.9 b, d). BT_{50} and ET_{50} estimated, however, quite well DT_{10} (Figure 3.9c). The differences between estimates given by different tests increased with frost hardening.

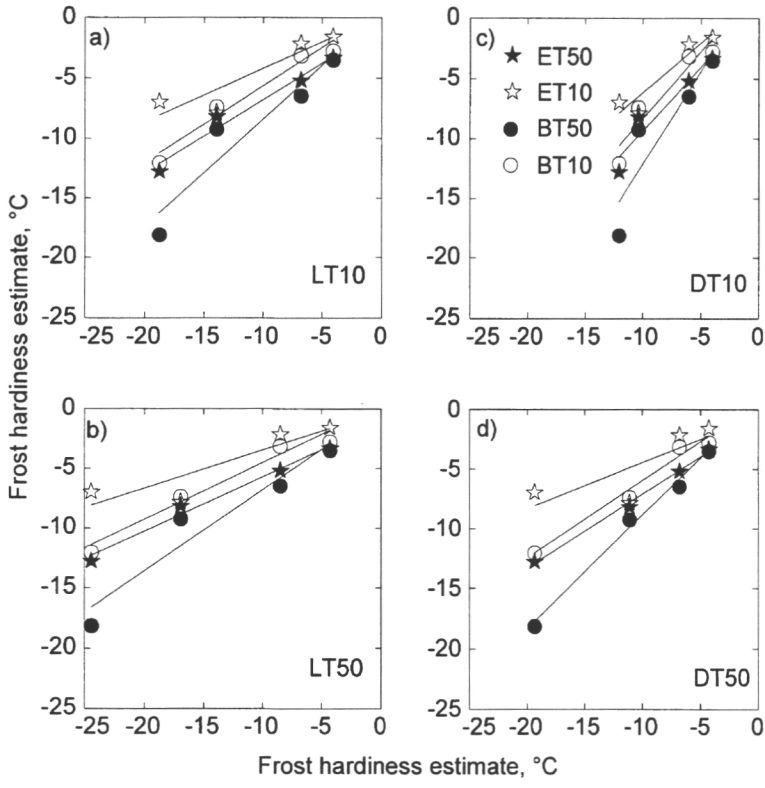


Figure 3.9 Correlations of frost-hardiness estimates by different assessment methods. The X-axis presents estimates for a) 10 and b) 50 percent lethal temperature (LT) or c) 10 and d) 50 percent damaging temperature (DT) assessed by whole seedling viability tests. In the Y-axis are the frost hardiness estimates from electrolyte-leakage (ET) or stem-browning (BT) tests.

3.2.4 Discussion

In this study all samples in a test were subjected to the same treatment conditions: the same chamber for samples at a given exposure temperature, the same shaker, all test tubes in the same water bath during the conductivity measurements etc. Thus, samples were pseudoreplicates. This might cause bias to the estimates of frost hardiness and its error variances. According to Repo and Lappi (1989), this does not cause bias in the estimated parameters of the response function, but their variances increase and are underestimated.

There were only three chambers and seedlings for each test and the number of test temperatures was limited. Thus, in order to decide on exposure temperatures, before freezing some assumptions were made about the frost hardiness of seedlings. In the second and third test the frost hardiness of the seedlings was assumed to be higher and the last time lower than it actually was. In all tests, there should have been enough temperatures so that both the upper (all samples dead, maximal *REL*) and lower limits (all samples alive, background level in electrolyte leakage) would have been reached (Anisko and Lindstrom 1995). Because this was not the case in the last test, fitting of reliable curves was difficult (browning test) (Figure 3.6j) or

impossible (electrolyte leakage) (Figure 3.6e). On the other hand, test temperatures should be so close each other that there are also values between asymptotes (partially damaged, only some seedlings dead) (Raymond et al. 1986). On the third date, there were too few temperatures between –5 and –10°C for reliable estimation of frost hardiness, especially by the electrolyte-leakage method (Figure 3.6c, h, m, q, Appendix I).

In early autumn, when the frost hardiness of seedlings was low, seedlings were damaged in a narrow temperature range and the response curve was steep. With frost hardening, the response curves became less steep due to decreased electrolyte leakage (Figure 3.7). The reduced leakage has been explained by cellular differentiation, lignification and suberization of cell walls (Sutinen et al. 1992, Repo et al. 1997) and decreased amount of free water in tissues (Levitt 1980). At the end of October, electrolyte leakage was so slight that it was not possible to fit the sigmoid curve (Figure 3.6e). Decreased leakage could not be caused by undamaged samples, since in the browning test most stem cuttings were scored as dead at the same exposure temperatures (Figure 3.6j). However, also in the stem-browning test, parameter estimation would have been more reliable if test temperatures had been lower. Difficulties in estimating frost hardiness by the electrolyte-leakage test during late autumn and winter have also been observed by Sutinen et al. (1992) using pine needles. According to them, low electrolyte leakage from hardy samples might be due to changes in the properties of the cell wall, which provide resistance to diffusion of electrolytes from cells into the extracellular water. Thus, the only suitable method used in this study for hardy seedlings was the stem-browning test.

According to the definition of Steponkus and Lanphear (1967), an ideal method of testing for frost hardiness "should eliminate bias associated with visual observations, be based on a quantitative system that could be analyzed statistically, utilize small quantities of tissue, be relatively quick, and be capable of predicting the future performance of the plant." The advantages and disadvantages of each method used for frost hardiness estimation are presented in Table 3.4. The electrolyte-leakage method fulfills all requirements for ideal methods, and the stem-browning test fulfills all except the requirement for elimination of visual observation. Observing the viability of whole seedlings does not fulfill any of Steponkus' and Lanphear's (1967) requirements except that it predicts the field performance of seedlings well. In this study, visual observation of viability of whole seedlings was used as the method that gives an estimate of the real frost hardiness of seedlings, as suggested by Stergios and Howell (1973) and Ritchie (1991); the frost hardiness estimates of the electrolyte-leakage and stem-browning tests were therefore compared to the results of whole-seedling viability test.

Both ET_{50} and ET_{10} were higher than BT_{50} and BT_{10}, but the differences were not statistically significant (Figure 3.9). The estimates of ET_{50} and ET_{10} might have been more appropriate if the samples exposed to freezing had been incubated in light in heated conditions some time before the pieces were cut into tubes for determination of electrolyte leakage. Palta et al. (1977) suggested that to give enough time for cell membranes to recover, measurements of conductivity should be made 1–2 weeks after exposure to freezing. In the present study, during five days of incubation the injuries to cell membranes probably recovered in water solution, but how much they recovered is uncertain. Thus it is possible that estimates of frost hardiness by both electrolyte leakage and stem-browing test could have been the same after two weeks incubation time before the electrolyte-leakage test. The electrolyte-leakage test identifies injury to tissues indirectly by measuring damage to cell membranes: how many electrolytes (mainly K^+) leak through the membranes or transport proteins embedded into plasma membranes to the apoplast and then into deionized water (Palta et al. 1977, Pukacki and Pukacka 1987). Therefore

Table 3.4 Advantages and disadvantages of electrolyte-leakage, stem-browning and whole-seedling survival tests for assessment of damage after freezing in silver birch seedlings, partially using the definition of an ideal method by Steponkus and Lanphear (1967).

	Advantages	Disadvantages
Electrolyte leakage	– relatively rapid (a week) compared to other tests	– laborious (2 measurements, several steps)
	– only 0.5 cm stem samples needed	– skills needed
	– could be analyzed statistically	– less suitable for hardy samples
	– large number of samples per test	– relatively expensive equipment needed
	– assessing early stages of injuries	– hardiness differences between different parts of seedlings
Stem browning	– relatively rapid (two weeks)	– visual observation
	– not laborious (only one relatively rapid measurement)	– difficulties in scoring damaged and living tissues
	– quantitative when injuries are measured as a length of brown tissues	– hardiness differences between different parts of seedlings, when using only a part of stem*
	– suitable for hardy samples	
	– small samples are sufficient (see *)	
	– could be analyzed statistically	
Whole-seedlings survival	– accurately predicts the mortality (chronic injuries) of whole seedlings	– not practical for large number of seedlings (space need in chamber, in storage or in greenhouse during incubation)
	– could be analyzed statistically	– weak separation of living/dead/damaged seedlings
	– not laborious (visual scoring once, storage needed)	– space needed in chamber (height of the seedlings)
		– time required before observation (rest and chilling period, months)
		– elimination of other stresses after exposure during storage, before evaluation

it is the most sensitive method for observing the minor injuries caused by freezing (Odlum and Blake 1996). Some of the injuries to cell membranes might be repaired and some be so minor that they do not kill the seedlings (Palta et al. 1977). A disadvantage of the electrolyte-leakage test is that it measures injuries in the whole sample. However, cambium is the most important tissue for survival of the plant; and even if it is dead, the other parts of the sample might be alive and decreasing the average leakage (Stergios and Howell 1973).

The stem-browning test measures damage in the cambium and phloem. It is also important to observe the position of injury in the stem tissues: cambial death, when it occurs in the terminal leader, is not lethal to the whole plant, whereas the same damage would be lethal if it occurred at ground level (Ritchie 1991). When damage was observed in dissected stems at the beginning of frost hardening, the most tender part of the stem was at ground level. However, the whole stem was usually damaged by freezing (Figure 3.8). These results in silver birch seedlings agree with observations made in conifer seedlings that seedlings harden from the top down (Ritchie 1991, Colombo et al. 1995).

A disadvantage of the stem-browning test is that it is subjective. Separation of colors in living (green) and dead (brown or yellow) tissues is not always easy. The green color is due to chlorophyll in the stem tissues, and a marked decline in chlorophyll levels is observed from the growing season towards winter (Kauppi 1991). This means that in late autumn the green color in the stem is not as bright as it is during summer and early autumn. Observations made with a microscope aid the assessment of injuries (Ritchie 1991), allowing the observer to view the breakdown of cells in addition to discoloration (Lindstrom 1992), but at same time the method would become more laborious.

The electrolyte-leakage and stem-browning tests underestimated frost hardiness compared to the survival test (Figure 3.9). The underestimation of the electrolyte-leakage test was also observed by Zhang and Willison (1987), Sutinen et al. (1992) and Odlum and Blake (1996). Although the stem-browning test describes the survival of stem tissues, some of the stem damage was moderate and/or not lethal and did not affect the survival of seedlings. If the seedlings exposed to freezing had been planted in the field and the viability observed after one season, those seedlings classified as damaged would probably have been dead and the estimates of lethal temperature and estimates of 50 % electrolyte leakage or stem browning more similar. According to Ryyppö et al. (1998b), survival of Scots pine seedlings was much lower at the end of the first season after exposure to freezing than prior to bud burst.

It was assumed that the ET_{50} and BT_{50} estimates of the electrolyte-leakage and the stem-browning test would estimate the LT_{50} values

and thus would not give useful prediction of frost hardiness for nursery managers, so estimates of ET_{10} and BT_{10} were calculated. ET_{50} or BT_{50}, however, predicted the temperatures causing damage of 10 % of seedlings quite well (Figure 3.9c). Thus by estimating the temperature causing 50 % electrolyte leakage in the observed range of relative electrolyte leakage or the temperature at which half of the stem tissues are damaged, it would be possible to predict the temperature at which only 10 % of seedlings are damaged. In the whole-seedling viability test, using the damaging temperature instead of the lethal temperature as an estimate of the viability of seedlings will give more accurate estimates of the damage due to freezing because frost affects not only mortality of seedlings but also suppresses growth (Raymond et al. 1986).

The method used for estimation of frost hardiness depends on the objective of the study. Odlum and Blake (1996) concluded that electrolyte leakage is a suitable method for assessing acute damage, whereas the whole-seedling method may be more appropriate for detecting chronic damage. If, for example, hardening methods are compared, the electrolyte-leakage or stem-browning tests, which give faster results, are suitable. When the aim is to evaluate the real survival of seedlings after hardening, assessment of the viability of entire seedlings gives more reliable and appropriate results.

None of the methods used in this study give estimates of frost hardiness just after exposure to freezing. Reliable estimation of frost hardiness in deciduous seedlings requires at least a week (electrolyte leakage). Results of tests for making decisions about nursery operations are needed much more rapidly, and therefore other methods should be evaluated. Other possible methods are, for example, impedance (Evert and Weiser 1971, Blazich et al 1974, Timmis and Fuchigami 1982, Glerum 1985, Jozefek 1989, Repo 1994, Repo et al. 1997) and chlorophyll fluorescence (Binder et al. 1996, Ögren 1999a). In the fertilizer experiment of Jozefek (1989), high pre-freezing impedance readings indicated frost hardy tissue and low readings indicated the opposite. In deciduous species, chlorophyll fluorescence is possible as long as there is chlorophyll in stem tissues (Binder et al. 1996). For example, Larcher and Nagale (1992) determined the photosynthetic activity of the stem tissues of beech (*Fagus sylvatica* L.) in winter using chlorophyll fluorescence. Ögren (1999a) also used this method in willow, with good results. Both methods might be useful if frost hardiness could be assessed without exposure to freezing. Promising results have also been obtained with impedance spectroscopy in willow seedlings (Repo et al. 1997).

3.2.5 Conclusion

Estimates of temperature-induced 50 % electrolyte leakage or injury to 50 % of dissected stem tissues would be useful for comparison of the effects of frost-hardening regimes on frost hardiness of silver birch seedlings. With these estimates, it is possible to predict the temperatures that induce 10 % of the seedlings to damage. The electrolyte-leakage test is more laborious, but it is quantitative and requires only small quantities of tissue. It is suitable for assessing frost hardiness in early autumn. When seedlings are hardy, the only suitable method (used in this study) for reliable assessment of frost hardiness is the stem-browning test.

4 Artificial control of growth and frost hardening of silver birch seedlings

4.1 Growth retardants

4.1.1 Background and study objectives

Growth retardants have been used to control stem growth (Gianfagna 1987) and studied for possible use in enhancing frost hardening, but with contradictory results (Carter and Brenner 1985). When Aphalo et al. (1997) studied the use of CCC (chlormequat chloride) and paclobutrazol in the production of silver birch container seedlings, height growth and accumulation of dry mass were partially inhibited after CCC application, but there were no negative effects on growth the following spring. Height growth in the nursery decreased after paclobutrazol application, but the growth of seedlings was also inhibited the following season. Aphalo et al. (1997) concluded that CCC could be a useful tool in nursery management, especially for controlling end-of-season growth during warm autumns. However, they did not study the influences of CCC on frost hardening or the long-term effects after planting.

Other compounds have also been used to control both height growth and frost hardening of birch seedlings. Cathey (1975) compared five growth retardants (ACPC, phosfon D, CCC, daminozide and ancymidol) in several plant species. According to his results, CCC retarded growth in most plants but not in paper birch (*Betula papyrifera* Marsh.). Application of daminozide (B-Nine), however, also retarded the height growth of paper birch. An advantage of daminozide, compared to CCC, was that it was nontoxic at all concentrations, while CCC induced temporary paling of the foliage and marginal burn (Cathey 1975).

There are also newer compounds, such as tetcyclasis and prohexadione calcium, which inhibit GA biosynthesis at later stages than CCC and ancymidol (Arteca 1996). Tetcyclasis acts like the triazoles (e.g. paclobutrazol, uniconazole), but is more effective than paclobutrazol in retarding height growth in one-year-old silver birches (Abod and Webster 1991a). Thus tetcyclasis might also inhibit the growth of seedlings the following year as paclobutrazol did in the experiment of Aphalo et al. (1997). The inhibition of GA biosynthesis

by prohexadione calcium is quite similar to the action of SDs (Junttila 1993).

Thus, the most potential retarding compounds are CCC and daminozide. There have been only a few reports on the effects of CCC and daminozide in controlling height growth of silver birch seedlings in nursery conditions (Aphalo et al. 1997) and, to my knowledge, none on control of frost hardening.

The objective of this part of the study was to compare the effectiveness of CCC and daminozide applications for retardation of height growth of silver birch seedlings. A further objective was to identify the concentration of these chemicals that would be effective in controlling height growth and hastening frost hardening of silver birch container seedlings, but without negative effects on vitality or after-effects on field growth.

4.1.2 Material and methods

Silver birch seeds were sown on peat-filled germination trays in a greenhouse on 2 May 1995. Germinants were transplanted on 22–26 May to Plantek 25 trays (25 cavities per tray, 380 cm^3 per cavity, 156 cavities per m^2, Lännen Plant Systems, Finland) filled with fertilized sphagnum peat (Vapo, XL, Finland). Seedlings were irrigated according to normal nursery practice by keeping the water content of peat at 30–60 percent by volume during the growing season. Seedlings were fertilized seven times with liquid fertilizer. The total amounts of nutrients (including basic and liquid fertilizer) given were 126 mg N, 56 mg P and 156 mg K per seedling plus micronutrients. Seedlings were grown in an unheated greenhouse until the plastic cover was removed on 20 June.

Daminozide (N-dimethylaminosuccinamic acid, trade name B-Nine, Alar 85, Uniroyal) was supplied in powder form with an active ingredient of 85.0 % ww^{-1}. CCC (chlormequat chloride, 2-chloroethyl-N,N,N-trimethyl-ammonium chloride, trade name Cycocel, Kemira Oy, Helsinki, Finland) was applied in an aqueous suspension at a concentration of 750 g l^{-1}. Seedlings were sprayed once (on 28 June) with daminozide dissolved in water at concentrations of 1.0, 2.0, 3.0, 4.0, 5.0 or 6.0 g pure daminozide per liter (the concentrations used were decided according to the results of Cathey (1975) and instructions for flowers) and twice (on 29 June and 27 July) with CCC dissolved in water at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 g of pure chlormequat chloride per liter (for CCC the concentrations were the same as there used by Aphalo et al. 1997). Control seedlings were sprayed with water. Each tray was sprayed inside a non-ventilated plastic-film hood for 20 s, which gave an applied amount of 200 ml per tray per date and about 8 ml per seedling per date.

Thirteen treatments, 30 trays with 25 seedlings in each treatment, were divided into six complete blocks, five trays of each treatment in each block, giving a total of 390 trays and 9750 seedlings. Height of the stem of the same randomly selected two seedlings per tray in all 30 trays in each treatment were measured weekly from 26 June to 18 September. Cessation of height growth was defined as the date when 90 percent of the seedlings had stopped growing. Water content was determined weekly from the uppermost five centimeters of 10 randomly selected seedlings per treatment from 27 June to 4 October.

In the autumn, after leaf abscission, 30 randomly selected seedlings per treatment were harvested, the height and diameter were measured, dry mass of shoots (stems and branches) was determined and amounts of nutrients were analyzed. Determination of root dry mass failed because of difficulties to separate the roots from the peat medium. For the nutrient analysis, stems were pooled by blocks into six samples per treatment. Nutrient concentrations in each treatment are presented in Appendix II.

On 11 October, 24 seedlings per treatment were randomly selected for tests of root growth potential (RGP); and 60 seedlings per treatment were selected for a planting experiment. Seedlings were packed in plastic bags and stored at –4°C until 19 February when the RGP seedlings were thawed in darkness for 4 d at +5°C followed by 4 d at +10°C. After thawing, the potted seedlings were divided into four blocks, six seedlings per treatment in each block in a heated greenhouse (day/night temperature: +20–+22°C/+15°C, with PAR 150 µmol $m^{-2}s^{-1}$ from metal halide lamps (HQI-400W Power Star, Osram), photoperiod: 18 h) for four weeks (until 27 March). In order to determine the timing of bud burst and leaf elongation, the length of the same three buds and after bud burst the leaves of each seedling were measured weekly.

On 22 May, 60 seedlings from each treatment were planted in the field in a randomized block design (six blocks, 10 seedlings per treatment per block). At planting and at the end of the first, second and third growing seasons the height was measured. Root collar diameter was measured at planting and at the end of the first and second growing seasons.

Statistical analysis. Height growth and water content were analyzed using a multivariate general linear hypothesis (MGLH) procedure for repeated measurements. Before analysis of variance, the diameter-to-height ratio of daminozide-sprayed seedlings in the autumn test and new roots in the RGP test were log-transformed to obtain normal distributions. Results shown in the figures and tables are back-transformed. After the analysis of variance, differences between applications were analyzed using Tukey's HSD multiple comparisons. ANOVA tables are shown in Appendix V.

4.1.3 Results

CCC induced chlorosis to the leaf edges at all doses sprayed except 0.5 g l^{-1} level, but the damage was later reversed. Later in the summer the leaves on both daminozide- and CCC-sprayed seedlings were darker green than the leaves on the control seedlings. Retardation of height growth by both compounds appeared two weeks after the applications (on 10 July) and continued for about four weeks (Figure 4.1).

Neither daminozide nor CCC affected cessation of height growth (all seedlings stopped growing by 20 August). In the middle of August, however, only 30–35 percent of the CCC-sprayed seedlings had stopped height growth. At the same time, only 42–48 percent of the control seedlings and seedlings sprayed with daminozide concentrations of 1.0–3.0 g l^{-1} and 57–62 percent of the higher daminozide concentrations had stopped their height growth.

Daminozide decreased the final height ($P<0.001$, Figure 4.2a), root collar diameter ($P=0.02$, Figure 4.2c) and dry mass of the shoots ($P=0.002$, data not shown); but the differences were significant only between the control seedlings and those with daminozide applied at 5 and 6 g l^{-1}. Daminozide concentrations over 2 g l^{-1} increased the diameter-to-height ratio compared to the controls and to a daminozide concentration of 1 g l^{-1} (Figure 4.2e).

CCC affected morphology less than daminozide did, and for all measured variables the differences were significant only between the control and the two highest (2.5 and 3.0 g l^{-1}) CCC concentrations (Figure 4.2b, d). CCC application did not affect diameter-to-height ratio ($P=0.68$, Figure 4.2f).

Concentrations of N, P, K, Ca, Mg, B, Cu, Zn in stems increased with increasing doses of CCC (statistically significant difference only between P concentration in the control and CCC doses of 1.0–3.0 g l^{-1}) (Appendix II). With daminozide at 3.0 g l^{-1}, daminozide increased concentrations of N, P, K, Ca, Mg, Mn, B, Cu, Zn in stems until the point of saturation was reached. With the two highest doses of daminozide, the difference in P, Ca, Mn, B and Zn concentrations between control and treated seedlings was significant. From a practical standpoint, however, these differences were not important.

In summer, during the three weeks after the first applications, the water content of the uppermost five centimeter of seedlings was decreased by both CCC and daminozide, but later there were no significant differences between treatments (Figure 4.3, Appendix V: Table 4.1.1).

Neither daminozide nor CCC affected leaf elongation ($P=0.106$), growth of new roots ($P=0.40$, Figure 4.4b) or height growth ($P=0.31$, Figure 4.4a) during the RGP test the following spring. Nor did the dry mass of old roots ($P=0.37$) differ between treatments. However, daminozide decreased the shoot-to-root (old plus new) ratio

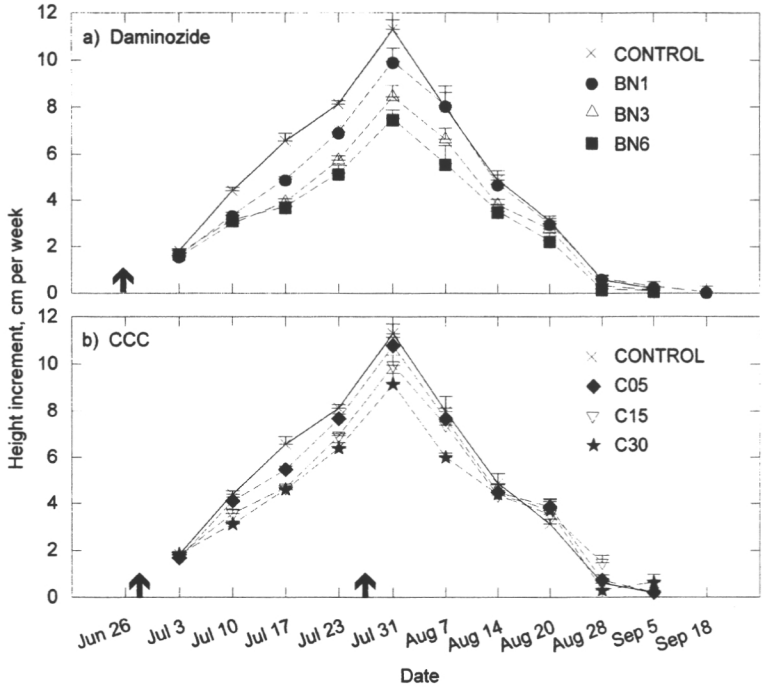


Figure 4.1 Increment in height (cm per week) of silver birch seedlings sprayed with **a)** 1.0 (BN1), 3.0 (BN3) or 6.0 (BN6) g l^{-1} doses of daminozide or **b)** 0.5 (C05), 1.5 (C15) or 3.0 (C30) g l^{-1} doses of CCC or water (control). Each curve shows the mean effect of the applications in 6 blocks (2 seedlings in 5 trays per block) at the given date, and vertical bars indicate the standard errors of the means. Arrows indicate the time of daminozide or CCC application.

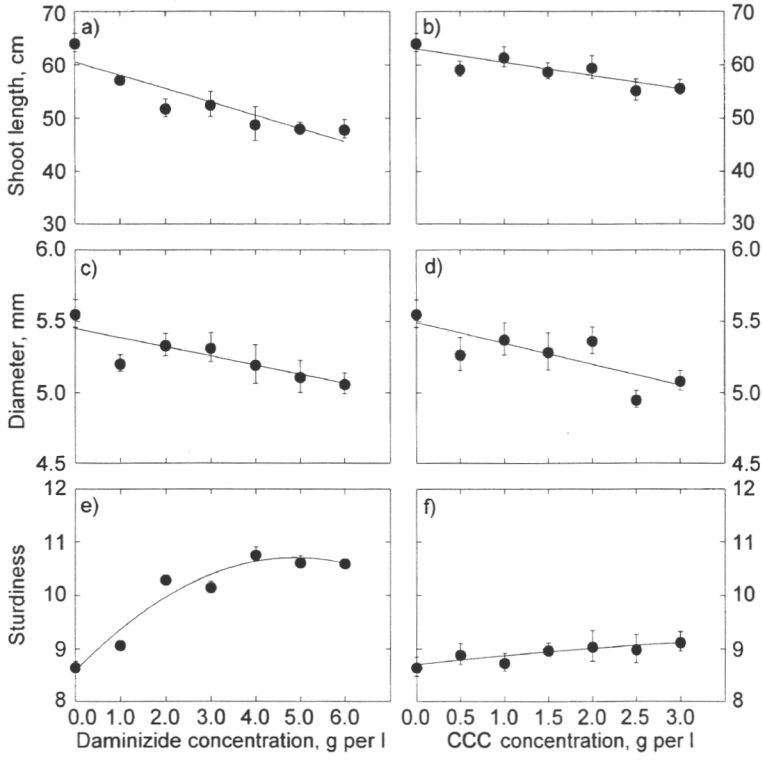


Figure 4.2 Effects of **a, c, e)** daminozide and **b, d, f)** CCC on morphology of silver birch seedlings. **a, b)** Height (cm), **c, d)** diameter (mm) and **e, f)** sturdiness [(diameter (mm))/height (m)] of seedlings sprayed with seven concentrations of daminozide or CCC. Five seedlings from each of the six blocks (total of 30 seedlings per application) were measured after leaf abscission in autumn. Vertical bars indicate standard errors of the means in six blocks.

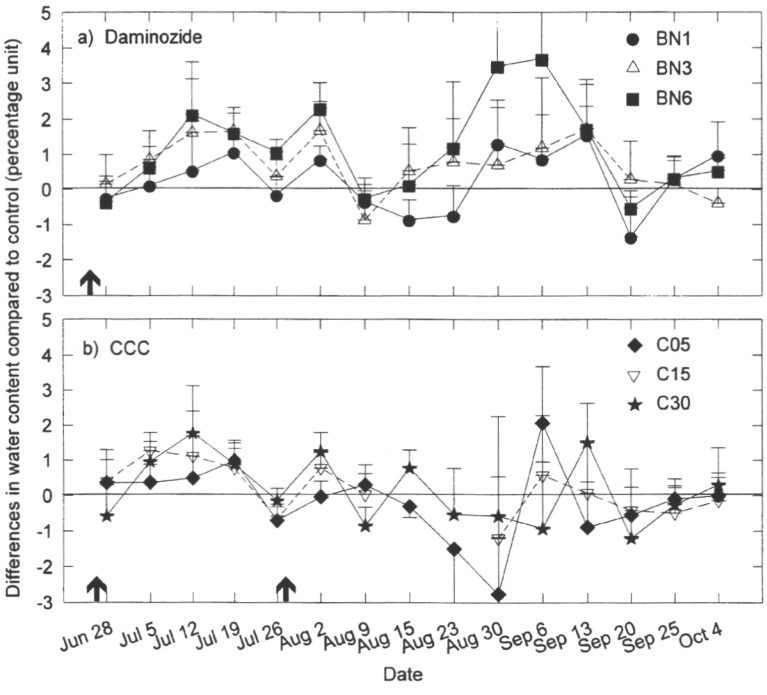


Figure 4.3 Differences in water content (percentage unit) between control and **a)** daminozide- or **b)** CCC-sprayed birch seedlings. Each symbol indicates the mean difference (control minus applied) of six block means, and the vertical bars indicate the standard errors of the means for each week. Positive values means that sprayed seedlings had lower water content than control seedlings. Explanations of treatments: arrows = spraying dates, BN1, BN3 and BN6 for 1.0, 3.0 and 6.0 g l^{-1} doses of daminozide, respectively; C05, C15, C30 for 0.5, 1.5 and 3.0 g l^{-1} doses of CCC, respectively, control = water sprayed.

(P=0.006). Due to its smaller influence on shoot growth, CCC did not affect the shoot-to-root ratio.

Neither daminozide nor CCC had after-effects on field growth (Appendix V: Table 4.1.3). After three years in the field there were no differences between control and daminozide or CCC sprayed seedlings in stem height, root collar diameter, diameter-to-height ratio or height and diameter growth (Figure 4.5). After the first summer in the field, seedlings sprayed with 5.0 or 6.0 g l^{-1} doses of daminozide were shorter than control seedlings or seedlings sprayed with 1.0 g l^{-1} concentration of daminozide. Later, however, there were no differences between applications.

4.1.4 Discussion

Both CCC and daminozide retarded the height growth of silver birch seedlings (Figure 4.1), but the effects on frost hardening could not be assessed. Both daminozide (11–25 % compared to water-sprayed control) and CCC (4–13 %) retarded height growth, but daminozide was more effective at the doses used. The differences in the effectiveness of these chemicals can be explained by the different responses of plants to the two compounds. The precise mechanism of daminozide action is unclear (Arteca 1996), but Rademacher (1991 cit.

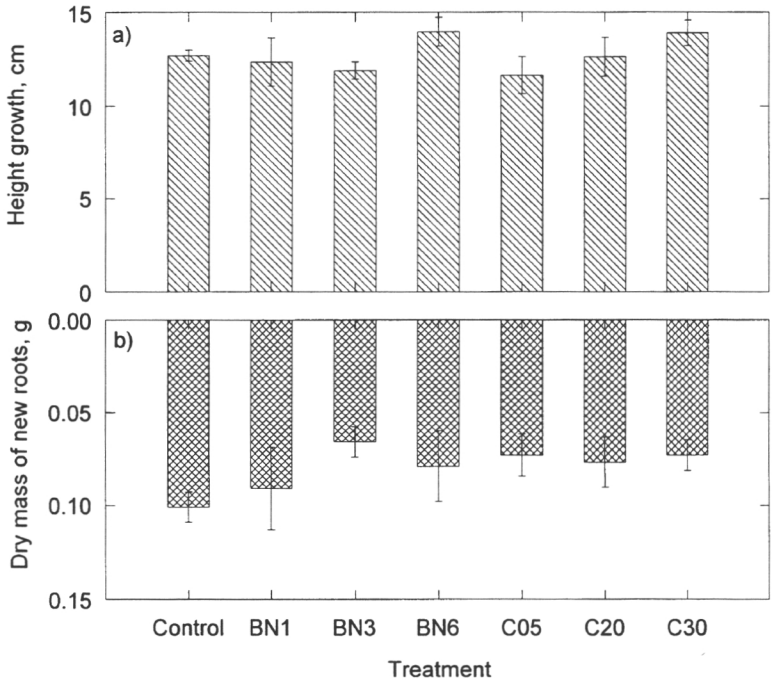


Figure 4.4 Effects of daminozide and CCC on root growth potential of silver birch container seedlings. **a)** Height growth and **b)** dry mass of new roots (roots grown out from the peat plug into the sand) of seedlings during the four-week growing period in a greenhouse in the spring of 1996. The control is water-sprayed seedlings; BN1, BN3 and BN6 are the seedlings sprayed with 1, 3 and 6 g l^{-1} daminozide doses and C05, C20 and C30 are the means of the seedlings sprayed with 0.5, 2.0 and 3.0 g l^{-1} doses of CCC in 4 blocks, respectively. The bars show the standard errors of means of four blocks (6 seedlings per treatment in each) of each treatment.

Cramer and Bridgen 1998) suggested that daminozide might reduce the translocation of GA to actively growing areas. CCC acts as an inhibitor of GA biosynthesis (Hedden 1990), thus inhibiting cell division and elongation of shoot meristems (Davies 1995).

As a response of seedlings to CCC and daminozide, reduced water content in the uppermost five centimeters of seedlings (Figure 4.3b) was observed a week earlier than the retardation in height growth (Figure 4.1b). Both compounds are able to decrease cell enlargement and the amount of intercellular space (Cathey 1975), which decreases the water-to-dry matter ratio in the top of the seedlings. A week after application, the decrease in water content was greater after CCC application than after daminozide application (Figure 4.3), indicating that seedlings responded faster to CCC than to daminozide. However, the retardation effect of CCC disappeared in four and three weeks after the first and second spraying applications, respectively. Junttila et al. (1991) also observed a brief reduction of height growth in willow after CCC application. Like here, Batjer et al. (1964) also noted that it required two weeks after daminozide application before any appreciable reduction in height growth became apparent.

GAs mediate processes that cause stem elongation in response to long days, but they affect root growth much less (Davies 1995). Diameter growth (division, elongation and differentiation of cells in cambium) is also more dependent on auxin and cytokinin levels than on

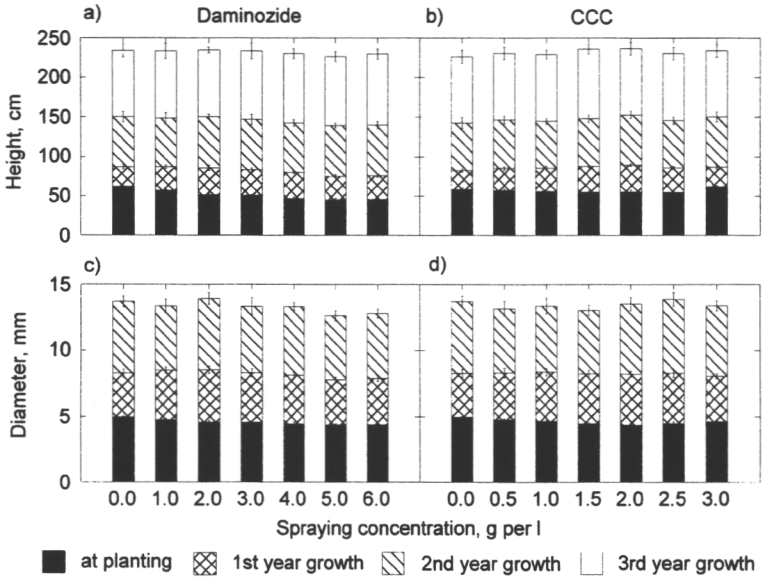


Figure 4.5 Field performance of silver birch container seedlings sprayed with seven doses of daminozide **(a, c)** or CCC **(b, d)**. Height **(a, b)** and diameter **(c, d)** at planting, after the first, second and third (diameter not measured) years in the field. Each bar is the mean of six blocks (10 seedlings in each). Vertical bars show the standard errors of the final height or diameter at the end of each year.

GAs (Aloni 1995). Thus the effects of compounds blocking GA biosynthesis on stem diameter and on dry mass of roots were less than the effects on stem height. Like silver birch in the present study (Figure 4.5a), the height growth of apple, pear, cherry and plum trees was also reduced, and growth the following year was unaffected by daminozide (Stahly and Williams 1967). In the present study the reduced diameter in autumn (Figure 4.5c) differed from the results of Stahly and Williams (1967), who found that daminozide had no influence on diameter.

CCC induced chlorosis at the leaf edges, which has also been noted by Cathey (1975), Kozlowski (1985) and Aphalo et al. (1997). Very high concentrations of daminozide (20 g l^{-1}) may cause chlorosis (Tinus 1974); but according to the results of Cathey (1975), at the concentrations used here chlorosis is not likely to occur. The darker green leaves later in summer might be caused by increased formation of chlorophyll, reduced cell size and smaller intercellular spaces in leaves induced by these compounds (Cathey 1975). As a consequence of reduced growth caused by daminozide and CCC, nutrients were concentrated in the stem (Appendix II). In tomato plants both compounds also increased the concentrations of N, P, Ca and Mg but decreased that of K compared to unsprayed plants when determined from stems and leaves six weeks after spraying (Knavel 1969).

Neither compound had significant post-planting effects. In this experiment no seedlings were killed by either CCC or daminozide. As found in the present study with silver birch, Stahly and Williams (1967) observed no after-effects in seedlings of apple, plum or cherry. According to Tinus (1974), deciduous species differ markedly in their

responses to daminozide. Some species could receive daminozide without after-effects, whereas in other species daminozide application could increase or decrease survival the following year, independently of the doses.

Because the freezing tests were unsuccessful, no reliable results were obtained concerning the effects of CCC and daminozide on the frost hardiness of seedlings. In autumn the water content of the treated and control seedlings did not differ significantly, although it was lower in seedlings sprayed with daminozide (Figure 4.3a). Previous results concerning the effects of growth-retarding compounds on frost hardiness of woody plants have been inconclusive. According to Irving and Lanphear (1968) and Kuzina et al. (1993), CCC and daminozide are effective in promoting the hardening of box-elder (*Acer negundo* L.) and birch seedlings. On the other hand, the results of Irving (1969) on box-elder and Young (1971) on *Citrus* showed that frost hardiness of seedlings was not increased by the application of either CCC or daminozide. The conditions of this study corresponded to those in the experiment of Irving (1969), where the treated seedlings were exposed to long days. Irving (1969) also studied the effects of five weekly applications of daminozide or CCC in short-day conditions following a three-week period at a temperature of +4.5 °C. In those conditions daminozide and CCC increased the frost hardiness of box-elder seedlings by about 3°C compared to short-day treatment alone. According to the results of Irving, it would not be possible to hasten the frost hardening of seedlings by the applying CCC or daminozide if they were applied during long days in June or July. However, immediately after a warm period (end of August or beginning of September) such application could be effective.

The summer of 1995, especially July, was cool and rainy, and there was no risk of excessive growth during autumn (Figure 2.1). If the weather conditions were more favorable for height growth, differences between spraying applications would probably be greater, as observed in the study of Aphalo et al. (1997). The final height of the control seedlings was only 64 cm (Figure 4.2), which is 10 cm shorter than in the experiment of Aphalo et al. (1997). The seedlings were first sprayed when they were about 20 cm tall. At this stage seedlings have young leaves, which are more effective at absorbing compounds sprayed on the foliage than mature leaves are (Sachs and Hackett 1972). According to Batjer et al. (1964), the growth is retarded most if daminozide is applied during the most rapid growth phase.

4.1.5 Conclusion

These results suggest that it would be possible to retard height growth with either daminozide or CCC. Suitable applications for this purpose, without negative effects on other morphological variables, could be a daminozide concentration of 4 g l^{-1} applied once and CCC concentration of 2 g l^{-1} applied twice. Higher concentrations would not produce more retardation in height growth or changes in sturdiness. In addition, dry mass of roots did not increase when the point of saturation was reached with CCC doses of 2 g l^{-1} (Aphalo et al.1997). However, the effects of later (end of July, beginning of August) dates of spraying on silver birch seedlings should be studied in order to avoid unnecessary sprayings.

4.2 Short-day treatment

4.2.1 Background and study objectives

Short-day (SD) treatment has been studied as a method for controlling the height growth and frost hardening of seedlings of some deciduous species (Kelly and Mecklenburg 1978, Nyström 1993, Calmé et al. 1995, Luoranen and Rikala 1997). In the study of Luoranen and Rikala (1997), SD treatment effectively stopped the height growth of silver birch seedlings when treatment was started at the end of June or in July. However, SD treatment started at the end of June stopped height growth only temporarily. When SD treatment was started in July, height growth stopped, dormancy was induced and frost hardening hastened.

The influences of SD treatment on diameter growth or on dry mass partitioning among shoot, roots and leaves during and after a SD period have been studied little. In most cases, root collar diameter has been measured only at the end of the experiment or growing season (e.g. Håbjørg 1972a, Heide 1974a, Eastham 1990, Major et al. 1994, Luoranen and Rikala 1997). Krasowski and Owens (1991) studied diameter growth in western red cedar seedlings during the SD period. Usually SD treatment did not affect diameter growth. In the study of Kelly and Mecklenburg (1978) on birch, Smit-Spinks et al. (1985) on Scots pine, Stahel (1972) on Sitka spruce (*Picea sitchensis* (Bong) Carr.) and Krasowski and Owens (1991) on western red cedar seedlings, SD treatment did not affect root growth either during or after treatments.

SD treatment has also been observed to affect nutrient concentration. In the study of Skre (1991c) and Bigras and D'Aoust (1993), SD treatment increased nutrient concentrations in the stems of birch and white spruce. After planting, higher nutrient concentrations, in

turn, have been shown to improve the field performance of seedlings (Timmer and Munson 1991, Malik and Timmer 1996, Bigras et al. 1996).

SD treatment has been found to have some after-effects. In several species, buds of SD-treated seedlings have burst earlier the following spring (Rosvall-Åhnebrink 1977, 1980, Sandvik 1980, Silim et al. 1989, Hawkins, C. et al. 1996). In a previous study on birch, the earlier seedlings were SD treated during the preceding summer the faster the buds burst (Luoranen and Rikala 1997). In conifer seedlings, the reported influence of SD treatment on dehardening has been variable (Bigras and D'Aoust 1992, 1993, Folk et al. 1994). To my knowledge, no studies concerning the effects of SD treatment on dehardening of birch seedlings have been published.

When Rikala (1996) studied planting of silver birch seedlings with leaves during the summer, the results were promising. So far, there is little information on summer planting and stress tolerance of birch seedlings with leaves (Salminen 1998, Perälä et al. 1999). Birch seedlings are, however, less tolerant to drought in summer than in spring. The stress tolerance of seedlings planted in summer could be increased by SD treatment (cessation of height growth, smaller size and sturdiness). SD treatment has not been shown to reduce root growth but it has been shown to increase height growth during the following seasons (Heide 1974b, Rosvall-Åhnebrink 1980, Odlum and Colombo 1988, Eastham 1991, Grossnickle 1992, Odlum 1992, Nyström 1993, Folk et al. 1994, Hawkins, C. et al. 1996). Thus SD-treated silver birch seedlings planted in summer might root well; and during the following seasons in the field growth will increase.

The objective of this part of the study was to determine possibilities to use SD treatment to control height growth and frost hardening of silver birch container seedlings. The purpose was also to seek suitable planting windows for both SD-treated and untreated seedlings grown in small containers. For evaluation of SD treatments and planting windows, SD treatment was investigated, in particular the effect of its timing on: 1) height growth, cessation of height growth, diameter growth and dry mass partitioning to leaves, stem and roots in the nursery, 2) frost hardening in autumn and dehardening the following spring, 3) ablity to root egress and root growth potential (RGP) and 4) field performance after summer or spring planting.

4.2.2 Material and methods

4.2.2.1 Seedling material and SD treatments

This part of the study consisted of two crops divided into two experiments: Seeds for the first crop were sown on 10 May 1996, and those for the second crop were sown on 18 June 1996. Timing of sowing, transplanting and SD treatments in both crops are presented in Table 4.1. Seedlings were grown in Plantek 64F trays (64 cavities per tray, 110^3 per cavity, 431 cavities per m^2, Lännen Plant Systems, Finland) filled with fertilized sphagnum peat (Vapo, XL, Finland). Seedlings were irrigated according to normal nursery practice by keeping the water content of the peat at 40–60 percent by volume during the growing season. Seedlings were fertilized with liquid fertilizer six times for the first crop (on 1, 8, 18, 24 and 30 July and 9 August) and two times for the second crop (on 30 July and 9 August). For the first crop the total amount of nutrients (including both base and liquid fertilizers) given was 51 mg N, 17 mg P and 58 mg K per seedling plus micronutrients. In the second crop each seedling received a total of 25 mg N, 10 mg P and 30 mg K per seedling plus micronutrients. Seedlings were grown in a greenhouse until they were transferred outside on 24 June (1st crop) or 26 July (2nd crop). Seedlings were sprayed against birch rust (*Melampsoridium betulinum* (Pers.) Kleb.) with Bayleton 25 (triadimefon 0.05 %) on 12 July and 1 August and against fungi that cause stem lesions with Tilt-250 ES (0.5 l per hectare) on 16 July.

Each crop was comprised of two treatments in five complete blocks, with 25 trays, 64 seedlings in each, per treatment, randomized to blocks, five trays per treatment in each block giving a total of 50 trays and 3200 seedlings. Photoperiod was shortened to 8 hours a day for three weeks both in SD 1 and SD 2 treatments (for timing see Table 4.1). The control treatment was natural day length. Seed-

Table 4.1 Dates for sowing and transplanting in the first and second crop of silver birch container seedlings. Also the dates, accumulated temperature sums (TS), and natural night lengths (NNL) at the beginning and end of the SD treaments (SD 1 or SD 2) and at the time when height growth ceased. Control 1 and 2 seedlings were grown under natural day lengths.

Crop	Treatment	Sowing date	Trans-planting date	SD period						Cessation of height growth		
				Start			End					
				date	TS d.d.	NNL hours	date	TS d.d.	NNL hours	date	TS d.d.	NNL hours
1st	SD 1	May 5	Jun 3	Jun 26	576	03:50	Jul 17	766	05:07	Jul 12	728	04:43
	control 1									Aug 22	1136	08:38
2nd	SD 2	Jun 18	Jul 1	Jul 29	580	06:13	Aug 19	832	08:20	Aug 5	644	07:55
	control 2									Aug 26	872	09:04

lings were treated from 1600 h until 0800 h every day by using light-reflecting cloth (Ludvig Svensson, PLS-Block Out Arbi cloth, aluminum-coated outside, white inside, maximum light transmission 0.7 μmol m^{-2} s^{-1} measured in 1200 μmol m^{-2} s^{-1} illumination) in a wooden frame (8 m length x 4 m width x 0.8 (0.6 m) height).

In both crops, blackout treatment affected mean daily temperatures only slightly: mean daily temperatures were +14°C for SD 1 and control 1 and +17°C and 16°C for SD 2 and control 2. The accumulation of temperature sum in the different treatments are presented in Table 4.1.

4.2.2.2 Measurement of morphology

Twelve seedlings in each block, a total of 60 seedlings per treatment, were randomly selected and the height of the stems of these seedlings was measured weekly from 11 June to 10 September (first crop) and from 5 July to 6 September (second crop). For the same seedlings the dates for cessation of height growth were determined (for method see section 2.6.2); and weekly color changes and abscission of leaves were scored according to the following classification: 1) all leaves green, 2) some of the leaves green, the rest yellow, 3) all leaves yellow, some of them fallen and 4) all leaves fallen.

For monitoring the development of diameter and dry mass of the shoots (stems and branches), leaves and roots, four randomly selected seedlings per treatment in each block (a total of 20 seedlings per treatment per day) were harvested each week from 11 June to 15 October (except on 3 and 10 September) in the first crop and from 26 July to 11 October in the second crop. Nutrients were analyzed from five pooled leaf samples per treatment at the end of the SD period (on 16 July in SD 1 and on 23 August in SD 2) and from five pooled shoot and five pooled root samples at the last harvesting time in autumn.

4.2.2.3 Estimation of frost hardening

In both crops, the frost hardening of seedlings was monitored by weekly determination of water content in the shoot apex, and in the second crop by exposing whole seedlings to –10°C temperature weekly or stem samples to freezing temperatures three times during autumn. For determination of water content, in the first crop the apices from two of the harvested seedlings per block were cut weekly from 25 June to 15 October. In the second crop the apices were cut from all harvested seedlings from 26 July to 11 October.

In the first crop, for the evaluation of mortality at a test temperature

of –10°C, eight seedlings from each block, a total of 40 seedlings per treatment were randomly selected each week from 26 August to 14 October. Color changes and shedding of leaves were classified as described above; and seedlings were randomized into 10 polystyrene trays (four cavities per tray), two seedlings from each treatment in each tray. Roots were protected from freezing by covering them with small polystyrene beads (about 0.5–1 cm on peat surface). Half of the trays were kept in outdoor growing area in natural weather conditions as a control. Seedlings in five trays were exposed to the –10°C test temperature in an air-cooled chamber (Weiss, type Bioclim 1600 Sp-Pa-S, Lindenstruth, Germany) for three hours. The initial temperature of the champer was between +15 and +5°C depending on the outdoor temperature at each test date, but at the end the temperature was +10°C at all dates. The rates of cooling and warming were 2°C/h and 5°C/h, respectively. After exposure, seedlings were transferred to Plantek 64F trays and kept in an unheated greenhouse, where they were watered as needed until 17 October. Trays were packed in plastic bags and stored at –4°C. From 5 to 10 February, seedlings were thawed in darkness at a temperature of +5°C and then transferred to +15°C in a naturally illuminated greenhouse. On 17 February the greenhouse was heated and illuminated artificially (day/night temperature: +20 – +22 °C/ +15°C, with photon flux density (PAR) 150 $\mu mol^{-2}s^{-1}$ from metal halide lamps (HQI-400W Power Star, Osram), photoperiod: 18 h). Seedlings were watered daily with tap water. Fourteen days after thawing, on 3 March, seedlings were classified as dead if they had no leaves or only a few leaves on the lower three centimeters of the stem, damaged if leaves were smaller than the leaves on the unexposed seedlings, or undamaged if leaves were normal in size and had no other damage on the stem.

Frost hardiness of the seedlings in the second crop was assessed three times in autumn and once in spring: on 19 August, 11 September, 2 October 1996 and 14 May 1997. Twenty randomly selected seedlings per treatment were sampled from each block, a total of 100 seedlings at each test date. Each seedling was divided into two parts: from the uppermost five centimeters water content was determined; the rest of the seedling was exposed to freezing temperatures. The samples were exposed in air-cooled chambers to the test temperatures presented in Table 4.2. Air temperatures in chambers were controlled by the external alcohol circulation system of Lauda Ultra-Kryomat K6 together with programmer PM350 (MGW, Lauda, Germany). On all test dates the control temperature was +4°C. Both the initial and end temperature was +20°C. The rates of cooling and warming were 2°C/h and 3°C/h, respectively. The duration of minimum temperatures varied from 3 to 12 hours, depending on the temperature.

Table 4.2 Exposure temperatures (°C) used in frost-hardiness tests in autumn 1996 and spring 1997.

Test date	Exposure temperature, °C																		
	+4	–2	–4	–6	–8	–10	–12	–14	–16	–18	–20	–22	–24	–26	–28	–30	–32	–34	... –50
19 Aug 1996	x	x	x	x	x	x	x	x				x				x			
11 Sep1996	x		x	x	x	x	x	x		x				x				x	
2 Oct 1996	x		x		x		x	x		x	x		x			x		x	
14 May 1997	x		x	x		x			x		x				x				x

The frost hardiness of seedlings was determined with the relative electrolyte-leakage (*REL*) method and with visually determined browning of phloem and cambium (*RB*), as described in Section 3.2, but with slight modifications. After freezing, the middle part of the stem sample was set on boxes filled with watered quartz sand. Samples were kept wet by watering the sand as needed. Relative conductivity was measured as in Section 3.2 by using an incubation time of 120 hours for samples exposed to freezing.

4.2.2.4 Performance tests for summer planting

In both crops, root-egress tests and outplanting were used to evaluate the field performance of seedlings. In the first crop, four seedlings per treatment in each block (a total of 20 seedlings per treatment) were randomly selected for a test each week from 25 June to 27 August. Seedlings planted in 0.75 l plastic pots were divided into five blocks, four seedlings per treatment in each block, and placed in a greenhouse, where the daily mean temperature was about 21°C and the photoperiod decreased from 20 to 14 hours from the first to the last test period.

Control seedlings were planted into the field test from 3 July to 6 August, and SD-treated seedlings were planted from 16 July (after the SD period) to 27 August. Each week 72 randomly selected seedlings per treatment were planted into a randomized block design (4 blocks, 18 seedlings per treatment per block, 0.75 m between seedlings in a row, 1 m between rows). During the first autumn, the color and abscission of leaves were observed as in the nursery experiment. Height and diameter were measured at planting and at the end of each of three growing seasons. Because of hares, the test area was fenced in at the beginning of September in the year of planting.

4.2.2.5 Performance tests for spring planting

In the second crop, seedlings for the RGP test and for the planting experiment the next spring were packed into plastic bags and stored at –4°C on 14 October 1996. On 15 April 1997, cooling of frozen storage was stopped and the temperature increased about 0.4°C per day to +3.5°C until 3 May. After that, it increased +1°C per day to 6.5°C, being +6.5°C by 27 May. Seedlings for RGP test were stored until 7 May and spring-planted seedlings until 26 May. From 7 to 14 May, RGP seedlings were thawed in darkness (4 d at +6°C). Thawed seedlings were grown in a greenhouse (day/night temperature: +20 ... +22 °C/ +15°C, photon flux density (PAR) 150 µmol · m^{-2} · s^{-1} from metal halide lamps (HQI-400W Power Star, Osram), photoperiod: 18 h) for four weeks. In order to determine the timing of bud burst and leaf elongation, the length of the same three buds and after the bud burst the leaves of each seedling were measured every third day.

The outplanting experiment was designed as six randomized blocks with two treatments as plots. In each block, 10 seedlings per treatment were planted on 26 May. Height and root collar diameter were measured at planting and at each autumn.

4.2.2 6 Statistical analysis

The first crop. The homogeneity of group variances was analyzed with the Bartlett test and the normality of distribution with the Lillefors test. Before analysis of variance, root growth (dry mass of new roots) in root-egress tests, diameter growth in the second year, height growth in the first year and diameter at the end of the second year in the summer planting experiment were transformed in order to normalize the distributions. Differences between root-egress test periods within a treatment were analyzed using Tukey's test. Because no transformation was found to transform the distributions to normal and to homogenize the variances of weekly measured water content, dry mass of stem, roots and leaves, the effects of treatment on these weekly measured variables were analyzed by using Kruskal-Wallis one-way analysis each week. Because of the unequal variances, differences in Cu and Mn concentrations in stems between treatments were also analyzed with the Mann-Whitney U-test. For other nutrients the differences were tested with a t-test. ANOVA tables are presented in Appendix V. Tables 4.2.1–4.2.5 are connected with the first crop.

The second crop: Water content was analyzed with an MGLH procedure for repeated measurements. Before analysis of variance, weekly measurements of water content and of height growth in the RGP

test were transformed in order to stabilize the variances and transform the distributions to normal. ANOVA tables are presented in Appendix V. Tables 4.2.6–4.2.8 are connected with the second crop. Differences in Cu and Zn concentrations in stems, P, K, Ca, Mg, Mn, Fe, B and in Zn concentrations in roots between treatments were also analyzed with the Mann-Whitney U-test, and differences in other nutrients were analyzed with a t-test.

4.2.3 Results

4.2.3.1 Early SD treatment and summer planting

Morphology. SD treatment started on 26 June stopped growing in height after two weeks (Figure 4.6, Table 4.3). Half of the SD-treated seedlings, however, resumed height growth after the SD period. Regrowth of SD 1 seedlings did not affect the date when height growth was determined to cease (Table 4.3), but it increased the variability in height from 5 % at the beginning of SD to 19 % at the end of August. The control 1 seedlings stopped growing on 22 August. At the end of August the difference in mean height between SD 1 and control 1 was significant ($P<0.001$, Figure 4.6).

SD treatment did not affect diameter or root growth (Table 4.3, Figure 4.7b,e). The highest rate of increase in dry mass of shoots was at the end of July and in the second week of August for SD 1 and control 1 seedlings, respectively. In both treatments (at the beginning and in the middle of September for SD 1 and control 1, Table 4.3), increment in dry mass of shoots ceased about a month after the time of highest growth. In September, leaves began to be shed two weeks earlier in SD 1 seedlings than in control 1 seedlings (Table 4.3, Figure 4.9a,c). All leaves were shed by the first and second week of October for SD 1 and control 1 seedlings, respectively (Table 4.3).

At the last harvesting date, due to the unaffected diameter ($P=0.10$) and dry mass of the roots ($P=0.54$) but shorter stem ($P<0.001$) and less dry mass of the shoots ($P<0.001$) (Figure 4.7), SD 1 seedlings were sturdier ($P<0.001$) and their shoot-to-root ratio was lower ($P<0.001$) than those control 1 seedlings.

Nutrient concentrations. Concentrations of N, P, K, Mn, B, Cu, Fe and Zn in the leaves of treated seedlings in the end of the SD period were increased by SD 1, but the concentrations of Ca and Mg were the same in both treatments (Appendix III). When nutrients were analyzed after leaf abscission in October, SD 1 treatments increased concentrations of N, P, Mg, Mn, B, Cu, Fe and Zn in stems and concentrations of N, P, K, Mg, Mn, B and Zn in roots.

Table 4.3 Estimated parameters of the growth curves for height (Equation 2.4), diameter (Equation 2.3) and dry mass of shoot and roots (Equation 2.4) and the parameters of the developmental curves for the dry mass of leaves (Equation 2.15) in seedlings from the first (control 1, SD 1) and second crop (control 2, SD 2) in 1996. Control seedlings were grown under natural photoperiod. SD 1 and SD 2 seedlings were subjected to short-day treatment from 26 June to 17 July and from 29 July to 19 August, respectively. Dates and accumulated temperature sums at the age of maximum rate of growth (parameter c) and at the age when the growth ceases ($x_{0.95}$) for each variable and the dates and accumulated temperature sums at the age when leaves began to be shed (r) and when all leaves were shed ($x_{0.05}$) for the dry mass of leaves.

Parameters of estimated growth curves

Variable	Treatment	m	b	c	$x_{0.95}$	l
Height	control 1	39	0.07	62	104	
	SD 1	13	0.16	45	63	
	control 2	21	0.11	43	69	
	SD 2	12	0.28	37	48	
Diameter	control 1	4.0	0.06	66	113	0.2
	SD 1	3.6	0.07	68	110	0.5
	control 2	1.8	0.21	59	74	1.2
	SD 2	1.4	0.19	60	75	1.3
Shoot	control 1	1.5	0.08	90	129	
	SD 1	0.7	0.09	81	116	
	control 2	0.5	0.11	65	91	
	SD 2	0.3	0.13	58	82	
Roots	control 1	1.0	0.08	87	122	
	SD 1	1.1	0.09	89	122	
	control 2	0.4	0.09	65	98	
	SD 2	0.4	0.09	64	97	

Parameters of estimated development curves for dry mass of leaves

Variable	Treatment	m	b	c	$x_{0.95}$	k	h	r	$x_{0.05}$
Leaves	control 1	0.8	0.12	62	87	–0.32	145	109*	154
	SD 1	0.5	0.12	58	83	–0.27	139	122	150
	control 2	0.6	0.08	53	89	–0.20	98	95	113
	SD 2	0.4	0.09	51	83	–0.38	100	94	107

Dates and accumulated temperature sums at the estimated ages

		Max. growth rate c		Cessation of growth $x_{0.95}$		Beginning of shedding r		All leaves shed $x_{0.05}$	
Variable	Crop	Date	d.d.	date	d.d.	date	d.d.	date	d.d.
Height	control 1	11 Jul	714	22 Aug	1136				
	SD 1	23 Jun	554	12 Jul	728				
	control 2	31 Jul	591	26 Aug	872				
	SD 2	25 Jul	533	5 Aug	644				
Diameter	control 1	17 Jul	764	25 Aug	1161				
	SD 1	24 Jun	566	28 Aug	1190				
	control 2	16 Aug	763	31 Aug	925				
	SD 2	17 Aug	804	1 Sep	969				
Shoot	control 1	8 Aug	963	16 Sep	1278				
	SD 1	30 Jul	882	3 Sep	1251				
	control 2	22 Aug	839	17 Sep	981				
	SD 2	15 Aug	777	8 Sep	994				
Roots	control 1	5 Aug	933	9 Sep	1266				
	SD 1	7 Aug	954	9 Sep	1276				
	control 2	22 Aug	839	24 Sep	987				
	SD 2	21 Aug	860	23 Sep	1002				
Leaves	control 1	11 Jul	714	5 Aug	933	23 Sep	1282	11 Oct	1314
	SD 1	7 Jul	679	1 Aug	899	9 Sep	1276	7 Oct	1306
	control 2	10 Aug	692	15 Sep	981	21 Sep	983	9 Oct	1015
	SD 2	8 Aug	680	9 Sep	994	20 Sep	1000	3 Oct	1012

* the change point (r) in this case does not express the age of the beginning of leaf shedding. In stead, beginning of leaf shedding was determined by fitting the curve by substituting equations (2.7) or (2.9) with values of 0.95 or 0.05 into the second part of equation 2.15.

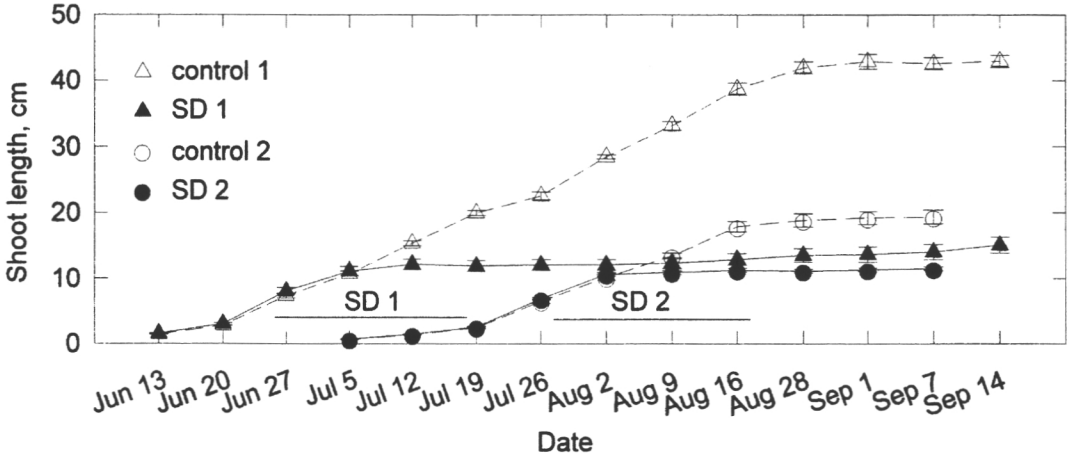


Figure 4.6 Shoot length of birch seedlings sown on 10 May (SD 1 or control 1) and on 18 June (SD 2 and control 2). Horizontal bars indicate SD periods from 26 June to 17 July and from 29 July to 19 August for SD 1 and SD 2, respectively. Vertical bars show the standard error of block means (n=5). Solid lines connect the means of SD and broken lines the means of control blocks.

Frost hardening. In July and August, SD 1 treatment decreased the water content in the uppermost 5 cm of seedlings compared to the control seedlings (Figure 4.8). In September, there were no statistically significant differences between treatments. After the SD period the variability in water content (coefficient of variation 3–16 %) in treated seedlings increased due to regrowth of some seedlings. In both treatments, yellowing of leaves began on 26 August and abscission of leaves on 1 October; but total abscission was hastened by SD (Figure 4.9a,c).

In the root-egress tests in summer, control 1 seedlings grew at least 0.05 g of new roots until the test period started in the middle of August, when the root growth slowed down (Figure 4.10a). In the middle of July root growth was temporarily weak. During the first three test periods, height growth was strong; but after mid-July it slowed down (Figure 4.10c). Diameter growth was strongest in the beginning of August (Figure 4.10b). In late summer, root growth decreased with declining height and diameter growth.

The dry mass of the new roots in SD 1 seedlings was greater during the test period started on 2 July (only one week in SDs) than during the other test periods (Figure 4.10a). It was also higher than that in control seedlings at the same date (P=0.01). Root growth of SD 1 seedlings declined continuously from 2 July (except for a low test result on 16 July and a high test result on 20 August). Height growth during the test period decreased during the SD treatment and stopped after that, but increased after 23 July (Figure 4.10c). After the SD period, the dry mass of the new roots in control seedlings was higher than that in SD seedlings, although there was a statisti-

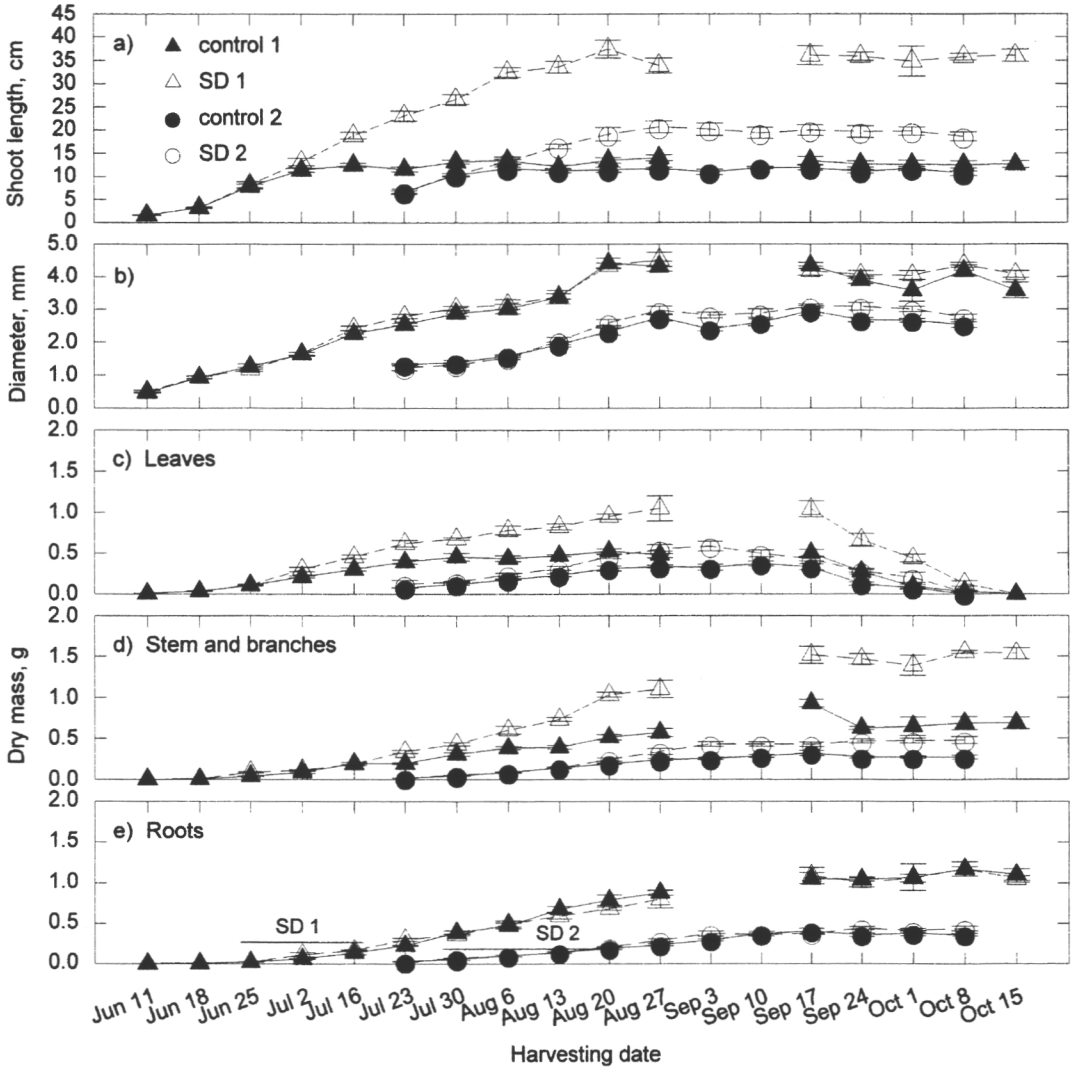


Figure 4.7 Development of dimensions and dry mass in seedlings sown on 10 May (SD 1 or control 1) and on 18 June (SD 2 and control 2). **a)** Shoot length (cm), **b)** diameter (mm), dry mass (g) of **c)** leaves, **d)** stem and branches and **e)** roots of SD 1, control 1, SD 2 or control 2 seedlings harvested from 11 June to 15 October. Horizontal bars indicate SD periods. Control seedlings were grown under natural photoperiod.

cally significant difference only in the test started on 30 July (P=0.03).

Summer planting. During the first (planting) summer, the earlier the seedlings were planted the more seedlings were eaten by hares; when seedlings were SD-treated before planting (Table 4.4), the more treated seedlings were damaged (possibly by hares) during the winter and the following growing season.

The later control 1 seedlings were planted the taller (P<0.001) and thicker (P<0.001) they were at planting and the less they grew the first year in the field (1st year growth, P<0.001) (Figure 4.11b). Dur-

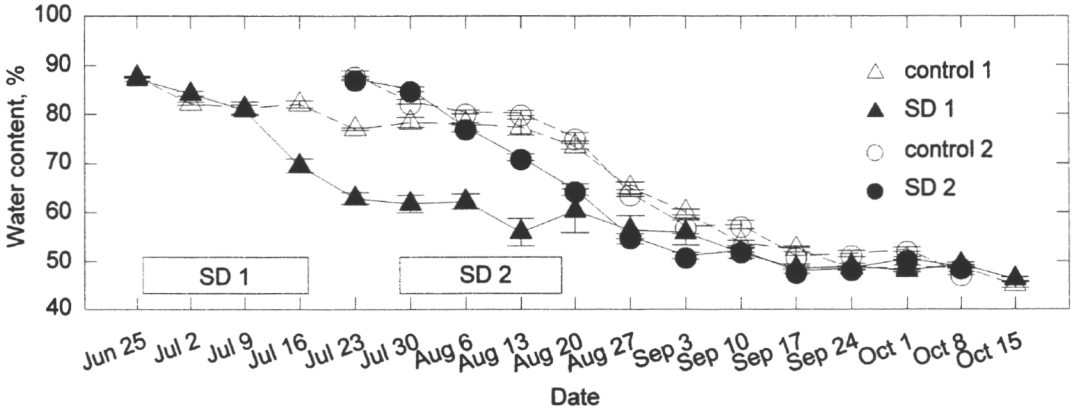


Figure 4.8 Water content of the uppermost 5 cm of the stems of birch seedlings sown on 10 May (solid lines) and on 18 June (broken lines) determined from 25 June or 26 July to the middle of October. Explanations of lines, bars, and symbols are as in Figure 4.6.

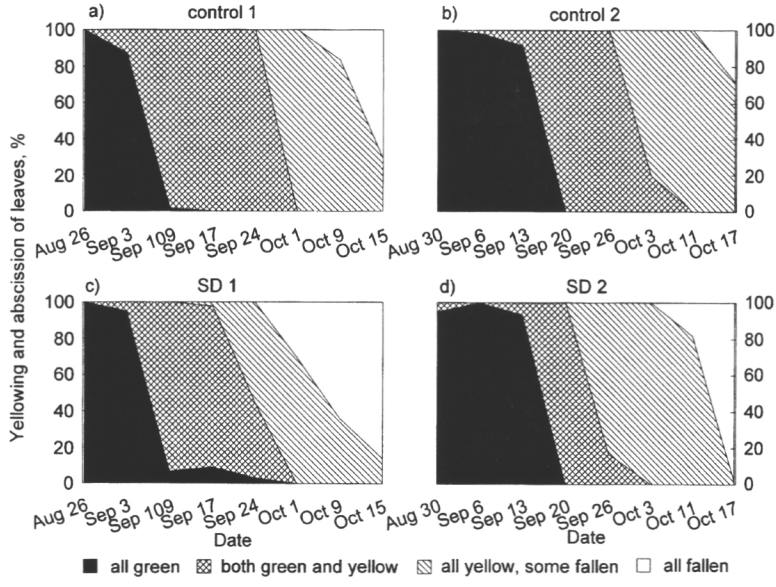


Figure 4.9 Yellowing and abscission of leaves in autumn 1996 in seedlings sown on 10 May (SD 1 or control 1) and on 18 June (SD 2 and control 2). In both crops, 60 seedlings in each treatment (control/SD) were scored weekly and classified into four classes (shown as proportion of seedlings in each class).

ing the first summer (Figure 4.11d), planting date did not affect diameter growth (P=0.03). During the second season, the height and diameter growth of seedlings planted on 16 and 30 July was less than that of seedlings planted at other dates. After the third year, total height (P=0.25), diameter (P=0.21) and growth (P=0.97 and P=0.99 for height and diameter growth, respectively) did not differ significantly between planting dates.

At planting, the height of the SD 1 seedlings was the same in all weeks (P=0.63, Figure 4.11a), but the later seedlings were planted the greater their diameter (P<0.001) (Figure 4.11c). Differences in

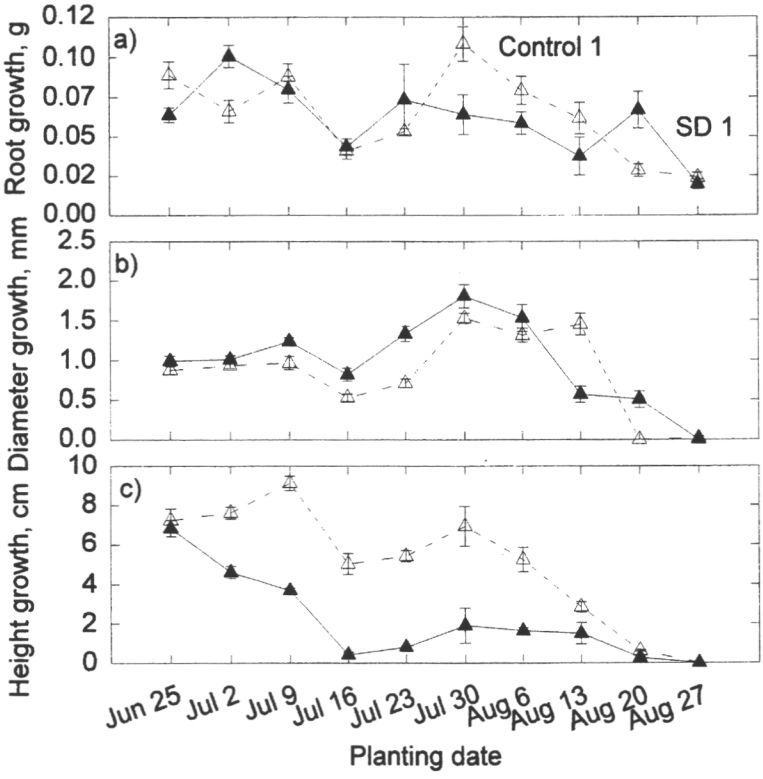


Figure 4.10 Changes in ability of seedlings sown on 10 May 1996 to root egress. **a)** Root growth (as dry mass of new roots grown out from the peat plug into sand), **b)** diameter growth or **c)** height growth of SD-treated seedlings (SD1) and seedlings grown under natural photoperiod (control 1) during the three-week test period in an unheated and unlighted greenhouse in summer 1996. Seedlings were SD-treated from 26 June to 17 July. On the horizontal axis are the dates when the seedlings were planted into the sand-filled pots. Bars indicate the standard errors of means on each date. n = 5 block means (4 seedlings per block).

height were statistically significant between the three earliest (on 16, 23 and 30 July) and the two latest planting dates (on 20 and 27 August). As occurred in the nursery, some seedlings continued to grow in height after planting (Table 4.5). For all seedlings, the earlier they were planted the more they grew in diameter ($P<0.001$) after planting (Figure 4.11c,d). There were no differences in total height ($P=0.65$, $P=0.16$) or diameter ($P=0.02$, $P=0.16$) between planting dates at the end of the first (planting summer) and second seasons (Figure 4.11). At the end of the third season the seedlings planted after 13 August were shorter than other seedlings ($P=0.004$–0.05). When the SD-treated and untreated seedlings planted during the same week are compared, the SD-treated seedlings were shorter at planting and at the end of the first year, but later there were no differences between treatments.

4.2.3.2 SD treatment of late sown seedlings

Morphology. SD 2 seedlings stopped growing in height during the SD period (SD ended on 5 August) (Figure 4.6). The height growth of control 2 seedlings stopped later (Table 4.3). Due to the earliest cessation of height growth, the SD 2 treatment affected the timing of the

Table 4.4 Total proportion of damaged and dead birch seedlings (%) in the field experiment in which the SD 1 and control 1 seedlings were planted in summer of 1996. Damage and mortality were checked on 3 September 1996 (in parentheses) and 10 September 1997. SD 1 seedlings were subjected to short-day (26.6.–17.7.) treatment before planting. Control 1 seedlings were grown under natural photoperiod before planting.

	Planting date								
Treatment	2 June	9 July	16 July	23 July	30 July	6 August	13 August	20 August	27 August
SD 1			(17) 50	(10) 44	(17) 68	(18) 67	(18) 77	(7) 73	(1) 67
control 1	(6) 23	(18) 39	(11) 33	(1) 22	(8) 21				

Table 4.5 Proportion of undamaged birch seedlings (%) which grew more than 0.5 cm after planting in summer. For explanations see Table 4.4.

	Planting date								
Treatment	2 June	9 July	16 July	23 July	30 July	6 August	13 August	20 August	27 August
SD 1			9	0	23	4	12	37	44
control 1	97	98	93	89	85	40			

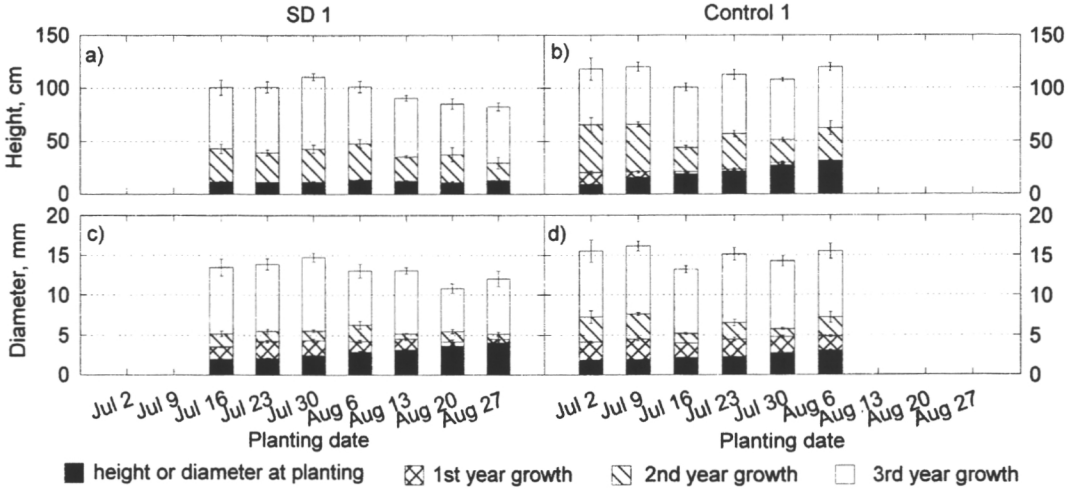


Figure 4.11 a, b) Height (cm) and **c, d)** diameter (mm) of seedlings subjected to short-day (SD 1) treatment (**a, c**) and seedlings grown under natural photoperiod (control 1) (**b, d**) outplanted weekly to the nursery field in summer 1996 and measured at planting and at the end of the first (1996), second (1997) and third (1998) seasons. Seedlings were sown on 10 May 1996, and SD-treated from 26 June to 17 July. Each bar indicates the mean of four block (20 seedlings in each per treatment) means and the vertical bars the standard errors of the means for each date.

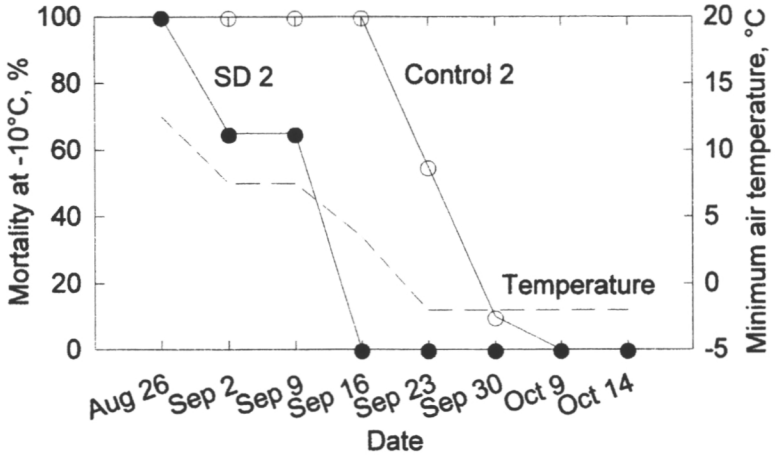


Figure 4.12 Mortality of seedlings subjected to short-day (SD 2) treatment from 29 July to 19 August and seedlings grown under natural photoperiod (control 2) and exposed weekly to −10°C temperature from 20 August to 8 October 1996. Damage to exposed seedlings was evaluated after frozen storage in February 1997 by growing the seedlings for 14 days in a heated and lighted greenhouse. The broken line indicates minimum air temperature on the seedling level obtained until each test date.

maximum growth rate of seedlings: the highest rate of height growth and increase in dry mass of shoots were about a week earlier in SD 2-treated seedlings than in control 2 seedlings (Table 4.3). Diameter growth had ceased by the end of August in both treatments. In addition to height ($P<0.001$), SD 2-treated seedlings also had less dry mass of shoots ($P=0.003$) at the last date of harvesting (Figure 4.7a,d).

Nutrient concentrations. Accumulation of nutrients in leaves during the SD 2 treatment was smaller than in the SD 1 treatment. However, at the end of the SD 2 period there was a significant increase in treated seedlings compared to controls only for N, Mn and Zn. After leaf abscission in October, SD 2-treated seedlings accumulated more N, P, Ca, Mg, Mn, B, Cu, Fe and Zn in the stems and more N, P, K, Mg, Mn, B and Zn in the roots than control seedlings did. In the second crop, seedlings in both treatments had higher nutrient concentrations in the stem and roots than seedlings of the first crop had (Appendix III).

Frost hardening. Reduction in water content was hastened by SD treatment started on 29 July (Figure 4.8). On the other hand, SD treatment did not affect the timing of leaf yellowing (yellow leaves were first observed on 6 September in both treatments), but it hastened the abscission of leaves by two weeks (Figure 4.9b,d). All SD-treated seedlings survived at −10°C for the first time on 16 September and all control seedlings on 9 October (Figure 4.12). SD-treated seedlings with green or yellow leaves survived at −10°C, but control seedlings did not survive until all the leaves had been shed (Figure 4.13). SD treatment increased the frost hardiness (expressed as either ET_{50} or BT_{50}) of seedlings by 2–6°C, depending on the test date (Figure 4.14). On 21 August and 11 September, leaves in all frost-exposed seedlings were green and on 2 October they were yellow or had fallen.

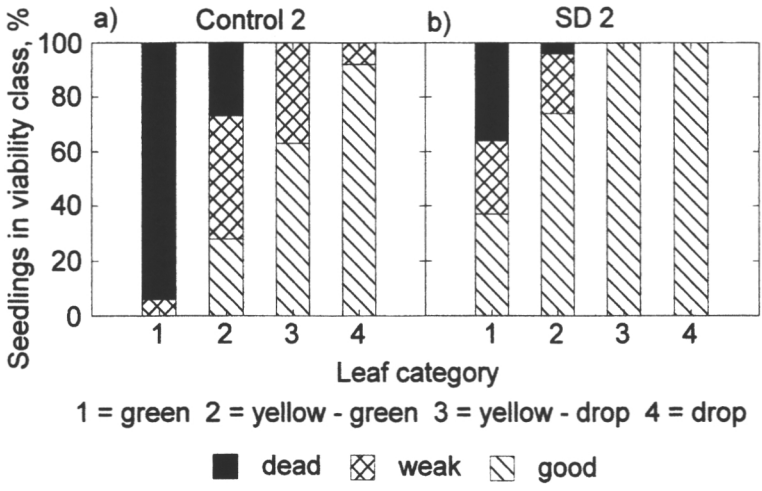


Figure 4.13 Relationship between yellowing and abscission of leaves and frost hardiness in a) seedlings grown under natural photoperiod (control 2) and b) seedlings short-day treated from 29 July to 19 August, 1996 (SD 2). Each leaf category includes the proportion of good, weak and dead seedlings according to injuries caused by exposure to −10°C weekly from 20 August to 8 October. Leaf class was scored before the frost test.

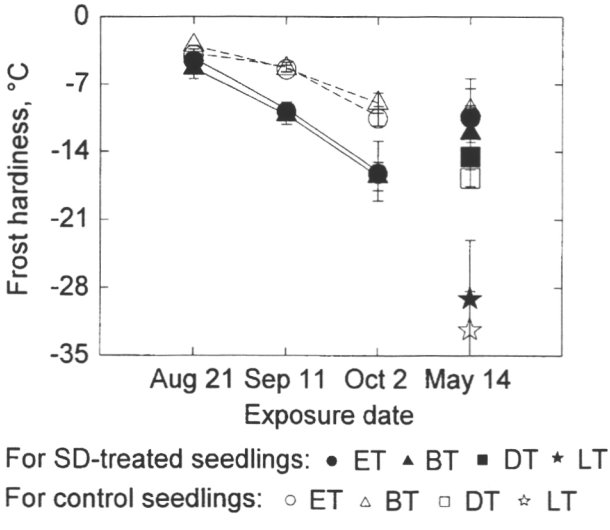


Figure 4.14 Frost hardiness of seedlings short-day treated (SD) from 29 July to 19 August 1996 or grown under natural photoperiod (control) in autumn 1996 and spring 1997. Frost hardiness was determined by an electrolyte-leakage (ET_{50}) or stem-browning test (BT_{50}), and in spring also by a test of whole-seedling viability as proportion of damaged (DT_{50}) or dead (LT_{50}) seedlings. Vertical bars indicate asymptotic standard errors of frost hardiness estimates.

Root growth potential in spring. SD treatment started in the end of July the previous year did not affect the timing of bud burst or leaf elongation of seedlings in the RGP test the following spring. However, SD treatment increased the number of unflushed buds so that 16 percent of all buds and 24 percent of the top buds did not flush. In control 2 seedlings, only three percent of all buds and four percent of the top buds were unflushed.

At planting in the RGP test, SD-treated seedlings of the second crop were shorter (height $P<0.001$) and thinner (diameter $P=0.01$) than the control seedlings of the same crop (Figure 4.15a,b). After four weeks of the RGP test period, the SD-treated seedlings were still shorter ($P=0.001$), but the diameter ($P=0.20$) was the same in

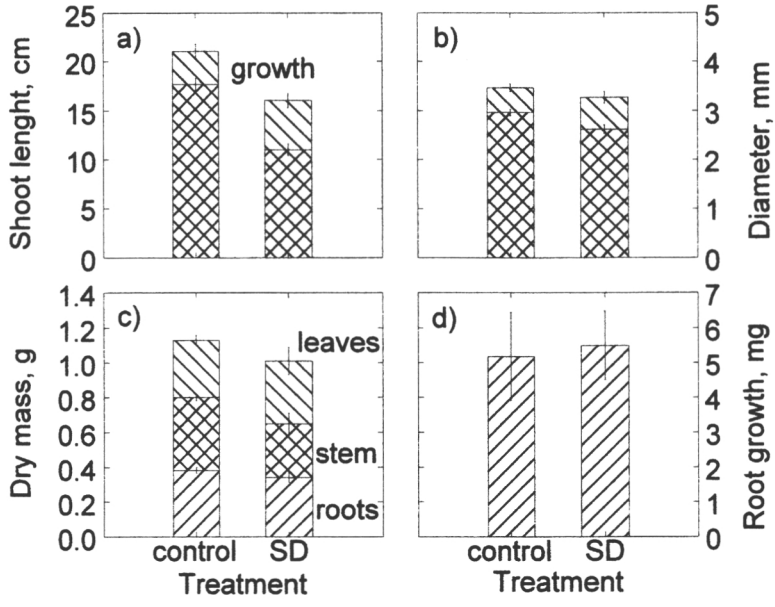


Figure 4.15 Effects of SD treatment (from 29 July to 19 August 1996) on root growth potential (RGP) the following spring in seedlings sown on 18 June 1996 (the second crop). **a)** Shoot length at planting (crossed lines) and height growth, **b)** diameter at planting and diameter growth **c)** dry mass of leaves, stem (including branches) and roots and **d)** root growth (dry mass of new roots grown out from peat plug) in a four-week RGP test in spring 1997. Seedlings were sown on 18 June 1996 and control seedlings were grown under natural photoperiod. Each bar is the mean of 5 blocks (5 seedlings in each).

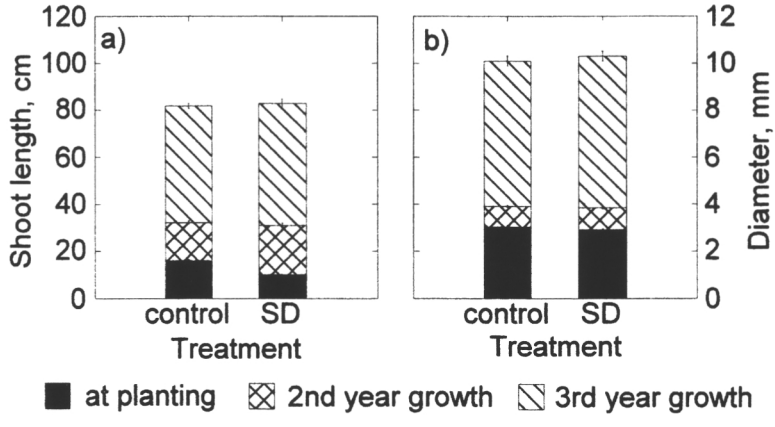


Figure 4.16 a) Shoot length (cm) and **b)** diameter (mm) at planting and height and diameter growth of seedlings subjected to short-day treatment (SD) from 29 June to 19 August in 1996 and seedlings grown under natural photoperiod (control) and planted on 26 May in 1997 and measured at planting and the end of second and third growing season. Seedlings were sown on 18 June 1996. Each bar is the mean of six blocks (10 seedlings in each). Only living and healthy seedlings were included.

both treatments. SD treatment did not affect the root growth potential (P=0.75), dry mass of new roots, (P=0.88; Figure 4.15d) or diameter growth (P=0.12; Figure 4.15b), but did increase the height growth of the seedlings (P<0.001; Figure 4.15a). The diameter (P=0.04) and height (P<0.001) growth relative to the height or diameter at planting was also increased by SD 2.

Spring planting. After the first growing season in the field, 87 percent of the control and 78 percent of the SD-treated seedlings in the second crop were alive and healthy. SD-treated seedlings had more (10 %) stem lesions than the control seedlings (3 %). Hares had eaten a few more SD-treated seedlings (12 %) than control (8 %) seedlings. In the first growing season (diameter at planting, P=0.20) and in the following seasons, the diameter growth (1st P=0.68, 2nd P=0.40) of the seedlings was not affected by SD (Figure 4.16). In contrast, the height of the stem at planting was lower with SD treatment compared to the controls (P<0.001), and height growth was 5 cm greater (P=0.01) in SD 2 seedlings the following season than in the controls, so that control and SD-treated seedlings were the same size (P=0.28) at the end of the first summer in the field (Figure 4.16). Height growth (P=0.27) and final height of the stem in the second (P=0.67) and third (P=0.94) summer in the field were the same in both SD 2 and control 2 treatments.

4.2.4 Discussion

4.2.4.1 Morphology and growth

SD treatment started in the end of June did not cause bud dormancy, only a temporary cessation of height growth (Figure 4.6). However, seedlings sown later but the same age (about 6 weeks) and seedlings treated later in the summer stopped growing (Table 4.3). Luoranen and Rikala (1997) studied the effects of SD treatments on cessation of height growth of seedlings sown on the same date (2 May), but treated with a three-week SD period at different times of the summer. In that study, SD treatments started in the middle or end of July stopped height growth; but after the SD period started in the end of June, some seedlings begun to grow again.

One reason for different responses to SD treatments done at different times or with different age birch seedlings can be the interaction of temperature sum and night length (Koski and Selkäinaho 1982, Koski and Sievänen 1985). Height growth of different birch origins could be stopped by critical night length, when about two-thirds of the average temperature sum (threshold +5°C) of the provenance had been accumulated (Koski and Sievänen 1985). Both in a previous study (Luoranen and Rikala 1997) and in the present one, the seeds used

were from the same seed orchard (SV 379). The average temperature sum of the origins in the seed orchard was 1130 d.d. [range 1040–1220 d.d. (Pielavesi–Varkaus)]. Thus, according to the rule, the temperature sum that induced cessation of height growth accumulated until the beginning of the SD period was about 750 d.d. (690–810 d.d.). In the preliminary study, the needed temperature sum was accumulated for the SD periods starting in the middle (782 d.d.) and end of July (968 d.d.), but not for the period starting in June (644 d.d.) (Luoranen and Rikala 1997 Table 1). In the present study, the required temperature sum was not accumulated until the beginning of the SD periods (Table 4.1). However, all seedlings treated from the end of July stopped growing in height during the SD period (Figure 4.6).

On the other hand, the reason, why seedlings subjected to SD treatment from the end of July (SD 2) stopped growing in height during the SD period but seedlings subjected SDs from the end June did not may be the natural photoperiod conditions after the SD periods. According to Håbjørg (1978), at 63° latitude (Suonenjoki) the critical night length for cessation of the height growth of silver birch seedlings is about 8 hours, which in natural photoperiod conditions occurs in the middle of August (on 16 August) (Figure 1.3a). During the SD periods, night length was clearly shorter than that; but after the SD 1 period started in June, the nights were longer (Table 4.1). After the three-week SD 2 period started in the end of July, the natural photoperiod was short enough to cause permanent cessation of height growth.

Environmental factors such as photoperiod and previous temperature conditions (accumulated temperature sum) also affect cessation of height growth. During the SD 1 period started in the end of June, both the night and day temperatures were lower (13–14°C) than during the later period (16–19°C). Differences in day and night temperatures have been shown to hasten the response of downy birch seedlings to long nights (Junttila 1980). On the other hand, high night temperatures may delay the cessation in growth caused by long nights (Håbjørg 1972a, Downs and Bevington 1981). Longer than three weeks of SD treatment or higher day temperatures during the first treatment period could have stopped the height growth of all seedlings. Dickson and Nelson (1982) showed that in cottonwood (*Populus deltoides* Bartr. ex. Marsh.) seedlings, leaf function changed markedly about the fourth week under SD. If this is also the case in silver birch seedlings, a longer SD period may affect cessation of height growth differently.

SD treatment started in June did not affect diameter growth; but after the SD period started later, the treated seedlings were thinner than the controls (Figure 4.7b). The present results agree with the hypothesis presented by Håbjørg (1972a) and Heide (1974a) that photoperiod probably has no direct influence on diameter growth. According to

Heide (1974a), after cessation of height growth, diameter growth of Norway spruce seedlings ceases several weeks later. He concluded that photoperiodic effects on cambial activity are mediated indirectly through the buds and thus delayed in time. In this study, the differences between treatments might be explained by temperatures (Håbjørg 1972a) and availability of carbohydrates for growth.

As expected, dry mass to leaves and shoots (Figure 4.7c,d) was decreased due to the shorter seedlings (Figure 4.7a). A hypothesis was also that SD treatment did not affect root growth: during the early SD treatment and after both early (SD 1) and late (SD 2) started treatments, however, the mean dry mass of roots was decreased slightly but did not differ significantly from untreated seedlings (Figure 4.7e). Although SD did not affect dry mass, it was not possible to say whether there were any effects on the amount, length, diameter and/or dry-matter content of roots. In the growth-rhythm studies in Section 3.1, however, it was shown that root growth was possible as long as there were leaves on the seedlings. According to Stahel (1972), SD treatment may decrease the rate of root growth during the treatment period. Smit-Spinks et al. (1985) suggested that this is more likely a response to availability of carbohydrates than a direct effect of photoperiod. Under SD conditions, in sugar maple seedlings when the light intensity was reduced, root elongation also decreased (Webb 1976).

SD increased nutrient concentrations in the stem and roots (Appendix III). In the roots of white spruce SD treatment has also been reported to increase Mg and Ca, but not other nutrient concentrations (Bigras and D'Aoust 1993). According to Skre (1991c), the shorter the day length in which seedlings are grown the more N and P the shoots and roots of silver birch contain. Compared to nutrient levels in seedlings raised according to 'normal' practices (see Table 3.1), nutrient concentrations in the stem and roots of seedlings in the second crop were higher in both treatments, although seedlings were given less nutrients in fertilizer.

In the present study, the leaves were green until the beginning of September, independently of treatment (Figure 4.9). In general, after height growth ceases, the most obvious signal for leaf yellowing is lower temperature. Both photoperiod and temperature determine leaf abscission (Thomas and Vince-Prue 1997), although suddenly shortened photoperiod does not induce leaf yellowing as rapidly as gradually diminishing photoperiod does (van Huystee et al. 1967). The important aspect of timing of leaf shedding, as in all phases of growth in plants, is the order of events. The responses of seedlings to different environmental factors change with seedling age. This might explain the differences in timing of leaf yellowing and abscission in different crops (Figure 4.9). In younger seedlings (the second crop) the leaf senescence started later, independently of treatment, compared to the older seedlings (first crop).

4.2.4.2 Frost hardening

Frost hardening of seedlings was estimated by several methods: in both experiments by scoring yellowing and abscission of leaves and water content in seedling apices; in the second crop also by weekly exposure to –10°C and by assessment of freezing damage. According to these results, SD 2 treatment started in the end of July hastened frost hardening by about two weeks (Figures 4.12 and 4.14), but the earlier SD 1 period did not affect the frost hardening of seedlings (Figures 4.8 and 4.9). The results obtained here are comparable to those found for conifer seedlings: frost hardening may occur two to six weeks earlier, depending on timing of the SD period (Aronsson 1975, Timmis and Worrall 1975, Christersson 1978, D'Aoust and Hubac 1986, Bigras and D'Aoust 1992, Folk et al. 1994, Hawkins, C. et al. 1996).

After both SD periods, the water content in the stem apices decreased faster than in control seedlings (Figure 4.8). This agrees with results reported for other deciduous (Calmé et al. 1995) and conifer seedlings (Rosvall-Åhnebrink 1977, Calmé et al. 1993). However, a decline in water content just after the SD periods might not mean a big change in frost hardiness, but may rather be due to retardation of height growth.

Another indirect method for estimating frost hardening was to score leaf yellowing and abscission. According to the results for the second crop, however, frost hardiness of seedlings cannot be predicted by leaf yellowing and abscission. Leaves in SD-treated seedlings were still green when seedlings had little or no damage after exposure to –10°C (Figure 4.13). In untreated seedlings, however, all leaves had to be shed before the seedlings could tolerate –10°C without damage. In the first crop, frost hardening of seedlings was estimated only indirectly by scoring yellowing and abscission of leaves and by determining water content. Thus, the frost hardiness of SD seedlings could not be predicted with certainty.

4.2.4.3 Field performance and planting windows

For the selection of planting windows for SD-treated and control seedlings, it is important to know the after-effects of treatments and field performance of seedlings. Both early (SD 1) and late (SD 2) treatments had some after-effects that affect their suitability for nurseries.

SD 1 treatment started at the end of June increased the risk of hare damage during the planting summer and the following winter (Table 4.4). In addition, the reliability of the results in the summer planting experiment was reduced by hare damage. For some planting dates, only a few seedlings remained undamaged. Shading has been obser-

ved to decrease the number of resin droplets on the bark of birch seedlings and increase the palatability of seedlings to hares (Rousi et al. 1993). Planting date also affected palatability, since the earlier seedlings were planted the more seedlings in both treatments were eaten (Table 4.4). Resistance to hares might also decrease due to the unlignified stems and higher nutrient concentrations during summer. At the end of the treatment that was started early (SD 1) the nutrient concentrations in leaves were higher in SD-treated seedlings than in the controls (Appendix III).

The most negative effect of SD treatment started at the end of July was that it increased the mortality of top buds during the first winter. This has also been observed in studies on coniferous species (Rosvall-Åhnebrink 1977, Hawkins and Draper 1991, Odlum 1992, Dormling 1993, Krasowski et al. 1993). According to Krasowski et al. (1993), the number of unflushed buds in white and Engelmann spruce (*Picea engelmannii* Engelm.) seedlings was associated with the length of the SD period: the longer the night length the more buds remained unflushed. In the preliminary study, in winter no dead buds were observed in the RGP test; but after spring planting, the earlier seedlings were SD treated the previous summer the more seedlings there were with multiple tops at the end of the first season (Luoranen and Rikala 1997). In the present study, unflushed buds were scored in the RGP test done in May. Thus, buds may be damaged during the last part of storage. Lengthening the storage period has been observed to increase the death of top buds in Norway maple (*Acer platanoides* L.), red oak, European mountain ash (*Sorbus acuparia* L.) and paper birch seedlings (Englert et al. 1993).

SD treatment started at the end of July the previous year did not affect timing of bud burst or leaf elongation of birch seedlings the following spring. In the preliminary study, SD treatment hastened bud burst in seedlings treated from the end of June to the middle of July (Luoranen and Rikala 1997). From the standpoint of practical forestation, however, the difference in flushing between seedlings with SD treatment from the end of July and that of untreated seedlings was insignificant. In conifers, SD treatment hastened bud burst by a few days compared to control seedlings (Rosvall-Åhnebrink 1980, Sandvik 1980, Colombo 1986, Odlum and Colombo 1988, Silim et al. 1989, Hawkins and Draper 1991, Bigras and D'Aoust 1992, 1993, Krasowski et al. 1993, Luoranen et al. 1994, Hawkins, C. et al. 1996). Differences between conifers and deciduous seedlings are due to different growth patterns. In conifers, part of the growth is already predetermined in the second growing season, in which case conditions during bud formation affect bud burst the following spring and the amount of growth during the following season (Heide 1974b). In silver birch seedlings, growth is not predetermined; and only conditions during the present season affect the amount of growth and its

timing. In their study Koski and Selkäinaho (1982) also observed that the differences in growth rhythm of birch seedlings disappeared during the dormant period.

In general, SD treatment hastens frost hardening in autumn, but the differences disappear by the following spring (Bigras and D'Aoust 1992, 1993, Folk et al. 1994). This was also true in the present study (Figure 4.14). In the experiment of Bigras and D'Aoust (1992), SD treatment hastened dehardening in both black and white spruce seedlings (intact seedlings) as well as in the needles, but it did not affect dehardening of the stem and root tips.

SD treatment improved the first year growth if seedlings were planted in spring (SD 2) (Figure 4.16) but had no effects on the growth of seedlings planted in summer (SD 1) (Figure 4.11). In later years, there were no differences in growth between treatments. Nyström (1993) reported increased growth after spring planting in SD-treated birch seedlings. Height growth of conifer seedlings treated with SD treatment and planted in spring usually increases during the following season (Heide 1974b, Rosvall-Åhnebrink 1980, Odlum and Colombo 1988, Eastham 1991, Grossnickle 1992, Odlum 1992, Folk et al. 1994, Hawkins, C. et al. 1996).

The improved growth of SD-treated seedlings after spring planting may be explained by increased nutrient concentrations. SD-treated seedlings had higher N concentrations in both the roots and stems (at least in autumn) (Appendix III). Heiskanen and Rikala (1998) showed that N concentrations in silver birch seedlings before planting correlated with rooting and with the dry mass of leaves grown during a five-week period. In black spruce, seedlings containing more N, P and K (nutrient-loaded) grew more after planting than did seedlings with lower nutrient levels (Timmer and Munson 1991, Malik and Timmer 1996, Bigras et al. 1996).

The present results suggest that there was no need to treat seedlings with SD treatment before planting. Summer planting increased the growth in later years independently of previous treatment, with the amount of increased growth being dependent on how early seedlings were planted (Figure 4.11). This contradicts the results of Nyström (1993). In his study, SD-treated seedlings planted in July grew more during the following season than untreated seedlings did. On the other hand, SD treatment the previous year increased mortality and damage to seedlings but did not markedly affect RGP (Figure 4.15) or growth after planting (Figure 4.16).

4.2.5 Conclusion

In silver birch container seedlings, SD treatment after mid-July induced earlier cessation of height growth and hastened frost hardening by about two weeks compared to untreated seedlings. If the SD period was started earlier, it stopped height growth only temporarily and did not affect frost hardening. SD treatments did not influence diameter or root growth, timing of bud burst and leaf elongation or dehardening the following spring; but they did increase susceptibility to damage after both summer and spring planting. With SD treatment, it is possible to stop height growth and hasten frost hardening of silver birch seedlings if the SD period is started after mid-July.

5 Summer planting of silver birch container seedlings

5.1 Background and study objectives

A way to avoid the problem of how to control shoot elongation of silver birch seedlings is to shorten the growing period in the nursery. This would be possible either by sowing later in summer (the second crop in section 4.2) or by planting during the summer when seedlings are sown. It has been recommended that birch seedlings be planted in spring or early September (Vastamäki 1993). According to Raulo (1981) and Raulo et al. (1994), the time for planting of birch seedlings can be prolonged by using cold or frozen storage; and dormant seedlings can be planted until midsummer. If dormant seedlings are planted after that, the risk of frost injury increases in autumn as the growing season becomes too short for cessation of height growth and frost hardening of the seedlings (Raulo et al. 1994). In some silver birch plantations in central Finland, promising results have been achieved when actively growing silver birch container seedlings have been planted in summer (A. Ruha and M. Suihkonen, personal communications).

In an experiment in a nursery field, silver birch seedlings planted in summer were shown to grow well (Rikala 1996). The shortest (youngest) seedlings at the time of planting usually grow the most, and the differences in height between planting dates (summer, autumn, or the following spring) either disappear during the following seasons or summer-planted seedlings grow more than autumn- or spring-planted seedlings (Rikala 1996). The results of Rikala (1996) and a growth-rhythm study on this topic showed that, during the season they are sown, silver birch seedlings have the highest capacity for root growth in the middle of July; after that root growth slows down linearly toward autumn. In July and early August soil temperature is also near its seasonal maximum (Figure 1.3b). The good performance of summer-planted, actively growing seedlings might be because they are capable of recovering rapidly from the shock caused by planting and/or unfavorable weather conditions compared to seedlings planted in autumn or spring.

Information on seedling field performance in the study of Rikala (1996) was based on results from only one experimental field in summers with average weather conditions. Before a recommendation

could be made about planting leaf-bearing container-grown silver birch seedlings during the growing season, it was necessary to study summer planting on different sites over several years. Compared to dormant seedlings, actively growing seedlings might be more vulnerable to injury during lifting, transporting and planting. Thus, summer-planted seedlings may have an increased risk of stem lesions. The risk of injurious drought stress may also increase due to the higher transpiration of leaf-bearing seedlings.

To my knowledge, there are no reports on how summer planting affects frost hardening of the seedlings of any species. Moderate mechanical and drought stress increase the frost hardiness of seedlings (Levitt 1980). On the other hand, too severe drought stress may also disturb frost hardening (Glerum 1985). Thus, stresses caused by lifting, transporting and planting might increase frost hardiness of summer-planted silver birch seedlings or, if too severe, decrease frost hardiness later in the autumn.

This part of the study had the following objectives: 1) to determine the best planting window (the largest growth with the smallest risk for damage) for summer planting of silver birch container seedlings (the field-performance experiment) and 2) to ascertain the effects of summer and early autumn planting on the frost hardiness of seedlings at the time of early autumn frosts in September (frost-hardiness experiment).

5.2 Material and methods

5.2.1 Field-performance experiments

In May 1995 and 1996, silver birch seeds (for origins see Table 5.1) were sown at the Suonenjoki Research Station (62°39'N, 27°03'E, altitude 142 m asl) and Itä-Suomen Taimi Oy in Lapinlahti (63°20'N, 27°25'E, 110 m asl), Tuusjärvi (62°53'N, 28°19'E, 102 m asl), Onkamo (62°13'N, 30°10'E; 111 m asl) and Syrjälä (61°53'N, 29°11'E, 90 m asl) nurseries. The seeds were sown and thinned or transplanted into Plantek 25 trays (25 cavities per tray, 380 cm^3 per cavity, 156 cavities per m^2, Lännen Plant Systems, Finland), and were cultured according to normal practices of each nursery (Table 5.1) until lifting for planting in summer. Seedlings for spring planting were packed in plastic bags and placed in frozen storage (Suonenjoki) or lifted, bundled and stored outdoors (other nurseries).

Seedlings grown in each nursery were planted in nursery fields in 1995 and 1996. In each area the soil was prepared for planting by harrowing and tilling. In Suonenjoki, weeds were mowed late summer every season, but at the other areas there was no weed control. In addition, in 1996 an experimental area was established in a clear-

Table 5.1 Seedlots, date for sowing, thinning or transplanting and moving out from the greenhouse to an outdoor area of silver birch seedlings used in each nursery in the summer planting experiments in 1995 and 1996. SV means the seeds were collected from a seed orchard and B2 that the seeds were from an accepted seed collection stand.

Nursery	Year	Seedlot	Sowing	Thinning	Transplanting	Moving out
Suonenjoki	1995	SV 379, M29-92-0001	12 May		6 Jun	3 Jul
	1996	SV 379, M29-92-0001	2 May		23 May	20 Jun
Lapinlahti	1995	B2 T3-89-35	20 May			28 Jun
	1996	B2 1089 T-93-24	14 May			27 Jun
Tuusjärvi	1995	SV 694	15 May	5 Jun		26 Jun
	1996	SV 373, R1-95-19	6 May	3 Jun		26 Jun
Onkamo	1995	B2 T3-93-0026	16 May		2 Jun	28 Jun
	1996	B2 1273 T3-93-36	15 May		10 Jun	8 Jul
Syrjälä	1995	SV 378, R01-93-006	15 May		6 Jun	29 Jun
	1996	SV 378, R01-93-006	6 May		29 May	24 Jun

Table 5.2 Particle size distribution and nutrient concentrations of nursery fields and a forest area in the experiments of summer planting.

	Suonenjoki		Lapinlahti		Tuusjärvi		Onkamo		Syrjälä		forest
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1996
Clay, %	1	1	4	3	6	3	2	3	4	4	2
Silt, %	1	1	10	8	14	85	9	11	7	8	8
Fine sand, %	19	19	57	59	69	59	36	39	29	31	26
Sand, %	32	29	8	16	7	24	21	14	27	19	18
Gravel, %	47	50	21	14	4	7	32	33	33	38	46
Organic matter	7	8	7	3	4	4	8	6	23	29	8
N, mg g^{-1}	1.4	1.6	1.4	0.7	0.8	0.9	1.3	1.2	3.9	5.2	1.6
P, mg kg^{-1}	24	26	20	15	20	13	9	7	9	14	4
K, mg kg^{-1}	17	20	108	83	56	71	47	38	160	185	39
Ca, mg kg^{-1}	223	174	243	305	119	229	432	109	939	1070	76
Mg, mg kg^{-1}	24	25	34	53	15	29	60	8	133	162	15
pH	4.6	4.6	5.0	5.6	5.0	5.6	5.6	5.3	4.8	5.0	4.6

cut, scarified forest site in Suonenjoki (later referred to as forest 96). In this experiment, weeds were trampled down in late summer 1997. A total of 11 experiments were established. Each experiment consisted of 6 randomized blocks with 11 planting dates (treatments). In each experiment 72 seedlings were planted weekly, 12 seedlings in each block (0.75 m between seedlings in a row, 1.0 m between planting dates (=rows)) from week 29 (on 17–21 July, 1995 or on 15–19 July, 1996) to week 38 (on 19–23 September 1995, on 16–20 September 1996) and in May the following spring.

The height of each seedling was measured to the nearest 0.5 cm from ground level to the top of the seedling at planting and at the

end of September in the first, second, third and fourth years. Diameter (2 cm above the soil surface) was also measured (0.01 mm) and survival evaluated in September each year. From each block in each experimental area, a few soil samples were taken with a soil drill in autumn 1995 or 1996. Before analysis, block samples were pooled into one sample per experiment. Particle-size distribution was analyzed as described by Elonen (1971). Nitrogen concentration was determined with a LECO CHN-600 analyzer (Leco Co, USA); and concentrations of exchangeable Ca, K, Mg and soluble P in soil samples were determined from an acidic solution of ammonium acetate (Halonen et al. 1983) using plasma emission spectrophotometry analysis (ICP, ARL 3800). Particle-size distribution and nutrient concentrations in each experiment are presented in Table 5.2.

Temperature was measured at each area using a thermometer set in the soil at a depth of 10 cm (bottom of the planting hole). In addition, in experiments planted in Suonenjoki 96 and forest 96, soil temperature was monitored with a thermograph (Lambrecht) during the summer from the middle of May to the end of September.

5.2.2 Assessment of frost hardiness

For evaluation of the effects of planting date on frost hardiness, the frost hardiness of summer-planted seedlings was assessed by using an electrolyte-leakage test at Suonenjoki Research Station in 1997. The material used is presented in Section 3.1 (the crop sown in May 1997). Seedlings were planted three times (on 10 July, 12 August and 8 September) in a field trial in Suonenjoki. Five trays were randomly selected for each planting date, and randomly selected seedlings were planted in 6 blocks, 10 plots (for 7 test temperature and 3 extra) in each, and 3 subplots (planting date) in each plot. Height of the seedlings was measured after each planting date and just before sampling of seedlings for the frost hardiness test. Summer 1997 was warm (Figure 2.1) and dry; soil moisture (determined by weighing and drying the soil samples) at the time of planting on 12 August and 8 September was 13 and 9%, respectively. Between planting and the frost-hardiness test, 28 % of the seedlings planted on 12 August were killed by drought.

On 16 September, the frost hardiness of planted seedlings was compared to that of seedlings still growing in the nursery. Six seedlings, one from each block, were cut and divided into six parts, which were put into plastic bags and sprayed with water. Samples of the nursery-grown seedlings were obtained the same way, except that there were 8 seedlings instead of the 6 used for planted seedlings.

The bags were exposed to six freezing temperatures (–4, –8, –13, –15, –20 and –36 °C) in air-cooled chambers and to a control tem-

perature (+4°C). The temperature inside the chambers was lowered –2°C per hour, kept at the minimum temperature (–20 or –36°C) for 3 to 10 hours, depending on the test temperature, and raised 5°C per hour. After exposure, 0.5 cm was cut from the middle of each sample and put into distilled water (18 ml) in test tubes (6 pieces in each tube, 6 tubes from each test temperature and planting date, a total of 182 tubes). Then the tubes were shaken at +4°C in the dark for 120 hours. The viability of seedlings was determined by measuring electrolyte leakage from the cut pieces. The procedures for assessment and calculation are presented in section 3.2.

5.2.3 Statistical analysis

For statistical analysis, block means of seedlings were calculated for survival for each planting date, height at planting, and height and diameter at the end of each season and height- and diamater growth (seedlings with severe injuries were excluded when the means for height and diameter variables were calculated). Differences between planting dates were analyzed by analysis of variance (MGLH procedure). Each nursery raised seedlings according to their normal practices from seeds of origin recommended for use in their area. This means that in each experiment, in addition to different soil types (soil fractions and nutrient concentrations), geographical location and weather conditions, variation in growth was potentially caused by differences in planting height, stock quality and origin of seedlings. The objective was to study the effects of planting date. Thus, to control error, the experiment was included as a factor in the analysis and all experiments were analyzed at the same date. In Onkamo in 1996, however, hares damaged seedlings during the first winter and this experiment was excluded from the analysis. In some experiments (Onkamo 95 and 96, Lapinlahti 95, Tuusjärvi 95 and 96), spring planting was not done or there were other uncertainties in spring planting, so summer- and spring-planted seedlings could be compared in only 6 experiments. Differences between planting dates in summer were analyzed in 10 experiments (Onkamo 96 was excluded). Differences between planting dates were determined by Tukey's HSD test. Mortality and reasons for damage were analyzed separately in each experiment by Kruskall-Wallis one-way analysis. Before analyses, growth data were transformed in order to normalize the distribution and to homogenize the variances. The results presented in the figures and tables are back-transformed values.

5.3 Results

5.3.1 Field performance

After planting on the Suonenjoki 96 site, drought damaged 7, 32, 71, and 15 percent of seedlings planted in weeks 33, 34, 35 and 36, respectively (results not shown). In the Onkamo 95, 96 and Suonenjoki 96 experiments, some seedlings were eaten by hares, but there were no statistically significant differences in damage between planting weeks. When damage was recorded in the autumn of 1996, stem lesions were observed at all planting dates in Suonenjoki 96 (about 2 %) and at the forest site (about 7 %), but there were no differences in frequency of stem lesions between planting dates. During the first winter (before spring planting), hares ate 89 % of the seedlings planted in Onkamo 96 and voles ate 9 % of the seedlings planted in the Syrjälä 95 experiment (results not shown). In later years there was also damage caused by voles or hares, but the amount of damage did not differ between planting dates. At the end of the third season, there were more stem lesions in the seedlings planted in August in Suonenjoki 96 and in seedlings planted in September in both the 95 and 96 Syrjälä experiments (Table 5.3).

When combined together, seedlings planted in all experiments in August or September had lower survival than seedlings planted earlier (Figure 5.1). However, mortality differed in different experiments (Table 5.3), and there was no clear relationship between planting dates and experiments. In Syrjälä 95 and 96, Suonenjoki 96 and the forest site 96, mortality was higher than in other experiments. Some of the seedlings damaged by voles, hares, lesions or for other reasons recovered later. For instance, in Onkamo 96 only 4 % of the seedlings damaged by hares died.

Planting date did not affect diameter growth during the planting summer, and height growth declined after planting compared to seedlings growing in the nursery (Figure 5.2). Thus the earlier in the summer and the younger the seedlings were planted, the shorter and sturdier they were at the end of the first season (planting summer) (Figure 5.2). In following years, the earlier seedlings were planted the more diameter- and height growth increased (Figures 5.2 and 5.3). Differences in sturdiness decreased with time; and after three seasons, there were no differences between planting dates (data not shown). Due to the differences in planting material, sites and location, there were also small differences in responses to planting date (interaction between experiment and planting date was statistically significant) (Table 5.4). The growth of seedlings planted in August or September did not differ from the growth of spring-planted seedlings; but seedlings planted before mid-August grew more in the following seasons (Figures 5.2 and 5.3).

Table 5.3 Mortality of seedlings planted in summer 1995/1996 and spring 1996/1997 and percentage of seedlings eaten by voles or hares, with stem lesions or damaged and dead due to other (drought, freezing, or unknown) reasons in each experiment (nursery and year) and at each planting week (calendar week) at the end of the third season [in the autumn of 1997 (planted 1995) or 1998 (planted 1996)]. In the last column are the probabilities of differences between weeks given by Kruskall-Wallis one-way analysis of variance and in the last row after each section is the mean of all experiments in each week.

	Planting week											
	July			August				September				
Experiment	29	30	31	32	33	34	35	36	37	38	spring	p-value
Mortality at the end of the third season												
Suonenjoki 95	0	0	1	4	8	3	3	3	1	1	1	0.595
Lapinlahti 95	0	0	0	0	0	0	0	0	1	0	0	1.000
Syrjälä 95	7	8	15	1	11	10	13	31	22	18	13	0.014
Tuusjärvi 95	0	0	1	0	1	1	1	0	0	0	0	0.693
Onkamo 95	1	0	0	1	1	1	0	1	3	1		0.823
Suonenjoki 96	13	11	10	11	17	49	60	15	18	15	1	<0.001
Lapinlahti 96	4	0	4	4	8	7	10	11	11	12	3	0.087
Syrjälä 96	11	7	7	3	4	15	22	26	46	36	4	<0.001
Tuusjärvi 96	3	1	0	0	3	4	4	3	1	17	3	0.001
Onkamo 96	3	0	0	0	0	4	1	8	7	18		0.003
forest 96	8	8	8	17	22	25	32	26	21	15	14	0.066
mean	5	3	4	4	7	11	14	11	12	12	4	
Hares and vole damage												
Suonenjoki 95	0	0	1	1	7	1	0	0	0	0	0	0.319
Lapinlahti 95	0	0	0	0	0	0	0	0	0	0	1	0.440
Syrjälä 95	4	4	1	0	3	6	1	8	4	7	1	0.354
Tuusjärvi 95	0	0	0	0	0	0	0	0	0	0	0	
Onkamo 95	3	7	8	4	1	8	6	11	7	3		0.141
Suonenjoki 96	1	7	4	8	6	10	6	3	11	4	1	0.748
Lapinlahti 96	3	0	0	4	7	7	6	6	6	4	0	0.213
Syrjälä 96	0	0	1	1	0	0	1	3	3	4	0	0.500
Tuusjärvi 96	0	0	0	0	0	0	0	0	1	1	3	0.218
Onkamo 96	92	96	94	82	94	92	93	78	90	78		0.071
forest 96	1	3	0	1	0	3	0	1	0	0	0	0.507
mean	9	11	10	9	11	12	10	10	11	9	1	
Stem lesions												
Suonenjoki 95	0	0	1	4	3	1	3	1	1	3	1	0.860
Lapinlahti 95	0	0	0	0	0	0	0	0	0	0	0	
Syrjälä 95	1	4	8	1	7	1	7	19	22	8	14	0.001
Tuusjärvi 95	8	3	3	1	6	1	7	1	1	3	0	0.219
Onkamo 95	1	3	0	4	1	1	0	0	1	1		0.832
Suonenjoki 96	7	1	3	1	8	21	43	11	4	3	0	<0.001
Lapinlahti 96	0	1	0	0	0	0	0	0	1	0	0	0.519
Syrjälä 96	0	3	10	6	6	12	8	8	50	47	19	<0.001
Tuusjärvi 96	0	0	0	0	0	3	4	3	8	15	0	0.001
Onkamo 96	0	0	0	0	0	3	1	4	3	7		0.643
forest 96	14	25	11	17	33	25	33	33	32	19	24	0.114
mean	3	4	3	9	6	6	10	7	11	10	6	
Other damage												
Suonenjoki 95	0	0	0	0	0	0	0	0	0	0	0	
Lapinlahti 95	0	0	0	0	0	0	0	0	0	0	0	
Syrjälä 95	1	0	7	3	8	8	8	10	1	6	6	0.134
Tuusjärvi 95	0	0	0	0	0	0	0	0	0	0	0	
Onkamo 95	1	0	0	0	0	0	0	1	4	1		0.083
Suonenjoki 96	6	3	3	0	4	18	8	1	6	8	1	<0.001
Lapinlahti 96	1	0	4	0	4	0	4	6	4	10	3	0.010
Syrjälä 96	0	0	0	0	0	0	0	1	7	3	7	0.001
Tuusjärvi 96	3	1	0	0	3	3	0	1	4	0	1	0.283
Onkamo 96	0	0	0	0	0	0	0	0	0	0	0	
forest 96	0	0	1	1	1	0	0	0	0	3	3	0.351
mean	1	0.5	1	0.5	2	3	2	2	2	3	2	

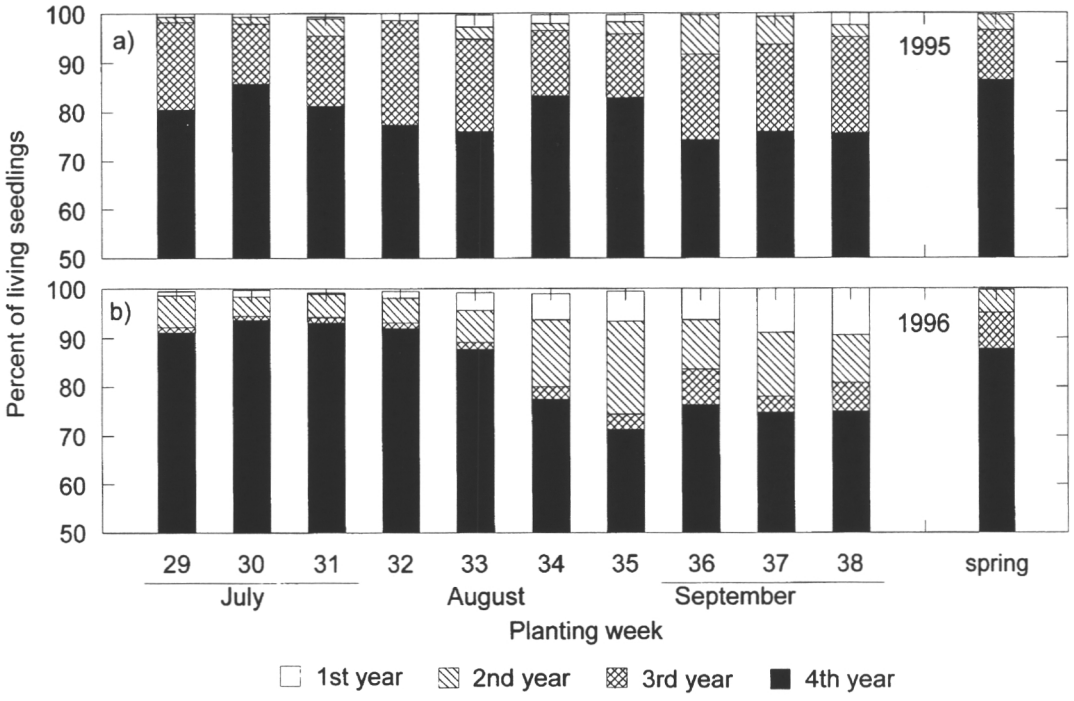


Figure 5.1 Survival of seedlings at the end of the first (planting summer) to fourth seasons after planting when birch seedlings were planted weekly (weeks 29 to 38 and in spring) a) in summer 1995 (or spring 1996) or b) 1996 (or spring 1997). Bars indicate the proportion of living seedlings scored undamaged, alive but weakened or alive but the top of the seedling damaged or dead. Each bar is the mean of five experiments in 1995 and six experiments in 1996. Each experiment consists six blocks with 20 seedlings in each.

Figure 5.2 a) Shoot length (cm) and **b)** diameter (mm) of birch seedlings planted weekly from the middle of July (week 29) to the end of September (week 38) in 1995 and 1996 and the next spring (1996, 1997). Each section in bars indicates final shoot length at planting and shoot length and diameter at the end of each season (1st, 2nd and 3rd year) in six experiments with six blocks with 20 seedings in each.

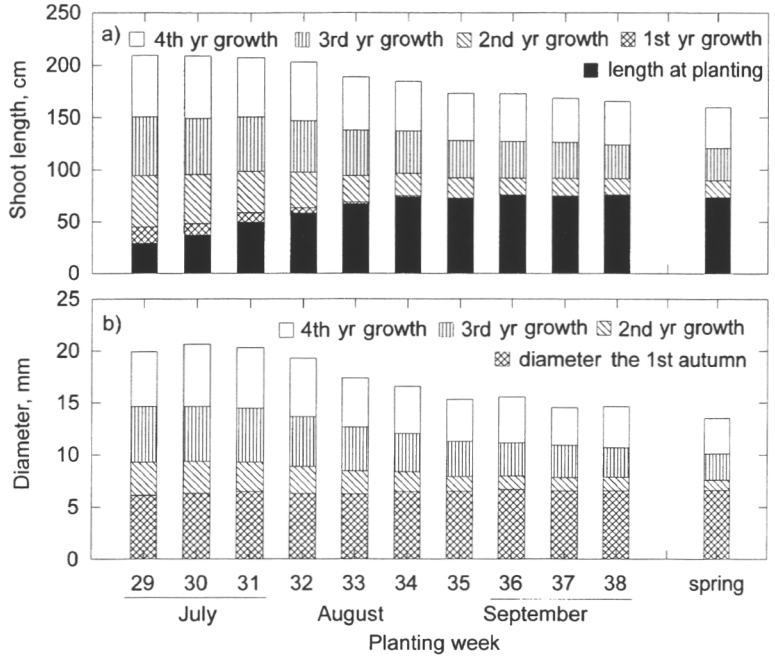


Table 5.4 Statistical differences (p values for analysis of variance) in height, diameter, height- or diameter growth at planting and at the end of the first, second, third and fourth growing seasons between summer-planted experiments. Seedlings were planted weekly from week 29 to 38 and in spring to ten field experiments. From the second to fourth columns are the propabilities given by ANOVA when experiments with appropriate spring planting (differences between all planting weeks) and from the fifth to seventh columns are the probabilities when all experiments with summer planting (differences between planting weeks in summer) were analyzed. n = 6 blocks in each week and experiment, W11 = 11 weeks, W10 = 10 weeks, R6 = 6 experiments, R10 = 10 experiments.

	Week W11	Experiment R6	W11xR6	Week W10	Experiment R10	W10xR10
Height						
at planting	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1st year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2ndyear	<0.001	<0.001	<0.001	<0.001	0.003	0.004
3rd year	<0.001	<0.001	0.344	<0.001	<0.001	0.022
4th year	<0.001	<0.001	0.951	<0.001	<0.001	0.576
Height growth						
1st year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2nd year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3rd year	<0.001	<0.001	0.086	<0.001	<0.001	0.012
4th year	<0.001	<0.001	0.995	<0.001	<0.001	0.885
Diameter						
1st year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2nd year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3rd year	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
4th year	<0.001	<0.001	0.782	<0.001	<0.001	0.054
Diameter growth						
2nd year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3rd year	<0.001	<0.001	0.018	<0.001	<0.001	0.184
4th year	<0.001	<0.001	0.986	<0.001	<0.001	0.885

5.3.2 Frost hardiness

At the time of the frost-hardiness test on 14 September, the mean heights of the seedlings were 47, 81 and 79, when planted on 10 July, 12 August and 8 September, respectively, while nursery-grown seedlings were 83 cm tall. Seedlings planted in July grew about 13 cm after planting, but seedlings in later plantings no longer grew. The differences in frost hardiness (ET_{50}) between planting dates (–9.5, –10, –8.5 °C) were not significant, but planted seedlings were slightly hardier (not significant) than nursery-grown (–8°C) seedlings (Table 5.5).

Neither the leakage range (parameter *a*) nor the background level of leakage (*d*) differed between nursery-grown seedlings and seedlings planted in September. But the leakage ranges (*a*) increased in seedlings planted in August compared to other treatments (Table 5.5). There was also a clear trend in level of background leakage: the earlier the seedlings were planted the lower the values of *d*.

Table 5.5 Estimated parameters (with their Wald 95 % confidence intervals) for the fitted sigmoid curves in the frost hardiness test of seedlings planted in summer (planted on 10 July, 12 August or 8 September 1997) and seedlings grown in the nursery. *d* is the lower asymptote of the fitted curve (the lowest electrolyte leakage), *a* is the range of electrolyte leakage, *b* is the slope of the curve and *c* is the inflection point of the curve (temperature where electrolyte leakage is 50 % of the observed leakage range).

Planting date	*d*	*a*	*b*	$c = ET_{50}$
Nursery	0.44 (0.42...0.47)	0.25 (0.21...0.28)	–2 (–12...– 8)	–8 (–9...–7)
on 10 July	0.31 (0.28...0.35)	0.30 (0.25...0.35)	–0.5 (–1... 0)	–9.5 (–11...–8)
on 12 Aug.	0.33 (0.30...0.38)	0.42 (0.37...0.48)	–1 (–3 ...0.5)	–9 (–11...–7)
on 8 Sept.	0.41 (0.38...0.44)	0.30 (0.27...0.34)	–8 (–8... 5)	–8.5 (–10...–7)

Table 5.6 Survival (%, lowest and highest value in all experiments in both years) in the third autumn after planting observed in this study (three first rows) compared to survival levels (%) observed in other studies (1st column) on silver birch seedlings in Finland. In the last three columns are given the stocktype (container or bareroot seedlings), time of the investigation and the planting site (field or forest) used in each study.

Author/s	Survival, %	Stocktype	Years after planting	Planting site
In the present study				
Experiments in 1995	69–100	container	3	nursery
Experiments in nurseries 1996	40–100	container	3	nursery
Experiment in forest 1996	68–92	container	3	forest
Leikola 1976	15–75	bareroot	3–4	field
Parviainen 1979	61	bareroot	3	forest
Raulo and Rikala 1981	26–67	bareroot	5	forest
Heinonen and Lukkari 1987	92–98	bareroot	3	forest
Valtanen and Engberg 1987	68–82	bareroot	11	forest
Parviainen et al.1989	60–96	both	3	forest
	94–95*	bareroot	3	forest
	89–94*	container	3	forest
Hytönen 1991	29–44	bareroot**	15–16	field
Valtanen 1991	69	bareroot**	4	field
Ferm et al. 1993	84***	both	3	field
Hytönen 1995	63***	both	5	field
Hytönen 1998	32–59***	both	7	field

* Survival levels cannot be divided by seedling type; the level of viable seedlings is given to help the comparison between seedling types.

** In these inventory studies it was not mentioned whether seedlings were bareroots or containers. Planted seedlings were most probably bareroot seedlings because fields on which the inventories were made, were planted in 1973–1977. During these years most of the seedlings delivered from nurseries were bareroots (Anonymous 1976, 1977).

*** Same areas

5.4 Discussion

5.4.1 Field performance of summer-planted seedlings

In this study, large variation in survival was observed between experiments, but there were no differences between planting dates unless there was a period of drought that continued a few weeks before seedlings were planted into easily drying soil (coarse sandy soil in Suonenjoki). Survival of birch seedlings a few years after planting has varied greatly in different studies in southern Finland (Table 5.6). In the present study, survival (40–100%) after four seasons (Table 5.3, Figure 5.1) was near the highest survival levels observed in the studies mentioned in Table 5.6.

In spring planting, the mortality of short silver birch seedlings has been observed to be as much as double compared to tall seedlings (Hytönen 1995). Height growth of surviving short seedlings has also been reported to be minor (Hytönen 1995). Although summer-planted seedlings grow more than spring-planted ones (Figure 5.2), mortality might increase if they are planted on sites with strong weed growth. Even with careful soil preparation, ground vegetation develops rapidly on reforestation sites (Raulo and Rikala 1981). Thus in a forestation area, the best results might be achieved if silver birch container seedlings are planted on sites that have been prepared during early summer and planted the same summer. In this way, seedlings will have an advantage over weeds.

Because of the exceptionally warm and dry period in August 1996, in Suonenjoki mortality increased after planting in weeks 34–35. On the other sites the differences in mortality or damage between planting dates were not significant. In Suonenjoki, seedlings on the forest site were planted the same day as seedlings planted into the nursery field, but survival at the forest-site did not decrease after planting. In the experimental field in Suonenjoki, the soil (coarse sandy) has poor water-retention characteristics (Heiskanen and Rikala 2000). In spring planting, on the other hand, Heiskanen and Rikala (1998) observed that particle size and water content of the soil strongly affect root egress of seedlings. They also observed that, when comparing coarse and fine sandy soils, the number of out-grown roots is fewest in dry, fine, sandy soil. On mineral soil, an increase in the fine soil fractions (fine sand, silt and clay) reduces the size (Saramäki et al. 1991, Niemistö et al. 1997) and quality of silver birch seedlings (Niemistö et al. 1997). Silt and clay soils also become hard when they dry. One must avoid planting silver birch seedlings in soils dominated by very coarse or fine particles because the risks of drying may increase, especially in summer planting. To minimize damage, one must also avoid planting seedlings into dry soils. However, survival should be

high if well-watered seedlings are planted in wet soil in July or early August (rapid root egress) and the soil dries after that (Salminen 1998).

In the Syrjälä 95 experiment, voles damaged some seedlings during the first winter. It might, however, be difficult to avoid such damage. In spring-planted seedlings, Henttonen (1991) recommended the use of repellents together with weed control or seedling shelters to control or prevent vole damage. Pesticides (e.g. Ersa) can be used in summer, but they do not effectively prevent summer damage (Henttonen et al. 1995). According to Henttonen (1991), seedlings with stem diameters over 4 cm are safe from voles. Thus rapidly growing, summer-planted seedlings may pass the risk phase faster than slower growing seedlings planted in spring and fall (Figure 5.2). Regardless of planting date, the best method for minimizing vole damage may be weed control (Henttonen 1991, Ferm et al. 1994).

At the end of the summer of 1996, stem lesions were observed for all planting dates at Suonenjoki 96 and for the forest site (data not shown). Most likely, seedlings planted on these sites were infected at the nursery, but stem lesions had not been observed before planting. Later the number of lesions increased more at the forest site than in the nursery field. The difference between sites could be caused by weeds. In the nursery field, weeds were cut every season; but on the forest site, weeds were only trampled down. Due to weeds, the conditions around the seedlings were more humid, which increases the risk of fungus infection (Poteri 1999).

Increased numbers of stem lesions at the end of the third season were partially caused by previous damage by mammals (Table 5.3). When damage was scored after the first winter and at the end of each season, the amount of mammalian damage did not differ between planting dates. However, there were more infections in seedlings planted in late August and September than in those planted in July or early August. As seedlings planted earlier in the summer grew more in later years, they also had a greater ability to heal wounds. In previous studies Hytönen (1995) observed that on afforestation sites, stem lesions appeared from the third year after planting and even 49 percent of seedlings were infected. Compared to that, the present experiments had few infected seedlings on most sites and at most planting dates.

In this study, the soil temperatures (15–20°C at the depth of 10 cm) were highest during July and early August (Figure 5.3b). These temperatures favored the growth of seedlings (Figurte 5.3e,f). In Germany, Lyr (1996) observed that the soil temperature for optimal growth of silver birch seedlings is about +15°C and the range of temperature tolerance for this species is +5 ... +25°C (Lyr 1996). In the present study, however, during the planting summer, the height growth of seedlings was retarded; but diameter growth was not af-

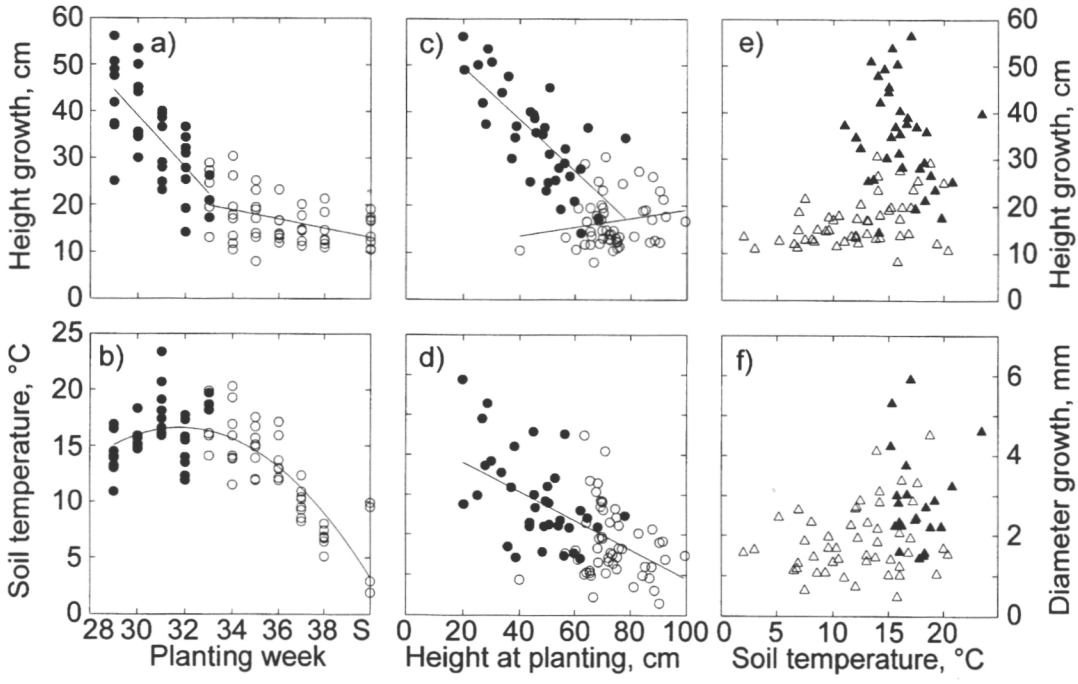


Figure 5.3 Correlations between **a, b)** planting week, **c, d)** height at planting or **e, f)** soil temperature at planting and **a, c, e)** second year height growth, **b)** soil temperature or **d, f)** second year diameter growth of birch container seedlings planted to 10 nursery fields and a forest regeneration site in summer 1995 and 1996 or spring 1996 and 1997. To help identify planting week in figures, the symbols have been colored black in block means for seedlings planted before 15 August in both years [upper lines: linear regression in figures a) and c)]. Open symbols indicate seedlings planted after that (lower lines). S = spring planting.

fected (Figure 5.2). As the leaves of the planted seedlings were still green and assimilation was possible, it was assumed that the seedlings were allocating carbohydrates to diameter and root growth. The increased growing space (increased light, decreased competition between seedlings) and the slight stress in the field also favor root- and diameter growth instead of height growth (Mexal and Landis 1990). Furthermore, as some drought stress is often combined with planting (Burdett 1990), after planting, root growth (not measured in the present study) was assumed to be favored instead of shoot growth.

If root growth was increased after planting, this may have been the reason for the increased shoot growth during the following years (Figures 5.2 and 5.3a,c–e). Shoot growth in the later years increased until planting date in mid-August, after that, i.e., at time of conventional autumn and spring planting, the observed growth was the same at all planting dates. This agrees with the results of Nyström (1993) and Rikala (1996), who observed that root growth is slow in autumn and spring. As a result of this, growth during the following seasons decreased compared to summer-planted seedlings. According to these

results, the best planting window for silver birch seedlings seems to be time from the middle of July to the middle of August.

5.4.2 *Frost hardiness of summer-planted seedlings in autumn*

The results indicate that planting dates and nursery-grown seedlings did not differ in frost hardiness in mid-September (Table 5.5). Summer 1997 was warm and dry. Especially at the time of August planting, the soil was very dry (soil moisture 13 %). When the drought period continued after planting, 28 percent of the seedlings planted at this time died.

In the seedlings planted in August and exposed to freezing in September, electrolyte leakage was increased by freezing and probably also by drought stress caused by planting and the warm and dry weather. Water stress can injure the cell membranes the same way freezing stress does (Levitt 1980, Zwiazek and Blake 1990, Kuhns et al. 1993). Planting retarded the height growth of seedlings, and the earlier the seedlings were planted the more lignified they were in September. Lignification is associated with frost hardening of plant cells (Levitt 1980), which might increase resistance to electrolytes leakage from cells (Sutinen et al. 1992). Here, it is not clear whether a reduction in background level (parameter *d*) was also combined with increasing frost hardiness. Although only living and vigorous-looking seedlings were sampled for frost hardiness, some injuries could have been caused by drought stress after planting rather than by freezing. Thus the estimates of the frost hardiness of seedlings planted in August are not completely accurate.

According to the results reported here (Table 5.5), it could not be determined whether problems with early autumn frosts could be avoided by planting seedlings in summer. It was assumed that water stress could increase the frost hardiness of summer-planted seedlings. This could be true if after planting the soil had not been as extremely dry as it was. Increased frost hardiness is induced by moderate and short-lived water stress (Chen et al. 1977). In the planting experiments in 1995 and 1996, damage caused by frost was not observed in the autumn of the planting year. During planting years 1995 and 1996, however, there was no severe early frost at any site.

5.5 Conclusion

Summer planting is a promising method for avoiding excessive growth of birch seedlings in nurseries. A clear increase in the growth of seedlings was also observed with summer planting (between mid-July and mid-August) compared to fall or spring planting. Increased growth improves the performance potential of seedlings in later years. Only 72 seedlings, however, were carefully lifted and planted at each time and on each site. The planting areas were also near nurseries so there was no need to pack the seedlings for transporting. Thus, the survival results presented here cannot be generalized. However, the results of studies on tolerance of silver birch seedlings to drought (Salminen 1998) and other stresses (Perälä et al. 1999) and the preliminary results from more extensive experiments have also confirmed that it would be possible to enlarge planting windows by planting seedlings at least between mid-July and mid-August, or perhaps even earlier.

6 Concluding discussion

6.1 Development of first-year silver birch container seedlings

6.1.1 Growth rhythm

To make appropriate decisions concerning production and planting, the annual developmental cycle of seedlings has to be known. An understanding of the growth rhythm and the frost hardening of seedlings and factors affecting these attributes is especially important when growing methods and/or planting windows are changed.

The annual cycle of woody species can be divided into an active period and dormancy (Sarvas 1972, 1974). Landis et al. (1999) divided seedling growth and development in the nursery into three phases: establishment, rapid growth and hardening. Landis et al. (1999) separate the growth cycle according to how active height growth is. Cessation of height growth and induction of bud dormancy does not, however, mean that active growth in other tissues of the seedlings (diameter and roots) does not continue (Landis et al. 1999).

In this study, seedling development was monitored beginning with the rapid growth phase, i.e., after germinants were transplanted into containers. The rapid growth phase can be divided further into phases: slowly increasing, linear and decreasing growth. These phases can, in turn, be separated according to each organ (stem, roots, leaves, height growth) (Skre 1991a). I have considered the active period according to rates of height growth, since for operational use in nurseries this is easiest. In addition, when the active period is divided according to height growth, the same phases also describe the sensitivity of seedlings to photoperiod-induced dormancy (Koski 1990). Provenances might respond to light (both intensity and photoperiod) and temperature conditions in different ways (Skre 1991b). Thus, the results presented here represent the responses of a central Finnish provenance (temperature sum 1130 d.d.).

During the first phase, growth rate increases (Figure 6.1) and photoperiod-induced cessation of height growth is not possible even when nights are very long (Koski 1990). In this phase, the height and diameter increments are rapid (Figure 6.3a), but when the water contents of both stems (Figure 6.3c) and roots are high (Skre 1991b), their dry mass is low (Figure 6.3b). This means that most of the total dry mass of a seedling is in the leaves (Figures 3.2, 6.2 and 6.3). Root elongation and initiation, however, are strong, although the dry mass

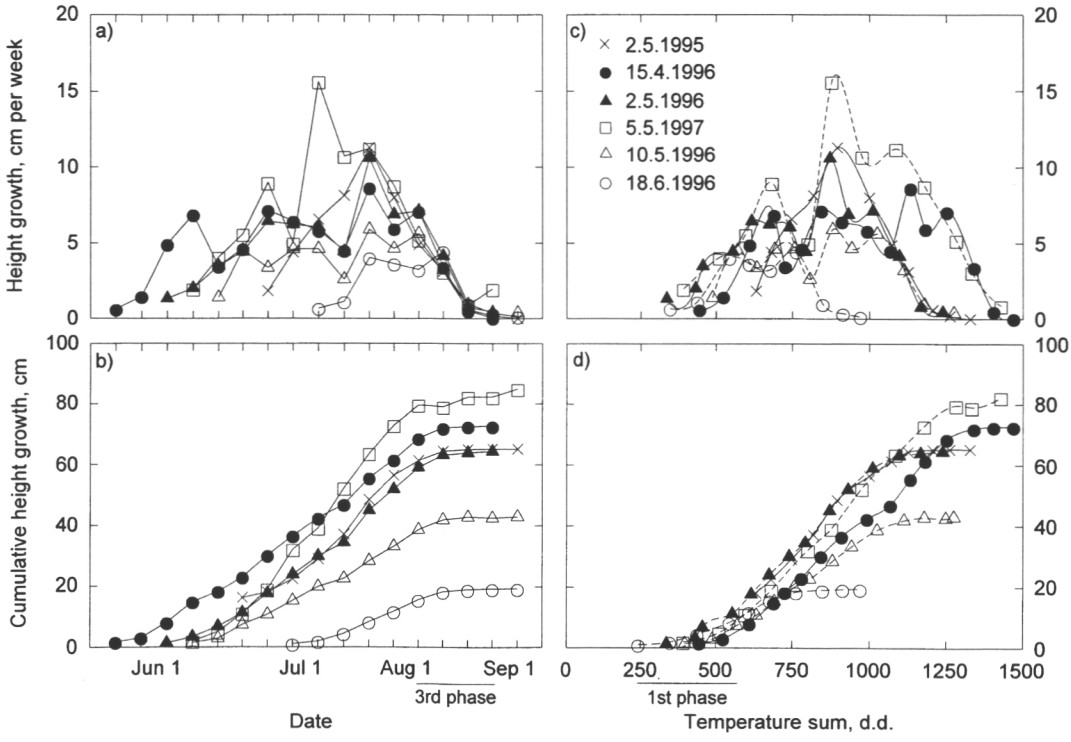


Figure 6.1 a, c) Weekly growth in height (cm week^{-1}) and **b, d)** cumulative height growth as a function of **a, b)** time or **c, g)** accumulated temperature sum (d.d.) in seedlings sown at different times of summer at Suonenjoki Research Nursery during the 1995–1997 growing seasons. Straight lines under the dates and temperature sums indicate the timing of the first and third growth phases.

of roots is low (Figures 6.2c and 6.3c).

Duration of the first phase of growth depends mainly on temperature: the higher the temperature (up to a certain level) the shorter the first phase (Figure 6.1d). When a temperature sum of about 500–600 d.d. (with base temperature of +5°C) has accumulated, height growth is nearly linear (Figure 6.1b). At the end of the first phase, the height of seedlings is about 10 cm.

In the second phase, growth is linear and height growth stops in response to very long nights. According to Koski and Sievänen (1985), silver birch will stop growing and dormancy be induced when two-thirds of the total temperature sum for the normal growing season of that provenance has accumulated and the dark period is long enough. This means that, for central Finnish provenance, height growth cannot stop before a temperature sum of 750–800 d.d. has accumulated (Figure 6.1c,d). In this phase, in addition to large increment in size, lignification begins and thus the dry mass of the stem and roots increases. Root initiation and elongation are also strong (not measured), and the ablity of seedlings to root egress rapidly is at its seasonal maximum (Figures 6.1–6.3).

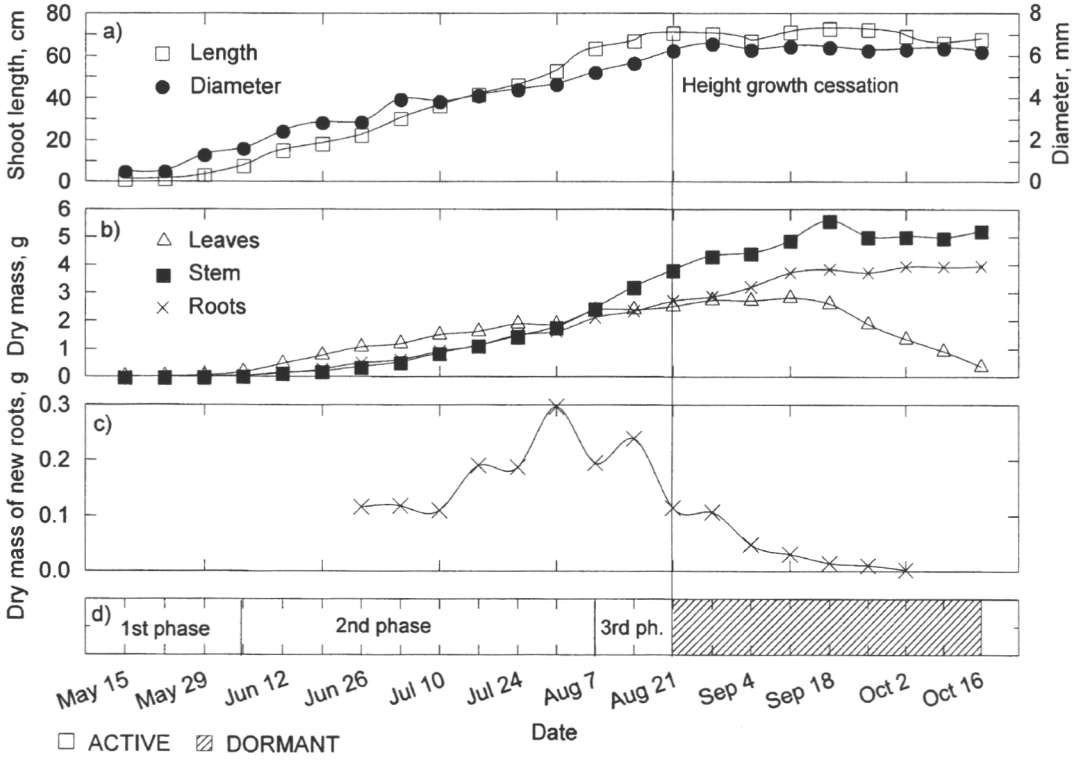


Figure 6.2 Cumulative **a)** shoot length (cm) or diameter (mm) growth, **b)** increment in dry mass (g) of leaves, stem (including branches) or roots and **c)** root growth (g) (as dry mass of roots grown out from a peat plug during a three-week period in the root-egress test) and **d)** their timing related to active growth phases and induction of dormancy in seedlings sown in April and grown in large containers [data presented in section 3.1 (APRIL96 crop)].

Several factors, for example, temperature, nutrients, moisture and light, strongly affect growth and partitioning of assimilates to plant parts (Rook 1991, Walters et al. 1993). In particular, the supply of nitrogen affects growth rate (Ingestad 1979). In the beginning of this linear growth phase, birch seedlings have usually been transferred from a greenhouse to an outdoor growing area. This means that in open field conditions, weather conditions during the growing season are very important for growth and dry-mass partitioning. For example, during this study, weather conditions differed substantially from one summer to the next: the early summer of 1995 was cool and rainy; on the other hand, the whole summer of 1997 was warm and dry (Figure 2.1). Due to these differences, control seedlings in the growth retardant experiment in the summer of 1995 (Section 4.1) were short and the seedlings of the crop sown in May 1997 (Section 3.1) in the study of growth rhythm were tall (Figure 6.1b), even although seedlings grown in 1997 received less fertilizer. In an outdoor growing area, nutrients and water can be manipulated to some extent, but

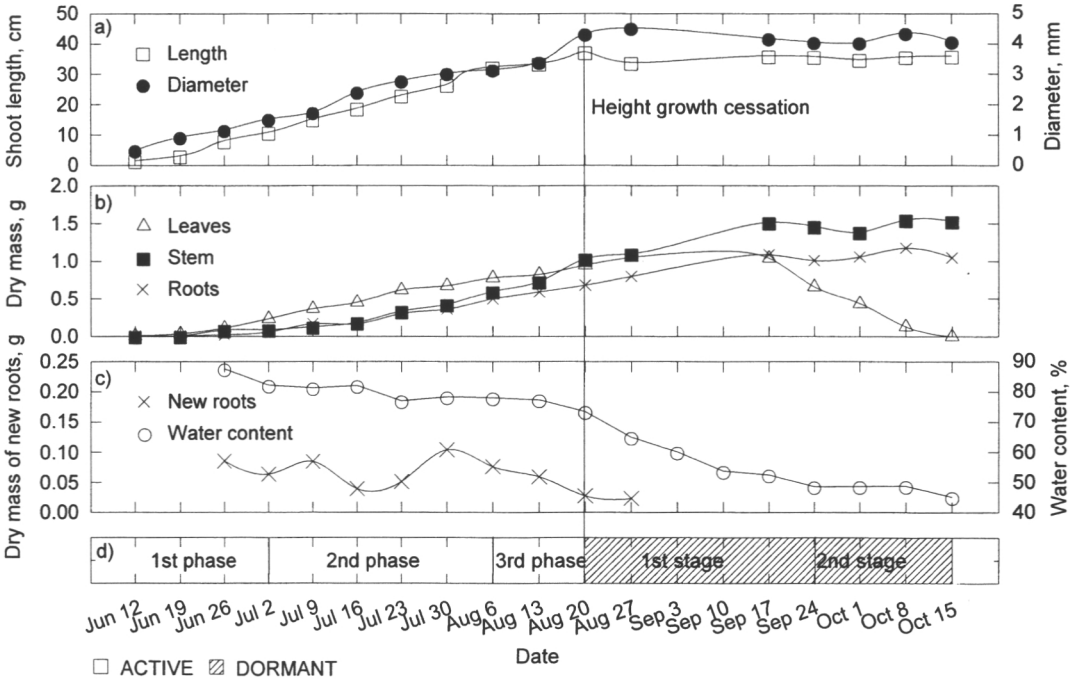


Figure 6.3 Cumulative **a)** shoot length (cm) or diameter (mm) growth, **b)** increment in dry mass (g) of leaves, stem (including branches) or roots and **c)** root growth (g) (as dry mass of roots grown out from the peat plug during a three-week period in the root-egress test) or water content of apices (%) and **d)** their timing related to active growth phases, induction of dormancy and frost hardening stages in seedlings sown in May and grown in small containers [data presented in section 4.2 (the first crop in 1996)].

temperature conditions cannot. In a cool summer, growth rate can be increased by fertilization; but in a warm summer, the growth rate needs to be retarded, although this may be difficult.

In long photoperiod, increasing day and night temperatures lead to an increasing number of leaves and lengthening of the stem (Håbjørg 1972a). In addition, high temperatures are associated with higher light intensities in the field. Thus, in warm conditions, increasing light intensity also increases the height growth and dry-matter production of birch seedlings (Håbjørg 1972b). Light intensity affects the rate of photosynthesis and causes great fluctuations in root growth (Webb 1976, Noland et al. 1997). This may explain the fluctuations in root egress during the second phase of growth (Figures 6.2c and 6.3c).

Temperature conditions also affect the nutrient economy of seedlings. Rate of nitrogen uptake by the roots is faster at higher temperatures, and thus the growth rate is affected more by temperatures in growth medium than by its nutrient levels (Karlsson and Nordell 1996). This may explain the increased growth of seedlings grown in 1997 (Section 3.1). Partitioning of dry mass between shoot and roots

can be altered with fertilizer, since heavy applications of fertilizer, especially N, change allocation of growth toward the shoot, and nutrient deficiency favors root growth (Rook 1991, McDonald et al. 1996).

In an average summer, temperature and light are limiting factors for growth [see Section 3.1 (the crops in year 1996) and Section 4.1 (control seedlings)]. During a warm growing season, it is difficult to control growth in nurseries since nitrogen uptake is efficient, and temperature and light conditions increase the growth rate, thus increasing the final height of the stem. High air temperature also favors shoot growth compared to roots (Skre 1991b), and thus the risk of seedlings becoming too tall relative to the volume of the peat plug increases when the summer is warm.

During the third phase of growth, under natural photoperiod the growth rate declines and height growth stops. At this stage increasing night length is the major factor inducing a decline in the rate of height growth (Figure 6.1a). Increasing amounts of dry mass are allocated to the stem and roots (Figure 3.2). The ability of seedlings to root egress is still high, although declining (Figures 6.2c and 6.3c).

In a cool summer, the growth of seedlings may be retarded by lower temperature early in the summer (for example, control seedlings in the experiment in a growth retardants, Section 4.1). In nurseries, the temptation to fertilize with nitrogen to increase the final height of seedlings may be great. However, heavy fertilization during the third phase may cause excessive growth and delay the cessation of height growth, thus increasing the risk of frost damage from early autumn frosts (Glerum 1985). After a favorable summer for growth, seedlings may be tall (for example, the seedlings grown in 1997, Section 3.1). In the third phase, control of height growth is possible with the SD treatment. In the study of Luoranen and Rikala (1997) SD treatment started in this phase decreased the final height by about 10 cm compared to the height of the untreated birch seedlings. On the other hand, there are no studies of the use of growth retardants to control of height growth in the third phase.

Cessation of height growth initiates frost hardening. There are, however, several factors that modify the cessation of height growth. Previous conditions during the growing season (temperature, night length), as well as seedling age, affect reactions later on (Dormling 1993).

The time of cessation of height growth varies in different years and crops. In the present study, for example, height growth ceased on 14 August 1997 in the crop sown on 5 May (Section 3.1) and on 26 August 1996 in the crop sown on 18 June (Section 4.2) (Figure 6.1). When excessive nutrition is avoided, the time of cessation of height growth, however, can be predicted according to prevailing temperature conditions (i.e., accumulation of temperature sum). Cessation of height growth is based on the joint effect of increasing night

length and temperature sum (Koski and Selkäinaho 1982, Koski and Sievänen 1985). In average summers, height growth stops on 15–20 August in northern latitudes (Junttila and Nilsen 1993). This is true, however, only if seedlings are sown before the end of June (unpublished data of Luoranen). In the middle of August, at the latitude of Suonenjoki (62°39'N, 27°03'E) the night is about 8 h long (Figure 1.3a). In this study, the accumulated temperature sum for all crops stopping height growth at that time was at least 700–800 d.d., which is two-thirds of the average temperature sum of the region of the provenance (1130 d.d.) used. In a warmer summer, height growth stops earlier, for example, in 1997 the accumulated temperature sum was 1300 d.d and natural night length about 7.5 hours at the time when height growth ceased. In short night conditions, however, seedlings of silver birch continue to grow more or less indefinitely if temperature conditions are favorable for growth (Thomas and Vince-Prue 1997).

After cessation of height growth, diameter growth continues. It seems obvious that cessation of diameter growth is induced by factors other than photoperiod. In SD experiments, diameter increased until the end of August – in the beginning of September independently of treatments (Section 4.2). According to Heide (1974a), the effect of photoperiod on the cambial activity of Norway spruce seedlings is indirect, mediated through the effects on the bud and thus delayed in time, which means 2–3 hours longer critical night length for cessation of diameter growth.

The present results showed that, after cessation of height growth, the dry mass of the stem and roots increased until the dry mass of the leaves started to decline (Figures 6.2b and 6.3b). Accumulation of dry mass in the overwintering parts stops when leaf abscission begins (Figure 4.7). With leaf shedding, the transportation of compounds from leaves to stem and roots stops (Abod and Webster 1991b). There could, however, be a slight increase in root dry mass after that, since Calmé et al. (1994) observed increased dry mass of the roots in yellow birch, red oak and sugar maple seedlings in autumn as long as the temperature in the growing medium was above 0°C. This is explained as being due to nutrient uptake of the roots, which can occur as long as soil temperature does not drop below 0°C (van den Driessche 1984).

Although root dry mass continues to increase after cessation of height growth, this does not mean that the ability of seedlings to root egress also increases. Increasing root dry mass is due mainly to root lignification and transport of carbohydrates from leaves to roots (Abod and Webster 1991b). The ability of seedlings to root egress decreases rapidly with declining height growth (Figures 6.2 and 6.3). When planting windows are selected for silver birch container seedlings, the general rule for seedlings grown under natural photoperiods is

that the ability to root egress is fastest until cessation of height growth, i.e., until the middle of August. Growing density and container size also affect root egress. Too small container and high growing density weaken the root growth of seedlings after the maximal height for the available root volume is attained (compare Figures 6.2 and 6.3).

6.1.2 Control of frost hardening

6.1.2.1 Development of frost hardiness

In many tree species, warm short days are important for hardening (Irving and Lanphear 1967, van Huystee et al. 1967, Weiser 1970, Howell and Weiser 1970, Fuchigami et al. 1971, Cannell 1990). This is also the case for silver birch seedlings. By monitoring the seedlings' development, it is possible to separate two frost hardening stages. The first stage starts from the time height growth ceases and the second one from the point when frost hardiness increases rapidly. The first stage of frost hardening can be related to declining metabolic activity of tissues (Weiser 1970) and, for example, a decrease in the water content of seedling apices (Figure 6.4).

In the third phase of active growth, the frost hardiness of birch seedlings is about –3°C (Figures 6.4a and 6.5d). This is comparable to Norway spruce seedlings in the same phase (Christersson and von Fircks 1990), whereas Scots pine seedlings, for example, can tolerate a temperature as low as –6°C during active growth (Rikala and Repo 1987, Christersson and von Fircks 1990). During the first stage of hardening, frost hardiness slowly increases a few degrees, usually to about –10°C (Cannell 1990) (Figure 6.5d).

Duration of the first stage of hardening seems to be flexible. It is not possible to predict the shortest duration of the first stage of hardening by using the data obtained here, but studies of other deciduous species suggest that it lasts at least four weeks (Fuchigami et al. 1971, Hietala et al. 1998). In 1997, the second stage started at the beginning of October (Figure 6.4c). In 1996 for the crop sown on June it was not possible to determine the start of this stage, since frost hardiness was assessed only three times, the last time at the beginning of October. In these seedlings the second stage was most probably induced at that time. Although the frost hardiness of SD-treated seedlings of that crop was as high as –10°C on 11 September and –16°C three weeks later, the increment does not, however, inevitably mean induction of the second hardening stage (Fuchigami et al. 1982).

The most rapid increase in frost hardiness (the second hardening stage) usually starts until the first night frosts (<0°C) in autumn (van Huystee et al. 1967, Howell and Weiser 1970, Weiser 1970, Kacperska 1993). Before that, however, the first stage must last long

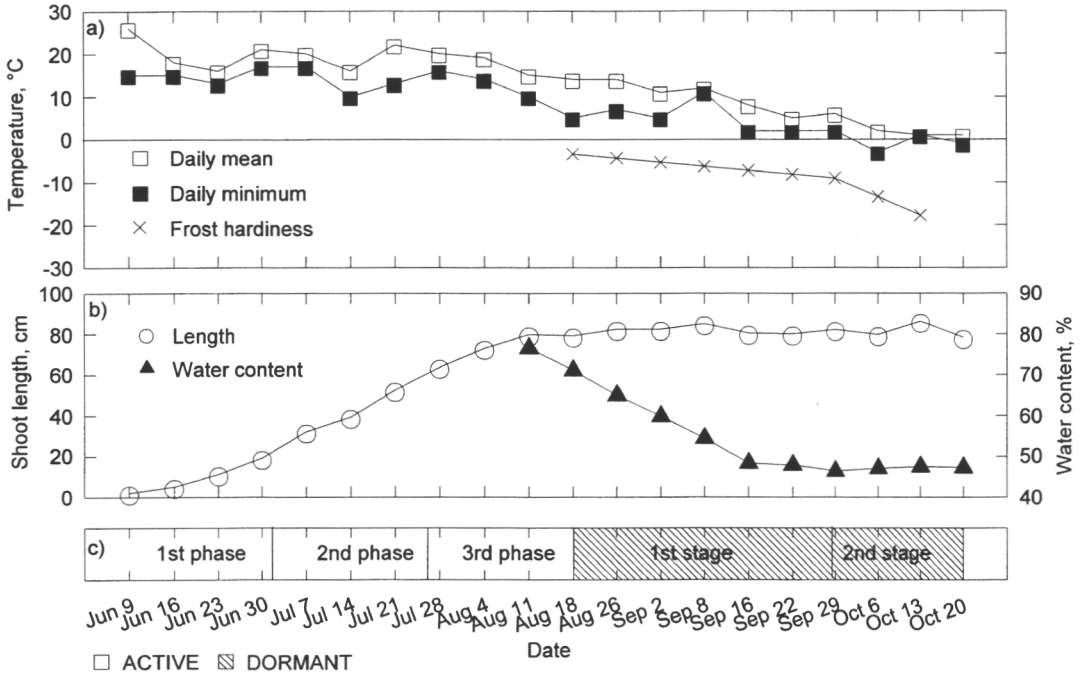


Figure 6.4 Relationship of **a)** mean and minimum air temperatures, frost hardiness (°C), **b)** shoot length (cm), water content in the uppermost 10 cm of seedling apices (%), **c)** growth phases and frost hardening stages. [Data presented in chapter 3 (MAY97 crop)].

enough. If the temperature decreases too early during the first stage, there can be a lag phase before frost hardiness increases (Howell and Weiser 1970, Fuchigami et al. 1971, Ketchie 1985, Burr et al. 1989). Low temperatures too early may delay or inhibit frost hardening. Low temperatures may damage leaves and cause early leaf abscission (Kozlowski 1991). Freezing temperatures are not necessary for triggering the second stage of frost hardening, but exposure to freezing hastens frost hardening (Howell and Weiser 1970, Fuchigami et al. 1982). Repo (1992), for example, observed that the rate of hardening in adult spruce and pine trees was rapid when daily mean temperature fell near or below +5°C. The same was also true for silver birch seedlings (Figure 6.4a).

Since the first stage of hardening is associated with changes, for example, in water status (Pellet and White 1969, McKenzie et al. 1974, Fuchigami et al. 1982, Hietala et al. 1998), the progress of hardening can be monitored by determining these changes. The first stage lasts, for example, until the water content stabilizes to a certain species-specific and ecotype- and treatment-dependent level, which is the induction point of the second stage of hardening. During the second stage the water content does not change, but frost hardiness increases rapidly (Figure 6.5i). This was also reported by Hietala et

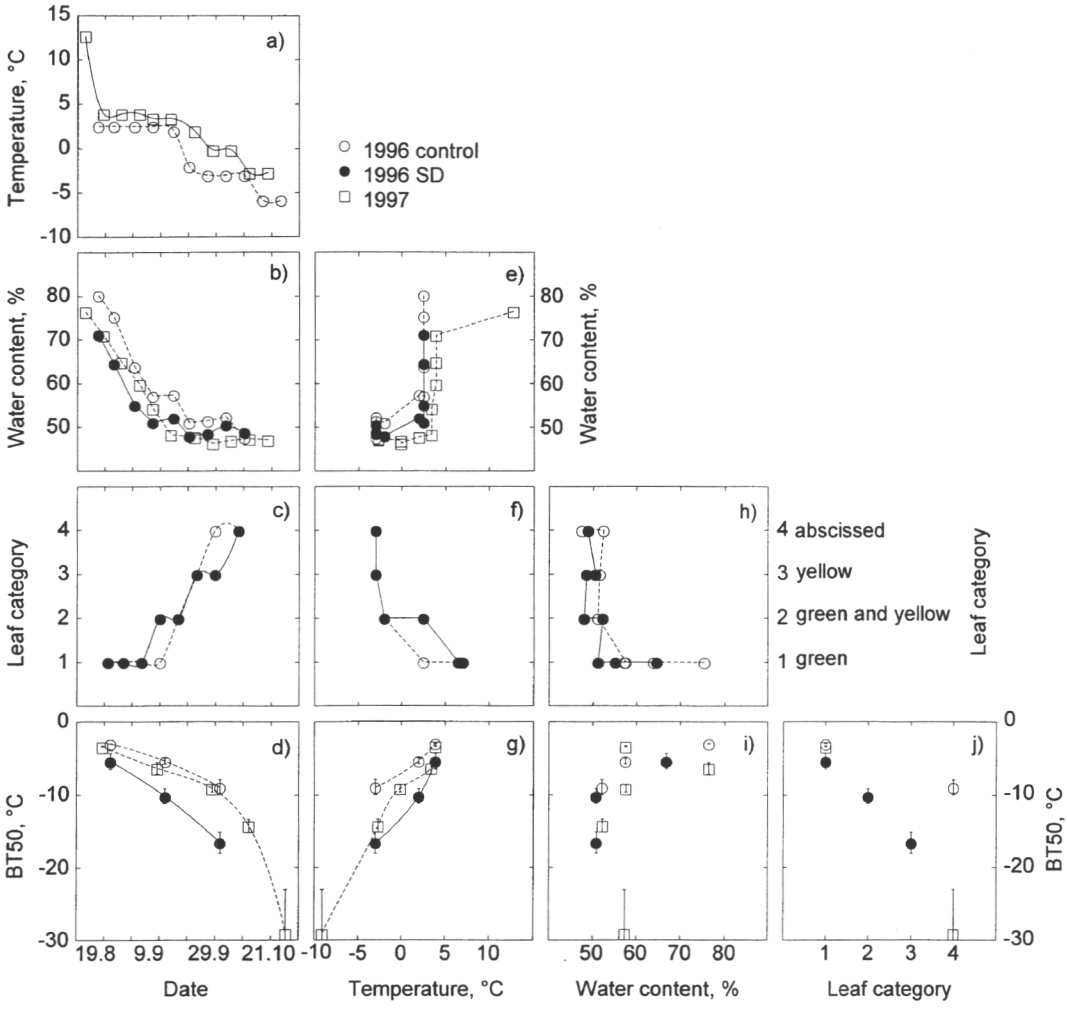


Figure 6.5 Relationships between date, minimum air temperature, water content in seedling apices, median leaf category and frost hardiness (BT_{50}) of birch seedlings sown on 18 June and subjected to short-day treatment from 29 July to 19 August (1996 SD) and natural photoperiod (1996 control) in autumn 1996 (presented in section 4.2) or seedlings sown on 5 May 1997 and grown under natural photoperiod (1997) in autumn 1997 (presented in chapter 3). Minimum air temperature indicates the daily minimum temperature on the seedling level attained each time. Leaf yellowing and abscission was recorded according to the following classification: 1) all leaves green, 2) some of the leaves green, the rest yellow, 3) all leaves yellow, some of them fallen, 4) all leaves fallen. BT_{50} was estimated after exposure to freezing and 14 days incubation time by scoring the length of brown cambium and phloem in stem samples.

al. (1998) and by Ögren (1999b) for willow seedlings. Thus, according to water content monitoring in the seedling crops presented here, the duration of the first stage was 3–6 weeks, depending on year and when height growth ceased; and induction of the second stage of hardening occurred in the latter part of September or in the beginning of October.

6.1.2.2 Assessment of frost hardening in a nursery

For nursery managers it is important to determine the stage of frost hardening for the decisions about nursery practices and for timing of frozen storage. Thus, one purpose of this study was to seek methods to evaluate the frost hardening and frost hardiness of seedlings in autumn. For that purpose, several methods that might be suitable for operational use were used to monitor the frost hardening of silver birch seedlings. These methods were: 1) scoring leaf yellowing and abscission, 2) determining water content in the seedling apices, and 3) exposing the seedlings to –10° C weekly. An evaluation of these methods is presented in Table 6.1.

Leaf yellowing and abscission were monitored in the experiments on growth retardants (Section 4.1), SD treatments (Section 4.2) and summer planting (Section 5). In the SD experiments the same randomly selected seedlings were monitored weekly and the leaf class was scored. In the other experiments the leaf class was observed weekly as the average in each block. There were problems with both scoring methods, but the more reliable method was to score the same seedlings individually each week. If scored by tray or block average, the yellowing and abscission of lower leaves were difficult to observe. Yellowing of leaves starts from the older, lower leaves. Furthermore,

Table 6.1 Evaluation of the studied methods for monitoring the growth and frost hardening of birch seedlings in nurseries. The methods evaluated were regular height growth measurements, scoring of leaf yellowing and abscission, determination of water content, viability test after exposure to –10°C and the combined method, in which cessation of height growth is determined with height growth measurements, frost hardening during the first hardening stage and the end of the stage is assessed with weekly determination of water content and the risk of frost damage is evaluated with temperature monitoring. 'Yes' indicates that the method can be used to determine the subject of the criterion or the method used to fulfill the criterion; 'no' indicates that the method is not suitable for use as mentioned in the criterion or it does not fulfill the criterion.

Criterion	Height growth	Leaf scoring	Water content	–10°C exposure	Combined
Timing of nursery practices	yes	no	yes	yes	yes
Planning of growing schedules	yes	no	yes	yes	yes
Induction of frost hardening	yes	no	no	no	yes
Correlate with frost hardening	no	yes	yes	yes	yes
Predict frost hardiness of seedlings	no	no	no	no	no
Previous growing conditions affect the relation to frost hardening	no	yes	yes	no	yes
Does not require expensive equipment	yes	yes	no	no	no
Non-destructive	yes	yes	no	no	no
Quantitative, no visual observation	yes	no	yes	no	yes
Rapid	yes	yes	yes	no	yes
Inexpensive	yes	yes	yes	no	yes
Requires regular monitoring	yes	yes	yes	yes	yes

leaf abscission induced by stresses causes additional difficulties. In nurseries, yellowing and abscission of the lower leaves might be due to the reduced light intensities when tall seedlings are grown densely, even though this kind of shedding of leaves does not indicate the start of overwintering. Several nutrient-deficiency symptoms also affect color of the leaves, and after drought stress the leaves may be shed earlier. Pathogens, for example, birch rust (*Melampsoridium betulinum* (Pers.) Kleb.), and leaf spots (leaf spot fungi: *Asteroma microspermum* (Pk) Sutton, *Gloeosporium betulinum* Westd., *Marssonina betulae* (Lib.) Sacc.) may also cause yellowing, necrotic spots or early shedding of leaves (Kurkela 1994).

There is some regularity in the timing of leaf yellowing and abscission. During active growth, the leaves are green until height growth ceases. Then the leaves start to yellow as the air temperature decreases (Figure 6.5f) and the photoperiod shortens naturally (Salisbury and Marinos 1985, Westergaard and Eriksen 1997). Velling (1979) observed that leaves begin to yellow when night length is 10–11 h – with no differences between provenances from Latvia to central Finland. Håbjørg (1972a) observed that the time from induction of dormancy (cessation of height growth) to the beginning of autumn colors was strongly affected by temperature. High temperatures (Westergaard and Eriksen 1997) and high nitrogen and calcium concentrations in seedlings (Arteca 1996) may delay leaf abscission. Sarvas (1972) observed that the leaf abscission of birch seedlings was accelerated by temperatures below +4°C for more than three hours, if such temperatures do not occur at too early a stage of development. Low temperatures induce ethylene production in seedlings, which then induces formation of the abscission zone (Addicott 1970). At the same time, compounds in leaves (e.g. soluble sugars and N-rich compounds) are converted to mobile forms and then transferred to storage in the stem and root tissues (Dickson and Nelson 1982, Chapin and Kedrowsky 1983, Abod and Webster 1991b, Nordell and Karlsson 1995). This mobilization can also be observed in the pattern of dry-mass allocation. After height growth ceased and the dry mass of the leaves stabilized, the dry mass of the stem and roots still increased (Figures 3.3f,g, 6.2b and 6.3b). During yellowing and abscission of the leaves, the water content in the seedling apices no longer decreased (Figure 6.5h), but the frost hardiness of seedlings increased rapidly (Figure 6.5i). Thus in deciduous species, induction of the second stage of hardening is also associated with the beginning of leaf abscission (Christersson and von Fircks 1990).

Monitoring the yellowing and abscission of leaves is not a reliable method for predicting the frost hardiness of silver birch seedlings, because the frost hardiness of seedlings in the same leaf class differ if the seedlings have been treated in different ways (Figure 6.5j). In

the second crop of 1996, the frost hardiness of the stems of SD-treated seedlings increased without changes in leaf color (Section 4.2). On the other hand, the frost hardiness of seedlings grown under natural photoperiod was low, even though the leaves were abscised (Figure 4.13).

In this study, *water content* decreased from 80–90 % in mid-August to 45–50 % in October (Figure 6.5b). The decrease in water content was most rapid during the two weeks after cessation of height growth, which agrees with the results of Timmis and Fuchigami (1982) and those of Wolbert and Howell (1985, 1986). In SD-treated one-year-old black spruce seedlings, water content decreased linearly during growth cessation and bud formation, and was correlated both with frost hardiness and with the percentage of seedlings that suffered needle damage in frozen storage (Colombo 1990). In willow, a linear relationship between water content and LT_{50} values has also been observed until frost hardiness reached about –15°C (Ögren 1999b).

When water content is monitored already before cessation of height growth, the declining water content does not describe the big changes in frost hardiness. For example, CCC application retarded height growth and decreased the water content in the apices of seedlings compared to control seedlings (Section 4.1). In that part of the study, water content was determined from the uppermost 5 cm of the shoot. In the most rapid phase of growth, birch seedlings grew over 10 cm a week (Figure 6.1a). Thus for each weekly measurement, 5 cm of the newest stem tissues was cut, which in control seedlings could be less than week-old partially lignified tissue but in seedlings with CCC applications comparable 5-cm samples contained older, lignified cells.

The frost hardiness of shoots of seedlings (LT_{50}) cannot be predicted by measurement of water content. However, during the first stage of hardening, decreasing water content describes the improvement in frost hardiness but as Repo et al. (1997) noted, water content alone is not reliable for predicting frost hardiness. Nursery practices, provenance and tree species affect the relationship between water content and frost hardiness (Toivonen et al. 1991, Krasowski et al. 1994). On the other hand, Calmé et al. (1995) observed that the relationship between frost tolerance and dry matter content (DW/FW ratio) in hardwood seedlings was independent of both photoperiod and species. In larch seedlings, when water content decreased below 70%, stem viability was over 90% after exposure to –10°C (Konttinen 1999). In the present study, the same water content did not express the same level of frost hardiness in SD-treated and untreated seedlings (Figure 6.5i).

Weekly exposure of whole seedlings to –10°C test temperature was also used to monitor the frost hardening of seedlings in the SD 2 experiment (Section 4.2). This method provides good prediction of

the development of frost hardiness. The declining proportion of dead seedlings described the frost hardening of seedlings well (Figure 4.12).

In conifers, this method has been used with good results, when seedlings have been exposed to –18°C (Simpson 1990). Needle damage, however, can be evaluated after a few weeks incubation time (Binder et al. 1996). Seedlings have no growth competence during the rest period in autumn and early winter. After a period at chilling temperatures, growth can be initiated and damage can be scored, but not until mid-winter. Information on hardiness is needed in the fall, and thus a more useful method for nurseries is to expose only stems or stem samples and to score the viability as damage in phloem and cambium (see Section 3.2). Earlier the equipment for freezing exposure was expensive, but recently less expensive methods for freezing exposure using conventional freezers and a standard PC equipped with a temperature sensor and control equipment have been used in Sweden (Sundblad and Granlund 1997). A disadvantage is that the minimum time from test to results is about two weeks.

The operational approach for predicting the frost hardening of silver birch seedlings is to determine the induction of both the first and the second stages of frost hardening. This is possible by measuring height growth regularly during active growth and determining water content in seedling apices (Figure 6.4). Frost hardening starts from the time height growth ceases. The first stage of hardening and the active metabolic processes connected with increasing hardiness last until the water content reaches a stable minimum value. This is the starting point of the second stage of hardening and the rapid increase in frost hardiness. In addition, monitoring of air temperature is needed to evaluate the risk of frost damage during early autumn frost. If height growth continues late in the autumn (risk after cool summer, late sown crops, seedlings fertilized too late and too heavily) and the temperature decreases to <0°C one to three weeks after growth ceases, we can be quite sure that the seedlings will be damaged. When the air temperature decreases slowly and the duration of the first hardening stage is long enough, the time when air temperatures drop below 0°C for the first time is the starting point of rapid increase in frost hardiness (usually at least –10°C). However, it should be remembered, that with this method it is not possible to predict the actual frost hardiness of seedlings, which depend on earlier growing conditions, fertilizer, etc.

6.2 Evaluation of seedlings vigor with the root-growth potential test

In birch, the RGP test is not very useful as an operational method for evaluating the vigor of seedlings, especially the roots. In spring, birch seedlings must first grow fully expanded leaves before they can use photosynthates for root growth (Eliasson 1968, Abod and Webster 1991b). On the other hand, leafless seedlings have a slight ability to grow roots in autumn (Section 3.1). It is possible to identify damaged seedlings with the RGP test, which indicates the general health and vigor of seedlings (Burdett 1987, Ritchie and Tanaka 1990). However, it is questionable whether, at the time of seedling delivery, RGP test results indicate seedling vigor. In addition, birch seedlings seldom have root damage that significantly affects the field performance of seedlings: birch seedlings are usually in a frozen storage or stored in a field by covering the root plugs with sand. This means in most cases that the damage observed in the RGP test is, in fact, shoot damage. If shoot damage is suspected, a more practical method for evaluating the vigor of the shoot might be to grow seedlings under optimal temperature and light conditions without planting them in sand or other growing media (see Section 3.2, whole-seedling viability test).

It is recommended that RGP testing be done near the time of planting (Simpson and Ritchie 1997). In birch seedlings, to obtain results for delivery in May, a four-week RGP test should be started in April. In several evergreen conifer species the RGP test period can be shortened to 7–14 days (Ritchie and Tanaka 1990) but this is not possible in deciduous seedlings, where bud burst and fully expanded leaves are needed for root growth. In birch seedlings it takes about two weeks for leaves to grow to full size (sections 4.1 and 4.2). If the vigor of the seedlings deteriorates near the end of storage, as observed in the study of Luoranen and Rikala (1997), the results of the RGP test will not describe the vigor of the delivered seedlings.

With the modified RGP test, it is possible to avoid delivering seedlings damaged by autumn frosts or during winter storage. Simpson and Ritchie (1997) called the modified RGP test the seedling vitality (SV) test. An example of the SV test is the test of whole-seedling viability described in Section 3.2. If the SV test had been used in nurseries in spring 1994, planting of seedlings from crops damaged in the early autumn frost of 1993 (Kivivuori 1994 a, b) would have been avoided. The test period for evaluation of shoot damage can be shortened to 14–20 days. For example, in the test of whole-seedling viability (Section 3.2) the incubation time was 17 days. After that the dead and damaged (small leaves) seedlings could be separated from the undamaged ones.

If a nursery has changed the method for growing seedlings, it is possible with the RGP test to evaluate the effects of this change on field performance potential (Simpson and Ritchie 1997). A more reliable prediction of the field performance potential can be obtained if the RGP test is done under simulated planting-site conditions (Folk and Grossnickle 1997). The actual field conditions are seldom optimal. Grossnickle et al. (1991a, b) give an example: in their study, western hemlock seedlings treated with SD and LD (long day) grew equally well in optimal conditions, but when soil temperature was +5°C, the SD-treated seedlings had better field performance potential. Another example from the study of Rikala and Puttonen (1988) also indicates that damage in seedlings becomes more apparent under stress conditions: under optimal soil temperature (+23°C), Scots pine seedlings with desiccated roots grew, but at a soil temperature of +12°C they did not.

Ritchie (see Simpson and Ritchie 1997) argued that the RGP test does not describe the field performance potential of seedlings, since the soil during the planting season (meaning spring planting) is too cold to support root growth and the level of RGP is irrelevant. This argument does not concern summer RGP to the same extent as RGP determined in spring or autumn. In summer, the soil temperature is +15–20°C (Figure 1.3b) and the roots can grow immediately after the seedlings are planted (Sections 3.1 and 4.2). However, RGP tests under optimal moisture conditions overestimate the performance potential of seedlings in stressed field conditions. The modified RGP test, called here the root-egress test, done in summer, however, forecasts field shoot growth potential at different times of the growing season quite well (Figure 6.6e,f). The more roots that grew out from the peat plugs the greater was seedling growth during the second growing season. In this study, root egress was tested in summer under natural photoperiod and light conditions; but temperature and soil moisture were more optimal than in field conditions. In this way it was possible to investigate the ability of seedlings to root egress at different planting dates. However, a root-egress test done under drought-stress conditions will predict field performance potential of summer-planted seedlings under stressed field conditions better than a test done in near-optimal conditions.

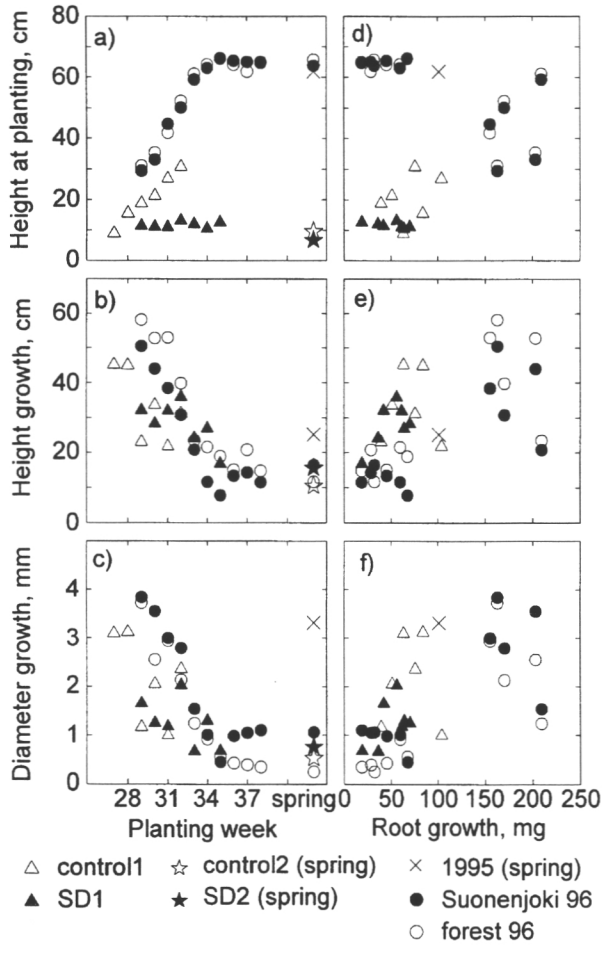


Figure 6.6 Relationships between planting week, amount of outgrown roots, seedling height at planting, second-year height and diameter growth of seedlings planted in different experiments in Suonenjoki. Each symbol indicates the mean value for each planting week in a field or a forest area and greenhouse root-egress or RGP test. Explanations for treatments: Control1 = seedlings grown under natural photoperiod and planted weekly from the end of June to the beginning of August, SD1: seedlings subjected to three-week SD period from 26 June and planted weekly from the middle of July to the end of August (the first crop in Section 4.2), control2 and SD2 = seedlings grown under natural photoperiod or subjected to three-week short-day treatment from 29 July and planted in spring (the second crop in Section 4.2), 1995 (spring) = water-spayed seedlings planted in spring (data presented in Section 4.1), Suonenjoki 96 and forest 96 = seedlings planted weekly from the middle of July to the end of September, and the last time the following May to the nursery field (Suonenjoki 96) or forest site (forest 96) (both the same day) (data presented in chapter 5). All seedlings in the root-egress or RGP test were planted in the greenhouse on the same day as to the field, except in 1995; instead, the RGP test was done in March 1996.

6.3 Root growth and summer planting

The performance of seedlings after planting depends strongly on their capacity to grow roots into the surrounding ground and guarantee water and nutrient uptake (Kozlowski and Davies 1975). After planting, root growth is affected by the growth phase of the seedlings (Figure 6.2), soil moisture, and the temperature and nutrient conditions in the soil (Lopushinsky and Max 1990, Noland et al. 1997).

Seedlings often suffer from water stress after planting (Burdett 1990). The reason for this is thought to be poor root–soil contact and thus reduced water uptake (Kozlowski and Davis 1975). High potential for root growth plays a major role in determining the ability of newly planted seedlings to avoid water stress (Burdett 1987). Because the capacity to take up water and nutrients is a major determinant of early survival and growth (Burdett 1990), seedlings planted in summer have better performance potential than seedlings planted in fall and spring (Figure 3.5).

Ability of first-year silver birch container seedlings to rapid root egress is high until the mid-August. After that, root growth slows down (Figures 6.2c and 6.3c) and planted seedlings are not able to root well before winter. In autumn, after leaf abscission, roots do not grow even if the soil is warm (Section 3.1). Spring-planted, leafless seedlings also have weak ability to root egress (Figure 3.5, Figure 6.6d–f). Leafless seedlings use their carbohydrate reserves for leaf growth, and root growth is possible when the leaves have reached the full size (Abod et al. 1991, Kozlowski 1991), which takes several days (Sections 4.1 and 4.2; RGP tests in spring). This retards the root egress of seedlings and increases the risk of water stress in spring-planted seedlings. When seedlings have some fully-sized leaves, they have, however, few or no new roots. Evaporation may be quite strong in early summer, and thus spring-planted seedlings may suffer water stress. Later, slow or retarded root egress may also decrease the growth of seedlings. In addition, in spring, low temperatures impede water uptake by the roots, which also increases the risk of water stress (Keller 1972).

In spring, seedlings planted and rooted the previous summer were assumed to have a larger root system compared to spring-planted seedlings. In conifer seedlings, spring-planted seedlings have been observed to have higher optimum soil temperature (20–25°C) for root growth than established seedlings (15–20°C) (Söderström 1974). If this is also true for birch seedlings, summer-planted seedlings are more acclimated to low temperatures than spring-planted seedlings. This means that summer-planted seedlings are able to take up nutrients at lower soil temperatures in spring (Clarkson et al. 1988). Hence, summer-planted seedlings have more efficient water and nutrient

uptake in spring and early summer, and growth may increase during the first season after planting (Figure 6.6).

Rapid rooting of summer-planted seedlings can be prevented if seedlings are planted into dry soil or if the peat plug of planted seedlings is too dry (Salminen 1998). Drought stress, if not so severe that it kills seedlings, may lead to shedding of leaves (Fort et al. 1998). This reduces all growth parameters and might reduce the rooting of seedlings, which may also affect growth in later years. The water stress of seedlings could be minimized by planting only well-watered seedlings; however, moisture differences between wet container media and the surrounding soil disappear within days or hours after planting (Day and Skoupy 1971, Heiskanen and Rikala 2000). Although dry container media absorb water from the soil, they remain drier for several days than those watered before planting (Heiskanen and Rikala 2000). Thus, drought stress can, to some extent, be prevented by watering seedlings before planting (Salminen 1998).

In addition, height of a seedling affects its ability to tolerate drought. For summer planting this is important. When the leaves-to-outgrown roots ratios of seedlings at the end of the root-egress test periods were compared, the ratios were low in small seedlings that were planted earlier in July (data not shown). This means that, compared to taller older seedlings, short young seedlings have greater water-uptake ability in relation to the transpiring shoot. Accordingly, in summer, one should avoid planting very tall (over 45–50 cm) seedlings, due to the higher risk of drought stress.

The prerequisite for planting growing, leaf-bearing seedlings is that seedlings be grown in small cells, since root systems should be large enough to bind peat plugs during handling. The usefulness of planting very young seedlings might also be limited by low stress tolerance of unlignified seedlings; these seedlings may be damaged severely during lifting, transporting, storage and planting. Wounds, even small ones, in turn, increase the risk of stem lesions (Lilja et al. 1997).

In the present study, the first seedlings were planted when they were six weeks old in small cells and at 10–11 weeks old in large cells. It would be interesting to examine the performance of younger seedlings. However, the earliest time to plant birch seedlings is the beginning of the second growth phase (Figures 6.1–6.3), when a temperature sum of about 500–600 d.d. has accumulated (Figure 6.1d).

An interesting, but so far uninvestigated, possibility might also be to plant young (5–8 weeks old) leaf-bearing, actively growing seedlings in spring or early summer. These seedlings would have high ability to root egress, but how much soil temperature would affect the field performance of such seedlings is not known. In one-year-old aspen (*Populus tremuloides* Michx.) seedlings, the transfer of growing seedlings from 20 to 5°C greatly reduced water flow to roots,

stomatal conductance and net photosynthesis (Wan et al. 1999). However, in younger, first-year seedlings the response to transfer might be weaker, especially if the temperature difference were not so large (20°C in greenhouse, 8–12°C in soil in late-May, see Figure 1.3b).

Height of the seedlings at planting does not affect growth during the following years as much as planting date does (Figures 5.3 and Figure 6.6). Seedlings sown on 10 May and grown under natural photoperiod were planted from the beginning of July to the beginning of August (Section 4.2) and seedlings in the summer-planting experiment from the middle of July to the end of September (Section 5). When the seedlings of the same size at planting were compared, seedlings planted earlier grew more the following season (Figure 6.6a–c) due to high soil temperature (Figure 5.3e,f) and quick root egress.

If only the relationships between height at planting and the following seasons growth from summer planting experiments are examined, this may give the idea of planting winter-stored short seedlings in spring. However, spring-planted leafless seedlings have a weak ablity to root egress, which means lower growth than short seedlings planted in summer (Figure 6.6d–f). Furthermore, one-year-old overwintered seedlings which have fully expanded leaves should not be planted in early summer: Rikala (1996) showed that this kind of seedling, when planted in the middle of June, had as poor growth as did leafless seedlings planted a month earlier.

In conclusion, first-year silver birch container seedlings can be planted from the time when a temperature sum of 500–600 d.d. is accumulated until height growth cessation. During this time, roots grow rapidly from the peat plug into the surrounding ground. For this reason the field performance of summer-planted seedlings is at least the same as, but probably better than, seedlings planted in May or September (recommended for planting dates at present (Vastamäki 1993).

6.4 Comparison of control methods

Height growth can be controlled by both methods investigated (application of CCC or daminozide, and SD treatment), but frost hardening can only be controlled with SD treatment. The evaluation of methods is presented in Table 6.2.

Both daminozide and CCC retarded height growth of seedlings, but daminozide was more effective (Section 4.1). There were no aftereffects for either of the compounds. A disadvantage concerning the use of chemical growth retardants is that at the time of application (as used in this study) it is not possible to predict whether there is a

Table 6.2 Evaluation of methods for production of silver birch container seedlings. The four first rows present the methods. The effectiveness of methods in controlling development of seedlings in nurseries, their effects on field performance and their influences on the production and planting practices are compared to present practices in nurseries. 'Yes' and 'no' indicate whether the method affects the criterion or not.

Sowing time	early May	early May	early May	early May	mid-June	mid-June	early May	early May
Cavity volume (cm^3)	380	380	110	110	110	110	380	380
Treatment	CCC	B9	–	SD	–	SD	SD*	–
Timing of treatment	end of June + end of July	end of June		3 weeks from end of June		3 weeks from end of July	after mid-July	
Time for response	1–2 weeks	1–2 weeks		2 weeks		2 weeks	2 weeks	
Control of development in a nursery								
Retardation of height growth	yes	yes	no	yes	yes	yes	yes	
Earlier cessation of height growth	no	no	no	no	no	yes	yes	
Increased sturdiness	no	yes		yes		yes	yes	
Hastened frost hardening	no	no	no	no	no	yes	yes	
Planting and field performance								
Planting date	spring	spring	summer	summer	spring	spring	spring	summer
Increased RGP	no	no	yes	yes	no	no	no	yes
Decreased survival after planting	no	no	no	no	no	no	no	no
Increased growth after planting	no	no	yes	yes	no	no	no	yes
Production and planting of seedlings								
Enlarged planting window	no	no	yes	yes	no	no	no	yes
Decreased seedling costs	no	no	yes	no	yes	no	no	yes
Decreased planting costs	no	no	yes	yes	yes	yes	no	yes
Increased flexibility of seedling production	no	no	yes	yes	yes	yes	no	yes

* According to Luoranen and Rikala 1997

risk of excessive height growth or not. In this study, the second application of CCC was made at the end of July. In that time it is possible to notice the need to control height growth. The CCC application, however, was not effective. In addition, neither of the compounds affected the frost hardening of seedlings. Thus the method for artificial control of growth and frost hardening of birch seedlings with more potential is SD treatment.

Two crops of silver birch seedlings can be raised in small cells (100–200 cm^3) in the same greenhouse during a growing season (Section 4.2). However, there might be problems with field-performance in seedlings of the second crop. As in the present study, seedlings in the first crop (Section 4.2, control 1 seedlings sown in the middle of May) should be planted when they are six weeks old (at the end of June or beginning of July). The second crop can be sown in the early part of June and transplanted when seedlings in the first crop are

moved outdoors. The first hardening stage can be induced by SD treatment. This treatment, however, cannot be started until the second growth phase. If it is started earlier, frost hardening does not progress because the nights are too short. After the three-week SD treatment started at the end of June, seedlings did not frost harden (Section 4.2). If SD treatment is started after the middle of July, frost hardening can be hastened about two weeks with this treatment (Section 4.2, Luoranen and Rikala 1997).

Without any hardening regime, frost hardening of seedlings grown at higher density may be delayed because of later cessation of height growth (unpublished results of Luoranen). It has also been observed that seedlings grown at lower densities are more frost-hardy, due to exposure to stress, compared to those grown at higher densities (Mexal and Landis 1990).

Compared to untreated seedlings, SD-treated seedlings are more vulnerable to damage caused by hares and pathogens (stem spots) (Section 4.2). In addition, the height growth of spring-planted seedlings was not improved by SD treatment and these seedlings may be weaker competitors with weeds than are taller seedlings raised without SD treatment.

In the studies of Luoranen and Rikala (1997) and J. Nurminen (personal communication) the after-effects of SD treatments were minor when seedlings older than those in the present study were treated. Luoranen and Rikala (1997) observed that seedlings sown in early May and treated in either mid- or late July had fewer unbroken buds than seedlings treated earlier, and their growth was comparable to that of untreated seedlings. In addition, according to Nurminen, SD treatment did not affect the mammalian palatability of birch seedlings when the SD period was started after two-thirds of the average temperature sum in the region of the provenance was accumulated. However, seedlings that are about 2 1/2 months old have to be grown in large cells (250–400 cm^3, 150–230 cells per m^2) to ensure enough space for shoot and root growth. This means a large growing space. Because SD treatment facilities in a greenhouse are expensive, it might not be economical to treat this kind of seedling with SD.

The most effective method for avoiding the risk of excessive height growth of silver birch seedlings is summer planting. When the nursery growing period of these seedlings is short, there may not be problems with excessive growth. When seedlings are planted in July or before mid-August, the growth of the seedlings improves, and survival is the same as for traditional spring- or autumn-planted seedlings. The alternative growing schedules for silver birch container seedlings are presented in Figure 6.7.

More studies on summer planting are needed. For instance, in this study there were no experiments in which small seedlings were planted in mid-June.

In conclusion, problems with growth and frost hardening of silver birch container seedlings can be avoided by shortening the duration of nursery growing and planting seedlings when they are a few months old (Figure 6.7a, b). This production method makes it possible to respond better to fluctuating demand for seedlings and gives lower seedling and planting costs. In conventional production of one-year old birch container seedlings, based on the results of several years results of monitoring seedling growth and development during nursery growing, growing schedules and practices can be changed so that seedlings are grown without risk of excessive or late height growth and thus with a low risk of delayed frost hardening (Figure 6.7c).

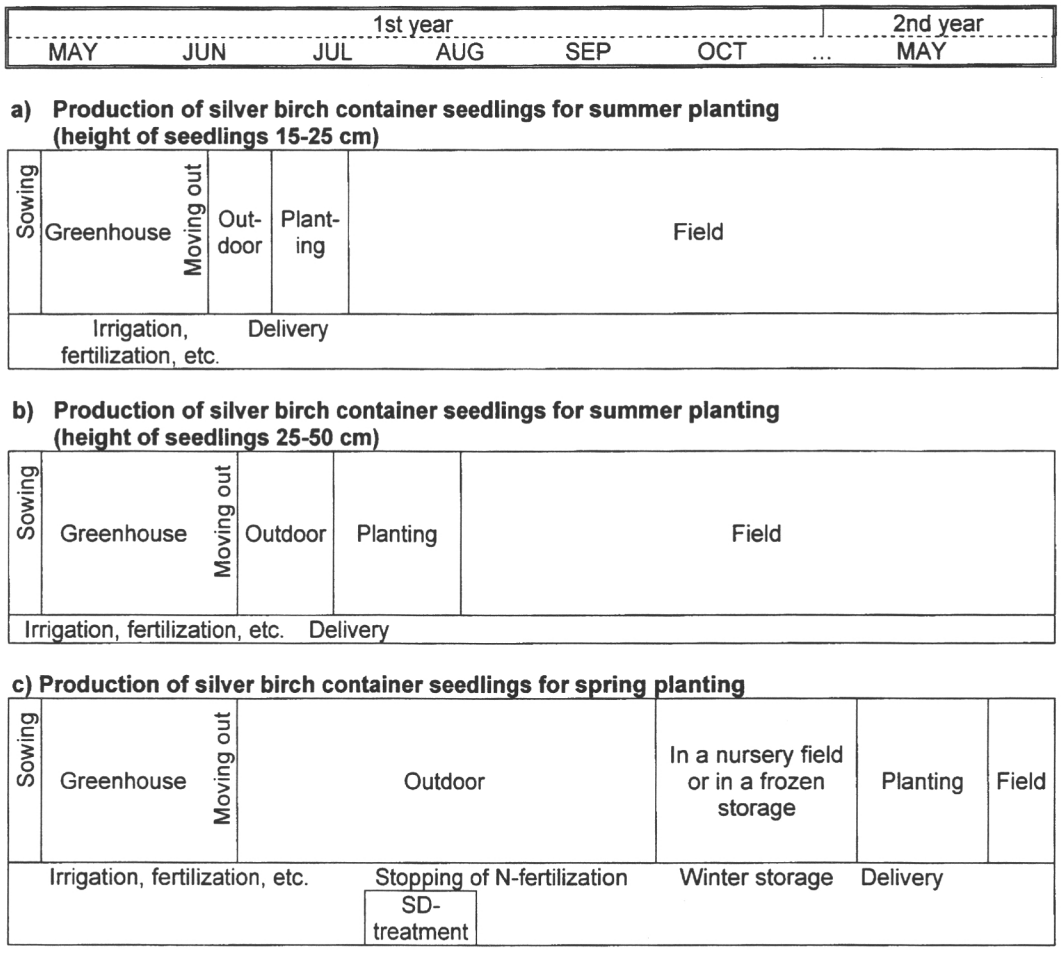


Figure 6.7 Schematic presentation of possible production and planting schedules of silver birch container seedlings according to the results of this study.

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Appendix I. Estimated parameters in functions estimation of frost hardiness of silver birch seedlings. Parameters are presented with their 95 percent confidence intervals, asymptotic standard errors (ASE) for logistic equation $y = d + \frac{a}{1+e^{b(c-x)}}$ and its reparametrization (calculation of x).

Date		$c = T_{50}$			ASE	b			ASE	a			ASE	d			ASE	$x = T_{10}$			ASE
Aug. 18	ET	–4	**–3**	–3	0.28	–2.0	**–1.26**	–0.5	0.38	0.27	**0.33**	0.38	0.03	0.31	**0.36**	0.40	0.02	–3	**–2**	–0.4	0.57
	BT	–3	**–3**	–3	0.11	–3.7	**–2.97**	–2.2	0.38									–3	**–3**	–2.3	0.18
	DT		**–4**		0.00		**–9.7**		0.00												
	LT		**–4**		0.00		**–9.7**		0.00												
Sept. 8	ET	–6	**–5**	–4	0.48	–1.1	**–0.72**	–0.3	0.21	0.32	**0.37**	0.42	0.02	0.32	**0.36**	0.40	0.02	–4	**–2**	–1	0.76
	BT	–7	**–6**	–5	0.41	–0.9	**–0.69**	–0.5	0.09									–4	**–3**	–2	0.64
	DT	–9	**–7**	–5	0.97	–20	**–2.85**	14	8.55												
	LT	–9	**–8**	–8	0.31	–1.7	**–1.31**	–0.9	0.19												
Sept. 29	ET		**–8**		0	–11	**–3.06**	4.9	3.98	0.23	**0.27**	0.31	0.02	0.43	**0.46**	0.49	0.01		**–7.8**		0
	BT	–10	**–9**	–9	0.26	–1.6	**–1.18**	–0.8	0.20									–8	**–7**	–6	0.38
	DT	–11	**–11**	–10	0.40	–27	**–2.97**	21	11.9												
	LT	–18	**–17**	–16	0.54	–1.0	**–0.73**	–0.4	0.1												
Oct. 13	ET	–15	**–13**	–11	0.92	–0.6	**–0.38**	–0.2	0.10	0.26	**0.33**	0.39	0.03	0.30	**0.34**	0.38	0.02	–10	**–7**	–4	1.56
	BT	–20	**–18**	–16	0.96	–0.6	**–0.37**	–0.2	0.09									–16	**–12**	–8	2.16
	DT	–16	**–12**	–9	1.74	–0.4	**–0.30**	–0.2	0.06												
	LT	–27	**–24**	–22	1.43	–0.6	**–0.39**	–0.2	0.09												
Oct. 27	BT	–35	**–29**	–23	3.04	–0.2	**–0.11**	–0.1	0.03									–18	**–9**	–0.5	4.29

Appendix II. Nutrients in stems of birch seedlings to which daminozide and CCC were applied. Nutrient concentrations in seedlings given six doses of daminozide once or six doses of CCC (chlormequat chloride) twice during the growing season. Control seedlings were sprayed with water. Nutrients were analyzed from pooled stem samples taken after leaf abscission in the autumn (n = 6). Underlined numbers indicate statistically significant ($P < 0.05$) differences between control and treated seedlings according to Tukey's HSD-test.

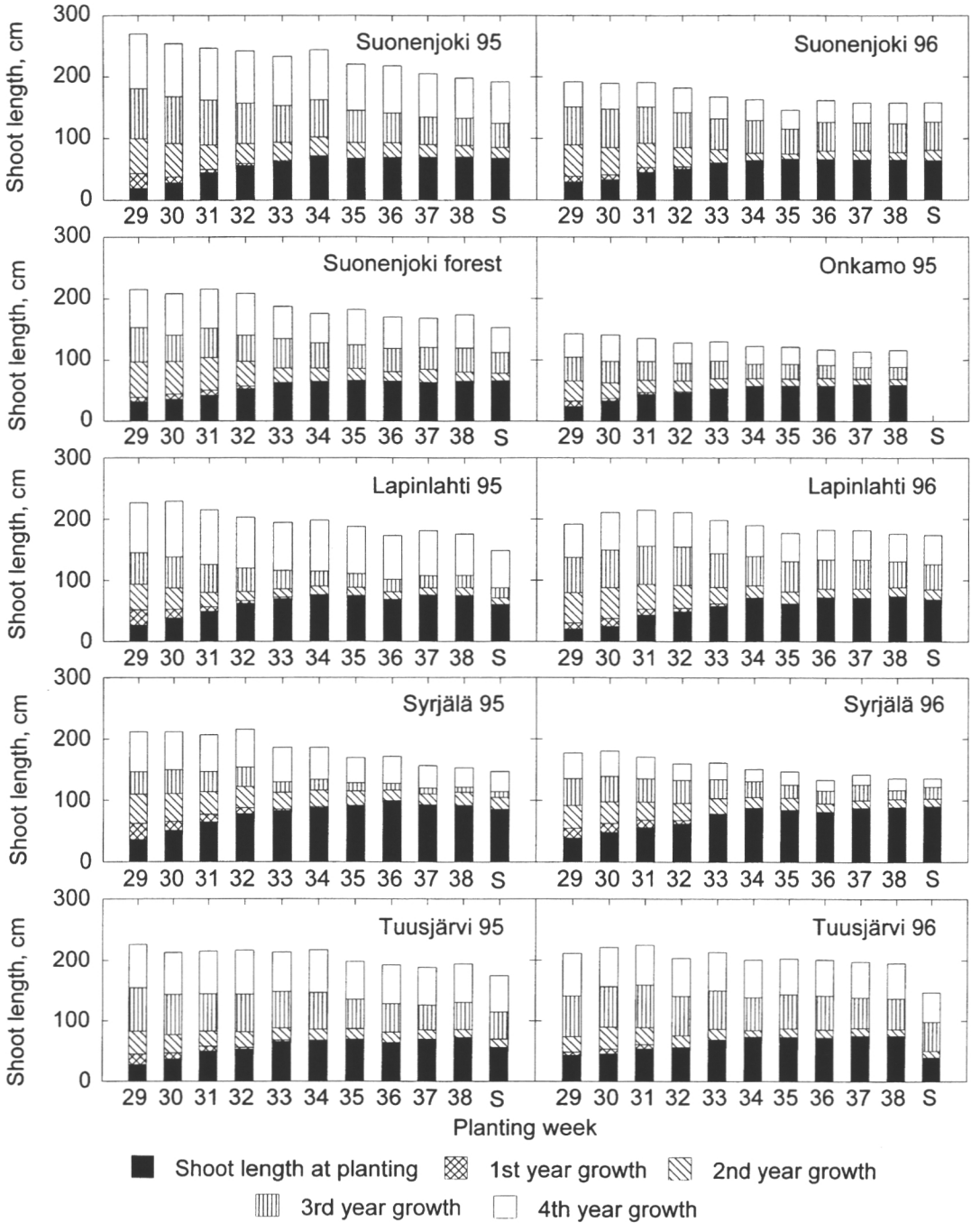
	Control	Daminozide, g l^{-1}						CCC, g l^{-1}					
Nutrient	0	1	2	3	4	5	6	0.5	1.0	1.5	2.0	2.5	3.0
N, mg g^{-1}	9.6	10.5	11.0	10.7	10.9	11.3	11.1	10.1	10.7	10.5	10.5	10.5	11.2
P, mg g^{-1}	1.5	1.6	1.7	1.7	1.7	1.7	1.7	1.5	1.6	1.6	1.6	1.6	1.7
K, mg g^{-1}	4.4	4.7	4.7	4.7	4.7	4.8	4.8	4.6	4.6	4.6	4.6	4.6	4.7
Ca, mg g^{-1}	1.7	1.8	2.0	1.9	2.0	2.1	2.1	1.8	1.9	1.8	1.9	2.0	1.9
Mg, mg g^{-1}	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.9
Mn, mg kg^{-1}	55	67	66	67	69	67	70	66	60	60	61	62	57
Cu, mg kg^{-1}	2.7	2.9	3.2	3.4	3.5	3.4	3.4	3.7	2.9	3.1	3.1	3.0	3.3
Zn, mg kg^{-1}	61	68	76	73	80	77	75	72	66	73	67	70	70
Fe, mg kg^{-1}	31	30	33	34	31	40	33	31	29	32	29	34	31
B, mg kg^{-1}	8	8	10	9	9	9	10	9	8	9	8	9	9

Appendix III Nutrients in stems and roots of birch seedlings subjected to short-day treatment (from 26 June to 17 July for SD 1 and from 29 July to 19 August for SD 2) or natural photoperiod (control 1, control 2). Nutrient concentrations were analyzed from leaves at the end of the treatment period [17 July (SD 1) or 19 August (SD 2)] and from stems and roots in the end of the growing season (15 October). Differences in means between treatments were analyzed by using a t-test and in the case of heterogeneous variances (marked with [1]) by using non-parametric Mann-Whitney U test. n = 5 samples (each pooled from 4 seedlings).

NUTRIENT	PART	SD 1*	Control 1*	P	SD 2**	Control 2**	P
Nitrogen	leaf	28	18	<0.001	24	17	0.005
(mg g^{-1})	stem	17	9	<0.001	15	10	0.003
	roots	12	8	0.001	11	10	0.005
Phosphorous	leaf	3.3	2.3	0.001	3.1	2.8	0.60[1]
(mg g^{-1})	stem	1.8	1.3	<0.001	2.0	1.6	0.001
	roots	2.2	1.6	<0.001	3.1	1.9	0.009[1]
Potassium	leaf	17	14	0.01	18	16	0.28
(mg g^{-1})	stem	4.7	22	0.07	4.6	4.7	0.60
	roots	5.7	4.0	<0.001	7.4	5.1	0.05[1]
Calcium	leaf	3.4	3.6	0.12	4.8	4.6	0.25[1]
(mg g^{-1})	stem	2.2	1.8	0.10	2.3	1.7	0.02
	roots	2.8	2.9	0.83	3.5	2.5	0.07[1]
Magnesium	leaf	2.2	2.3	0.10	2.8	2.9	0.47
(mg kg^{-1})	stem	1.1	0.9	0.001	1.3	1.0	0.001
	roots	1.5	1.3	0.04	2.2	1.5	0.02[1]
Manganese	leaf	794	622	<0.001	1324	1008	0.003
(mg kg^{-1})	stem	157	65	0.009[1]	338	212	0.004
	roots	98	66	0.03	287	164	0.08[1]
Copper	leaf	7	3	0.004	6	5	0.55
(mg kg^{-1})	stem	5	2	0.009[1]	18	5	0.05[1]
	roots	14	13	0.58	33	25	0.29
Zinc	leaf	148	87	<0.001	191	144	0.001
(mg kg^{-1})	stem	131	78	0.001	162	96	0.01[1]
	roots	90	66	<0.001	149	100	0.03[1]
Iron	leaf	129	77	<0.001	100	121	0.20
(mg kg^{-1})	stem	59	32	<0.001	47	29	0.002
	roots	190	197	0.80	170	120	0.18[1]
Boron	leaf	14	11	0.001	13	13	0.38
(mg kg^{-1})	stem	13	8	<0.001	10	7	<0.001
	roots	10	8	0.002	15	10	0.03[1]

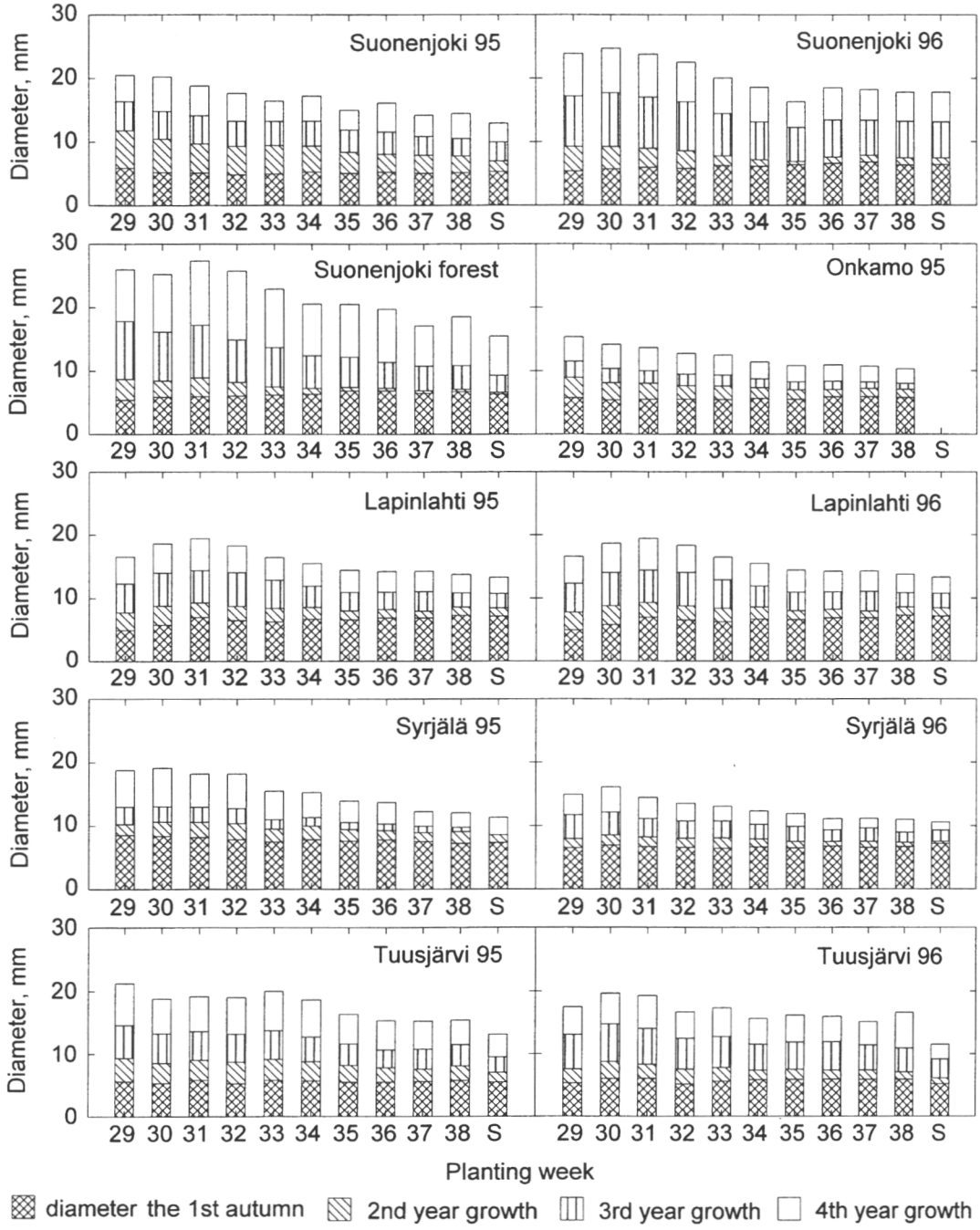
* SD 1 and control 1 seedlings were sown on 10 May 1996

** SD 2 and control 2 seedlings were sown on 18 June 1996



Appendix IV a)

Appendix IV Results of summer planting in experiments established on nursery fields in Suonenjoki, Lapinlahti, Syrjälä (Kerimäki) and Tuusjärvi in 1995 and 1996. In both years seedlings were also planted to the nursery field in Onkamo, but only results of 1995 are presented. Hares damaged most of seedlings in the experiment established in 1996. An additional experiment was established on a forest site in Suonenjoki



Appendix IV b)

(Suonenjoki forest) in 1996. In each experiment, seedlings were planted weekly from week 29 (15–21 July) to week 38 (16–23 September) and the following May (S). **a)** Shoot length and **b)** diameter of silver birch container seedlings in different experiments (nursery, planting year) at time of planting, or after the first, second, third and fourth year following planting.

Appendix V ANOVA tables.
ANOVA tables connected to Section 4.1 Growth retardants

Table 4.1.1 Analysis of variance showing the influence of seven daminozide and seven CCC levels of concentration on height growth, development of water content and morphology of silver birch container seedlings.

Variable	Source	df	MSE	F	P
Height growth	Between				
Daminozide	Concentration (C)	6	1083	11.7	<0.001
	error	35	925		
	Within				
	Date (D)	11	10108	3362	<0.001
	D x C	66	40.4	13.4	<0.001
	error	385	3.0		
CCC	Between				
	Concentration (C)	6	382	5.5	<0.001
	error	35	69		
	Within				
	Date (D)	11	13577	8.5	<0.001
	D x C	66	10.4	6.5	<0.001
	error	385	1.6		
Water content	Between				
daminozide	Concentration (C)	6	15.5	3.8	0.005
	error	25	4.1		
	Within				
	Date (D)	12	8049	2854	<0.001
	D x C	72	3.9	1.4	
	error	420	2.8		
	Between				
CCC	Concentration (C)	6	0.9	0.126	0.992
	error	35	6.8		
	Within				
	Date (D)	12	7780	2597	<0.001
	D x C	72	3.9	1.3	0.064
	error	420	3.0		
Morphology at the end of the first year					
Final height					
daminozide	Concentration	6	210	9.3	<0.001
	error	35	23		
CCC	Concentration	6	58	3.1	0.015
	error	35	19		
Diameter					
daminozide	Concentration	6	0.16	2.86	0.023
	error	35	0.06		
CCC	Concentration	6	0.23	3.96	0.004
	error	35	0.06		
Dry mass of shoot					
daminozide	Concentration	6	0.98	4.29	0.002
	error	35	0.23		
CCC	Concentration	6	0.80	4.73	0.001
	error	35	0.18		
Sturdiness					
daminozide	Concentration	6	4.1	6.7	<0.001
	error	35	0.6		
CCC	Concentration	6	0.2	0.7	0.678
	error	35	0.3		

Table 4.1.2 Analysis of variance showing the influence of treatment with daminozide and CCC on root growth potential and timing of leaf elongation of silver birch container seedlings the following spring. Treatments were spraying concentrations of 1.0, 3.0 and 6.0 g daminozide per liter and 0.5, 2.0, 3.0 g chlormequat chloride (CCC) per liter or water spraying.

Variable	Source	df	MSE	F	P
Root growth	Treatment	6	0.001	1.1	0.397
	error	25	0.001		
Dry mass of roots	Treatment	6	0.044	1.1	0.369
	error	25	0.039		
Dry mass of	Treatment	6	1.072	6.6	<0.001
shoot	error	25	0.163		
Dry mass of	Treatment	6	0.047	1.0	0.47
leaves	error	25	0.049		
Height at planting	Treatment	6	152	17.6	<0.001
	error	25	8.6		
Final height	Treatment	6	145	18.1	<0.001
	error	25	8		
Height growth	Treatment	6	3.2	1.3	0.306
	error	25	2.5		
Diameter	Treatment	6	2139	3.6	0.011
	error	25	597		
Shoot:root ratio	Treatment	6	0.087	4.4	0.003
	error	25	0.02		
Leaf elongation	Between				
	Treatment (T)	6	62.9	1.8	0.106
	error	161	35.3		
	Within				
	Date (D)	3	88685	7140	<0.001
	D x T	18	65	5	
	error	483	12		

Table 4.1.3 Analysis of variance showing the influence of seven daminozide and seven CCC concentrations on field performance of silver birch container seedlings.

Variable	Source	df	MSE	F	P
Height at the end of the first year in the field					
daminozide	Concentration	6	0.5	1.2	0.001
	error	35	0.4		
CCC	Concentration	6	28	1.0	0.44
	error	35	27		
Height at the end of the second year					
daminozide	Concentration	6	129	0.8	0.58
	error	35	162		
CCC	Concentration	6	64	0.4	0.85
	error	35	162		
Height at the end of the third year					
daminozide	Concentration	6	55	0.16	0.99
	error	35	338		
CCC	Concentration	6	86	0.25	0.96
	error	35	340		
Diameter at the end of the first year in the field					
daminozide	Concentration	6	0.5	1.23	0.316
	error	35	0.4		
CCC	Concentration	6	0.1	0.13	0.99
	error	35	0.5		
Diameter at the end of the second year					
daminozide	Concentration	6	1.2	1.02	0.43
	error	35	1.2		
CCC	Concentration	6	0.5	0.35	0.91
	error	35	1.4		
First-year height growth					
daminozide	Concentration	6	58	1.5	0.19
	error	35	37		
CCC	Concentration	6	83	2.7	0.03
	error	35	30		
Second-year height growth					
daminozide	Concentration	6	10	0.09	1.0
	error	35	109		
CCC	Concentration	6	17	0.18	0.98
	error	35	94		
Third-year height growth					
daminozide	Concentration	6	24	0.34	0.91
	error	35	71		
CCC	Concentration	6	15	0.22	0.97
	error	35	65		
First-year diameter growth					
Daminozide	Concentration	6	0.3	0.60	0.73
	error	35	0.5		
CCC	Concentration	6	0.2	0.39	0.88
	error	35	0.6		
Second-year diameter growth					
Daminozide	Concentration	6	0.4	0.54	0.78
	error	35	0.9		
CCC	Concentration	6	0.6	1.02	0.43
	error	35	0.5		

ANOVA tables connected to Section 4.2 Short-day treatment

Table 4.2.1 Analysis of variance showing the effects of short-day treatment on morphology of silver birch container seedlings at the end of the first year. Seedlings were sown on 10 May 1996 subjected to short-days from 26 June to 17 July or grown under natural photoperiod.

Variable	Source	df	MSE	F	P
Final height	Treatment	1	137	249	<0.001
	error	8	6		
Diameter	Treatment	1	0.6	3.52	0.01
	error	8	0.2		
Dry mass of shoot	Treatment	1	0.8	72	<0.001
	error	8	0.03		
Dry mass of roots	Treatment	1	0.007	0.41	0.54
	error	8	0.016		
Shoot:root	Treatment	1	1.8	103	<0.001
	error	8	0.02		
Sturdiness	Treatment	1	775	42	<0.001
	error	8	18		

Table 4.2.2 Analysis of variance showing the influence of short-day treatment on root egress, height growth and diameter growth of silver birch container seedlings during three-week root-egress tests carried out weekly from the end of June (Week 26) to the end of August (Week 35). Seedlings were sown on 10 May 1996 and subjected to short-day treatment from 26 June to 17 July 1996 or grown under natural photoperiod.

Variable	Source	df	MSE	F	P
Root growth (dry mass of new roots)					
Week 26	Treatment	1	0.001	7	0.03
	error	8	<0.001		
Week 27	Treatment	1	0.003	12	0.01
	error	8	0.001		
Week 28	Treatment	1	<0.001	0.5	0.51
	error	8	<0.001		
Week 29	Treatment	1	<0.001	0.2	0.70
	error	8	<0.001		
Week 30	Treatment	1	0.001	0.8	0.40
	error	8	0.001		
Week 31	Treatment	1	0.004	7	0.03
	error	8	0.001		
Week 32	Treatment	1	0.001	3	0.11
	error	8	<0.001		
Week 33	Treatment	1	0.001	2	0.16
	error	8	0.001		
Week 34	Treatment	1	0.003	10	0.01
	error	8	<0.001		
Week 35	Treatment	1	<0.001	1	0.36
	error	8	<0.001		
Height growth					
Week 26	Treatment	1	0.4	0.3	0.59
	error	8	1.3		
Week 27	Treatment	1	23	51	<0.001
	error	8	0.5		
Week 28	Treatment	1	74	191	<0.001
	error	8	0.4		
Week 29	Treatment	1	53	72	<0.001
	error	8	0.7		
Week 30	Treatment	1	54	257	<0.001
	error	8	0.2		
Week 31	Treatment	1	64	14	0.006
	error	8	4.5		
Week 32	Treatment	1	33	32	<0.001
	error	8	1		
Week 33	Treatment	1	4.5	5	0.06
	error	8	0.9		
Week 34	Treatment	1	0.6	18	0.003
	error	8	0.02		
Diameter growth					
Week 26	Treatment	1	0.3	2.0	0.20
	error	8	0.02		
Week 27	Treatment	1	0.02	1.7	0.23
	error	8	0.009		
Week 28	Treatment	1	0.18	7.8	0.02
	error	8	0.02		
Week 29	Treatment	1	0.21	9.5	0.02
	error	8	0.02		
Week 30	Treatment	1	0.94	33	<0.001
	error	8	0.03		
Week 31	Treatment	1	0.19	3.0	0.12
	error	8	0.06		
Week 32	Treatment	1	0.12	1.3	0.29
	error	8	0.09		
Week 33	Treatment	1	1.9	27	0.001
	error	8	0.07		
Week 34	Treatment	1	0.64	24	0.001
	error	8	0.03		
Week 35	Treatment	1	0.008	1.5	0.25
	error	8	0.005		

Table 4.2.3 Analysis of variance showing the influence of planting date on field performance of silver birch container seedlings sown on 10 May 1996 and grown under natural photoperiod. Seedlings were planted weekly from 2 July to 6 August 1996.

Variable	Source	df	MSE	F	P
Height at planting	Date	5	223	110	<0.001
	error	17	2		
Height at the end of the 1st year	Date	5	83	16	<0.001
	error	17	5		
Height at the end of the 2nd year	Date	5	299	5	0.007
	error	17	64		
Height at the end of the 3rd year	Date	5	231	2	0.16
	error	17	126		
Diameter at planting	Date	5	0.83	82	<0.001
	error	17	0.01		
Diameter at the end of the 1st year	Date	5	0.4	4	0.02
	error	17	0.1		
Diameter at the end of the 2nd year	Date	5	4.1	6	0.004
	error	17	0.7		
Diameter at the end of the 3rd year	Date	5	4.4	1.5	0.24
	error	17	2.9		
First-year height growth	Date	5	59	24	<0.001
	error	17	2		
Second-year height growth	Date	5	416	7	0.001
	error	17	57		
Third-year height growth	Date	5	12	0.17	0.97
	error	17	76		
First-year diameter growth	Date	5	0.34	3.3	0.03
	error	17	0.11		
Second-year diameter growth	Date	5	3.3	4	0.01
	error	17	0.8		
Third-year diameter growth	Date	5	0.2	0.11	0.99
	error	17	1.6		

Table 4.2.4 Analysis of variance showing the influence of planting date on field performance of SD-treated silver birch container seedlings. Seedlings were sown on 10 May 1996 and subjected to short-day treatment from 26 June to 17 July 1996 and planted weekly from 16 July to 27 August 1996.

Variable	Source	df	MSE	F	P
Height at planting	Date	6	3.3	0.73	0.63
	error	20	4.5		
Height at the end of the 1st year	Date	6	3.0	0.71	0.65
	error	20	4.2		
Height at the end of the 2nd year	Date	6	125	1.75	0.16
	error	20	72		
Height at the end of the 3rd year	Date	6	385	4.11	0.008
	error	20	94		
Diameter at planting	Date	6	2.17	26	<0.001
	error	20	0.08		
Diameter at the end of the 1st year	Date	6	0.44	3.37	0.02
	error	20	0.13		
Diameter at the end of the 2nd year	Date	6	0.58	1.74	0.16
	error	20	0.33		
Diameter at the end of the 3rd year	Date	6	6.25	2.81	0.04
	error	20	2.22		
First-year height growth	Date	6	1.03	5.74	0.002
	error	20	0.18		
Second-year height growth	Date	6	137	1.99	0.12
	error	20	69		
Third-year height growth	Date	6	175	1.42	0.26
	error	20	123		
First-year diameter growth	Date	6	1.49	11.15	<0.001
	error	20	0.13		
Second-year diameter growth	Date	6	0.91	2.41	0.065
	error	20	0.38		
Third-year diameter growth	Date	6	6.53	3.12	0.03
	error	20	2.10		

Table 4.2.5 Analysis of variance showing the influence of short-day treatment and planting date from 16 July to 6 August (the dates when seedlings from both treatments were planted) on field performance of silver birch container seedlings. Seedlings were sown on 10 May 1996 and subjected to short-day treatment from 26 June to 17 July 1996 or grown under natural photoperiod.

Variable	Source	df	MSE	F	P
Height at planting	Treatment (T)	1	1244	506	<0.001
	Date (D)	3	39	28	<0.001
	T x D	3	44	18	<0.001
	error	23	2		
Height at the end of the 1st year	Treatment (T)	1	1760	562	<0.001
	Date (D)	3	56	18	<0.001
	T x D	3	38	12	<0.001
	error	23	3		
Height at the end of the 2nd year	Treatment (T)	1	839	17	<0.001
	Date (D)	3	174	3	0.03
	T x D	3	105	2	0.13
	error	23	51		
Height at the end of the 3rd year	Treatment (T)	1	374	4.4	0.05
	Date (D)	3	150	1.8	0.18
	T x D	3	182	2.1	0.12
	error	23	85		
Diameter at planting	Treatment (T)	1	0.33	14	0.001
	Date (D)	3	1.27	53	<0.001
	T x D	3	0.01	0.4	0.732
	Error	23	0.2		
Diameter at the end of the 1st year	Treatment (T)	1	1.47	14.5	0.001
	Date (D)	3	1.07	10.6	<0.001
	T x D	3	0.05	0.5	0.68
	error	23	0.10		
Diameter at the end of the 2nd year	Treatment (T)	1	2.35	5.09	0.04
	Date (D)	3	3.12	6.74	0.002
	T x D	3	0.48	1.03	0.40
	error	23	0.46		
Diameter at the end of the 3rd year	Treatment (T)	1	4.13	1.79	0.19
	Date (D)	3	2.43	1.05	0.39
	T x D	3	3.39	1.47	0.25
	error	23	2.31		
First-year height growth	Treatment (T)	1	51.1	249	<0.001
	Date (D)	3	3.0	14	<0.001
	T x D	3	0.7	3	0.04
	error	23	0.2		
Second-year height growth	Treatment (T)	1	163	3.8	0.07
	Date (D)	3	71	1.7	0.21
	T x D	3	91	2.1	0.13
	error	23	43		
Third-year height growth	Treatment (T)	1	93	1.05	0.32
	Date (D)	3	64	0.72	0.55
	T x D	3	83	0.94	0.44
	error	23	88		
First-year diameter growth	Treatment (T)	1	0.41	4.25	0.05
	Date (D)	3	0.56	5.82	0.004
	T x D	3	0.06	0.59	0.63
	error	23	0.10		
Second-year diameter growth	Treatment (T)	1	0.10	0.21	0.65
	Date (D)	3	1.59	3.24	0.04
	T x D	3	0.64	1.30	0.30
	error	23	0.49		
Third-year diameter growth	Treatment (T)	1	0.25	0.14	0.71
	Date (D)	3	2.22	1.24	0.32
	T x D	3	1.64	0.92	0.45
	error	23	1.79		

Table 4.2.6 Analysis of variance showing the effects of short-day treatment on morphology and development of water content of silver birch container seedlings. Seedlings were sown on 18 June 1996 subjected to short-days from 29 July to 19 August 1996 or grown under natural photoperiod.

Variable	Source	df	MSE	F	P
Morphology at the end of the first year					
Final height	Treatment	1	159	54	<0.001
	error	8	3		
Diameter	Treatment	1	0.15	4.2	0.08
	error	8	0.04		
Dry mass of shoot	Treatment	1	0.110	17	0.003
	error	8	0.006		
Dry mass of roots	Treatment	1	0.015	5.5	0.047
	error	8	0.003		
Shoot:root	Treatment	1	0.30	25	0.001
	error	8	0.01		
Sturdiness	Treatment	1	198	46	<0.001
	error	8	4		
Water content					
	Between				
	Treatment (T)	1	0.02	75	<0.001
	Error	8	<0.001		
	Within				
	Date (W)	11	0.095	656	<0.001
	T x W	11	0.002	13	<0.001
	error	88	<0.001		

Table 4.2.7 Analysis of variance showing the influence of short-day treatment on root growth potential and timing of leaf elongation of silver birch container seedlings the following spring. Seedlings were sown on 18 June and subjected to short days from 29 July to 19 August or grown under natural photoperiod.

Variable	Source	df	MSE	F	P
Height at planting	Treatment	1	111	59	<0.001
	error	8	2		
Diameter at planting	Treatment	1	0.29	10	0.01
	error	8	0.03		
Final height	Treatment	1	63	23	0.001
	error	8	3		
Final diameter	Treatment	1	0.10	1.99	0.20
	error	8	0.05		
Height growth	Treatment	1	0.019	175	<0.001
	error	8	<0.001		
Diameter growth	Treatment	1	0.05	3	0.12
	error	8	0.02		
RGP	Treatment	1	0.07	0.11	0.75
	error	8	0.67		
Relative height growth	Treatment	1	1757	146	<0.001
	error	8	12		
Relative diameter growth	Treatment	1	156	6	0.04
	error	8	27		

Table 4.2.8 Analysis of variance showing the influence of short-day treatment on field performance of spring-planted silver birch container seedlings. Seedlings were sown on 18 June 1996 and subjected to short-day treatment from 29 July to 19 August 1996 or grown under natural photoperiod.

Variable	Source	df	MSE	F	P
Height at planting	Treatment	1	106	135	<0.001
	error	10	0.8		
Height at the end of the 1st year	Treatment	1	4.90	0.95	0.35
	error	10	5.15		
Height at the end of the 2nd year	Treatment	1	3	0.20	0.67
	error	10	15		
Height at the end of the 3rd year	Treatment	1	14	0.35	0.57
	error	10	40		
Diameter at planting	Treatment	1	0.04	1.89	0.20
	error	10	0.02		
Diameter at the end of the 1st year	Treatment	1	0.014	1.59	0.24
	error	10	0.009		
Diameter at the end of the 2nd year	Treatment	1	0.12	0.45	0.52
	error	10	0.27		
First-year height growth	Treatment	1	65	11	0.01
	error	10	6		
Second-year height growth	Treatment	1	16	1.38	0.27
	error	10	12		
Third-year height growth	Treatment	1	30	1.90	0.20
	error	10	16		
First-year diameter growth	Treatment	1	0.008	0.183	0.68
	error	10	0.042		
Second-year diameter growth	Treatment	1	0.22	0.79	0.40
	error	10	0.28		

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