Effects of seed origin and sowing time on timing of height growth cessation of Betula pendula seedlings

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Summary We studied the effects of seed origin and sowing time on height development and timing of height growth cessation of first-year silver birch (Betula pendula Roth) seedlings in a greenhouse experiment. Seeds of seven origins ranging in latitudes from 58° to 67° N were sown at 1–2-week intervals eight times from May 21 to July 30, 2001. The day/night temperature in the greenhouse was set at 20/10 °C, but lighting was natural and day length varied accordingly. Seedling height was measured twice a week. The interaction term between seed origin and sowing date was significant, but the pattern of height development and timing of growth cessation depended systematically on latitude of seed origin and sowing date. As seed origin became increasingly northern, growth cessation began earlier and resulted in shorter growth periods. Later sowing dates delayed growth cessation but also shortened the growth period. Final seedling height systematically decreased with increasingly northern origins and with later sowings. Linear regression analysis predicted timing of growth cessation, night length at growth cessation, length of growth period and final seedling height with high precision when the latitude of seed origin, origin and sowing time were predictor variables. The timing of seedling height with high precision when the latitude of seed origin, origin and sowing time were predictor variables. The timing of growth cessation, night length and developmental stage of the seedlings.

Keywords: annual rhythm, climatic adaptation, critical night length, growth period, photoperiod, silver birch, stage of development.

Introduction Trees native to northern latitudes survive seasonal changes and unfavorable wintertime conditions through well-timed acquisition of dormancy and frost hardiness. Cessation of height growth is the first visible component of the frost hardening process and is necessary for its further development (Weiser 1970). Changes in the photoperiod (i.e., an increase in night length) act as the predominant trigger for cessation of height growth (Fuchigami et al. 1982). The influence of the photoperiod on growth and dormancy of woody plants has been shown in a number of studies (Garner and Allard 1923, Wareing 1956, Nitsch 1957). The photoperiodic control of vegetative growth is particularly important in species with a free growth pattern, such as juvenile birches, which grow indefinitely under long days but stop growing and set terminal buds under short days (Nitsch 1957, Howe et al. 1996). The effect of photoperiod is determined by the length of the dark period and not by the length of the day (Nitsch 1957, Howe et al. 1996, Thomas and Vince-Prue 1997).

Cessation of vegetative growth in response to a decreasing photoperiod at the end of the growing season is an indirect adaptation to the seasonal change in temperature (Vaartaja 1959, Ekberg et al. 1979). Tree species having a wide geographic distribution have photoperiodic ecotypes that respond to different critical night lengths; northern ecotypes react to shorter critical night lengths compared with southern ecotypes (Sylvén 1940, Pauley and Perry 1954, Vaartaja 1954, 1959, Heide 1974, Ekberg et al. 1979). Håbjörg (1972a) demonstrated the existence of photoperiodic ecotypes in Betula pubescens Ehrh. and later in Betula pendula Roth among other Scandinavian tree and shrub species (Håbjörg 1978).

Photoperiodic regulation of growth cessation is modified by several external and internal factors. Temperature is the most important external factor (Dormling et al. 1968, Håbjörg 1972a, 1972b, Heide 1974, Koski and Selkämaa 1982, Koski and Sievänen 1985, Li et al. 2002), but soil water and water stress (Li et al. 2002), air humidity (Håbjörg 1972a) and nutrient availability (Landis et al. 1999) also affect the growth cessation of tree seedlings. Internal factors such as seedling size (Junttila 1976) and physiological stage of development may also affect the photoperiodic response of seedlings (Hari et al. 1970, Hari 1972, Luoranen and Rikala 1997, Landis et al. 1999, Luoranen 2000). A different selection of plants in growth chamber experiments may result in various photoperiodic responses and critical night lengths within a certain population (Junttila 1976, Håbjörg 1978, Ekberg et al. 1979). Precise characterization of birch seedling materials related to
growth cessation timing and critical night length is important to a variety of studies, such as gene mapping of photoperiodism and frost hardening, and nursery production of seedlings for forest cultivation (Luoranen 2000, Luoranen and Rikala 2001).

In this study, we examined how seed origin, sowing time and a naturally changing photoperiod affect the timing of growth cessation, length of growth period and final height of first-year silver birch (Betula pendula Roth) seedlings. An experimental design in which seeds of each origin were sown at different times during the summer yielded seedlings at different stages of development that were subjected to lengthening night conditions at the end of summer.

**Materials and methods**

*Seed origins*

Silver birch seeds were obtained from seven different latitudes (i.e., from locations differing in photoperiodic regime) (Table 1). The southernmost origin was from Viljandi in southern Estonia (58°10' N) and the northernmost from Kittilä in northern Finland (67°44' N). Each origin consisted of one selected stand. All stands originated from natural regeneration, except for Pudasjärvi, which was cultivated with seeds of local origin. At each stand, a mixture of seeds from open pollination of several mother trees was taken.

*Experiment*

A completely randomized factorial experiment with two fixed factors (eight sowing times and seven origins) was carried out in a greenhouse at Haapastensyrjä Tree Breeding Station, Loppi, southern Finland (60°37' N, 24°26' E) in 2001. The experiment was conducted under natural light and photoperiod during the growing season (Figure 1). All origins were sown eight times at 1–2-week intervals during the summer. The sowing dates were: May 21, June 4, 18, 25, July 2, 9, 16 and July 30. Each treatment (origin and sowing time combination) was replicated twice with 12 seedlings in each plot.

For the duration of the experiment, the greenhouse temperature was kept at 20 °C during the day (0800–1800 h) and 10 °C at night (2000–0600 h). The temperature changed during the intervening times at a steady rate of 5 °C h⁻¹. The temperature was recorded once a minute.

Seeds were sown in Kekkilä M6NCW40 peat in TAKO 1210 styrox trays (12 cavities tray⁻¹, 880 cm³ cavity⁻¹, 50 cavities m⁻², Metsä-Serla, Tampere, Finland). The peat had been treated with long-acting (40 days) Nutricote fertilizer and moisture retainer. After germination, seedlings were thinned to one per cavity. The seedlings were irrigated during the summer according to normal nursery practice.

*Measurements and statistical methods*

Seedling height was measured twice a week (Monday and Thursday). Measurements of the seedlings from the first sowing (May 21) began in the first week of July, and in the later sowings, soon after the seedlings had reached the upper surface of the seedling tray. Seedlings were measured until the height remained constant over three successive measurements (growth cessation). Height was measured from the upper surface of the seedling tray to the estimated growing point of the seedling. For statistical analysis, the mean value of 12 seedlings in the plot was the observation.

The response variables analyzed were the time of growth cessation, night length, heights of seedlings at growth cessation and length of growth period. Results were evaluated with a two-way analysis of variance. The equality of the effects of different sowing dates within origins and the equality of the effects of different origins within sowing dates were assessed with simple contrast F-tests. Regression models of response variables on seed origin and sowing date were determined by replacing the seed origin factor with the interval scale variable of latitude, and by replacing the sowing time factor with the interval scale time variable.

*Results*

**Seedling height development**

The general pattern of height development of the seed origins at different sowing times is shown in Figure 2, where seedling growth curves from the first (May 21), the fourth (June 25) and the last (July 30) sowing times are illustrated. The pattern depended clearly and systematically on the latitude of the seed origin and the sowing time.

Table 1. Silver birch seed origins and mean annual temperature sums of the original growing sites from 1965–1995.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (m)</th>
<th>Temperature sum (&gt; 5 °C, degree days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estonia, Viljandi</td>
<td>58°10' N</td>
<td>25°32' E</td>
<td>70</td>
<td>1430</td>
</tr>
<tr>
<td>Finland, Tuusula</td>
<td>60°27' N</td>
<td>24°58' E</td>
<td>50</td>
<td>1335</td>
</tr>
<tr>
<td>Finland, Ruovesi</td>
<td>62°03' N</td>
<td>24°15' E</td>
<td>100</td>
<td>1209</td>
</tr>
<tr>
<td>Finland, Viitasaari</td>
<td>63°14' N</td>
<td>26°07' E</td>
<td>140</td>
<td>1116</td>
</tr>
<tr>
<td>Finland, Pudasjärvi</td>
<td>65°14' N</td>
<td>27°36' E</td>
<td>130</td>
<td>957</td>
</tr>
<tr>
<td>Finland, Rovaniemi municipality</td>
<td>66°52' N</td>
<td>24°55' E</td>
<td>140</td>
<td>872</td>
</tr>
<tr>
<td>Finland, Kittilä</td>
<td>67°44' N</td>
<td>24°51' E</td>
<td>200</td>
<td>758</td>
</tr>
</tbody>
</table>

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Timing of height growth cessation and night length at growth cessation

Timing of height growth cessation varied according to seed origin and sowing time (both $P < 0.001$). Despite the interaction between seed origin and sowing time ($P < 0.001$), there was a systematic linear clinal pattern in the timing of growth cessation from southern to northern origins, as well as a linear trend from early to late sowing times (Figure 3). Generally, the more northerly the origin, the earlier the cessation of growth occurred, and therefore, the shorter the night length at growth cessation. However, a delay in sowing time delayed growth cessation into autumn, and thus increased the night length at the time of growth cessation within each origin. Growth cessation of seedlings from the first sowing (May 21) varied from August 20 (Kittilä) to September 19 (Viljandi), compared with September 11 (Kittilä) to September 25 (Viljandi) for seedlings from the last sowing (July 30).

Because of the interaction, differences in date of growth cessation among seed origins were greater for seedlings sown earlier than for seedlings sown later and decreased with sowing time ($P < 0.001$ in all sowings). The difference in date of growth cessation between the northernmost (Kittilä) and the southernmost (Viljandi) origins was 30 days for the first sowing and 14 days for the last sowing. Conversely, differences in date of growth cessation among sowing times were greatest for seedlings of northern origin and lowest for seedlings of southern origin ($P < 0.017$ within Viljandi, $P < 0.001$ within all other origins).

Length of growth period

The total length of the growth period from sowing to growth cessation varied according to the seed origin and sowing time, and there was a statistically significant interaction between these variables (all $P < 0.001$). The observed change in growth period length was systematic: increasingly northerly origins and later sowing times shortened the growth period (Figure 4). For the seedlings sown first (May 21), the growth period of Kittilä origin was 90 days and that of Viljandi origin was 121 days. For the seedlings sown last (July 30), the growth period of Kittilä origin was 43 days and that of Viljandi origin was 58 days.

Because of the interaction between origin and sowing date, differences among seed origins in length of growth period were largest within the earliest sowings and decreased as sowing date advanced ($P < 0.001$ within all sowings) (Figure 4). The difference in the length of growth period between the northernmost (Kittilä) and southernmost (Viljandi) origin was...
31 days for the first sown seedlings and 15 days for the last sown seedlings. Conversely, differences between sowing times in the length of growth period were the largest for seedlings of southern origin and decreased with increasing latitude \((P < 0.001\) within all origins) (Figure 4).

The temperature sum in the greenhouse accumulated linearly as a function of time. Thus, the temperature sums of the growth period (Table 2) produced the same pattern of variation as the length of the growth period (Figure 4). Increasingly northerly origins and later sowing times resulted in lower temperature sums accumulated during the growth period.

**Final height of the seedlings**

Heights of seedlings at the end of the experiment varied according to the seed origin and sowing time, and there was an interaction between seed origin and sowing time \((all P < 0.001)\). Final heights of the seedlings decreased in a curvilinear pattern as a function of the sowing time, and southern origin seedlings were always taller than northern origin seedlings (Figure 5). The seed origin differences in final height decreased with sowing time: the differences were significant \((P < 0.001)\) for the first five sowing dates, but not for the last two \((P = 0.334 and P = 0.989\), respectively). Sowing time had a significant \((P < 0.001\) within all origins) effect on the final seedling height of each origin.

**Regression models of timing of growth cessation, night length at growth cessation, length of growth period and final height of seedlings**

The dependence of timing of growth cessation (TGC; number of days from the beginning of the year) on the sowing time (ST; number of days from the beginning of the year) and the latitude of seed origin \((L; ^\circ)\) was evaluated by regression analysis. The second-order regression model:

\[
TGC = 418.04 - 1.073ST - 0.05088L + 2(0.02066ST)L
\]  

explained the variation in timing of growth cessation with high precision \((s\text{ (residual standard deviation)} = 3.11, R^2 = 0.92)\); and all coefficients were highly significant \((P < 0.001)\). According to the model (Equation 1), the timing of growth cessation increased by 0.24 days per 1-day delay in sowing date at the mean latitude \((63.39^\circ)\) and decreased by 2.78 day per 1° increase \((\approx 110\text{ km})\) in latitude of seed origin at the mean sowing date \((177.75\text{ days})\). The pattern of estimated regression surface was slightly curved because of the interaction (Figure 6).

The relationship between night length \((NL; h)\) and TGC was nearly linear \((r > 0.99)\), and thus NL and TGC contained practically the same information, but on different scales. The equation for night length at growth cessation was:

\[
NL = 25.78 - 0.0988ST - 0.004686 + (0.00190ST)L
\]  
in which \(s = 0.29\) and \(R^2 = 0.92\) and all coefficients were significant \((P < 0.001)\). Night length increased by 1.3 min for every 1-day delay in sowing date at the mean latitude and decreased by 15.4 min per every 1° increase in latitude of seed origin at mean sowing date (Equation 2).

The length of growth period (LGP), which is related to timing of growth cessation as \(LGP = TGC - ST\), is given by:

\[
LGP = 418.04 - 2.073ST - 0.05088 + 2(0.02066ST)L
\]  
in which \(s = 3.11\) and \(R^2 = 0.97\) and all coefficients were significant \((P < 0.001)\). The growth period shortened by 0.76 days for every 1-day delay in sowing date at the mean latitude and shortened by 2.78 days for every 1° increase in latitude of seed origin at mean sowing date (Equation 3).

Comparing Equations 1 and 3 shows that later sowing dates delayed growth cessation but shortened the growth period. The length of growth period describes the age of the seedlings at growth cessation. Thus, seedlings were younger at growth cessation when sown later. To summarize, the growth cessation process of first-year birch seedlings depended on the interaction between seed origin, night length and the developmental stage of the seedlings created by different sowing times.

The regression model for seedling final height \((H)\) at growth cessation was:

\[
H = 1787.40 - 10.678ST - 20.499L + 0.0098ST^2 + (0.0990ST)L
\]  
in which \(s = 4.64\) and \(R^2 = 0.96\) and all coefficients were significant \((P < 0.001)\). Unlike the other response variables, the dependence of seedling final height on sowing time was strongly curvilinear (Figure 5).
The pattern of height development and timing of growth cessation depended on the latitude of seed origin and sowing time. Among origins, there was a clinal trend from south to north. Our results are in accordance with growth chamber studies showing differentiation between tree populations in terms of the response to photoperiod and critical night length; northern populations have shorter critical night lengths compared with southern populations (Håbjørg 1972, 1978, Heide 1974, Ekberg et al. 1979). Because photoperiodic conditions change gradually with latitude, the critical night length and photoperiodic response of the tree populations also follows a gradual clinal pattern rather than being sharply ecotypic (Langlet 1959, Eriksson and Ekberg 2001). Sharp differentiation between birch populations is unlikely because of long-distance dispersion of pollen, wide overlapping flowering times of distant birch stands, wide dispersion of seeds and thus effective gene flow (Hjelmroos 1991, Luomajoki 1999, Eriksson et al. 2003). The clinal variation of growth cessation, as well as the negative correlation between timing of growth cessation and latitude, has also been reported in nursery and common garden experiments (Clausen 1968, Sharik and Barnes 1976, Velling 1979).

Critical night length for growth cessation is the minimum night length causing cessation of extension growth (Thomas and Vince-Prue 1997). We examined the night length in a naturally changing photoperiod at growth cessation, rather than the critical night length inducing growth cessation, making it difficult to compare our results with earlier growth chamber studies.

Håbjørg (1978) reported a mean profile of critical day length in relation to latitude in different tree species. From his results and Figure 1, we estimated the critical night length and its timing at Loppi (Figure 1) for the origins used in our study as: Viljandi 8.5 h (August 17), Tuusula 8 h (August 12), Ruovesi 7.5 h (August 6), Viitasari 7 h (July 31), Pudasjärvi 6 h (July 18), Rovaniemmi 5 h (June 29) and Kittilä 4 h (not reached at Loppi). In growth chamber studies, the length of the induction period from the beginning of short-day treatment to the cessation of height growth of birch seedlings varies from about 1 week to several weeks (Håbjørg 1978, Rinne et al. 1997). Faster responses to short-day treatments occur with

### Table 2. Mean temperature sums (> 5 °C, degree days) over the growth period for silver birch for each seed origin and sowing date in the experiment and the mean (1965–1995) annual temperature sum of their original growing sites.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Sowing date</th>
<th>Mean 1965–1995</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May 21</td>
<td>June 4</td>
</tr>
<tr>
<td>Viljandi</td>
<td>1386</td>
<td>1292</td>
</tr>
<tr>
<td>Tuusula</td>
<td>1248</td>
<td>1202</td>
</tr>
<tr>
<td>Ruovesi</td>
<td>1312</td>
<td>1155</td>
</tr>
<tr>
<td>Viitasaari</td>
<td>1221</td>
<td>1102</td>
</tr>
<tr>
<td>Pudasjarvi</td>
<td>1140</td>
<td>1043</td>
</tr>
<tr>
<td>Rovaniemmi</td>
<td>1075</td>
<td>978</td>
</tr>
<tr>
<td>Kittila</td>
<td>1042</td>
<td>967</td>
</tr>
</tbody>
</table>

![Figure 5: Mean final height of silver birch seedlings by seed origin as a function of sowing date.](image-url)

![Figure 6: Time of growth cessation of silver birch seedlings as a function of latitude of seed origin and sowing time.](image-url)
more northerly origins and shorter day lengths (Håbjørg 1978). Differences between the date of critical night length according to Håbjørg (1978) and our observed date of growth cessation for the first sowing (May 21) of the various origins were: Viljandi 33 days, Tuusula 26 days, Ruovesi 38 days, Viitasari 35 days, Pudasjärvi 40 days and Rovaniemi 53 days. The longer induction times of the northern origins in our study seem to contradict the results of Håbjørg (1978); however, they may be explained by differences in the stage of development between the origins, because northern origins reach their critical night length earlier than southern origins.

Timing of seedling growth cessation varied within an origin according to sowing date and no unambiguous night length at growth cessation could be shown for any origin. This is in accordance with the findings of Luoranen and Rikala (2001), who reported that, in a nursery experiment with one seed orchard seed origin of *B. pendula*, later sowing times caused poorer height growth and later growth cessation.

We used an experimental design in which seeds were sown at different times during the summer to obtain seedlings at different developmental stages that were then subjected to lengthening nights at the end of summer. The number of days between the attainment of the critical night length according to Håbjørg (1978) and the cessation of height growth in our study was, for sowings 1–8, 26, 35, 35, 40, 39, 43, 41 and 44 days for the Tuusula origin and 53, 58, 65, 68, 67, 69, 73 and 77 days for the Rovaniemi origin. Thus, delay in sowing delayed growth cessation following attainment of the critical night length. Growth and development of first-year nursery seedlings can be divided into different phases according to Landis et al. (1999), the main three being the establishment, rapid growth and hardening phases. The effects of environmental factors on a seedling vary in each phase (Landis et al. 1999), and therefore the response to the long night signal can differ (Luoranen 2000), which is in accordance with our results. Early sown seedlings were in the rapid growth phase and rather tall when they surpassed the critical night length, whereas seedlings from late sowings were small, having hardly reached the establishment phase and therefore were slower to respond to the critical night length.

In agreement with our observations, delay in sowing time delayed growth cessation of first-year silver birch seedlings in the studies of Koski and Selkäinaho (1982), Koski and Sievänen (1985) and Partanen (2004). These authors concluded that the timing of growth cessation was determined by a joint effect of night length and accumulated temperature sum, the higher the temperature sum, the shorter the night length required to induce growth cessation. We observed a similar interaction in our study (Table 2, Figure 3). However, in the simulation study by Hänninen et al. (1990) based on long-term meteorological data, a model based on night length predicted growth cessation more efficiently than a model based on a factor combining night length and temperature sum or temperature sum alone because of the year-to-year variation in temperature sum.

Sensitivity to night length increases with seedling development (Koski and Selkäinaho 1982, Koski and Sievänen 1985 and Partanen 2004). This relationship may be interpreted in two ways. First, the critical night length varies in relation to the stage of seedling development, as stated by Koski and Selkäinaho (1982) and supported by the results of Ekberg et al. (1979). Alternatively, the critical night length is unaffected by sowing date, but the speed of the response depends on the stage of seedling development. Junttila (1976) reported that in *Salix caprea* L. and *Salix pentandra* L., both species with a free growth pattern like birch, the rate of response to short day treatment in a phytotron depended on seedling size; small seedlings were slow to respond to short days, and the time from the start of short-day treatment to apical growth cessation decreased with increasing initial seedling size (Junttila 1976). This physiological difference between small and large seedlings may be related to leaf number and the production of inhibitors under short-day regimes (Wareing 1954, Eagles and Wareing 1963, 1964).

The final height of seedlings decreased curvilinearly in relation to sowing time, although the length of the growth period decreased linearly (Figures 4 and 5). Thus, differences in growth of seedlings from the different sowings are a result of differences in light conditions and in length of the growth period. Seedlings sown on the first three dates began their development during lengthening days, whereas the later sown seedlings faced shortening days from the beginning (Figure 1). The rate of shoot elongation increases rapidly with increasing photoperiod above the critical day length for shoot elongation (Junttila and Nilsen 1993). Thus, the curvilinearity in the relationship between height and sowing date was most pronounced in the seedlings of southern origins, which were grown during long days (Junttila and Nilsen 1993).

Our experiment was carried out in controlled conditions in a greenhouse, but the timing of growth cessation would presumably vary according to growth conditions. Temperature, in particular, has a strong modifying effect on the photoperiodic response. Different responses to photoperiod have been shown between low and high temperatures (Dormling et al. 1968, Håbjørg 1972α, Heide 1974, Li et al. 2002), between fluctuating and constant temperatures (Heide 1974) and between high and low night temperatures (Håbjørg 1972α, Heide 1974). Other environmental factors, like soil water and water stress (Li et al. 2002), air humidity (Håbjørg 1972α) and nutrients (Landis et al. 1999) may also affect growth cessation of seedlings. However, the role of seedling age in the regulation of growth cessation is poorly understood. This study was carried out with first-year birch seedlings that manifest free growth and are entirely controlled by environmental conditions of the given year (Landis et al. 1999). The growth of mature birches is partly predetermined (Kennedy and Brown 1984), and as trees mature, endogenous conditions are presumably increasingly important in regulating growth cessation (Junttila and Nilsen 1993, Thomas and Vince-Prue 1997).

In conclusion, height growth cessation of silver birch seedlings in the year of sowing is controlled by the interaction between night length and stage of seedling development and varies with seed origin. Thus, the use of a single value of a night length parameter is insufficient to characterize the
growth cessation of birch seedlings of differing seed origins, and the stage of seedling development should be integrated.

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