Growth Regulation and Cold Hardening of Silver Birch Seedlings With Short-Day Treatment

Jaana Luoranen and Risto Rikala

Doctoral student and senior research specialist, Finnish Forest Research Institute, Suonenjoki Research Station, Suonenjoki, Finland

Height growth and cold hardening of silver birch—Betula pendula *Roth—seedlings was controlled with short-day treatment at different times in summer. If the goal is both to retard height growth and to hasten cold hardening, the seedlings should be treated after midsummer; and if the goal is only to hasten cold hardening, seedlings should be treated in late summer. Earlier flushing in the spring following the treatment may expose treated seedlings to late spring frost and cold.* Tree Planters' Notes 48 (3/4): 65-71; 1997.

The production of silver *birch—Betula pendula* Roth—seedlings in Finland increased from 3.6 million to 16.0 million seedlings/year from 1980 to 1994, and the proportion of container seedlings from 5 to 78%/year (Aarne 1995). The target height of the container birch seedlings for outplanting is normally 50 to 80 cm (20 to 32 in). Due to different weather conditions, however, it is difficult to decide the right sowing time and growing schedule to obtain seedlings of this size. In warm summers, seedlings tend to grow too tall compared to the size of the container. Sometimes seedlings harden too late, and autumn frosts kill millions of seedlings.

Manipulation of the irrigation and fertilization regime (Landis and others 1989) and regulation of photoperiod (Landis and others 1992) have been used to retard height growth and to initiate hardening of container seedlings. The use of synthetic growth regulators has also been studied as a method of regulating height growth (Aphalo and others 1997; Weston and others 1980). The most promising method is the use of regulated photoperiod. In Canada and in Sweden, photoperiod control, also called short-day (SD) treatment, is used routinely in growing conifer seedlings. As far as we know, SD treatment has not been used in the production of deciduous seedlings. Nystrom (1992, 1993) presented preliminary results that showed that SD treatment of silver birch seedlings resulted in cessation of height growth. Photoperiod regulation may also have other consequences. In several conifers, early flushing after SD treatment has been reported (for example, Bigras and D'Aoust 1993; Dormling and others 1968). According to Grossnickle and others (1991), SD treatment also increased the root growth capacity of western hemlock—Tsuga *heterophylla* (Raf.) Sarg—seedlings at low root temperature, although there were no differences in the root growth capacity at the optimum root temperature.

The purpose of this study was to determine whether SD treatment could be used to stop excessive height growth and hasten hardening of birch seedlings at different times during summer without negative effects on further development after planting. The hypothesis was that SD treatments for 3 weeks stop height growth but not diameter or root growth of silver birch seedlings, and that SD treatment does not affect the root growth capacity the following spring.

Material and Methods

Silver birch seeds (seed orchard 379, M29-92-0001) were sown on peat-filled flats in a greenhouse at Suonenjoki Research Nursery, which is located at 62°39'N, 27°03'E, altitude 142 m asl (= above mean sea level) in Finland, on May 2. Germlings were pricked on May 22 to 26 to Plantek 25 trays (25 cavities/tray, 380 ml/cavity, 156 cavities/m^2; from Lännen Plant Systems, Finland) filled with fertilized sphagnum peat (Kekkilä, Finland). Seedlings were irrigated according to normal nursery practice by keeping the water content of peat at 30 to 60% by volume during the growing season. Seedlings were fertilized 7 times with liquid fertilizer. The total amount of nutrients (including basic and liquid fertilizers) given was 126 mg N, 56 mg P, and 156 mg K (plus micronutrients) per seedling. Seedlings were grown in a greenhouse until the plastic cover was removed on June 20. At the beginning of August, short-day (SD)-treated seedlings showed spores of birch *rust—Melampsoridium betulinum* (Pers.) Kleb. All seedlings were sprayed twice with triadimefon 0.05% (Bayleton 25), on August 3 and 17.

Short-day treatments (8 hours a day for the 3 weeks) were started on June 29 (SD 1), on July 17 (SD 2), and on July 31 (SD 3). The control treatment was natural day length (CO). The natural day lengths at the beginning and the end of SD-treatment periods are listed in table 1. Seedlings of SD 1 were treated by using a plastic hood

Table 1— *The dates, the heat sums (dd), and the natural day lengths (hours) at the beginning and the end of short-day (SD) treatment periods and at the time of height growth cessation*

Treatment	Begin			End			Growth cessation	
	Date	dd	ndl	Date	dd	ndl	Date	ndl
SD1	June 29	644	20:07	July	823	18:42	Aug 29	12:50
SD2	July 16	782	18:57	Aug 7	1107	16:58	July 26	09:14
SD3	July 31	968	17:41	Aug	1237	15:33	Aug 9	10:83
CO							Aug 16	11:37

Note: dd = degree-days; ndl = natural day length.

(1.5 mx1mx1 m, black inside, white outside, with a photon flux density 0.85 mmol/m^2/sec inside the hood under 1,700 mmol/m^2/sec of sunlight). Seedlings in SD 2 and SD 3 were moved to the greenhouse, where the seedlings were SD-treated automatically using a shade cloth (LS-100, a photon flux density 0.6 mmol/m^2/sec under 1,300 mmol/m^2/sec of sunlight). There were 8 trays with 25 seedlings in each treatment, giving a total of 32 trays and 800 seedlings. The accumulation of heat sum—threshold value > 5 °C (41 °F)—was calculated from the time of sowing. Heat sums at the beginning and the end of the SD treatment periods and at the time of cessation of height growth are presented in table 1.

Shoot lengths of the same randomly selected 8 seedlings/tray in all 8 trays were measured weekly from June 27, except SD 3 from July 5, to September 14. Cessation of growth was defined as the date when seedlings reached 95% of their final height. Leaf abscission, defined as when leaves had dropped, was recorded weekly.

In the autumn, after the leaf fall, 10 seedlings/treatment were harvested, and their heights and diameters at 2 cm (.8 in) above the container surface were measured. Stems and roots were separated, the roots were washed, and both roots and shoots were dried for 48 hours at 60 °C before weighing. Stems were pooled into 1 sample/treatment for the nutrient analysis. Nitrogen concentration was determined with a LECO CHN-600 analyzer (Leco Co, USA) and concentrations of P, Ca, K, Mg, Cu, and B were determined from dry-digested (2 M HCl) samples (Halonen and others 1983) using plasma emission spectrophotometric analysis (ICP, ARL 3800).

The procedures used for determination of water content were as described in Rosvall-Ahnebrink (1977) for conifer seedlings and Calmé and others (1995) for hardwood seedlings, but with slight modifications. The seedlings were watered a day before sampling. The uppermost 5 cm (2 in) of 10 randomly selected seedlings/treatment were cut in the morning between 7:30 and 9:00 a.m. Cut apices were put into plastic bags and weighed without leaves within 1 hour (fresh weight), then put into paper bags, dried in an oven for 24 hours at 105 °C (221 °F) and weighed again (dry weight). Water content was expressed as the ratio of fresh weight minus dry weight to fresh weight. Water content was determined weekly from July 19 to October 11.

The cold hardiness of the seedlings was determined using the relative conductivity method (or freeze-induced electrolyte leakage test) described in Aronsson and Eliasson (1970) with modifications. Cold hardiness was tested twice on August 28 and September 25. Fourteen randomly selected seedlings were sampled from each treatment. Each seedling was divided into 3 parts: from the uppermost 5 cm (2 in), water content was determined; the next 16 cm (6.3 in) was divided into two 8-cm (3.1-in)—long samples. Both of the latter samples were cut into four 1-cm (.4-in)—long pieces, which were washed in deionized water and put into 2 test tubes with 0.5 ml of water. There were 4 test tubes/test temperature per treatment: 2 upper and 2 lower parts of the stem in each. On the first testing date the samples were placed in air-cooled chambers to 0, –4, –8, –12, –15.5, and –19.5 °C (32, 24.8, 17.6, 10.4, 4.1, –3.1 °F) test temperatures, and on the second date to –6, –12, –18, and –24 °C (21.2, 10.4, –4, –11.2 °F) test temperatures. Temperatures in the freezers were lowered 6 °C /hour, kept 3 hours at the minimum, and then raised 6 °C (6.5 °F)/hour to 20 °C (68 °F). On both testing dates the control temperature was +4 °C (39 °F).

After freezing and return to 20 °C (68 °F), 11.5 ml water was added to the tubes, which were then shaken in a to-and-fro motion (110/min) for 22 hours at room temperature. The conductivity of the bathing solution was then measured in a radiometer (CDM 83, with a cell constant of 1.008 mS at room temperature). The samples were then held in a water bath at 90 °C (194 °F) for 20 minutes and retested. The second conductivity measurement was made after another 22-hour shaking period. The values for relative conductivity were calculated by dividing the conductivity after freezing by the conductivity after killing. Due to problems with measuring cold hardiness we could not calculate the LT_{50} values. According to the results of others (for example,

Aronsson and Eliasson 1970) the lower the relative conductivities, the hardier the seedlings.

On October 11, 10 seedlings/treatment were randomly selected for root growth capacity (RGC) tests and 32 seedlings/treatment for a planting experiment. These were packed in plastic bags and stored at —4 °C (24.8 °F) until February 19 when the RGC seedlings were thawed in darkness for 4 days at +5 °C (41 °F) followed by 4 days at +10 °C (50 °F). After thawing, the seedlings were planted in 0.75-liter plastic boxes filled with sand and randomized in a heated greenhouse (day/night temperature: +20 to +22 °C (69 to 71.6 °F)/ +15 °C (59 °F), with supplemental illumination of 150 pmol/m^2/sec from metal halide lamps (HQI-400W Power Star, Osram), photoperiod: 18/6 hours) for 4 weeks. The seedlings were hand-watered daily with tap water. At planting, the height of the shoot was measured. Flushing was checked weekly by measuring the length of the same 3 leaves of each seedling. On March 27, all seedlings were harvested, and their heights and diameters were measured. Roots that had grown out from the peat plug into the sand ("new" roots) were cut and washed, and the shoot was divided into stem and leaves. The roots in the peat plug ("old" roots) were separated from the peat. All plant parts were then dried in an oven for 48 hours at 105 °C (221 °F) and weighed.

On May 22, 8 seedlings from cold storage were planted in the field in a randomized block design (4 blocks, 8 seedlings/treatment/block). At planting and at the end of the first growing season, the heights and diameters were measured.

Height growth was analyzed using an MGLH procedure for repeated measurements in Systat 5.05. Before analysis of variance, all the measured morphological variables, except height to diameter ratio in autumn and height growth in the RGC test, were log-transformed to obtain normal distribution. Results shown in the figures and in the tables are back-transformed. Because the variances were unequal, the estimates of water content were analyzed using Kruskal-Wallis one-way analysis of variance. Frost hardiness and nutrients were not analyzed statistically because there were too few independent samples. Difficulties with the relative conductivity method will be discussed later.

Results

The first visible signs that seedlings responded to the SD treatments were that the uppermost leaves turned from reddish to pale green. All leaves turned dark green about 1 week after the end of the treatment period. In the autumn, the leaves of the SD 2 seedlings fell first (September 28) and the leaves of the SD 3 seedlings fell a week later. In the SD 1 treatments and control seedlings, the leaves did not fall until October.

In SD 2 and 3 treatments, the height growth of the seedlings stopped during the 3 weeks of treatment. In treatment SD 1 some seedlings continued their height growth later in August (table 1, figure 1). The earlier the blackout treatment, the shorter the seedlings ($P < 0.001$, figure la).

Early blackout treatment (SD 1) did not result in a difference in diameter and root growth as compared to the control. SD 2 and 3 treatment resulted in significantly smaller diameter and less root growth than SD 1 and control seedlings (table 2). The earlier the photoperiod was shortened, the lower was the height-to-diameter ratio and the shoot-to-root ratio (table 2). The regrowth of the SD 1 seedlings increased the variability in all variables measured compared to other treatments, except for dry mass of the roots in the SD 2 treatment.

Concentrations of N, P, K, Ca, Mg, Cu, and B were higher in the SD-treated seedlings (table 3). The nutrient concentration in control seedlings was only half that in the SD-treated seedlings.

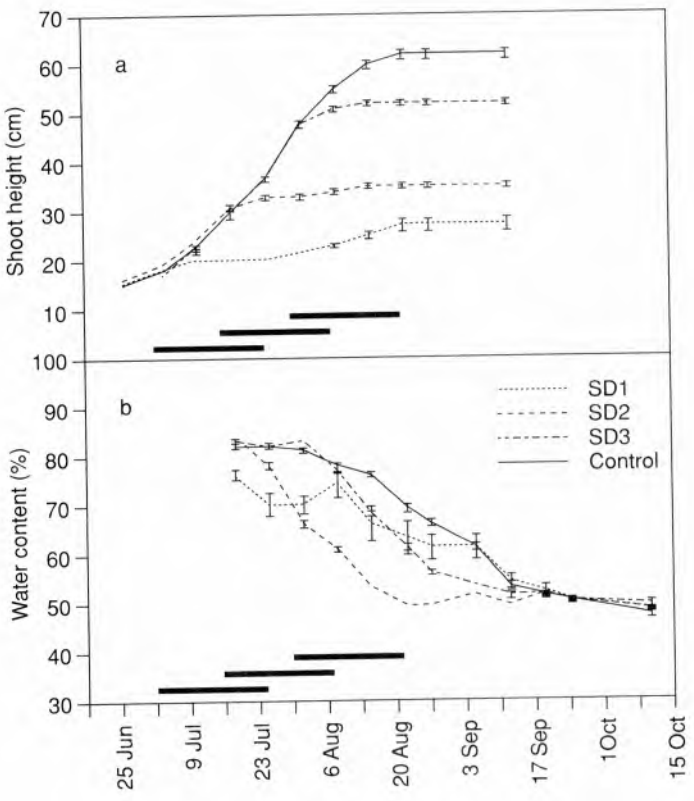

Figure 1— *Mean weekly height growth (a) and water content (b) of the uppermost 5 cm of silver birch seedlings in short-day (SD)—treated and control seedlings during the autumn 1995. The vertical bars are the standard errors of the means. Horizontal bars indicate blackout treatments.*

Table 2 —*Mean height, diameter, shoot, and root dry mass of 64 silver birch seedlings blacked out on different dates in 1995 (height-to-diameter and shoot-to-root ratios are also shown for 10 seedlings sampled in autumn)*

Treatment	Height (cm)		Diameter (mm)		Shoot (g)		Roots (g)		Height:diam		Shoot:root	
	mean	CV %	mean	CV %	mean	CV %	mean	CV %	mean	CV %	mean	CV %
SD 1	27 a	35	5.5 a	11	1.73 a	38	2.81 a	22	4.3 a	25	0.61 a	26
SD 2	34 b	11	4.2 b	7	1.45 a	15	2.30	24	7.9 b	13	0.65	19
SD 3	52 c	10	5.0 c	7	2.48 b	24	2.04 b	12	10.3 c	11	1.21 b	20
CO	62 d	11	5.8 a	7	4.05 c	15	2.83 a	12	10.9 c	7	1.44	17
P	<.001		<.001		<.001		<.001		<.001		<.001	

Note: CV represents coefficient of variation; differences in mean values followed by the same letter are not statistically significant at $P < 0.05$ as determined by Tukey's HSD test.

Table 3—*Nutrient concentration in harvested short-day-treated (SD 1, SD 2, & SD 3) and control (CO) stems; pooled samples are consisted of 10 seedlings*

Treatment	N (%)	P (g/kg)	K (g/kg)	Ca (g /kg)	Mg (g/kg)	Cu (mg/kg)	B (mg/kg)
SD 1	1.20	1.81	4.61	2.33	1.01	5.3	13.3
SD 2	1.61	2.38	4.79	3.00	1.50	5.9	12.3
SD 3	1.78	2.19	4.95	2.50	1.20	4.4	9.7
CO	0.84	1.39	4.24	1.79	0.79	2.4	6.7

Water content of seedlings decreased rapidly after the treatment period, especially in SD 2 (figure 1b). The regrowth of some SD 1 seedlings after the treatment period increased the water content. The water content of SD 2 and SD 3 seedlings reached a value of 55% within 2 weeks after the end of the SD treatments (August 16 and September 6, respectively), but the water content of SD 1 and control seedlings did not reached that value until September 20.

Cold hardiness of the seedlings differed between treatments (figure 2, results shown only for the test of August 28). On both testing dates, the SD 2 seedlings were slightly hardier than the SD 3 seedlings, and both were hardier than the control or the SD 1 seedlings. The SD 1 seedlings sampled to —16 °C (3.2 °F) temperature had low relative conductivity because they did not continue their height growth after blackout as did the seedlings sampled to other test temperatures.

The following spring the SD 1 and SD 2 seedlings flushed earlier in the RGC test than did the SD 3 or control seedlings. After 2 days in the greenhouse, all SD 1 and SD 2 seedlings and 80% of the control and SD 3 seedlings showed signs of flushing. However, during the first week in the greenhouse, the leaves of the control seedlings grew faster than those of the seedlings in other treatments. After 2 weeks, the leaves of the SD 3 seedlings were longer than those of other seedlings. The SD treatments decreased the root growth capacity of the seedlings, but increased the height growth the following spring (table 4). The earlier the seedlings were treated, the higher was their relative growth rate for height.

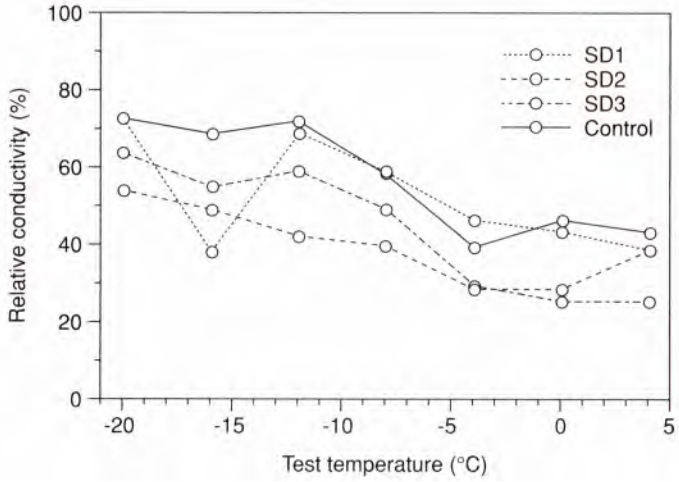

Figure 2— *Relative conductivity values after the first freezing test at 7 test temperatures on August 28-29, 1995. Lower values indicate greater cold hardiness. Each symbol is the mean of 2 independent samples (2 seedlings).*

Table 4—*Mean height increment and new root dry mass of 10 RGC-tested silver birch seedlings in spring 1996*

Treatment	Height increment (cm)		New roots (mg)	
	mean	CV %	mean	CV %
SD 1	18 a	18	17	95
SD 2	16 ab	23	12	145
SD 3	14 ab	27	20	45
CO	13 b	20	28	101
P	0.023		0.109	

Note: CV represents coefficient of variation and differences in mean values followed by the same letter are not statistically significant at $P < 0.05$ as determines by Tukey's HSD test

Seedlings with a high relative growth rate for height had a low relative growth rate for roots.

During the first growing season in the field, the SD 1 seedlings grew most; but the difference was significant only for the SD 3 seedlings, which grew least. On the other hand, the diameter growth of SD 3 was greatest (table 5). SD treatment increased the number of leaders per seedling. The earlier the seedlings were treated, the more seedlings there were with multiple leaders (table 5).

Discussion

SD treatments that ended August 7 (SD 2) and August 21 (SD 3) stopped height growth during the 3-week treatment periods. Treatment ending 20 July (SD 1) resulted in some seedling regrowth in August, at 3 weeks after treatment. Koski and Sievänen (1985) suggested that the behavior of the birch seedlings during their first growing season was a combined effect of heat sum and night length. According to Koski and Selkäinaho (1982), the effect of the photoperiod seemed to increase gradually and to be proportional to night length. Koski and Sievänen (1985) predicted that the different provenances of birch stop growth after the accumulation of about two-thirds of the total heat sum for an average growing season. The heat sum for the provenance used here is about 1,100 degree-days. When the SD 1 treatment began, the heat sum was 644 degree-days (table 1). For permanent dormancy, the heat sum should have been about 750 degree-days at the beginning of the treatment period. In the other SD treatments, the heat-sum requirements suggested by Koski and Sievänen were fulfilled.

The diameter of the seedlings was decreased in SD 2 and SD 3 treatments, but not in SD 1. According to Håbjorg (1972), the maximum diameter growth of *Betula pubescens* occurrs at a photoperiod of 18 hours. After the SD 1 treatment, the photoperiod was still long enough for diameter growth. The later height growth stopped, the higher was the shoot–root ratio. During growth, shoots and roots compete for the same photosynthates. The carbohydrates produced could be allocated more to root growth, when height growth was stopped by SD treatment but the photosynthesis continued.

Almost all the nutrient concentrations measured were higher in the SD-treated seedlings than in control seedlings. The results of Skre (1991) agree with these findings: nitrogen and phosphorous concentrations were higher in seedlings growing in short days than those growing in longer days. Due to earlier cessation of growth in SD treatments, seedlings could accumulate more nutrient in their tissues.

SD treatment increased the height increment of the shoot, but decreased the root growth the following spring. The SD-treated seedlings flushed earlier and grew more than the untreated seedlings. Early spring flushing and increased height growth after the SD exposure have been reported previously in conifers (Bigras and D'Aoust 1993; Dormling and others 1968; Odium and Colombo 1988). Dormling and others (1968) also showed that the day length and temperature during bud maturation of Norway *spruce—Picea abies* (L.) Karst.—decisively influence time of initiation of flushing. In addition, nitrogen concentration has been shown to cause flushing earlier in the next spring (Benzian and others 1974).

The poor capacity for root growth after SD exposure found here is opposite to the results of Grossnickle and others (1991). In their research, new root growth of western hemlock—Tsuga *heterophylla* (Raf.) Sarg.—seedlings was greater after short-day treatment than in long-day treatment. As far we know, the root growth capacity of SD-treated deciduous seedlings the following spring is not reported in the literature.

We can only speculate as to the reasons for the SD-treated seedlings having multiple leaders after the first growing season in the field. In March, none of the RGC-tested seedlings had unflushed buds, so the top buds of SD-treated seedlings were damaged after that. Due to earlier flushing, SD-treated seedlings were more susceptible to spring frost, insects, winds, etc. Krasowski and others (1993) reported that photoperiod length was asso-

Table 5—*Mean height, diameter, and height and diameter growth of 32 seedlings per treatment at the end of the first growing season in the field*

Treatment	Height (cm)		Diameter (mm)		Height growth (cm)		Diameter growth (mm)		Leaders	
	mean	CV-%	mean	CV-%	mean	CV-%	mean	CV-%	2	> 2
SD 1	49 a	28	6.6 a	12	24 a	35	1.6 ab	48	16	25
SD 2	52 a	18	5.8 b	8	19 ab	44	1.9 ab	20	22	16
SD 3	67 b	11	6.5 a	10	16 b	45	2.1 a	28	19	3
CO	80 c	12	6.9 a	11	20 ab	40	1.6 b	54	3	3
p	<.001	<0.001	0.004	0.016						

Note: Seedlings with 2 or more leaders are expressed as a percentage of all seedlings in a treatment. Differences in mean values followed by the same letter are not statistically significant at $P < 0.05$ as determined by Tukey's HSD test.

ciated with the number of unflushed terminal buds of seedlings of Engelmann and white spruce-Picea *engelmannii* Parry ex Engelm. and *P. glauca* (Moench) Voss-the shorter the photoperiod, the greater the number of unflushed terminal buds. In our experiments, the photoperiod was 8 hours, shorter than any photoperiod length in the experiment of Krasowski and others (1993).

Evaluation of the hardening of conifer seedlings by determining the water content in the top of the seedlings has become routine in nursery practice in Sweden (Dunsworth 1997). For deciduous species this method has been studied by Calmé and others (1995) in seedlings of yellow birch *(B. alleghaniensis* Britton) and red and bur oak *(Quercus rubra* L. and *Q. macrocarpa* Michx.). The present findings were similar to theirs: water content was lower in SD-treated seedlings. They suggested that a dry matter content higher than 45% (water content lower than 55%) corresponded to seedlings with LT_{50} of -10 °C (14 °F) or less. Due to difficulties in assessing cold hardiness, we could not compare the values of cold hardiness and water content.

Short-day treatments increased the cold hardiness of the seedlings, but because of difficulties in the method of assessment, we could not interpret the results precisely. Since the work of Dexter and others (1930, 1932), the relative conductivity method has been widely used to measure the degree of cold hardiness of different plant tissues. In woody species, this method has been used in both deciduous (for example, Deans and others 1995; Wilner 1960) and conifer trees (for example, Aronsson and Eliasson 1970). According to Deans and others (1995), killing tissues in boiling water is not an effective method for destroying the cell membranes, and the incubation time should be at least 5 days. Because of the method we used, the conductivity values of the killed samples were only partial and the relative conductivities were higher than they would have been if all membranes had been destroyed. Unpublished data of the first author indicate that the leakage from hardened tissues is slower than that from unhardened tissues. This could explain the lower relative conductivities of SD 2 samples compared to the conductivities of the other samples.

Conclusion

Short-day treatment is a promising method for control of height growth and cold hardening of silver birch seedlings. In order to succeed in SD treatment, nursery managers should treat seedlings after two-thirds of the average heat sum for the provenance used is accumulated but before the critical night length is achieved naturally. SD treatment does not affect seedling growth after planting, but could cause multiple leaders in seedlings during the first growing season in the field. More studies are needed to determine the suitable length of treatment and examine the possibility of using a photoperiod that is longer than 8 hours or is gradually shortened.

Address correspondence to: Jaana Luoranen, Finnish Forest Research Institute, Suonenjoki Research Station, Juntintie 40, FIN-77600 Suonenjoki, Finland; email: » Jaana.Luoranen@metla.fi «

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