ARTICLES

Timing and Duration of Short-day Treatment of *Picea abies* Seedlings

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The effect of duration (1 to 4 weeks) and starting date (July 1 to August 28) of short-day (SD) treatment on first-year Norway spruce (*Picea abies* (L.) Karst.) container seedlings were studied. Shoot height, stem diameter, terminal bud formation, frost hardiness and the following spring bud burst were monitored and field performance was determined during the three growing seasons after planting. The earlier the SD treatment was started, the shorter the seedlings were in autumn. Duration of treatment did not affect seedling height, but with four-week treatments started on 1 and 10 July the diameter was reduced as compared to other duration treatments. In all SD treatments, frost hardiness improved; but it increased most when the duration was extended from one to four weeks. The following spring, the longer the duration of treatment was and the earlier the treatment was started, the earlier the terminal buds burst. Neither the timing nor duration of SD treatment affected the field performance of seedlings.

Key words: blackout, bud burst, bud formation, duration, field performance, frost hardiness, Norway spruce, shoot growth, short-day treatment, timing

Introduction

In Fennoscandia, the number of nurseries is reducing and thus the production volume and the range of provenances grown in a nursery are increasing. This means that seedlings of southern provenances may continue growing too late in autumn, their frost hardening may be delayed and the seedlings are susceptible to autumn frosts. The increase in night length is one of the most important environmental factors leading to cessation of height growth and hardening of Norway spruce (*Picea abies* (L.) Karst.) seedlings (Dormling *et al.* 1968). In Finland, controlling the growth and frost hardening of Norway spruce seedlings by short day (SD) (long night) treatment have been carried out between the middle of July and the end of August, the duration of treatment usually being three weeks. In order to utilize the same blackout equipment for several seedling crops, however, nurseries need to enlarge the operation time of the equipment. In addition, seedlings planted in late summer and early autumn are sensitive to early autumn frosts and to avoid frost damage SD treatments should be started earlier. Although the effects of SD treatment on the growth and frost hardening of Norway spruce seedlings have been studied largely (e.g. Dormling *et al.* 1968, Heide 1974a,b, Rosvall-Åhnebrink 1977, 1982, Christersson 1978), the effects of duration and timing of SD treatment have been studied slightly (Rosvall-Åhnebrink 1982). Results for other spruce species and in other conditions cannot always be applied in the conditions in Fennoscandia. In general, the longer the duration of SD treatment, the better is the frost hardiness of spruce species (Hawkins & Draper 1991, Coursolle *et al.* 1998). After a too short SD treatment, there is also the risk of reflushing of seedlings in late summer (D'Aoust 1981, Heide 1974a). According to Eastham (1991), in spite of the timing of treatment of Sitka × white spruce (*Picea sitchensis* (Bong.) Carriere) × *Picea glauca* (Moench) Voss) seedlings reflushed after a treatment period only lasting for one week. Timing of SD treatment may also affect seedling growth in the next year. This effect is based on the number of needle primordia formed in the bud (Junttila & Skåret 1990), which is affected by the temperature during bud formation (Pollard & Logan 1977).

Our objective was to investigate the effect of timing and duration of SD treatment on the growth, frost hardiness and field performance of Norway spruce container seedlings. In particular, we searched for answers to the following questions: (i) Is it possible to start SD treatment at the beginning of July in order to stop height growth and start the hardening? (ii) Does SD treatment started at or after the middle of August affect frost hardening? (iii) Is one-week SD treatment long enough to stop height growth and hasten frost hardening?

Materials and methods

Seedling material and treatments

Norway spruce (*Picea abies* (L.) Karst.) container seedlings were grown at Suonenjoki Research Nursery (62° 39' N, 27° 03' E, altitude 142 m a.s.l.) in central Finland. Seeds from the seed orchard for central Finland (62° N) were sown on April 24, 1998 into hard plastic trays (Plantek 81F trays, 81 cavities per tray, 85 cm^3 per cavity, 549 cavities per m^2, Lännen Plant Systems, Finland) filled with base-fertilized sphagnum peat (Vapo E, Finland). Trays were placed onto a plastic pallet 10 cm above the ground and grown in a greenhouse without artificial lights and heating. A net that reduced solar radiation by 30 % shaded the greenhouse. Seedlings were fertilized six times with liquid fertilizer from June 9 to July 28 (0.1-0.2 % solution; Super 9, Kekkilä Co, Finland). The total amounts of nutrients (base- and liquid fertilizers) given were 22 mg N, 8 mg P and 23 mg K per seedling. During the growing season the electrical conductivity of the peat-water extract varied between 0.6 and 1.3 mS cm^{-2}, declining towards autumn. The seedlings were grown in a greenhouse until the beginning of the SD treatments, when 42 container trays were moved under the blackout frame. Control seedlings were kept in a greenhouse until the middle of October 1998.

SD treatments were provided by extending the night length automatically by using a blackout curtain (LS-100, Ludvig Svenson, Sweden) that gave a photon flux density of 0.6 μmol m^{-2} s^{-1} under full day light (1300 μmol m^{-2} s^{-1}). For treatments starting before August 27 the night length was 14 hours (from 1730 h until 0730 h) then afterwards 16 hours (from 1530 h until 0730 h). The total number of treatments with different timing and duration was 21 (Table 1). Each treatment consisted of two randomly selected container trays (162 seedlings). During the SD treatment, the temperature was monitored at seedling level (about 10 cm above ground) with an HMP131Y sensor (Vaisala Oy, Finland). Accumulated temperature sum (degree days (d.d.), threshold value +5°C) at the beginning of each treatment and daily mean temperatures during the treatments are presented in Table 1. After each SD treatment, the seedlings were moved to an outdoor hardening area for the autumn and winter.

Table 1. Timing and duration of SD treatments, accumulated temperature sums (d.d.) at the beginning of each treatment and average daily temperatures during the three-week treatment periods under blackout curtains

SD treatment		Temperature sum	Daily temperature		
Beginning date	Duration weeks	d.d.	mean	min °C	max
1 July	2, 3, 4	690	18.2	13.5	23.3
10 July	1, 2, 3, 4	852	17.3	13.9	21.1
20 July	2, 3, 4	992	16.1	12.8	19.0
30 July	1, 2, 3, 4	1149	14.5	10.6	18.4
10 August	2, 3	1301	12.9	9.9	15.8
19 August	1, 2, 3	1412	12.2	9.6	16.4
28 August	2, 3	1500	12.8	9.6	17.4

Tests and measurements

Frost hardening of seedlings was determined by exposing whole seedlings to –8° C on September 2, 1998 and –10°C on September 22, 1998 in an air-cooled chamber (Weiss, type Bioclim1600 Sp-Pa-S, Lindenstruth, Germany). For the first freezing test on 2 September, all SD-treatments started between July 1 and August 10 and also the one- and two-week treatments started on August 19 were included. For the second freezing test, on September 22, only the treatments with injured seedlings in the first exposure as well as the three-week SD treatment started on August 19 and two- and three-week treatments started on August 28 were included. On each test date, 20 randomly sampled seedlings in each selected treatment were put into Plantek 81F trays, which were then placed in wooden boxes. The boxes were insulated with sawdust (cover) and polystyrene (bottom) to protect the roots from freezing. The temperature in the chamber was lowered 5°C per hour, kept three hours at the minimum temperature and then raised 5°C per hour to 20°C. After exposure, the seedlings were transferred to the greenhouse (day/night temperature 20/15 °C, supplementary light 400 μmol·m^{-2}·s^{-1} from high-pressure sodium lamps, photoperiod 8 hours). The seedlings were watered daily with tap water. After two weeks, freezing injuries were assessed visually by estimating the proportion of browned needles in each seedling in 10% classes.

The shoot length of ten seedlings in each treatment was measured at the start of each SD-treatment and on September 8, 1998. Terminal-bud formation on the same 10 seedlings was monitored visually twice a week from the start of each SD treatment until September 8. A terminal bud was considered to have formed when it was visible. For each treatment, it was considered that buds had formed when the terminal bud was visible on 50% of the seedlings. Final height and stem diameter of the same seedlings were measured on October 20. The seedlings were overwintered under the snow cover. In the spring of 1999, bud burst in 10 randomly selected seedlings was monitored every second day from May 5 until June 14. Buds were classified as burst when individual needle tips were visible. Unflushed buds were split on June 16 and examined with a microscope.

Planting experiment

In the spring of 1999, 60 seedlings from 11 treatments were randomly selected for the planting experiment. The treatments included in this experiment were all the three-week SD treatments, the one-, two- and four-week treatments started on July 10 and the control seedlings. The experiment was a randomised block design with four blocks. Fifteen seedlings per treatment were planted in each replication on the nursery field in Suonenjoki on May 15. After planting, bud burst of 20 seedlings (5 seedlings in each block) in each treatment was monitored every second day by using the same classification as that used in the nursery. Shoot height was measured after planting and at the end of the 1st, 2nd and 3rd growing seasons.

Statistical analysis

To analyse the differences in height and diameter between treatments after nursery phase, each seedling was assumed to be an independent observation although they grew together in two container trays. For each starting dates of SD-treatments, differences in height and stem diameter between duration treatments were tested by paired samples t-test in SPSS 10.0 for Windows. In the planting experiment, block means of height growth in each treatment was calculated and differences between treatments were analysed using one-way analysis of variance by the following statistical model:

$$Y_i = \mu + T_i + \varepsilon_i$$

where Y_i is an observation in the i^{th} treatment (i = SD treatment in planting experiment), μ is a general mean, T_i is the effect of the i^{th} treatment, and ε_i is the variation within treatment.

Results

Growth and hardening in nursery

The earlier the SD treatment was started, the shorter the seedlings were in autumn. In the treatments started in July, seedlings grew about 2 cm after the beginning of the treatment, whereas seedlings treated in August exhibited no height growth after the start of treatment (Table 2). Treatment duration (from one to four weeks) did not affect seedling height. Thus one week of treatment was enough to stop height growth. The earlier in July the SD treatment was started, the faster the buds formed (Table 2). On the other hand, buds formed earlier on seedlings in the SD treatments started on August 10 and 19 than on seedlings in the treatment started on July 30 (Table 2). In the former case, bud formation probably had already started in the greenhouse before SD treatment. Control seedlings grown in the greenhouse formed terminal buds before August 31. Reflushing of terminal buds was not observed in the seedlings of any treatment.

Table 2. Timing and duration of SD treatments, height and stem diameter of seedlings (mean and standard error) on 20 October 1998, height growth and duration of terminal bud formation (50 %) after the beginning of SD treatment of one-year-old Norway spruce container seedlings. Different letters after the means indicate differences (p<0.05) in height and diameters between different durations for each starting date, analyzed by paired samples T-test (N=10)

SD treatment		Heigth of seedlings cm		Stem diameter mm		Height growth cm	Terminal bud formation	
Beginning Date	Duration Weeks	Mean	SE	Mean	SE		Days	Date
1 July	2	8.5a	0.3	1.7a	0.06	1.8	19	20 July
	3	9.2a	0.6	1.8a	0.08	2.2	22	23 July
	4	9.2a	0.6	1.5b	0.06	2.1	22	23 July
10 July	1	13.6a	0.4	2.0a	0.05	2.9	24	3 Aug
	2	12.1b	0.7	1.8b	0.03	2.3	24	3 Aug
	3	11.5b	0.4	1.8b	0.05	2.2	27	6 Aug
	4	11.2b	0.4	1.4c	0.06	1.9	27	6 Aug
20 July	2	14.2a	0.5	1.8a	0.09	1.7	21	10 Aug
	3	15.4a	0.5	1.8a	0.07	2.0	28	17 Aug
	4	15.0a	0.8	1.7a	0.06	1.9	35	24 Aug
30 July	1	17.2a	0.7	1.8a	0.05	1.0	35	3 Sep
	2	17.1a	0.8	1.8a	0.12	0.9	42	10 Sep
	3	18.1a	0.6	2.0a	0.07	0.8	35	3 Sep
	4	17.4a	0.9	1.8a	0.09	0.8	35	3 Sep
10 Aug	2	18.3a	0.8	2.0a	0.09	0.3	17	27 Aug
	3	18.3a	0.9	2.1a	0.08	0.5	14	24 Aug
19 Aug	1	17.1a	0.9	2.1a	0.06	0.1	5	24 Aug
	2	18.9ab	0.6	2.0a	0.08	0.3	5	24 Aug
	3	20.0b	0.6	2.1a	0.05	0.3	5	24 Aug
28 Aug	2	18.2a	0.6	1.8a	0.05	0.3		28 Aug
	3	18.0a	0.7	2.0a	0.09	0.3	3	31 Aug

Means followed by the same letter are not significantly different (p<0.05).

Stem diameter differed only slightly among different starting dates of SD treatments in July, although there were large differences in the height of the seedlings (Table 2). Duration of treatment affected stem diameter only if the SD treatment was started on July 1 or 10. The seedlings treated for four weeks were thinner than these treated three weeks or less (p<0.05). The earlier in July the SD treatment was started, the sturdier the seedlings were in autumn.

The seedlings in all SD treatments had greater frost hardiness than the control seedlings did (Fig. 1a). The timing of SD treatment from July 1 to August 10 did not cause differences in the frost hardiness of seedlings at the first test date on September 2. On the other hand, when duration of SD-treatment was extended from one to four weeks, frost hardiness increased. If the control seedlings were excluded, the most serious needle damage was observed in the seedlings given SD treatment for two weeks beginning on August 19 (Fig. 1a).

Three weeks later, in the second freezing test on September 22, the differences between treatments disappeared and all the seedlings survived at −10 °C. The control seedlings were hardened even more than the seedlings treated with SD for one or two weeks (Fig. 1b).

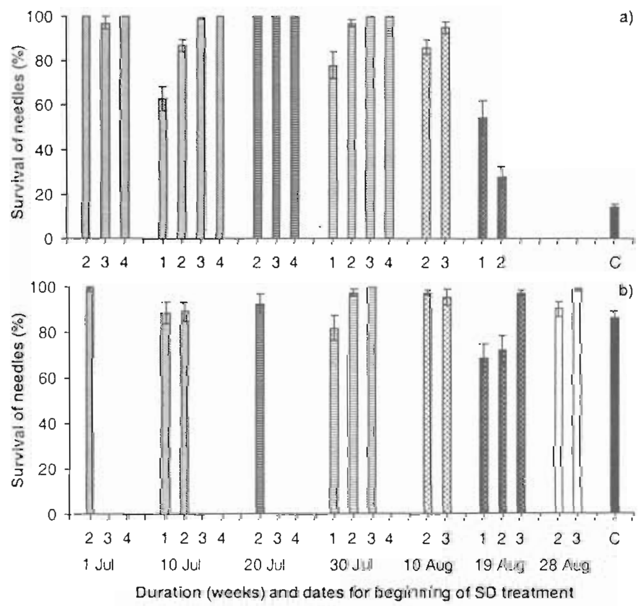

Figure 1. Survival of needles measured as the proportion of green, undamaged needles of SD-treated and control (C) Norway spruce seedlings after exposure to a) −8°C on 2 September or b) −10 °C on 22 September. The seedlings from treatments started in August or those, which damaged in the first test, were exposed to the second test (−10 °C) on 22 September. Each bar represents the mean of 20 seedlings, and vertical lines indicate the standard errors of the means

Bud burst and field performance

The longer the duration of SD-treatment was and the earlier the treatment was started, the earlier the buds burst in the next spring (Fig. 2ab). One-week SD treatment did not affect bud burst. Buds of seedlings in three- and four-week of SD treatments, started on July 1 or July 10, resulted four to five days earlier bud burst compared to the control seedlings. Buds of the seedlings for which SD treatment started on August 10 and 19 burst later than buds of the control seedlings. The latest SD treatment (started on August 28), however, did not affect the timing of bud burst (data not shown). Bud burst of the planted seedlings was similar to those kept for monitoring in the nursery.

About 7 percentage of the terminal buds were damaged and did not burst in seedlings kept in container trays (Fig. 2ab) or planted on field. Most of the unburst buds were found in those SD treatments with the earliest bud burst. The new leader shoots of the seedlings with unburst terminal buds usually grew from the uppermost lateral buds. Microscope examination revealed that under the bud scale the needle primordia of these buds were tanned.

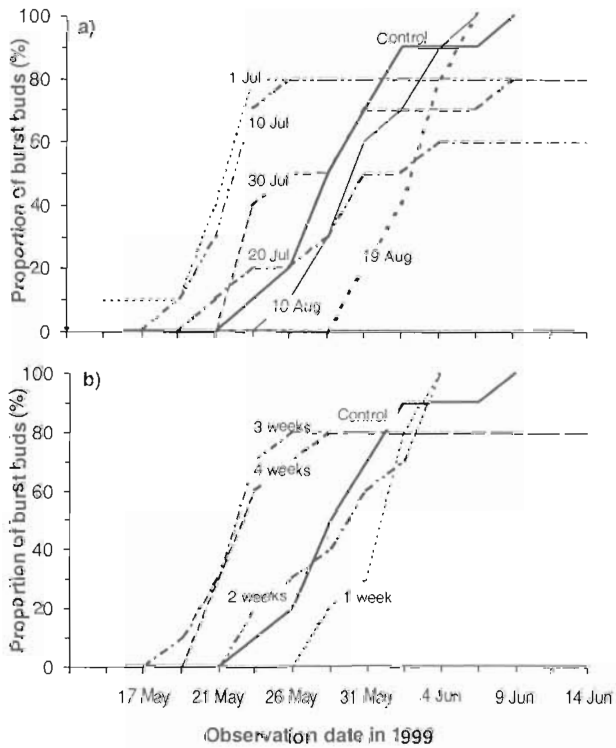

Figure 2. Burst of the terminal buds of SD-treated Norway spruce seedlings the following spring from treatment. a) Bud burst of three-week SD-treated seedlings in treatments started ten days intervals compared to bud burst of control seedlings. b) Bud burst of seedlings treated for one, two, three and four weeks started on 10 July compared to control seedlings. Bud burst was monitored from ten seedlings in each treatment

Neither the timing nor the duration of SD treatments affected the growth of first (p=0.11), second (p=0.28) or third (p=0.91) year shoots after planting. The differences in shoot height remained (p=0.06); and three years after planting the shortest seedlings were those for which SD treatment began on July 1 (Fig. 3). Survival of seedlings did not differ between treatments.

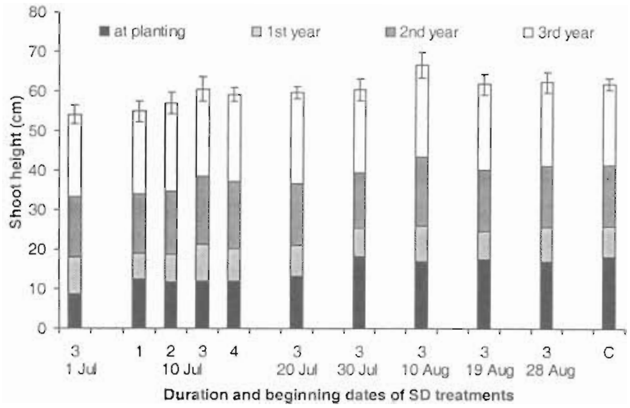

Figure 3. Shoot growth of SD-treated and control (C) Norway spruce seedlings during the three years after planting in the field. Seedlings of all three-week treatments from all starting dates and seedlings of the treatment started on 10 July were planted. Each bar represents the mean of four blocks (15 seedlings in each), and the vertical lines indicate the standard errors of the means

Discussion

Timing and duration of SD treatment

The timing of SD treatment affected the time required for bud formation. The earlier in July the SD treatment was started, the faster the buds formed (Table 2). Our study was not repeated in several years and the result may be affected by weather conditions in the study summer. Responses of seedlings in our study were quite similar as has been in other researches. It is known that the size of the buds and the time required for forming buds are dependent on the temperature during and after SD treatment (e.g. Pollard & Logan 1977). The temperature during bud formation affects the number of needle primordia (Dormling *et al.* 1968, Pollard & Logan 1977). For example, bud formation of black spruce (*Picea mariana* (Mill.) B.S.P.) and white spruce seedlings was faster at 25°C than at 20 or 15°C (Pollard Logan 1977). In Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) bud formation was speeded up only if the temperature during the eight-hour photoperiod exceeded 15°C (Timmis & Worrall 1975). In our study the daily average temperature was, as usual, lower in August than in July (Table 1), which, independing of SD treatments may have retarded bud formation. Accordingly, buds formed earlier on the control seedlings in the greenhouse than on seedlings SD treated from July 30 that grew outside at lower temperatures after treatment (Table 2).

According to Koski and Sievänen (1985), height growth of Norway spruce could be stopped by critical night length, when about two-thirds of the average temperature sum (threshold value +5°C) of the provenance used has accumulated. At the beginning of the first SD treatment (started on July 1) the accumulated temperature sum in the greenhouse was 690 d.d., which was 57 % of the average temperature sum (1199 d.d.) of the provenance. Thus, the growth cessation and frost hardening of these seedlings caused by SD treatment would not have been possible if the dates used had been earlier in June.

The duration of SD treatment did not affect the final height of seedlings, but even one week of treatment induced cessation of height growth (Table 2). This result supports the observation of Hawkins and Draper (1988, 1991) that duration of treatment from two to six weeks did not affect the height of seedlings of white, Engelmann (*Picea engelmannii* (Parry) Engelm.) or white × Sitka spruce. According to Eastham (1991), however, if SD treatment was extended from one week to three weeks, the final height of Sitka x white spruce seedlings was reduced.

The longest (four-week) SD treatments, which started on July 1 and 10, decreased the stem diameter of the seedlings (Table 2). Eastham (1991) also found that growth of the stem diameter of Sitka × white spruce seedlings was reduced when SD treatment was extended from one week to three or four weeks. Decreased diameter growth caused by extended SD treatment might be due to shorter daily photosynthetic production period.

The problem caused by too early and short SD treatment has been considered to be the risk of terminal buds reflushing in late autumn. According to Eastham (1991), one week of SD treatment caused reflushing of Sitka × white spruce; the earlier the treatment, the more buds reflushed. Eastham also noted that reflushing can vary from year to year. In our study, reflushing was not observed. The summer of 1998 was, however, cooler than normal. In 2001, in another experiment, one week SD-treatment started on July 6 caused 5 % reflushing (Konttinen 2002). In order to be certain that all buds become dormant and no reflushing appears after the treatment, SD treatment should last at least two (Eastham 1991) or three weeks (Dormling 1990ab).

The main objective of SD treatment is to hasten hardening of seedlings. Thus to ensure that seedlings have enough time to harden before the first frosts in the beginning of September, SD treatment should be started before mid-August. In this study, seedlings in all SD treatments that started before the middle of August tolerated –8°C at the beginning of September (Fig. 1a).

In the two-week treatment started on August 19 the needle damage caused by exposure to –8°C at the beginning of September was almost as serious as in the control seedlings (Fig.1a). The most probable reason for this was that the treatment ended on the day of the freezing test and although one- or two weeks treatment is enough to start the hardening, seedlings need one or two weeks more time to harden enough to tolerate -8...-10°C (Colombo *et al.* 1982, 1989, Coursolle *et al.* 1998). In the four weeks SD-treatment the seedlings harden already during the treatment (Colombo et al 1982).

If the aim of the treatment is to decrease the risk of frost damage occurring after the middle of September, shorter than three-week SD treatments are not beneficial, because control seedlings kept in the greenhouse in September also hardened rapidly (Fig.1ab).

Bud burst and shoot growth after planting

SD treatment has been found to cause earlier bud burst in Norway spruce seedlings during the following spring (e.g., Rosvall-Åhnebrink 1980). The stronger the SD treatment (the longer night length and duration) is, the earlier the buds burst (Hawkins & Draper 1991, Bigras & D'Aoust 1993, Krasowski *et al.* 1993). In our study, three-week SD treatments started before mid-July promoted bud burst, while one- and two-week SD treatments did not hasten it (Fig. 2ab). Surprisingly treatments started on August 10 or 19 delayed bud burst the following spring compared to control seedlings (Fig. 2a).

Some of terminal buds of SD-treated seedlings did not burst at all in the spring, which also has been observed in other studies (Rosvall-Åhnebrink 1977, Hawkins & Draper 1991, Odlum 1992, Krasowski *et al.* 1993). In our study, the reason for unflushed buds was probably the weather in the spring. April 1999 was exceptionally warm and buds of SD-treated seedlings started to grow (swell). In the first half of May for nine nights the temperature at ground level dropped between –5 and –10°C, which injured the swollen buds. Most of the unburst buds appeared in those SD treatments with the earliest bud burst (Fig. 2ab). Buds of SD-treated seedlings have a greater risk of damage during spring frost; and the stronger the SD treatment is, the greater the risk (Rosvall-Åhnebrink 1977, Krasowski *et al.* 1993).

Any of SD treatments did not affect the height development of seedlings after planting (Fig. 3). SD treatment may increase height growth after planting (Odlum and Colombo 1988, Eastham 1991, Odlum 1992, Hawkins *et al.* 1996), but this has not been found in many studies (for example, Rosvall-Åhnebrink 1982, Krasowski *et al.* 1993). According to Eastham (1991) Sitka × white spruce seedlings treated earlier grew better than the seedlings treated later. The greater height growth of SD-treated seedlings may be due to the higher temperature during bud formation of SD-treated seedlings compared to the lower temperatures during the later bud formation of untreated seedlings.

Conclusions

One-week SD treatment was enough to stop the height growth of Norway spruce seedlings. One-week treatment in July and at the beginning of August increased frost hardiness by the beginning of September, but only the three-week treatment increased frost hardiness in September compared to the control seedlings. Late SD treatments, started after the middle of August, affected very little frost hardiness. Long, three- or four-week SD treatment in the beginning of July caused earlier bud burst the following spring and exposed seedlings to early spring frost. Neither timing nor duration of SD treatment affected shoot growth of seedlings after planting. In order to hasten the frost hardiness of first-year Norway spruce seedlings (sown in the greenhouse at the end of April), SD treatment can be started any time from the beginning of July to August 10 and should last from two to three weeks.

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ВЛИЯНИЕ СРОКОВ И ПРОДОЛЖИТЕЛЬНОСТИ ЭКСПОЗИЦИИ КОРОТКОГО СВЕТОВОГО ДНЯ НА КУЛЬТУРЫ ЕЛИ *PICEA ABIES*

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Резюме

Изучалось влияние продолжительности (от 1 до 4 недель) и срока начала (с 1 июля до 28 августа) экспозиции короткого светового дня (КСД) на однолетние культуры ели европейской (*Picea abies* (L) Karst.). Были изучены: высота побега, диаметр ствола, формирование верхушечного побега, морозоустойчивость и последующее распускание верхушечного побега; также были рассчитаны ростовые характеристики в течение трех сезонов роста после посадки. Чем раньше проводилась экспозиция КСД, тем ниже была высота культуры осенью. Длительность экспозиции не влияла на высоту культуры, однако при четырехнедельной экспозиции, начатой 1 и 10 июля, диаметр был меньше, чем при экспозиции другой продолжительности. Во всех случаях экспозиция КСД повысила морозоустойчивость, однако, больше всего это проявилось при продлении экспозиции от одной до четырех недель., чем длительнее была экспозиция, и чем раньше она начинался, тем раньше следующей весной распускался верхушечный побег. Ни сроки, ни длительность экспозиции КСД не повлияли на ростовые характеристики культур.

Ключевые слова: затемнение, распускание верхушечного побега, формирование верхушечного побега, длительность, ростовые характеристики, морозоустойчивость, ель европейская, рост побега, экспозиция короткого светового дня, сроки