SILVA FENNICA

Silva Fennica 43(2) research articles

www.metla.fi/silvafennica · ISSN 0037-5330
The Finnish Society of Forest Science · The Finnish Forest Research Institute

Frost Hardening and Risk of a Second Flush in Norway Spruce Seedlings after an Early-Season Short-Day Treatment

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Luoranen, J., Konttinen, K. & Rikala, R. 2009. Frost hardening and risk of a second flush in Norway spruce seedlings after an early-season short-day treatment. Silva Fennica 43(2): 235–247.

There have been years in Finland when container seedlings of Norway spruce (*Picea abies* (L.) Karsten) planted in the summer have been damaged by early-autumn frosts. For August and September plantings, the seedlings can be hardened by means of short-day (SD) treatment, but little information is available about its usability for earlier plantings. We studied the effects of early-season SD treatment on the frost hardiness and risk of a second flush of Norway spruce seedlings. In three successive years, second-year seedlings were grown in a greenhouse or outdoors in the spring and early summer and then subjected to two or three-week SD treatment beginning on the second, third, or fourth week of June. We monitored the height growth cessation, bud formation, and frost hardiness of the seedlings in the nursery. All SD treatments made the height growth cease, but the risk of a second flush increased if the temperature sum was less than 300 d.d. before the beginning of the SD treatment or more than 450 d.d. between the end of the treatment and mid-August. Clearly, then, SD treatment reduced the risk of a second flush in seedlings that had been grown in a greenhouse in the spring. Early-season SD treatment increased the frost hardiness of both needles and stems for late July to early September in comparison with untreated seedlings. Later in the autumn, however, the differences disappeared. Before recommending the use of early-season SD-treated seedlings for summer planting, the method has to be tested in practical field conditions.

Keywords bud formation, container seedlings, frost hardiness, height growth cessation, *Picea abies*, second flush
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Received 9 May 2008 **Revised** 5 February 2009 **Accepted** 10 February 2009
Available at http://www.metla.fi/silvafennica/full/sf43/sf432235.pdf

1 Introduction

It is recommended that seedlings of Norway spruce (*Picea abies* (L.) Karsten) planted after the beginning of August should be short-day (SD) treated to improve their field performance potential under drought stress (Luoranen et al. 2007). The treatment also makes the seedlings less prone to autumn frost damage (Luoranen et al. 2006). According to the recommendation, the SD treatment has to last 3 weeks in order to increase frost hardiness in September (Konttinen et al. 2003). Furthermore, to prevent a second flush in late summer, the SD treatment should probably not begin before the seedlings have achieved a certain stage of development. At least for first-year seedlings, the required stage of development can be indicated by an origin-dependent temperature sum that needs to have accumulated before the SD treatment is begun (Koski and Sievänen 1985).

In British Columbia, the rearing of current-year seedlings of white spruce (*Picea glauca* (Moench) Voss) is begun in January or February in heated greenhouses equipped with photoperiodic lighting. The seedlings are SD-treated in June or July (Grossnickle and Folk 2003) and then planted in the summer (Revel et al. 1990, Grossnickle 2000, Paterson et al. 2001). This is also possible in Finland (Konttinen and Rikala 2006), although the high heating costs and a short photoperiod in late winter have restricted the use of this procedure. Another procedure used in British Columbia involves growing second-year seedlings in a nursery until an early start of SD treatment and then planting them in the summer (Grossnickle and Folk 2003).

Mechanized planting is increasing in Finland, as it is elsewhere in Fennoscandia. For economically efficient use of planting machines, it is important to extend the planting season and produce seedlings for summer plantings. Machines plant the seedlings deeper than forest workers do (Luoranen and Saarinen 2004). Too deep planting (with only the bud visible) reduces the seedlings' growth and chance of survival. However, when more than half of the shoot length is above the soil surface, the planting depth does not impair field performance (Huuri 1972). To perform properly after mechanized planting, the seedlings have to be tall enough and their SD treatment must not be started too early.

In the SD treatment, the seedlings grow only a few centimetres (Konttinen et al. 2003, 2007, Kohmann and Johnsen 2007). If the treatment is scheduled to start in June, the seedlings will have only a few weeks to grow before it. Accordingly, the seedlings have to be tall enough at the end of the first season, or alternatively, the growth period before the SD treatment has to be extended. The height of the seedlings can be controlled by changing the sowing time. On the other hand, growing them in a greenhouse in the spring accelerates their growth before the SD treatment. The sowing date has been shown to slightly affect the timing of the height growth cessation in second-year Norway spruce seedlings (Partanen 2004). Thus, the response of the seedlings to an early-season SD treatment may differ from their response to a treatment done later in the season, as in July. It is unclear, however, how much these differences in the timing of the height growth cessation affect the early-autumn frost hardening of the seedlings.

Previously, Kohmann and Johnsen (2007) and Fløistad (2007) have shown that Norway spruce seedlings have an increased risk of a second flush after an early-season SD treatment. The risk is especially high if the treatment lasts only one or two weeks. In addition, the frost hardiness of the buds and needles was lower in October when the SD treatment was done in June instead of July (Kohmann and Johnsen 2007). On the other hand, Tan (2007) found frost tolerance as early as mid-July in first-year white spruce seedlings that had been SD-treated in May or June. The time of the highest risk of frost damage, however, is the first autumn frosts, which usually come in late August or early September (Solantie 1987). To our knowledge, there are no previous studies on the frost hardiness of early-season SD-treated seedlings for that time period.

We set out to do an experimental study of the frost hardening of Norway spruce (*Picea abies* (L.) Karsten) seedlings after an early-season SD treatment. We hypothesized that an early-season SD treatment increases the frost hardiness of seedlings at the time of the early-autumn frosts in comparison with untreated control seedlings. Our second hypothesis was that the stage of development of the seedlings, as indicated by the accumulated temperature sum at the beginning of the SD period, affects the risk of a second flush after the SD treatment.

2 Material and Methods

2.1 The Seedling Material and the Growing Procedures

The study was conducted at the Suonenjoki Research Nursery of the Finnish Forest Research Institute (62°39'N, 27°03'E, altitude 142 m a.s.l.). The Norway spruce seedlings were grown in hard-plastic containers (Plantek PL64F: 64 seedlings per tray, volume 110 cm^3, growing density 432 cells m^{-2}, and Plantek PL81F: 81 seedlings per tray, volume 85 cm^3, growing density 546 cells m^{-2}, Lännen Corp., Iso-Vimma, Finland) equipped with air slits and filled with fertilized (0.8 kg m^{-3} of 16N:8P:16K soluble fertilizer with micronutrients) and limed (2.0 kg m^{-3}) medium-coarse sphagnum peat (Kekkilä Oyj, Eurajoki, Finland).

For the experiments in 2001 (hereafter referred to as Exp. 2001), the seeds were obtained from orchard no. 112 (61°54'N, 26°41'E) supplying Central Finland. For the experiments in 2003 and 2004 (hereafter referred to as Exp. 2003 and Exp. 2004), the seeds were obtained from orchard no. 177 (61°34'N, 26°05'E) supplying Central Finland. In each experiment, the seedlings were grown in a greenhouse under natural photoperiod conditions during the first growing season. They were irrigated regularly with a manually controlled mobile boom sprayer. On 20–22 October, the seedlings were moved to an outdoor growing area, where they over-wintered under the snow cover. During the second growing season, the seedlings were given a commercial fertilizer solution (Taimi-Superex, 19N:4P:20K + micronutrients; Kekkilä Inc., Eurajoki, Finland) 15 times in Exp. 2001, 17 times in Exp. 2003, and 14 times in Exp. 2004. All fertilizations took place between 2 June and 12 September.

In Exp. 2001, forty PL64F container trays were used, 20 of which were moved back to the greenhouse on 2 May, where they were kept until 20 June; this was the 'greenhouse' (GO) treatment. The other 20 trays were kept in the outdoor growing area; this was the outdoor (O) treatment until the beginning of the SD treatments. The SD treatments were carried out between 20 June and 11 July with 14-hour nights and 10-hour days under the SD-treatment frame (2.5 m × 3.5 m × 0.8 m) covered with a double black curtain (UV-proofed, black sheet-mulch, "LS groundcover", AB Ludvig Svensson, Sweden). A total of 20 seedling trays, 10 trays per treatment, were arranged randomly under the SD-treatment frame. The rest of the seedlings were kept in the outdoor growing area as a control (Co) for the SD-treatments (the O-Co stands for control seedlings grown outdoors and GO-Co for control seedlings kept in the greenhouse in the spring). For the timing of the growing phases, see Fig. 1.

The seedlings used in Exp. 2003 and Exp. 2004 had been sowed on three and two sowing dates, respectively (for the timing and the abbreviations used, see Fig. 1). The abbreviations used for the treatments in Exp. 2003 and Exp. 2004 were formed as follows: the first capital letter indicates the month of sowing (M for May and J for June) and the number after that (if there is one) the order of sowing in that month. The number after the slash indicates the year of the short-day (SD) treatments (03 or 04) or the photoperiod treatment (Co for untreated control seedlings), and the final number the order of the SD treatments in that year; the capital letter G before the SD indicates that seedlings were grown in a greenhouse in the spring before the SD treatment. The seedlings were grown outdoors in their second year except those for treatments J/03-G and J/04-G, which were moved back to the greenhouse on 2 May. The latter seedlings remained in the greenhouse until 20 June in 2003 and 18 June in 2004 for J/03-G and J/04-G, respectively.

In 2003 and 2004, the SD treatments were implemented by extending the night length automatically by using a blackout curtain (LS-100, Ludvig Svensson). After the SD treatments, all seedlings were moved to the outdoor growing area, where the seedling trays were randomly arranged on raised support bars (at a height of 20 cm) for air-pruning of roots.

In Exp. 2003, the SD treatments began on 13 June for treatment M1/03-SD1, on 19 June for

M1/03-SD2 and M2/03-SD2, and on 26 June for M1/03-SD3, J/03-SD3, and J/03-G-SD3. The SD treatment cycle (12-hour nights and 12-hour days) lasted for three weeks except in treatment M2/03-SD2-2wk, where it was two weeks. Meanwhile, the seedlings for the M1/03-Co and M2/03-Co treatments were grown outdoors. Three seedling trays were allocated to each treatment (M1/03 PL64F, M2/03, and J/03 PL81F).

In Exp. 2004, the SD treatments began on 18 June for treatments M/04-SD2, J/04-SD2, and J/04-G-SD2, and on 24 June for treatments M/04-SD3 and J/02-SD3. For all treatments in 2004, the SD-treatment cycle (14-hour nights and 10-hour days) lasted three weeks. For the controls, the seedlings for M/04-Co were grown outdoors. Again, three seedling trays (PL81F) were allocated to each treatment.

2.2 The Measurements

In Exp. 2001, the height of 4 randomly selected seedlings from each of the 5 photoperiod treatment trays (the term 'photoperiod treatment' is used here as a general term including both short-day (SD) and control (Co) treatments), totalling 20 seedlings per photoperiod treatment, was measured weekly from the beginning of the SD treatment until the beginning of September. In Exp. 2003 and Exp. 2004, the height of 5 randomly selected seedlings from each of the 3 treatment trays, totalling 15 seedlings per treatment, was measured at the beginning and end of each SD treatment and at the end of the season. In Exp. 2001 and Exp. 2003, the cessation of height growth was defined as the date when over 95% of the seedlings had attained 95% of their final height. To determine the date of cessation, the height of the measured seedlings was interpolated for each day between the measurements. In Exp. 2004, the cessation of height growth was not determined. In Exp. 2001, the bud formation was monitored on each date of height measurement. A bud was scored as formed when it had become visible. In all experiments, a possible second flush of the seedlings (including both terminal and axillary buds) was also monitored at the end of August.

Table 1. The dates of the freezing tests in autumn 2001, 2003, and 2004 and the test temperatures used.

Date	Temperatures, °C
2001	
26 July	5, –1.5, –3, –5, –7, –10, and –15
15 Aug	5, –2, –5.5, –7, –10.5, –15.5, and –25
3 Sep	5, –3.5, –5.5, –7.5, –10.5, –15.5, and –25.5
24 Sep	5, –4.5, –7.5, –10.5, –13, –17, and –30
15 Oct	5, –11, –21, –26, –31, –35.5, and –50.5
2003	
28 Aug	–4, –7, and –10
2004	
2 Sep	–4, –7, and –10

2.3 The Freezing Tests

The frost hardiness of the seedlings was tested on five occasions between late July and mid-October in 2001 and once in both 2003 and 2004 (Table 1).

On each of the five test occasions in 2001, the samples were exposed to a total of six freezing temperatures in three air-cooled chambers on two successive nights. The control temperature was +5°C for all of the tests. The temperatures were chosen according to the predicted level of frost hardiness attained. In 2003 and 2004, it was possible to use only three exposure temperatures, which were chosen as close to those used in 2001 and the predicted level of frost hardiness as possible. The air temperatures in the chambers were controlled by an external alcohol-circulating system (Lauda RUK90 Ultra-Kryomat combined with Lauda digital programmers R410 and PM351, MGM Lauda, Germany). The rate of cooling and warming the chambers was 3°C h^{-1} in 2001 and 5°C h^{-1} in 2003 and 2004. The duration of the minimum temperature varied between 2 and 12 hours because of the programming system of the test chambers, which stipulated that the lower the temperature, the shorter the duration of exposure. We assumed, on the basis of Levitt (1980) and Bigras et al. (2004), that the influence of the varying exposure time was minimal in comparison with the temperature itself.

In each test in 2001, 7 seedlings from each photoperiod treatment (one seedling per tray) were selected for each test temperature. The shoots of the selected seedlings were cut, placed in plastic bags, and moved to the chambers. After the freezing exposure, the basal 1 cm was cut from each shoot, whereafter the shoots were inserted upright into a holder in a water-filled plastic box. Then the boxes were moved to a greenhouse (20/15°C). There, natural light was supplemented with 400-watt high-pressure sodium lamps for 16 hours daily. The shoots were sprayed daily with tap water. After 14 days, frost damage was assessed visually from the stems, needles, and buds (if formed). Damage to the needles was scored visually at 10% intervals and damage to the stems by measuring the length of the damaged part after the stem was dissected. All parts, including the buds, were determined to be dead if they had turned brown.

In 2003 and 2004, ten seedlings from each treatment (3 or 4 seedlings per tray) were selected for each test temperature. The selected seedlings were randomly arranged in PL-81F trays, which were then placed in wooden boxes. The boxes were insulated with sawdust (cover and walls) and polystyrene (bottom) to protect the roots from freezing during the exposures. After the frost exposure, the trays were moved to a greenhouse (20/15°C) with the same maintenance protocol as in 2001. In 2003 and 2004, only damage to the needles was recorded.

2.4 Estimation of Frost Hardiness

In 2001, the seedlings that had not yet developed buds were excluded from the frost hardiness analyses. In 2003 and 2004, the needles were assumed to be uninjured at +5°C. That temperature was therefore counted as an additional temperature treatment in the estimation of frost hardiness. The frost hardiness of the seedlings was estimated by using the following logistic function (Repo and Lappi 1989):

$$y_i = f(x_i) + \varepsilon_i = \frac{1}{1+e^{b(c-x_i)}} + \varepsilon_i \qquad (1)$$

where y_i is damage to the needles, stems, or buds, x_i is the exposure temperature, b and c are parameters, and ε_i is the error term. The inflection point c is the temperature at which the change in the damage is maximal as the temperature decreases. This was used to estimate the temperature at which 50% of the needles, stems, or buds were damaged.

By means of the method described by Luoranen et al. (2004), the variances were homogenized by dividing both sides of Eq. 1 by the weight (w):

$$w = \sqrt{\hat{f}\left(1-\hat{f}\right)} + 0.01 \qquad (2)$$

where $\hat{f}$ is the current estimate of $f(x_i)$. On some exposure dates, the needles or stems were damaged within such a narrow temperature range that estimating the curves, especially parameter b, became difficult. Thus, to achieve a valid estimate of the inflection point c (LT_{50}), parameter b was set as fixed and only parameter c was estimated. The differences among the treatments in the estimated parameter c (frost hardiness) were tested by using the F-test

$$F = \frac{(\text{SSE of restricted model} - \text{SSE of full model}) / \left(p - p_1\right)}{\hat{\sigma}^2} \qquad (3)$$

where the SSE (error sum of squares) of the full model is the sum of the SSEs calculated separately for each tested treatment, p is the number of treatments multiplied by the number of parameters in the full model (p=4), p_1 is the number of parameters in the restricted model, and $\hat{\sigma}^2$ is the residual variance computed for the full model. The full model was estimated by using the model

$$y_i = \frac{1}{1+e^{b_1(c_1-x_i)i_1 + b_2(c_2-x_i)i_2}} + \varepsilon_i \qquad (4)$$

and the restricted model by using

$$y_i = \frac{1}{1+e^{b_1(c-x_i)i_1 + b_2(c-x_i)i_2}} + \varepsilon_i \qquad (5)$$

where b_1 and b_2 and c_1 and c_2 are the parameters b and c in treatments 1 and 2 respectively, and i_1 and i_2 are the dummy variables indicating the treatments. The weight w was calculated for model (4) and then also used for model (5). p_1 in model (3) is three. The frost hardiness calculations were carried out by using SPSS 15.0 for Windows.

2.5 The Statistical Analysis

For Exp. 2001, a four-way Anova (SPSS 15.0) was used to test for differences in seedling height between the SD and G treatments at the beginning and end of the SD treatment and at the end of the growing season. The following model was used:

$$y_{kij} = \mu + SD_k + G_i + SDG_{ki} + \beta_j + \varepsilon_{ijk} \quad (6)$$

where μ is the grand mean, SD_k is the fixed effect of the photoperiod treatment k, G_i is the fixed effect of the greenhouse treatment i, SDG_{ki} is the interaction of the photoperiod and greenhouse treatments, β_j is the fixed effect of the block (=seedling tray) j, and ε_{ijk} is the residual error.

In 2003 and 2004, the statistical analyses were done separately for each sowing date, and two- or three-way Anovas (SPSS 15.0) were used to test for differences in seedling height between the SD and G or W (duration of SD) treatments at the beginning and the end of growing season. Again, the seedling trays were used as blocks. The data was normal distributed and the homogeneity of variances was tested by Levene's test before Anovas. No transformations were needed. The differences between the treatments in the proportion of seedlings with a second flush were tested with the nonparametric Kruskall-Wallis test.

2.6 The Weather

The weather was monitored from the weather station at the Suonenjoki Research Unit. The temperatures, monitored at 2 metres, were near the long-term average in 2001 and 2003, except in July, which was warmer in both years (Table 2). In 2004, early summer was colder and late summer and autumn warmer than the average. The precipitation totals were roughly the same as the long-term average in 2001, lower in 2003, and higher in 2004 (Table 2). The minimum temperatures before each of the frost exposure dates were 7.4, 4.8, 0.9, and –3.7°C, respectively, for 15 August, 3 September, 24 September and 15 October 2001. In 2003 and 2004, the minimum temperatures before the freezing test dates were 7.3°C and 2.4°C. The first autumn frosts (–2.3, –0.1 and –1.2°C at 2 m, respectively) occurred on 25 September 2001, 3 September 2003, and 9 October 2004. The temperature sums at the beginning of the SD treatments were calculated from the temperature measurements at the weather station for the seedlings grown outdoors and from the temperatures monitored by the thermograph at the level of the seedlings for those first grown in a greenhouse.

Table 2. Monthly mean air temperature (°C) and precipitation (mm) at the Suonenjoki Research Unit in 2001, 2003, and 2004 and the long-term average (1972–2004).

	2001	2003	2004	1972–2004
		Temperature, °C		
June	14.2	12.0	10.6	14.1
July	18.7	19.9	14.8	16.5
August	14.6	14.3	16.2	14.2
September	10.6	10.1	12.4	9.1
		Precipitation, mm		
June	61	28	102	68
July	81	80	58	82
August	81	68	147	82
September	60	73	59	58

3 Results

3.1 Height Growth Cessation and Bud Formation

By the beginning of the SD treatments in 2001, the seedlings kept in a greenhouse during May had grown 91% of their annual growth, while the seedlings grown outdoors had grown 70% (Fig. 1). At the end of the SD period, however, no height differences between the treatments were observed (SD: $p=0.076$, G: $p=0.105$, SD×G: $p=0.196$; see Fig. 1 for estimates). Greenhouse growing in the spring hastened the height growth cessation and bud formation of the seedlings after the SD treatments: growth ceased on 27 June and 2 July, and buds formed on 31 August and 7 September for GO-SD and O-SD seedlings, respectively. In the control seedlings, growing in a greenhouse in the spring did not affect the height growth cessation or bud formation: height growth ceased

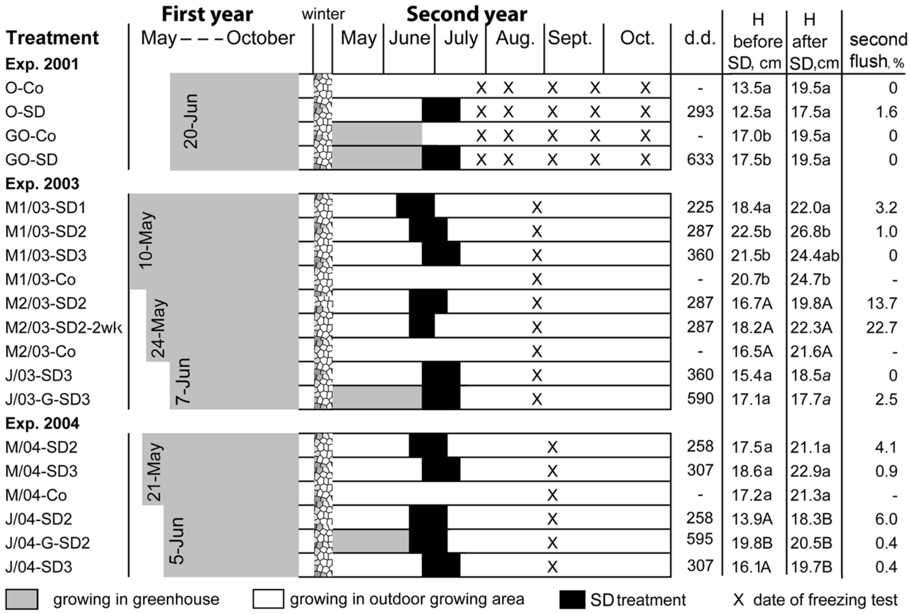

Fig. 1. Growing schedules of the different treatments in the experiments of 2001, 2003, and 2004. The sowing dates for each treatment are indicated under "First year." The abbreviations for the treatments are as follows: O = grown in an outdoor growing area during the second year, GO = grown in a greenhouse before the short-day treatment in the second year, SD = short-day treatment, Co = grown in an outdoor growing area as a control for the SD treatment, M = sown in May, and J = sown in June. The numbers in the names of the treatments indicate the year of the experiment (03 and 04), the order of sowing, or the SD treatment. "d.d." stands for the accumulated temperature sum and "H before SD" the mean height (cm) of the seedlings as they were at the beginning of the SD treatment. With the Co-seedlings, "H before SD" means the mean height (cm) at the beginning of the latest SD treatment for that sowing date. Correspondingly, "H after SD" stands for the mean height of the seedlings at the end of the SD treatment or, in the case of the Co-seedlings, at the end of the latest SD treatment for that sowing date. The column "second flush" indicates the percentage of seedlings with a second flush at the end of August. Different letters (small and capital letters for different sowing dates) in the "H before SD" and "H after SD" columns indicate significant differences according to Tukey's test (the test was done separately for each sowing date).

on 30 July and buds formed on 28 September in both GO and O seedlings. In the O-Co, GO-Co and GO-SD treatment seedlings, no second flush occurred, whereas some seedlings in the O-SD treatment had a second flush (Fig. 1).

In 2003, height growth ceased on 1, 7, and 12 July in SD1, SD2, and SD3, respectively, when the length of the SD treatment period was 3 weeks. When the treatment lasted only 2 weeks in SD2, height growth ceased as early as 30 June. Growing in a greenhouse in the spring hastened the height growth cessation in the SD3 seedlings; for the G-SD3 treatment, this date was 8 July. For the control (Co) seedlings sown in May, the dates of the height growth cessation were 5 and 10 August in M1/03 and M2/03, respectively. The sowing date in the previous year affected the second-flush risk of SD-treated seedlings ($p = 0.001$): more seedlings had a second flush in the M2/03 batches than in the other batches

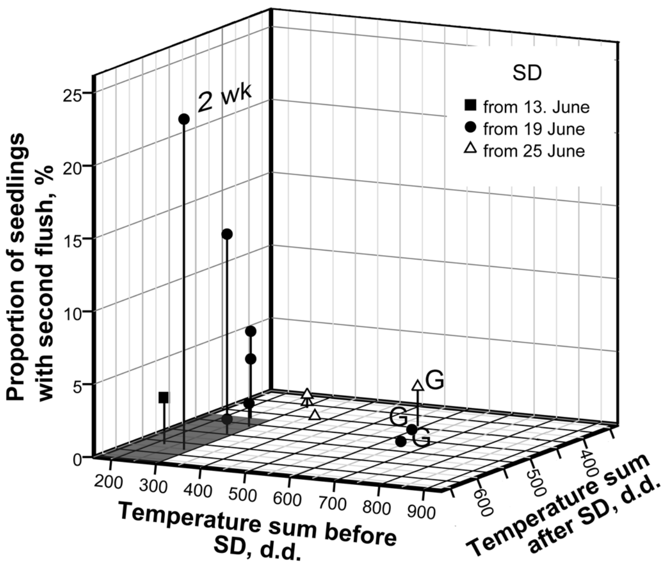

Fig. 2. The relationship between the incidence of second flush and the temperature sum accumulated before the SD period (calculated from the beginning of the growing season) and after the SD period but before 15 August (calculated from the end of the SD treatment to the 15 August). G = greenhouse growing before the SD treatment, 2-wk = two-week SD period. The visually determined grey area indicates the temperature sums at which the risk of a second flush is heightened.

(Fig. 1). A second flush was more common after a 2-week SD treatment than after a 3-week one (p = 0.007). In addition, when the SD treatment lasted 3 weeks, the trend was that the earlier the SD period was begun, the more seedlings with a second flush were observed (p = 0.054). At the end of the SD treatments, the seedlings sown the earliest in May and SD-treated the first in June (M1/03-SD1) were shorter than the seedlings of the other batches sown in May (Fig. 1). No differences in seedling height were observed between treatments M2/03 and J/03 (Fig. 1).

In 2004, more seedlings with a second flush were observed among the SD2 seedlings than among the SD3 seedlings grown outdoors in the spring (p = 0.006). On the other hand, greenhouse growing lowered the likelihood of a second flush, as can be seen by comparing the SD2 and the G-SD2 seedlings (p = 0.046) (Fig. 1). At the end of the SD treatments, the different treatments with the same sowing dates did not show any differences in seedling height (Fig. 1).

Considering all the experiments, we see that the risk of a second flush seemed to increase if the temperature sum was less than 300 d.d. at the beginning of the SD treatment or if it was more than 450 d.d. after the end of the SD treatment (but before mid-August), regardless of the beginning date of the SD treatment (Fig. 2).

3.2 Frost Hardening in Exp. 2001

In July, two weeks after the end of the SD treatment, the needles of the SD seedlings had already hardened to tolerate at least –12°C (Fig. 3a). No differences were observed among the different SD treatments in this respect. At the same time, the control seedlings tolerated about –6°C regardless of their growing conditions in May. Before early September, only small changes in frost hardiness were observed. The frost hardening of the control seedlings accelerated after 3 September, decreasing the differences in frost hardiness between treatments.

On the test dates in July, August and October, the stems of the SD-treated seedlings grown in a greenhouse (GO-SD) tolerated temperatures 3°C, 2°C, and 10°C lower, respectively, than the SD-treated seedlings grown outdoors (O-SD) (Fig. 3b). The frost hardiness of the stems among the control seedlings did not vary. In tests carried out on 26 July, 15 August, 3 September and 15 October, the stems of the SD-treated seedlings grown in a greenhouse (GO-SD) tolerated temperatures 8°C, 7°C, 3°C, and 17°C lower, respectively, than the stems of the control seedlings grown in a greenhouse (GO-Co). The effect of the SD treatment on frost hardiness was smaller in seedlings grown outdoors before the treatment: on August

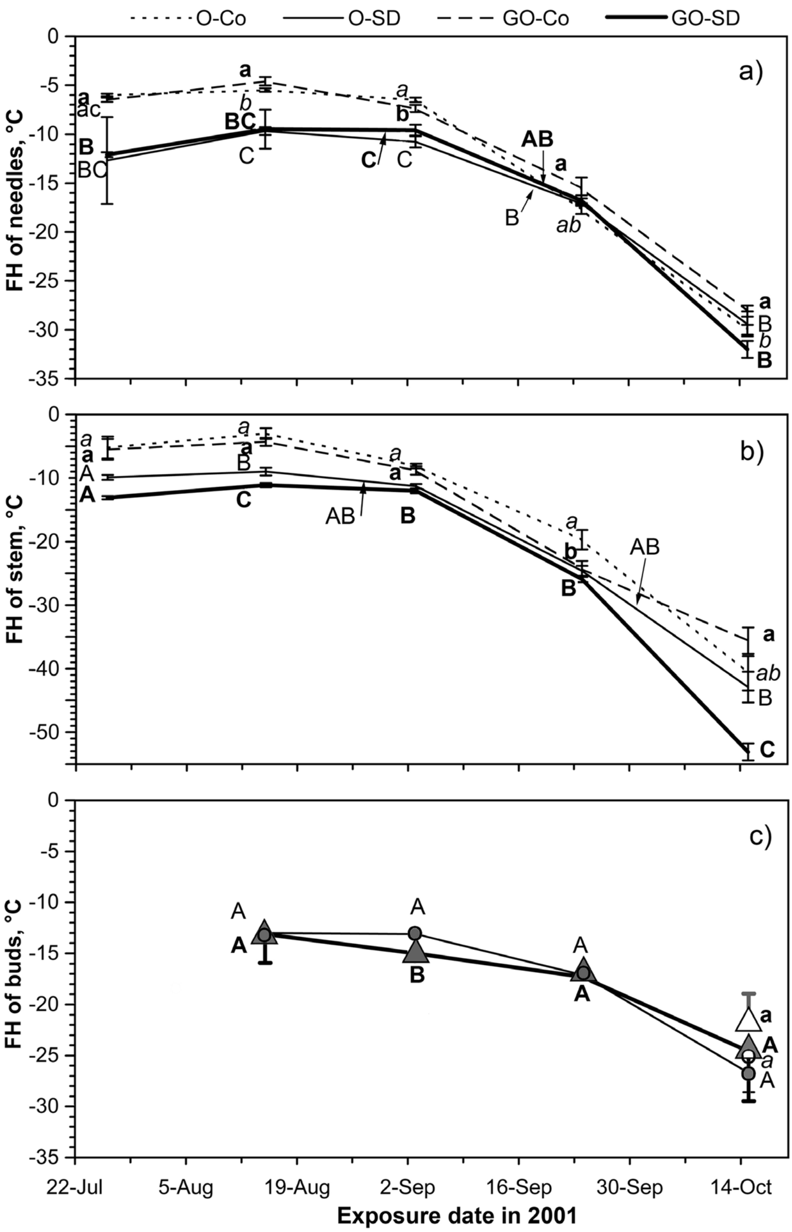

Fig. 3. The means (± s.e.) of the frost hardiness of a) needles, b) stems and c) buds in seedlings grown outdoors (O) or in a greenhouse (GO) in the spring and thereafter either short-day (SD) treated for 3 weeks (20 June–11 July) or grown outdoors (Co) under a natural photoperiod in 2001. Different letters indicate, separately for each exposure date, statistically significant differences among the treatments ($P < 0.05$). Capital letters are for SD, small letters for Co, and boldface letters for GO.

and September test dates, the stems of O-SD seedlings tolerated temperatures 6°C, 3°C, and 5°C lower than the control seedlings. The differences in frost hardiness among the treatments were smaller for the stems than for the needles. In September, when seedlings harden more rapidly, the hardening of the stems was faster than that of needles.

In July, no formed buds were observed yet, and in August and September it was possible to estimate the frost hardiness of the buds only for SD-treated seedlings. The frost hardiness of the buds stayed the same on 15 August, 24 September, and 15 October (Fig. 3c). On 3 September, the buds of the SD-treated seedlings grown in a greenhouse (GO-SD) had a better frost tolerance by 2°C than those grown outdoors (O-SD).

3.3 Frost Hardening in Exp. 2003 and Exp. 2004

At the end of August 2003, the frost hardiness of the needles in the SD-treated seedlings had improved in comparison with that of the control seedlings (Fig. 4a). The sowing date had only a slight effect on the frost hardiness of the needles: when the SD treatment was started on 19 June, the difference between the M1/03 and M2/03 seedlings was 0.5°C, and when the treatment was started on 26 June, there was no difference between the sowing dates in the frost hardiness of the needles (M1/03-SD3 and J/03-SD3). Growing in a greenhouse in May (J3/03-G-SD3) did not cause any differences in the frost hardiness of the needles between the batches whose SD treatments started on the same date (M1/03-SD3 and J/03-SD3). However, in comparison with treatments started earlier (M1/03-SD1 and M1/03-SD2), growing in a greenhouse improved frost hardiness by 1°C (Fig. 4a). The three-week SD treatment (M2/03-SD2) was more effective in improving frost hardiness than the two-week treatment (M/03-SD2-2wk). A second flush was observed in 3%, 16%, and 6% of the seedlings, respectively, sampled for freezing tests from batches M2/03-SD2, M2/03-SD2-2wk, and M2/03-Co.

At the beginning of September 2004, all SD treatments had improved the frost hardiness of the seedlings in comparison with that of the control seedlings (Fig. 4b). The hardening of the seedlings was not affected by the dates of sowing or of beginning the SD treatments, but growing in a greenhouse improved frost hardiness by 1°C in comparison with similar seedlings grown outdoors in May.

4 Discussion

Early initiation and short duration of the SD treatments increased the seedlings' risk of a second flush, which supports Fløistad's (2007) and Kohmann and Johnsen's (2007) results. In our study, the highest proportion of seedlings with a second flush was observed in the only two-week treatment included. Fløistad (2007) and Kohmann and Johnsen (2007) had several treatments of a one-week or two-week duration, and the number of seedlings with a second flush was always high. We may conclude, then, that in early summer, the duration of SD treatments must be longer than two weeks so as to avoid a second flush.

Kohmann and Johnsen (2007) showed that second-year seedlings carry a higher risk of a second flush in northern than in southern nurseries in Norway and that the risk is higher for southern provenances than for local or northern ones. In our study, the proportion of seedlings with a second flush was in between those reported by Kohmann and Johnsen (2007), which makes sense because the provenances in our study were local in all experiments and because the nursery we used was located in between the northern latitudes of the nurseries used in Kohmann and Johnsen's study.

Our results showed that the risk of a second flush was related to the stage of development of the seedlings as indicated by the temperature sum at the beginning and end of the SD treatment. When the temperature sum at the beginning of the SD treatment was more than 300 d.d. (Fig. 2), in other words, the current-year shoot of most seedlings had grown sufficiently tall before the SD treatment (Fig. 1), the risk of a second flush was low. It was also reduced when less than 450 d.d. had accumulated after the SD treatment by mid-August. Thus, in addition to the timing of the SD treatment, the weather conditions before and

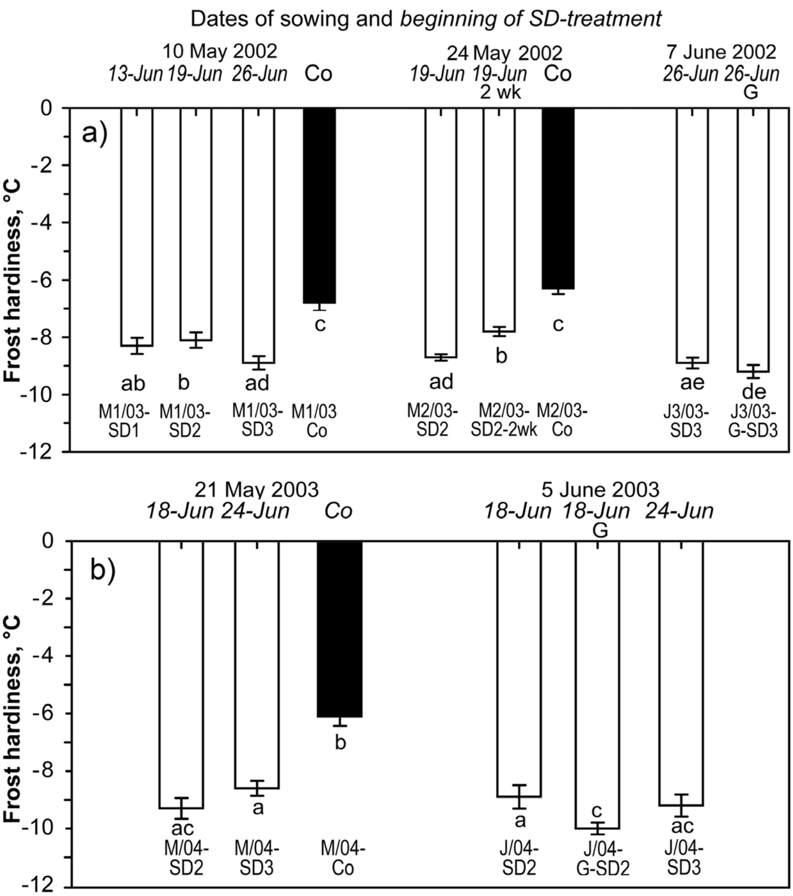

Fig. 4. Estimated (± s.e.) frost hardiness of the needles on a) 28 August 2003 and on b) 2 September 2004, shown for different sowing dates (upper dates) and different starting dates of the SD treatments (lower dates). The SD treatments are indicated with white bars and the controls (Co) with black bars. In a), "2-wk" indicates a 2-week treatment and G a treatment in which the seedlings were grown in a greenhouse in May. In all other cases, the duration of the SD treatments was 3 weeks and the seedlings were grown outdoors. Different letters indicate significant differences among the treatments ($P < 0.05$).

after the SD period also affect the risk of a second flush. Growing in a greenhouse in the spring before the SD treatment (treatments GO-SD, J/03-G-SD3 and J/04-G-SD2 in our study) accelerates the development of the seedlings as it provides for a greater temperature sum at the beginning of the SD treatment and thus decreases the risk of a second flush. In this study, second flushes were monitored from nursery-grown seedlings. Konttinen and Rikala (2006) have shown that a second flush in seedlings SD treated in July is more likely if the seedlings were grown in a nursery until autumn than if they were planted soon after the SD treatment. Therefore, before any final recommendations can be given, the risk of a second flush after an early-season SD treatment followed by a summer planting needs to be studied also.

Early-season SD treatments increased the frost hardiness of the needles and stems in late July and mid-August already (Fig. 3). This is in accordance with Tan's (2007) findings that in mid-July, the frost tolerance of the needles of current-year white spruce seedlings SD-treated for 15 days beginning in mid-May was higher than that of seedlings SD-treated for only 7 days in June, 3 days in July, or untreated seedlings.

By mid-October in Exp. 2001, no differences in the frost hardiness of buds could be observed among the treatments any longer. As regards needles, the frost hardiness of only the control seedlings grown in a greenhouse (GO-Co) was lower than that of all other seedlings. In this respect, our results do not support those of Kohmann and Johnsen (2007), who observed that in October the frost hardiness of the buds and needles of early-season SD-treated seedlings was lower than that of the control seedlings. The frost hardiness of the buds and needles of seedlings SD-treated in July, however, did not differ from that of the controls. In our study, the frost hardiness of the buds was lower than that of the needles and stems, especially in late autumn (Fig. 3), which accords with Kohmann and Johnsen's (2007) results.

Early-season SD-treated seedlings are meant to be planted in late summer, when the attainment of adequate frost hardiness for the hard early-autumn frosts (< –3 °C), which occur in early September every third year, on average, in southern Finland (Solantie 1987), is more important than the frost hardiness attained later in the autumn. In each experiment, the frost hardiness of the SD-treated seedlings during this critical time was better than that of the control seedlings (Figs. 3 and 4). This accords with previous results of Konttinen et al. (2003) and Luoranen et al. (2008). In this study, we tested the frost hardiness of seedlings grown in seedling trays in a nursery. The frost hardening of planted seedlings could be different on account of planting stress, for example, but in Norway at least, no differences in the frost hardiness attained with similar SD treatments were observed after planting (Kohmann & Sønsteby 2007).

The seedlings were at least 17 cm tall at the end of the SD period in all treatments (Fig. 1) and were therefore suitable for mechanized planting. As the average planting depth in mechanized planting is 6 cm (Luoranen and Saarinen 2004), the shoot height above the soil surface after planting was at least 11 cm. This was most likely sufficient to ensure proper growth in the years to come (Huuri 1972).

5 Conclusions

In early-season SD treatments, the frost hardiness of the seedlings increases immediately after the treatment, resulting in significantly better hardiness than in non-treated seedlings until the beginning of September. Later on, the differences in frost hardiness between SD-treated and control seedlings disappear. The seedlings have a risk of a second flush after early-season SD treatments, but that risk can be reduced by growing the seedlings in a greenhouse before the treatment. Before the use of early-season SD-treated seedlings for July plantings can be recommended, further studies are needed to assess the effects of planting stress on the second-flush risk and frost hardiness of the seedlings.

Acknowledgements

We should like to thank Auli Lehtinen, Ritva Pitkänen, Riitta Toivonen, and the staff at the nursery of Suonenjoki for their valuable technical assistance in the growing of seedlings and measurements of the experiments, Dr. Otso Huitu and Prof. Pekka Hirvonen for revising the English language of the manuscript.

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