From Forest Nursery Notes, Summer 2008

120. Growth and frost hardening of *Picea abies* seedlings after various night length treatments. Konttinen, K., Luoranen, J., and Rikala, R. Baltic Forestry 13(2):140-148. 2007.

BALTIC FORESTRY

GROWTH AND FROST HARDENING OF PICEA ABIES SEEDLINGS /.../

K. KONTTINEN ET AL.

Growth and Frost Hardening of *Picea abies* Seedlings after Various Night Length Treatments

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Konttinen, K., Luoranen, J. and Rikala, R. 2007. Growth and Frost Hardening of *Picea abies Seedlings after* Various Night Length Treatments. *Baltic Forestry*, 13(2): 140-148.

Abstract

We studied the effects of varying night length (NL: 16 h, 12 h or 10 h and ambient as control) treatments of three weeks duration on the morphology, shoot water content (WC) and frost hardiness (FH) of first-year Norway spruce (Picea abies (L.) Karst.) seedlings of northern (64° 40') and southern (60° 40') Finnish origins, and of first- and second-year Norway spruce seedlings of local origin at a nursery in Central Finland (62° 38'). We also studied the height growth of the seedlings after planting. NL was negatively associated with the increment of stem diameter and root dry mass of seedlings. The NL treatments increased FH from 4 to 7°C compared with control seedlings, but differences between NL treatments were small. No consistent relationship between WC and FH was found. NL treatments had only a minor effect on seedling height growth after planting. In conclusion, the shorter (10-12 h) night is recommended for use in a blackout treatment.

Key words: frost hardening, night length, Norway spruce, origin, Picea abies, photoperiod, short-day treatment

Introduction

Short-day treatment (SD) has been widely used to control height growth of seedlings and to harden seedlings to stand autumn frosts and freezer storage. With respect to the application of SD treatment, three factors must be determined for each seedling lot: timing, duration and night length within the treatment. The timing of the SD application is mainly governed by sowing time, target seedling size and planting date. The duration and night length applied in the SD treatment should be the shortest possible which will produce desired results (van Steenis 1992). In Finland, a three week duration has been considered sufficient and safe for spruce seedlings (Konttinen et al. 2003).

Night length (NL) is determined as the critical duration which is necessary to regulate height growth cessation and bud set. The critical NL varies in Scandinavian shrubs and trees from 6 to 8 hours for northern (66 °N) and southern (60 °N) origins, respectively (Heide 1974a, Håbjurg 1978). Thus, the more northerly the origin, the shorter the night that is needed to stop shoot elongation. The critical NL also varies naturally amongst individuals from a single origin. To achieve simultaneous growth cessation, homogenous size and sufficient frost hardening of seedlings the NL used must be long enough to cover the critical NL of all individuals in a seedling lot but not too long to

avoid drawbacks of the treatment. Too long a night length and duration of treatment could have negative impacts on seedling morphology, physiology and phenology (Hawkins and Draper 1991, Hawkins et al. 1994, Coursolle et al 1998). The length of the night commonly used in SD-treatments has been 16 hours.

The effects of NL in SD-treatments of Norway spruce seedlings have been studied extensively (e.g. Dormling et al. 1968, Christersson 1978, Sandvik 1980, Rosvall-Ahnebrink 1982, Dormling 1993). However, only few studies have focused on the effects of NL on growth and hardening of seedlings and these have been mainly carried out with first-year seedlings (Dormling et al. 1968, Heide 1974ab, Aronsson 1975). Norway spruce [Picea abies (L.) Karst.)] seedlings, in general, display improved hardening with increasing NL, however, if the night is longer than 18 h the hardening process becomes hampered (Aronsson 1975). Also, NL much longer than the critical NL (e.g., 12-16 h) may retard stem diameter and root growth of seedlings (Bigras and D'Aoust 1993). According to Hawkins and Draper (1991), the shoot growth of spruces can be controlled with no detrimental effects on diameter or root growth by using shorter (7-11 h) rather than traditional longer night (14-16 h) treatments.

NL may also affect seedling growth after planting. During bud formation, temperature affects the number of primordia (Pollard and Logan 1977). Because

2007, Vol. 13, No. 2 (25)

bud formation of seedlings previously exposed to long NL usually occurs earlier under warmer conditions than that of the control seedlings, this may result in an increase in shoot length during the following year (Heide 1974a). Results have been, however, contradictory. According to Eastham (1991) and Odlum (1992) NL-treatment improved shoot growth during the first growing season after planting while Rosvall-Åhnebrink (1982) and Konttinen et al. (2003) found no differences between the treatments.

It is important to monitor the hardening of seedlings in order to determine the proper timing of their shipment for planting or for frozen storage. Assessing directly the frost hardiness (FH) of seedlings is laborious and time consuming. Monitoring changes in the water content (WC) of seedling shoot tips has been recommended and used as an indirect indicator of FH (Rosvall-Åhnebrink 1977, Colombo 1990, Calme et al. 1993). However, there exist inconsistencies in the relationship between WC and FH amongst seedling lots treated with different cultural measures or from different seed origins (Toivonen et al. 1991, Krasowski et al. 1994).

Our aim was to study: 1) whether NLs of 10-12 h can be used to accelerate the hardening of Norway spruce seedlings without detrimental effects on stem diameter growth, root growth or planting performance; and 2) the usefulness of measuring WC of shoot tips to evaluate FH of seedling shoots. To achieve this, we carried out two experiments manipulating NL, seed source and seedling age to measure their effects on consequent seedling height, diameter, mass, frost hardening, as well as height growth of the seedlings after planting.

Materials and methods

Seedling material and night-length treatments

The experiments were carried out at the Suonenjoki research nursery (62° 38'N, 27° 04'E, 142 m asl) in the years 1999 (Experiment 1; E1) and 2000 (Experiment 2, E2). One-year-old Norway spruce seedlings were grown in hard plastic containers PL-81F (Lännen Plant Systems, Finland; 81 pots/tray, 549 pots m⁻², 85 cm³ pot⁻¹) and two-year-old seedlings in PL-64F (64 pots in each tray, 434 pots m⁻², 115 cm³ pot⁻¹) filled with pre-fertilized and limed light sphagnum peat (Forest nursery peat, Kekkilä Co., Finland). The seedling trays were irrigated by a mobile boom sprayer, and the moisture content of the peat was controlled by weighing and then irrigating the trays up to the target weight (response moisture content of 40–50 %, V/V) once a week.

In E1, two origins of stand-collected, 'selected' seeds, of southern (Lapinjärvi, 60° 40'N; M29-91-0118,

B3) and northern origins (Vaala, 64° 40°N; M24-95-0007, B3), were sown in 36 container trays (18 trays of each origin) in a greenhouse on 28 April 1999. The seedlings were fertilized 6 times during the growth period from 4 June to 30 July. Fertilization including pre-fertilizer consisted of 25 mg N, 9 mg P and 29 mg K per seedling plus micronutrients.

Seedlings for E2 were obtained from commercial seedling lots grown from 'qualified' seed-orchard seed (Sairila, seed orchard 177, 61°30', T03-98-0149) and intended for planting in Central Finland (area with average annual temperature sum of 1080-1280 day degrees (d.d.), threshold +5 °C). Seeds for the oneyear-old lot were sown in a greenhouse on 28 April 2000. They were fertilized 5 times during the growing period from 4 June to 28 July. Fertilization including pre-fertilizer consisted of 21 mg N, 8 mg P and 22 mg K per seedling plus micronutrients. Seeds for the twoyear-old seedling lot were sown in a greenhouse on 14 June 1999 and the seedlings were moved outdoors in mid-October. In the second season (2000), the seedlings were grown in an outdoor compound and they were fertilized 10 times from 25 May to 28 July with a total of 42 mg N, 11 mg P and 44 mg K per seedling plus micronutrients.

The NL treatments were conducted for a three-week period under two blackout frames (2.5 m x 3.5 m x 0.8 m) covered with a double black curtain (UV-proofed, black sheet-mulch, "LS groundcover", AB Ludvig Svensson). The PAR in the frame varied within 0.01-0.1 µmol s⁻¹ m⁻² compared to the outside PAR of 1170 µmol s⁻¹ m⁻² in the middle of a sunny day.

In E1, the NL treatments were started on 13 July 1999. The NLs lasted for 16 hours (NL16) from 1600 to 0800 or 10 hours (NL10) from 2100 to 0700. At the beginning of the NL treatment the temperature sum for the seedlings accumulated in the greenhouse was 1065 d.d. At that point in time, the natural NLs in Suonenjoki (the nursery), in Vaala (the location of the northern seed origin) and in Lapinjärvi (southern seed origin) were 5 h 13 min, 4 h 17 min and 5 h 56 min, respectively. The mean air temperature at 15 cm height in a blackout frame during the blackout treatment was 18.5 °C which was 0.3 °C higher than outdoor temperature. The number of seedlings in each origin and treatment was 486 (6 trays). The seedlings were moved under the blackout frames directly from the greenhouse. The control (untreated) seedlings (6 trays in each origin) were moved next to the blackout frames at a distance of 2 m ten days later, on 23 July.

In E2 the NL treatments were started on 20 July when ambient NL was 5 h 55 min. The NLs were 16 hours (NL16) from 1600 to 0800 or 12 hours (NL12) from 1900 to 0700. The first-year seedlings were moved un-

2007, Vol. 13, No. 2 (25)

GROWTH AND FROST HARDENING OF PICEA ABIES SEEDLINGS /.../

der the blackout frame from the greenhouse and the second-year seedlings from an open compound. In the case of the control seedlings, 8 trays of first-year seedlings and 9 trays of second-year seedlings were moved to the same field next to the blackout frames. At the beginning of the NL treatment, the temperature sum for the first-year seedlings accumulated in the greenhouse was 1190 d.d. and for the second-year seedlings outdoors 687 d.d. The mean air temperature at 15 cm height in a blackout frame during the blackout treatment was 16.0 °C (outdoor temperature 15.0 °C). After the NL treatment, all the seedling trays were randomized in an open compound until the freezing tests.

Seedling measurements

In E1, the heights of the same randomly selected 20 seedlings (4 seedlings in each tray) of each origin and NL-treatment were measured (to 1 mm) once a week or bi-weekly from 25 May to the end of the growing season. In E2 the shoot height was measured only at the beginning of the NL treatments and at the end of growing season. In October, four seedlings from each tray (block), totalling 20 seedlings in each treatment, were sampled randomly for their shoot height and stem diameter (to 0.01 mm, 10 mm above peat surface) as well as their dry mass (to 1 mg) of needles, stems and roots after drying (2 days at 60°C).

The seedlings were pooled by treatments for nutrient analysis. The needles were ground and the nitrogen (N) concentration was determined using a Leco-CHN-600 (Leco, St. Joseph, MI, USA), while the phosphorous (P) and potassium (K) from dry-digested (in 2 M HCl) samples (Halonen et al. 1983) were determined by means of plasma-emission spectrophotometry (ICP, ARL 3800, Fison Instruments, Valencia, CA).

In E1, the WC of shoot tips (2 cm pieces) was determined from 5 random seedlings per treatment by weighing them (1 mg) before and after drying (24 h at 105 °C). This was repeated every tenth day or once a week from July 13 to October 19. The WC was calculated as the ratio of fresh weight minus dry weight to fresh weight (×100).

Freezing tests

The FH of the seedlings was tested at four different times between late August and mid-October (Table 1). On each occasion, the seedlings (see below) were exposed to three freezing temperatures in aircooled chambers. The temperatures were chosen according to the expected level of FH. The air temperatures in the chambers were controlled by an external alcohol-circulating system (Lauda RUK90 Ultra-Kryomat combined with a Lauda digital programmer R410 and PM351 MGM Lauda Germany). The rate of cool-

Table 1. The exposure dates and minimum temperatures of the freezing tests

Experiment	1	Experimen	t 2
Date	Temperature °C	Date	Temperature °C
24 Aug.	-4, -9, -14	17 Aug.	-3, -6, -9
7 Sep.	-6, -11, -16	31 Aug.	-5, -9, -14
21 Sep.	-9, -14, -19	20 Sep.	-9, -14, -19
12 Oct.	-14, -20, -26	10 Oct.	-14, -19, -26

ing and warming of the chambers was 5°C h⁻¹. The durations of the minimum temperature varied in the treatments owing to the programming system of the test chambers. The durations in each test were 3, 4 and 6 hours, respectively, for the lowest, middle and highest test temperatures. We assumed, based on Levitt (1980) and Bigras *et al.* (2004) that the influence of varying exposure time was minute compared to the temperature itself.

In each test, 10 seedlings from each treatment (two seedlings from each tray) were sampled for each test temperature. The sampled seedlings were randomized (origins and treatments) either in PL-81F trays (1-year-old seedlings) or PL-64F trays (2-year-old seedlings), which were then placed in wooden boxes. The boxes were insulated with sawdust (cover) and polystyrene (bottom) to protect the roots from freezing during the exposures. After thawing, the seedlings were moved to a greenhouse (20/15°C); where the natural light was supplemented with 400 W high-pressure sodium lamps for 8 hours. The seedlings were watered with tap water whenever necessary. After two weeks the proportion of tissue browning was visually scored on the needles of each seedling at 10% intervals.

Field performance

To test the effect of NL on seedling shoot growth after planting, the seedlings were planted in Suonenjoki which is located in an area where average temperature sum differs less than 150 d.d. from the temperature sums of the locations of the southern and northern seed origins used in E1, and thereby deemed appropriate for the execution of the experiment. The seedlings of E1 overwintered outdoors under the snow cover and the seedlings of E2 in cardboard boxes in frozen storage (-2 °C), from which they were transferred outdoors at the end of April. 60 seedlings (from E1) and 100 seedlings (from E2) from each NL treatment were randomly selected for the planting experiments. The seedlings were not sprayed with insecticide before planting. The seedlings from the E1 were planted in a randomized block design with four blocks

2007, Vol. 13, No. 2 (25)

of 15 seedlings in each treatment in a sandy test field (E1F), on 15 May 2000. On 17 May 2001 the seedlings from E2 were planted in a clearcut mesic forest site that had been mounded in 2000. The one- and two-yearold seedlings were planted in separate but adjacent areas (E2F), both in randomized block design with 5 blocks of 20 seedlings in each treatment. The texture of the moraine soil was finer (fraction < 0.06 mm 21.8%) in the test area of the two-year-old seedlings than in that of the one-year-old seedlings (9.4%), and the ground-cover vegetation was more abundant in the area of the two-year-old seedlings. The height (1 mm) of the seedlings was measured at the time of planting and at the end of the first, second and third growing seasons after planting. Each autumn, mortality of the seedlings was also determined.

Statistical analysis

The means and standard errors of the variables were calculated for the treatment groups using SPSS 12.0.1 for Windows. Both for the data of the nursery phase and for the planting experiments, the analysis of variance (ANOVA) for a randomized block design was applied after testing for normality of distributions and homogeneity of the variances. The significances of the differences (p<0.05) among the group means were tested using Tukey's test. Mortality of seedlings among treatments were tested with Kruskal-Wallis test due to non-normal distribution of mortality.

The FH of the seedlings was estimated using the logistic function (Repo and Lappi 1989, Luoranen et al. 2004):

[1]
$$y_{i} = f(x_{i}) + \varepsilon_{i}$$

$$= \frac{1}{\frac{-\ln(\frac{1}{\alpha_{1}})}{1 + \alpha^{-\frac{1}{\alpha_{i}}}(c-x_{i})}} + \varepsilon_{i}$$

where y_i is the damage to the needles, x_i is the exposure temperature, x_{10} and c are parameters and e_i is the error term. Inflection point c is the temperature at which the change in damage is maximal as temperature decreases. It was used to express the temperature at which 50% of the needles were damaged. x_{10} estimates the temperature at which 10% of needles were damaged.

The variances were homogenized by dividing both sides of Eq. 1 by the weight (w) in accordance with the methods described by Luoranen *et al.* (2004):

[2]
$$w = \sqrt{\hat{f}(1-\hat{f})} + 0.01$$

where \hat{f} is the current estimate of $f(x_i)$. The differences between the estimated curves for the FH in the treatments were tested by means of F-tests and between the FH estimates by the overlapping of 95% confidence intervals. The FH calculations were carried out using SPSS 13.0 for Windows.

Results

The origin of seed (E1)

The shoot elongation ceased at the beginning of August, 2-3 weeks after the start of the NL treatments (data not shown). During the blackout period the seedlings grew 2-3 cm. The control seedlings of the northern and southern origins continued their elongation for 1 and 4 weeks, respectively, longer than the NL seedlings. However, the NL seedlings remained shorter only in the case of the southern rather than the northern seedlings (Table 2).

In comparison with NL10, NL16 caused a significant reduction in root dry mass and an increased shoot-to-root ratio of the seedlings. No significant differences in these variables between NL10 and the control seedlings were observed (Table 2). The foliar nutrient concentrations of the seedlings were low (N 10.5-13.1 g kg⁻¹, P 1.6-2.0 g kg⁻¹ and K 6.6-8.1 g kg⁻¹) but they represent common values in Finnish nurseries.

Table 2. Shoot height, stem diameter, dry mass of needles, stem and roots of the seedlings from southern (S; Lapinjārvi) and northern (N; Vaala) origins (E1) and first- (1y) and second-year (2y) (E2) Norway spruce seedlings by NL treatments (NL16, NL12, NL10). The various letters after the numbers indicate statistically significant (p<0.05) differences between NL-treatments within origin and seedling ages

Exp./ origin/ type	Treatment	Shoot height mm	Diameter	Dry mass			Shoot /root
EI				needles	stem	root	
	NL10	109a	1.6a	402a	164a	363b	1.6b
	Control	147b	2.1b	526b	314b	430ь	2.0a
N	NL16	99a	1.3a	290a	112a	206a	2.0a.
	NL10	100a	1.5a	346b	143a	331b	1.5b
	Control	97a	1.5a	286a	136a	301b	1.45
E2							
ly	NL16	156a	1.7a	581a	229a	252a	3.1a
	NL12	151a	1.9b	599a	272a	336b	2.7a
	Control	181b	2.2c	658a	405b	365b	3.0a
2у	NL16	267a	3.0a	1728a	943a	558a	4.8a
	NL12	265a	3.2ab	1596a	1022a	693ab	3.8b
	Control	307ь	3.4b	1478a	1335b	726b	3.9b

2007, Vol. 13, No. 2 (25)

The WC of the seedling shoot tips of both origins was reduced by both NL treatments (Fig. 1). The differentiation in the WC of the NL-treated and control seedlings started earlier in the seedlings of southern origin than in those of northern origin. The decrease in the WC of the NL-treated seedlings compared with the control seedlings was larger in case of the southern (about 10 percentage units) than of the northern (about 5 percentage units) seedlings. The absolute WC was reduced less in seedlings of the northern than of southern origin.

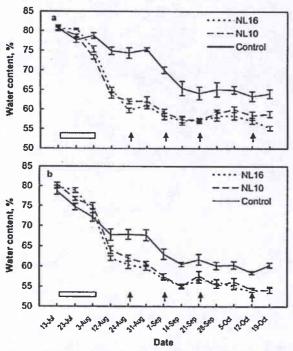


Figure 1. Mean (± s.e.; n=5) changes in the water content of shoot tips in first-year Norway spruce seedlings of (a) southern (Lapinjärvi), and (b) northern (Vaala) origins in various night length (NL) treatments (E1). The horizontal bar describes the time and duration of the NL treatments, and arrows indicate the dates of freezing tests

The control seedlings of the northern origin hardened faster and tolerated lower temperatures than seedlings of the southern origin (Fig. 2a and b). On the first two exposure dates, both NL treatments increased the FH by 4-6°C in comparison with the control seedlings of northern origin. On the last two exposure dates, the FH was difficult or impossible to estimate because the seedlings tolerated the lowest exposure temperatures (-19 and -26 °C) with no visual damage. Summarizing, the differences among the treatments disappeared when the frost hardening of the control seedlings also started to accelerate. In the case of the seedlings of southern origin, NL16 increased the FH by 5-6 °C compared to the controls on the first two exposure dates. Otherwise, there were no differences between the treatments in seedlings of the southern origin.

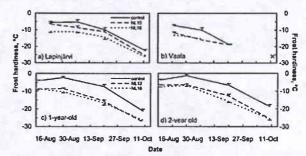


Figure 2. Frost hardiness (LT₁₀) of first-year Norway spruce seedlings of (a) southern (Lapinjärvi), and (b) northern (Vaala) origins, and of (c) first-year, and (d) second-year Norway spruce seedlings of Central Finnish origin in various night length (NL) treatments. The frost hardiness calculations are based on the temperature in which 10% of the needles were damaged. The vertical bars indicate asymptotic standard errors (ASE) for frost hardiness estimates. ASEs were estimated for all of the exposure dates, but in some cases they were too large to be shown in the Figure. On the final date in 1999 all of the northern origin seedlings tolerated the lowest exposure temperature and it was therefore impossible to estimate FH

The relationship between the WC and the FH of the seedlings differed according to the NL treatments and the seedling origins (Fig. 3). The control seedlings of the northern origin achieved FH of -20 °C with 2 percentage units lower WC than those of southern

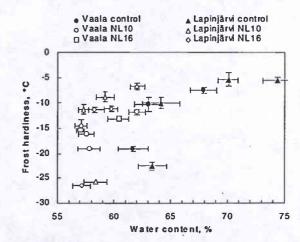


Figure 3. Correlation of water content (WC) and frost hardiness (LT₁₀) of seedlings from different origins and with varying night length treatments. Vertical and horizontal bars indicate ±SE of FH and WC estimates, respectively

2007, Vol. 13, No. 2 (25)

origin and NL-treated seedlings with approximately 5 percentage units lower WC than the control seedlings.

Seedling age (E2N)

After the start of the blackout, both 1- and 2-year old NL-treated seedlings grew 3 cm and the control seedlings 6 cm in height (data not shown). There were no differences between NL16 and NL12. In addition to reducing the shoot height, the NL treatments also reduced the dry mass of stems and roots in both first-and second-year seedlings (Table 2). However, the difference in root mass between the NL12 and control seedlings was minor and statistically insignificant. On the other hand, there were no clear differences in stem diameter or dry mass of seedlings in the responses to the NL treatments between the first- and second-year seedlings (Table 2). Foliar nutrient concentrations in seedlings varied for N from 12.7-1.46 g kg⁻¹, for P from 1.7-2.1 g kg⁻¹ and for K from 6.9-9.0 g kg⁻¹.

The frost hardening of the control seedlings did not differ between seedling ages (Fig. 2 c and d). In both seedling ages, the NL treatments both accelerated and increased the FH by 3-9 °C compared to the control seedlings, depending on the testing date. The NL16 seedlings hardened somewhat faster than the NL10 seedlings, but from a practical point of view there were no differences between the NLs.

Shoot growth after planting

The first-year shoot growth of the planted oneyear-old NL seedlings both in E1F and E2Fa did not differ significantly from the control seedlings though the height growth of NL treated seedlings was slightly bigger than that of the control seedlings (Fig. 4). Instead, the two-year-old control seedlings grew more than the NL16 seedlings in E2Fb. In both experiments no differences in shoot growth amongst the various treatments in later years were observed. The mortality of the seedlings planted at the forest site (E2F) was 8% for one-year-old seedlings and 29% for two-yearold seedlings. The mortality of the seedlings did not differ among the NL-treatments in one-year-old (Kruskal-Wallis test, p=0.464) or the two-year-old seedlings (p=0.109). The damage that they incurred was caused by large pine weevil (Hylobius abietis L.).

Discussion and conclusions

All of the NLs (from 10 h to 16 h) used for stopping shoot growth in this study were longer than the critical NLs for the origins used, and therefore no differences in height growth cessation existed among the treatments used (Table 2). Similarly, Eastham (1991) found no difference in the shoot growth of Sitka spruce

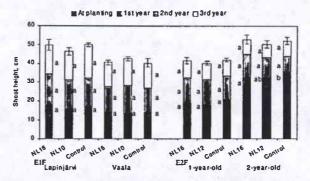


Figure 4. Initial height and height growth of one-year-old Norway spruce seedlings of southern (Lapinjärvi) and northern (Vaala) origins, and one- and two-year-old Norway spruce seedlings of seed orchard origin (for Central Finland), 3 years after planting. The stacked bars represent the block means (n=4 in E1F and n=5 in E2F). The vertical bars on the tops of columns indicate the SE of means of the height of seedlings and the same letters by the stacked bars indicate that differences in annual shoot growth are not significant (p<0.05) among NL-treatments separately in each origin and seedling age

(Picea sitchensis) (Bong.) Carriere) × white spruce treated with NLs of 15 h or 18 h. However, if the NL is shorter than the critical one for the origin, the height growth fails to stop (Heide 1974a, Hawkins and Draper 1991). The height growth of the control seedlings of southern origin continued for 3 weeks longer than that of northern origin. Thus, as Krasowski et al. (1993) have pointed out, a natural NL may be sufficient to induce the bud set of northern origin seedlings that are grown in southern nurseries.

NL16 reduced the stem diameter and root dry mass, and increased the shoot-to-root ratio compared with the control while NL10 or NL12 did not differ in these respects from the control seedlings. This can be explained by the different critical NLs for height growth and cambial growth (Heide 1974a, 1977) or differences in photosynthesis in seedlings between the treatments. Krasowski et al. (1993) have suggested that long night lengths could have negative effects on seedling morphology. Although Hawkins et al. (1994) showed that NL-treatment promotes photosynthesis after treatment it is not known whether this promotion compensates for the lower photosynthesis during the blackout period.

The seedlings of northern origin hardened faster and achieved higher FH than the seedlings of southern origin (Fig. 2) like many earlier studies have shown (Sandvik 1980, Johnsen 1989, Pulkkinen 1993). The NL-treated seedlings hardened earlier than the control seedlings in all cases (Fig. 2). The shorter NL used was long enough to harden the seedlings excluding

2007, Vol. 13, No. 2 (25)

the seedlings of southern origin, in which 16 h nights were more efficient than 10 h nights at the early stage of hardening. Similarly, in more southernly locations (<50 °N) with a shorter natural photoperiod, NL has been positively associated with frost hardening; e.g. white spruce achieved higher FH with NL16 than with either NL12 or NL14 (Bigras and D'Aoust 1993) and Douglas fir with NL16 rather than with NL12 or NL8 (van den Driessche 1969).

The WC of shoots decreases during the hardening of seedlings (Rosvall-Åhnebrink 1977, Colombo 1990) and NL treatment has been shown to accelerate this decrease (Rosvall-Ahnebrink 1977, Calme et al. 1993). In the present study, NL treatments also reduced the shoot WC in comparison with the control seedlings (Fig. 1). A critical value of WC for hardened (FH<-10 °C) seedlings has been regarded to be ca. 66-70% (Rosvall-Åhnebrink 1977, Calme et al. 1993). In this study, however, the seedlings were still non-hardened (FH >-10 °C) at a WC of 70% and especially NLtreatment seemed to affect the relation of FH and WC. However, also Rosvall-Ahnebrink (1977) mentioned that different cultural measures and origin of seed affect critical WC. Thus, there seems not to be one critical WC value which would reliably indicate the FH of seedlings, but rather, the effects of seed origin and cultural measures on the relation of shoot WC and FH must be known.

In the present study, the NL-treatments had no effect on the first year shoot growth of the one-year old seedlings. NL16 decreased the first year shoot growth in the two-year old seedlings relative to controls (Fig. 4). It is difficult to conclude the reasons for contradictions among the results which support (e.g. Odlum and Colombo 1988, Eastham 1991, Odlum 1992 and Hawkins et al. 1996) the increased shoot growth and which did not show any difference or even decreased shoot growth (Rosvall-Åhnebrink 1982, and Konttinen et al. 2003).

In conclusion, the advantage achieved with NL16, used traditionally, was only a minor increase in FH compared to the shorter NLs (10 and 12 h) with both one- and two-year-old seedlings and in both southern and northern origins. Furthermore, longer nights (NL16) even tended to retard the growth of stem diameter and root mass compared to shorter nights. Thus, the shorter night lengths (10-12 h) are recommended for use in blackout treatments. In nurseries, it appears particularly important to treat seedlings of more southern origins with blackout, since the photoperiod of their natural locale increases their susceptibility to autumn frost more so than seedlings of northern origin. The relationship between shoot WC and the FH of seedlings was shown to depend on the

night length treatments as well as on seedling origin. Thereby shoot WC should only be used as a predictor of seedling FH with reliance on sufficient background data. In this study only one of the three factors affecting the result of SD treatment was studied.

Therefore, we advocate further factorial experimentation to elucidate the separate and interactive effects of these treatments on the frost hardiness and further field growth performance of coniferous seedlings.

Acknowledgements

We should like to thank Ritva Pitkänen and Pekka Savola for assisting us with the experiments and field work, and Dr. John A Stotesbury and Dr. Otso Huitu for revising the English language of the manuscript.

References

- Aronsson, A. 1975. Influence of photo- and thermoperiod on the initial stages of frost hardening and dehardening of phytotron-grown seedlings of Scots pine (Pinus silvestris L.) and Norway spruce (Picea abies (L.) Karst. Studia Forestalia Suecica, 157: 1-47.
- Bigras, F. J. and D'Aoust, A. L. 1993. Influence of photoperiod on shoot and root frost tolerance and bud phenology of white spruce seedlings (*Picea glauca*). Canadian Journal of Forest Research, 23: 219-228.
- Bigras, F., C. Coursolle and Margolis, H. 2004. Survival and growth of *Picea glauca* seedlings as a function of freezing temperatures and exposure times during budbreak and shoot elongation. *Scandinavian Journal of Forest Research*, 19(3): 206 -217.
- Calmé, S., Margolis, H. A. and Bigras, F. J. 1993. Influence of cultural practices on the relationship between frost tolerance and water content of containerized black spruce, white spruce, and jack pine seedlings. Canadian Journal of Forest Research, 23: 503-511.
- Christersson, L. 1978. The influence of photoperiod and temperature on the development of frost hardiness in seedlings of *Pinus silvestris* and *Picea abies. Physiologia Plantarum*, 44: 288-294.
- Colombo, S. J. 1990. Bud dormancy status, frost hardiness, shoot moisture content, and readiness of black spruce container seedlings for frozen storage. *Journal of American Society for Horticulture Science*, 115(2): 302-307.
- Coursolle, C., Bigras, F.J., Margolls, H.A. and Hebert, C. 1998. Growth and hardening of four provenances of containerized white spruce (*Picea glauca* (Moench) Voss) seedlings in response to the duration of 16 h long-night treatments. *New Forests*, 16: 155-166.
- Dormling, I. 1993. Bud dormancy, frost hardiness, and frost drought in seedlings of *Pinus sylvestris* and *Picea abies*. In: Li, P.H. and Christersson, L. (Eds.), Advances in Plant Cold Hardiness. Boca Raton: CRC Press, p. 285-298.
- Dormling, I., Gustafsson, Å. and Wettstein, D. 1968. The experimental control of the life cycle in *Picea abies* (L) Karst. 1. Some basic experiments on the vegetative cycle. Silvae Genetica, 17: 44-64.
- Eastham, A. M. 1991. Timing of blackout application to regulate height in sitka x white spruce hybrid 1+0 contain-

2007, Vol. 13, No. 2 (25)

- er-grown seedlings. In 11th Annual Meeting of Forest Nursery Association of B. C. Prince George, B.C. Canada, p. 86-92
- Halonen, O., Tulkki, H. and Derome, J. 1983. Nutrient analysis methods. Finnish Forest Research Institute, Research Papers, 121, 28 p.
- Hawkins, C. D. B. and Draper D. A. 1991. Effects of blackout on British Columbia spruce seedlings at Red Rock Research Station. FRDA Report 170, 51 p.
- Hawkins, C. D. B., Eng, R.Y.N. and Krasowski, M.J. 1994. Short day nursery treatment promotes photosynthesis in Interior spruce seedlings: Summary of poster. In: Landis, T.D. and Dumroese, R.K. (Tech. coord.) National Proceedings, Forest and Conservation Nursery Associations. USDA Forest Service. General Technical Report RM-GTR-257: 268-270.
- Hawkins, C. D. B., Eastham, A. M., Story, T. L., Eng, R. Y. N. and Draper, D. A. 1996. The effect of nursery blackout application on Sitka spruce seedlings. Canadian J. of Forest Research, 26: 2201-2213.
- Heide, O. M. 1974a. Growth and dormancy in Norway spruce ecotypes (*Picea abies*). I. Interaction of photoperiod and temperature. *Physiologia Plantarum*, 30: 1-12.
- Heide, O. M. 1974b. Growth and dormancy in Norway spruce ecotypes. II. After-effects of photoperiod and temperature on growth and development in subsequent years. Physiologia Plantarum 31: 131-139.
- Heide, O. M. 1977. Photoperiodism in higher plants: An interaction of phytochrome and circadian rhythms. Physiologia Plantarum, 39: 25-32.
- Håbjørg, A. 1978. Photoperiodic ecotypes in Scandinavian trees and shrubs. Scientific reports of the agricultural university of Norway, 57(33): 1-20.
- Johnsen, Ø. 1989. Phenotypic changes in progenies of northern clones of *Picea abies* (L.) Karst. Grown in a southern seed orchard. I. Frost hardiness in a phytotron experiment. Scandinavian Journal of Forest Research, 4: 317-330.
- Konttlinen, K., Rikala, R. and Luoranen, J. 2003. Timing and duration of short-day treatment of *Picea abies* seedlings. *Baltic Forestry*, 9(2): 2-9.
- Krasowski, M. J., Letchford, T. and Eastham, A. M. 1993.
 Growth of short-day treated spruce seedlings planted throughout British Columbia. Forestry Canada and B.C. Ministry of Forests. FRDA report 0835-0752 209. 39 p.
- Krasowski, M. J., Caputa, A and Hawkins, C. D. B. 1994.
 Can foliage water content measurement replace freezer tests in determining a safe lifting time for frozen storage of conifer seedlings. In: Landis, T.D. and Dumroese, R.K. (tech. coords.). National Proceedings, Forest and Conservation Nursery Associations. Fort Collins CO: U.S. Department of Agriculture, Forest Service. Rocky Mountain Forest and Range Experiment Station. Gen. Tech. Rep. RM-257: 261-267.
- Levitt, J. 1980. Responses of plants to environmental stresses. Vol I. Chilling, freezing, and high temperature stresses. 2nd ed. Academic Press, New York, 497 p.

- Luoranen, J., Repo, T. and Lappi, J. 2004. Assessment of the frost hardiness of shoot of silver birch (Betula pendula) seedlings with and without controlled exposure to freezing. Canadian Journal of Forest Research, 34: 1108-1118.
- Odlum, K. D. 1992. Hardening and overwintering container stock on Ontario: Practices and research. In Proceedings of the 1991 Forest Nursery Association of British Columbia Meeting. Prince George, British Columbia, p. 29-35.
- Odlum, K. D. and Colombo, S. J. 1988. Short day exposure to induce budset prolongs shoot growth in the following year. In: Landis, T.D. (Ed.) Proceedings of Combined Meeting of the Western Forestry Nursery Association. USDA Forest Service. General Technical Report RM-167: 57-59.
- Pollard, D. F. W. and Logan, K. T. 1977. The effects of light intensity, photoperiod, soil moisture potential and temperature on bud morphogenesis in Picea species. Canadian J. of Forest Research, 7: 415-421.
- Pulkkinen, P. 1993. Frost hardiness development and lignification of young Norway spruce seedlings of southern and northern Finnish origin. Silva Fennica, 27(1): 47-54
- Repo, T. and Lappi, J. 1989. Estimation of standard error of impedance-estimated frost resistance. Scandinavian Journal of Forest Research, 4: 67-74.
- Rosvall-Ahnebrink, G. 1977. Artificial hardening of forest tree seedlings in plastic greenhouses. Swedish University of Agriculture, Institution for Forest Regeneration. Interna Rapporter, 14: 153-161. (In Swedish with English abtract)
- Rosvall-Åhnebrink, G. 1982. Practical application of dormancy induction techniques to greenhouse-grown conifers in Sweden. In Scarratt, J.B., Glerum, G. and Plexman, C.A. (Eds.), Proceedings of the Canadian containerized tree seedling symposium. Toronto, Ontario, Can. Ont. Joint For. Res. Comm. Proc. O-P-10: 163-170
- Sandvik, M. 1980. Use of controlled environment facilities. Environment control of winter stress tolerance and growth potential in seedlings of Picea abies (L.) Karst. New Zealand Journal of Forest Science, 10: 97-104.
- Toivonen, A., Rikala, R. Repo, T. and Smolander, H. 1991. Autumn coloration of first year *Pinus sylvestris* seedlings during frost hardening. *Scandinavian Journal of Forest Research*, 6: 31-39.
- van den Driessche, R. 1969. Influence of moisture supply, temperature, and light on frost-hardiness changes in Douglas-fir seedlings. Canadian Journal of Botany, 47: 1765-1772.
- Van Steenls, E. 1992. Short day treatment of conifer seedlings in British Columbia forest nurseries. In: Landis, T.D. (Tech. coord.) Proceedings Intermountain Forest Nursery Association. Park City, UT. Gen. Tech. Rep. RM., 211: 103-105.

Received 27 April 2007 Accepted 30 October 2007

2007, Vol. 13, No. 2 (25)

BALTIC FORESTRY

GROWTH AND FROST HARDENING OF PICEA ABJES SEEDLINGS /.../

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РОСТ И МОРОЗОУСТОЙЧИВОСТЬ САЖЕНЦЕВ *PICEA ABIES* ПОСЛЕ ВОЗДЕЙСТВИЯ ПЕРИОДА НОЧИ РАЗЛИЧНОЙ ПРОДОЛЖИТЕЛЬНОСТИ

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Резюме

Изучалось воздействие различной продолжительности ночного периода (ПН: 16 ч, 12 ч или 10 ч и окружающей среды в качестве контрольного) в течение трех недель на морфологию, содержание воды (СВ) в побеге и морозоустойчивость (МУ) однолетиих саженцев ели европейской (*Picea abies* (L.) Karst.) северного (64° 40') и южного (60° 40') финского происхождения и одно- и двухлетиих саженцев ели европейской местного происхождения в питомнике Центральной Финляндии (62° 38'). Также изучался рост культур в высоту после посадки. ПН отрицательно ассоциировался с приростом диаметра ствола и сухой массой корней. МУ под воздействием ПН увеличилась с 4 до 7°С в сравнении с юнтрольными саженцами, но разницы между воздействием ПН были малы. Не было замечено стойкой связи между СВ и МУ. Воздействие ПН имело лишь малый эффект на рост культур в высоту после посадки. В заключение, более короткая ночь (10 – 12 часов) рекомендуется для использования затемнения.

Ключевые слова: морозоустойчивость, период ночи, ель европейская, происхождение, *Picea abies*, фотопериод, короткий световой день

2007, Vol. 13, No. 2 (25)