Morphology and Phenology in *Picea abies* Seedlings in Response to Split Short-Day Treatments

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**Abstract**

Short-day (SD) treatment is used by forest nurseries to induce growth cessation in *Picea abies* seedlings. SD treatment may however increase the risk of refusling in autumn and earlier bud break the following spring. When the start of the SD treatment is early in order to control seedling height, the duration of the SD treatment should be longer in order to prevent refusling in autumn. However, due to the amount of manual work involved in the short-day treatment, increasing the number of days is undesirable from a practical point of view. Splitting the SD treatment could be a way to achieve both early height control and at the same time avoid autumn bud break with less workload. We tested how different starting dates and durations of SD treatment influenced on morphological and phenological traits. Regardless of timing and duration of the SD treatment, height growth was reduced compared to the untreated controls. Seedlings given split SD (7+7 days interrupted with two weeks in long days) had less height growth than all other treatments. Root collar diameter growth was significantly less in control seedlings than in seedlings exposed to early (7 or 14 days) or split (7+7 days) SD treatment. There were also differences in the frequency of refusling and bud break timing among the SD treated seedlings, dependent on duration and starting date. If the SD treatment started early, a continuous 14-day SD treatment was not sufficient to avoid high frequencies of refusling. However, by splitting the SD treatment into two periods of 7+7 days these negative effects were largely avoided, although spring bud break occurred earlier than in the controls.

**Keywords:** bud flush; height growth; lammas shoot; morphology; Norway spruce; *Picea abies*; phenology; root collar diameter; second bud break; short day treatment; sturdiness

**Introduction**

Short-day (SD) treatment is regularly used by forest nurseries at northern latitudes to promote growth cessation and to achieve sufficient frost hardiness (Dormling et al. 1968, Heide 1974a, Grossnickle 2000, Colombo et al. 2001). In forest nurseries such treatment is typically applied by giving 8-10 h day and 14-16 hight to promote an immediate reaction (Grossnickle 2000). In *Picea abies* (L.) Karst seedlings intended for autumn planting such treatment is standard routine in Norway (Ministry of Agriculture 1996, Floiastad and Granhus 2013), and due to increased frost hardiness, the SD treatment also improve storability of seedlings intended for planting in spring (Venn 1980, Colombo 1990, Jacobs et al. 2008, Wallin et al. 2017).

To preserve the good seedling quality obtained by the SD treatment, it is important to avoid autumn refusling (Kohmann and Johnsen 2007, Luoranan et al. 2009, Floiastad and Granhus 2013), and timing of the SD treatment should be synchronized with the critical night length of the provenances (Heide 1974a, Dormling 1993, Kohmann 1996). Both timing and duration of the SD treatment influence the risk of refusling in late summer (Eastham 1992, Konttinen et al. 2007, Luoranan et al. 2009), and, therefore, it is essential to adjust the SD routines accordingly. Temperature conditions may also influence the risk of autumn refusling, as a high temperature sum before and low temperature following the SD treatment has been found to prevent autumn refusling (Luoranan et al. 2009). In addition to the risk of frost damages in autumn planted seedlings, autumn refusling has been associated with greater risk of fungal infection during winter storage in the nursery (Sandvik 1976, Petäistö 2006).

When timing and duration of the SD treatment are properly adjusted (Konttinen et al. 2003, Floiastad and Granhus 2013) and optimal growing conditions are provided following the SD treatment, increased root collar diameter can be achieved (Bjørnseth 1977, Colombo 1997, Floiastad and Granhus 2010). In that way nursery cultural practice influence on the seedlings attributes and thereby affects seedling performance (Mattsson 1997, Grossnickle 2012, Grossnickle and MacDonald 2018). The SD
treatment has been shown to promote earlier spring bud break in several *Picea species*, such as *P. abies* (Heide 1974b, Sandvik 1980, Hannerz 1998), *Picea mariana* (Mill.) B.S.P. (Colombo 1986, Bigras and D’Aoust 1992) and *Picea glauca* (Moench) Voss (Bigras and D’Aoust 1992). High temperature during terminal bud formation is known to delay the timing of bud break (Sogaard et al. 2008, Granhus et al. 2009), and could thereby probably counteract the promoting effect of SD on bud break. However, temperature effects from altering the SD treatment during a few weeks are probably too small to have practical significance (Floistad and Granhus 2010).

An earlier study suggested that if the SD treatment started early in order to control height growth, the duration should be longer in order to prevent flushing in autumn (Floistad and Granhus 2013). However, due to the amount of manual work involved in the short-day treatment, increasing the number of days is undesirable from a practical point of view. Splitting the SD treatment could be a way to achieve both early height control and at the same time avoid autumn flushing with less workload. Therefore, the aim of the present study was to investigate how a split SD treatment influences growth and phenology responses in *P. abies* seedlings when the SD treatment is initiated early to control height growth. Our hypotheses were the following: (i) increased seedling diameter may be obtained if seedling height growth is terminated early; (ii) splitting the SD treatment may result in both control of height growth and avoidance of autumn bud flush, and (iii) the date of termination of the SD treatment influence more on the risk for autumn flushing than does the length of the treatment. In order to have control with the environmental conditions, the experiment was performed in a phytotron.

Materials and Methods

Seedling material and experimental conditions

Two lots of *Picea abies* seeds were sown on 25 June 2004 in limed peat, mixed with 25 % perlite, in multipot containers (75 cm² pots, 500 seedlings m⁻², 60 pots per container). One seed lot was collected in Buskerud County, South-Eastern Norway (60° N, 10° E, altitude 0-150 m. a.s.l.), while the other seed lot was collected in Hallen seed orchard, Norway (59°22’ N, 9°13’ E). Two seeds were sown in each pot and following germination thinned to one seedling per pot. Germination and the first-year growth phase took place in the greenhouse of a commercial forest nursery at Hokksund (59°46’ N, 9°53’ E), Buskerud County. During winter seedlings were stored outdoors and prior to the experiment, on 31 May 2005, seedlings were brought to the controlled environment of the phytotron at the University of Oslo.

The seedlings were placed into two phytotron rooms, which were initially programmed for long day conditions (21 h day (250 μmol), 3 h night) and day/night temperature 22/18 °C (12/12 h). Short-day (SD) treatment was given in a third phytotron room programmed for short day (10 h day, 14 h night) and day/night temperature 22/18 °C (12/12 h). The relative humidity was set to 70% in all phytotron rooms throughout the experiment.

Seedlings were exposed to SD treatment for a total duration of 7 or 14 days. The latter duration was tested either with starting date early (20 June), late (11 July), or as a split SD treatment with 7 + 7 days SD treatment separated by two weeks with 21 h day/3 h night (Figure 1). Control seedlings received continuously long day conditions (21 h day). For each seed lot, four multipot containers (replicates) were exposed to each of the five treatments.

![Figure 1. Time schedule for each of the four experimental short-day (SD) treatments with timing and duration (7 or 14 SD) of the treatments indicated. Control seedlings were not exposed to the SD treatment.](image)

For hardiness development, and to simulate outdoor conditions, the day length was gradually reduced from the long day condition (21 h) with 2h each week from 15 August. From 12 September light intensity was reduced to 70 μmol with day length 10 h, and the temperature was reduced to 10 °C. Then from 10 October the day length was 8 h and the temperature was reduced to 6 °C. From 2 November the day length was 6 h, until the seedlings were moved to dark, cold storage (0-1 °C) on 20 December. Following winter storage, the seedlings were transferred back to the phytotron room on 17 February with 21 h day (250 μmol), 3 h night and temperature at 22/18 °C (12/12 h).

The seedlings were watered and fertilized with a complete nutrient solution (60:40 Red Superba, Norsk Hydro and (NH₄)₂SO₄, 0.7 mS cm⁻¹) throughout the experimental period.

Measurements and phenological registrations

From the start of the experiment, seedling height and root collar diameter were measured twice a month on 10 randomly selected seedlings per treatment, seed lot and replicate. The same seedlings were measured each time. Seedling height was defined as the length of the
stem measured from the top of the container up to the terminal meristem (Mixel and Landis 1990).

From August and until the seedlings were placed in cold storage, registrations of autumn reflushing were made on terminal and lateral buds twice a month on all seedlings. During forcing following winter storage, the stages of bud break were assessed three times every week according to the following scale (Fløistad and Kohmann 2001): 0 = dormant buds; 1 = buds slightly swollen; 2 = buds swollen, bud scales still covering the new needles; 3 = bud scales diverging, no elongation of needles; 4 = elongation of needles to 5 mm, needles not yet spread; 5 = needle elongation 5–10 mm, needles spread; 6 = needle elongation 10–15 mm, needles spread; 7 = needle elongation >15 mm, needles spread.

**Statistical analysis**

Analyses of variance were performed using the GLM procedure in SAS (SAS Institute Inc. 2014) according to the model:

\[ Y_{ij} = \mu + t_i + c_j + e_{ij} \]  

where: \( Y_{ij} \) is the mean of all plants for each combination of the experimental factors considered, \( \mu \) is the total mean, \( t_i \) is the fixed effect of treatment, \( c_j \) is the random effect of container (replicate), and \( e_{ij} \) is the experimental error.

The effect of SD treatment on a second bud flush was assessed by calculating large-sample 95% confidence intervals (Agresti 1996) for the frequency of seedlings with the second bud flush upon termination of the experiment. The treatments were considered significantly different if confidence intervals did not overlap.

To calculate the mean number of days to bud break in 50% of the seedlings, linear regressions were developed to predict the proportion of seedlings that had reached bud break stage 3, with forcing days as the independent variable. Separate regressions were estimated for each combination of replicate and experimental treatment, and days to bud break were obtained from the estimated regression functions.

**Results**

There were no significant effects of provenances on seedling height, diameter growth, autumn reflushing or time of spring bud break, and therefore the two groups were calculated together when analysing effects of treatments.

**Seedling height and root collar diameter**

The height growth of the seedlings was significantly affected by the photoperiodic treatment (\( p < 0.0001 \)). Seedlings given the split SD treatment (7 + 7 days – Figure 2a) had significant less height growth than all other treatments (\( p < 0.0001 \)). There was significantly larger height growth in seedlings without SD treatment, than in SD treated seedlings (\( p < 0.0001 \)) regardless of timing and duration of the treatment. Otherwise, no significant differences in height growth appeared among the treatments. In seedlings given early SD treatment, resumption of height growth was evident after about three to four weeks following termination of the SD treatment, regardless of whether the SD duration was 7 or 14 days (Figure 2a). Diameter growth was significantly less in seedlings not exposed to the SD treatment than in seedlings exposed to early (7 days) or split SD treatment (\( p = 0.0062 \) for 7 days and \( p = 0.0052 \) for 7 + 7 days of treatment) (Figure 2b). Apart from this no significant differences appeared among seedlings exposed to the SD treatments of different duration and starting date.

**Reflushing**

Reflushing occurred with the highest frequency in seedlings exposed to early SD treatment, compared with seedlings having late SD treatment (Figure 3). The significantly highest frequencies of reflushing in terminal
and lateral buds, 94%, were observed among the seedlings given only 7 days of early SD treatment ($p < 0.05$). Among seedlings with 14 days early SD treatment, starting 20 June, 88% showed resprouting. Fourteen days with late (starting 11 July) or split (starting 20 June and 11 July) SD treatment resulted in only 20% and 23% of the seedlings with resprouting, respectively. Among seedlings in the two treatments resulting in the lowest number of resprouting, more than half of the cases were observed only in the lateral buds (Figure 3). However, among SD treatments with the highest frequencies of resprouting, it occurred in both terminal and lateral buds (Figure 3).

![Figure 3. Frequency of seedlings with autumn resprouting in terminal or lateral buds following SD treatment with different duration and starting dates.](image)

**Bud break after cold storage**

Following forcing in long days after cold storage, bud break appeared significantly earlier ($p < 0.0001$) in seedlings of all different combinations of SD treatment, compared with the control seedlings (Table 1). There were also notable differences among seedlings given different durations and starting dates of the SD treatment, with the earliest bud break in seedlings given 14 days of the early SD or split (7 + 7 days) SD treatment.

**Discussion and Conclusion**

As expected, the height growth of the seedlings was reduced significantly by the different SD treatments in accordance with previous findings (Dormling et al. 1968, Heide 1974a, Fløistad and Granhus 2013). The greatest reduction in height growth occurred following the early and split SD treatment. However, resumption of height growth in seedlings with only early SD, resulted in notable differences in seedling height between the early (7 or 14 days) and the split (7 + 7 days) SD treatments. While several of the seedlings given continuous early SD treatment of either 7 or 14 days duration resumed height growth upon return to the simulated ambient photoperiodic conditions, this was avoided with the split SD treatment. Thus, the best control of height growth was achieved with the latter treatment.

<table>
<thead>
<tr>
<th>Number of days to 50% bud break following SD treatment with different duration and starting dates</th>
<th>Number of days to bud break in spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early SD</td>
<td>20 June</td>
</tr>
<tr>
<td>Split SD</td>
<td>20 June and 11 July</td>
</tr>
<tr>
<td>14 + 14</td>
<td>20 June</td>
</tr>
<tr>
<td>14 + 14</td>
<td>11 July</td>
</tr>
<tr>
<td>No SD</td>
<td>-</td>
</tr>
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Although the effect of the different treatments on diameter growth was less pronounced, it is noteworthy that the smallest root collar diameter appeared in the untreated seedlings or following the late 14-day SD treatment and thus partly confirmed our first hypothesis. It is likely that this may be explained by patterns of carbohydrate partitioning within the seedlings, as less carbohydrates are available for diameter growth before cessation of height growth (Hawkins et al. 1994, Turner and Mitchell 2003, Lamhamedi et al. 2013, McKown et al. 2016). The differences in seedling heights resulting from the treatments may however also have affected the light conditions within the containers, causing a crowding effect that may have reduced the diameter growth in the taller seedlings. Regardless of the timing or duration of the SD treatment, the cessation of root collar diameter growth did generally not occur earlier in the SD treated than in the untreated seedlings (cf. Figure 2b). This suggests that an optimal timing and duration of the SD treatment could be designed to control the diameter to height ratio of seedlings and thus to improve the sturdiness and seedling quality prior to planting (Thompson 1985, Grossnickle 2012). Diameter growth after the SD treatment may however also depend on the day length during the treatment (Colombo et al. 2001), as well as on the nutrient supply and temperature regime following the SD period (Bjornseth 1977, Fløistad and Granhus 2010), which were not included as experimental factors in our study.

Our results support the findings of several other studies, who observed the highest frequencies of...
reflushing in seedlings given the early SD treatment (Eastham 1992, Luoranen et al. 2009, Fløistad and Granhus 2010, Fløistad and Granhus 2013, Luoranen and Rikalai 2015). The long day condition following the SD treatment in our experiment may have activated more reflushing than would have appeared in nursery conditions (Rostad et al. 2006, Fløistad and Granhus 2013). Still, the difference among treatments is interesting and corresponds to practical experience in forest nurseries when they are forced to start SD early to control seedling height. The frequency of seedlings flushing in autumn was substantially lower in the split SD (7 + 7 days) treatment and in seedlings given the continuous 14-day treatment with late onset, compared with both the early 7- and 14-day SD treatments and thus confirmed our second hypothesis. In the study of Luoranen et al. (2009) a higher frequency of autumn reflushing was observed following 14 days of SD treatment compared with three weeks of the SD treatment. With an early start of the SD treatment they observed however some reflushing even when the treatment was applied for three weeks. According to their results both a low temperature sum before and a high temperature sum after the SD treatment was associated with a high frequency of autumn reflushing in two-year-old seedlings. As termination of the SD period was latest with the treatments having the lowest frequencies of autumn reflushing in our study, our results are in generally in line with their findings and our third hypothesis. It may be noted however, that Fløistad and Granhus (2010) observed a higher frequency of autumn bud break when the seedlings were initially kept at 18 °C and ambient photoperiod after the 14-day SD treatment, compared with seedlings transferred to ambient photoperiod and 22 °C or 14°C. Further studies may be needed to clarify the complex and interacting effects of temperature and photoperiod on the susceptibility of seedlings to reflushing in autumn following the SD treatment.

Several studies have shown that SD treatment results in earlier bud break the following spring (e.g. Colombo 1986, Bigras and D’Aoust 1992, Konttinen et al. 2003, Fløistad and Granhus 2010, Luoranen and Rikalai 2015), which is in line with our results. Contrasting effects have however been reported as regards the effect of SD timing. For example, Konttinen et al. (2003) reported earlier bud break following the earlier onset of the SD treatment, while no such effect was found by Fløistad and Granhus (2010). Comparing the two continuous SD treatments in our study that were of similar duration (14 days), but with different starting dates (20 June or 11 July), we observed earlier bud break following the earliest SD. On the other hand, the number of forcing days required for spring bud break in seedlings given early SD of 7 days duration was as high as in seedlings given the late 14-day SD treatment. Still, considering the high frequency of autumn reflushing, it is evident that the short and early SD treatment did not result in the desired seedling traits. Morphological features of the bud and bud scale complex may be affected by the SD treatment and be at least partly responsible for the earlier onset of bud break in the SD treated seedlings (Luoranen and Sutinen 2017). In future work on different nursery SD treatments, it would therefore be of considerable value to include studies of the effects on the internal structure of buds.

The results support that timing and duration of the SD treatment may be used by forest nurseries to control height and diameter growth of seedlings. If the SD treatment started early, a continuous 14-day SD treatment was not sufficient to avoid height growth resumption and high frequencies of autumn reflushing. However, by splitting the SD treatment into two periods of 7+7 days these negative effects were largely avoided. The short-day treatment involves a high amount of manual work and reducing the number of days for the SD treatment is therefore desirable from a practical point of view. Splitting the SD treatment could be a way to achieve both early height control and reduced reflushing with less workload. According to our results this will also gain the seedling root collar diameter growth, thus leading to more sturdy seedlings for outplanting.

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References


Ministry of Agriculture 1996. Forskrift om skogstro og skogplanter [Regulations on forest seeds and forest plants], Oslo, Norway, 5 pp. (in Norwegian)


