Climatic control of bud burst in young seedlings of nine provenances of Norway spruce

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Summary Detailed knowledge of temperature effects on the timing of dormancy development and bud burst will help evaluate the impacts of climate change on forest trees. We tested the effects of temperature applied during short-day treatment, duration of short-day treatment, duration of chilling and light regime applied during forcing on the timing of bud burst in 1- and 2-year-old seedlings of nine provenances of Norway spruce (*Picea abies* (L.) Karst.). High temperature during dormancy induction, little or no chilling and low temperature during forcing all delayed dormancy release but did not prevent bud burst or growth onset provided the seedlings were forced under long-day conditions. Without chilling, bud burst occurred in about 20% of seedlings kept in short days at 12 °C, indicating that young Norway spruce seedlings do not exhibit true bud dormancy. Chilling hastened bud burst and removed the long photoperiod requirement, but the effect of high temperature applied during dormancy induction was observed even after prolonged chilling. Extension of the short-day treatment from 4 to 8 or 12 weeks hastened bud burst. The effect of treatments applied during dormancy development was larger than that of provenance; in some cases no provenance effect was detected, but in 1-year-old seedlings, time to bud burst decreased linearly with increasing latitude of origin. Differences among provenances were complicated by different responses of some origins to light conditions under long-day forcing. In conclusion, timing of bud burst in Norway spruce seedlings is significantly affected by temperature during bud set, and these effects are modified by chilling and environmental conditions during forcing.

Keywords: chilling, dormancy, forcing, light conditions, Picea abies.

Introduction

Survival of northern tree species depends on synchronization of their annual growth–dormancy cycle with local climate. Cessation of growth in the fall and initiation of growth in the spring are key processes in this cycle. In species with a free growth pattern, growth cessation and cold acclimation are induced by short days and low temperatures in fall (Dormling et al. 1968, van den Driessche 1970, Weiser 1970, Håbjørg 1972, Heide 1974*a*, Aronsson 1975, Christersson 1978, Junttila 1980, Jonsson et al. 1981, Junttila and Kaurin 1990, Welling et al. 1997). After growth cessation, bud dormancy develops gradually and a state of true dormancy may result after 6–8 weeks (Junttila 1976, Junttila et al. 2003)—we define true dormancy as the inability of a bud to burst at normal growth temperatures in long days (cf. Junttila et al. 2003). After dormancy induction, bud burst depends on dormancy release by chilling (Romberger 1963) and accumulation of thermal time above a species-specific threshold.

The developmental cycle from bud set to bud burst consists of several sub-processes—dormancy induction, dormancy release and forcing—each with different responses to climatic conditions. The temperature during dormancy induction affects the timing of bud burst in some angiosperm tree species, e.g., black alder (*Alnus glutinosa* (L.) Moench) and birch (*Betula* spp.) (Heide 2003, Junttila et al. 2003). In these species, increased temperature in autumn enhances the chilling requirement and delays bud burst, lowering the risk of premature bud burst in late winter and early spring, a risk that may increase in the event of climate warming (Saxe et al. 2001, Hänninen et al. 2007). A similar response was indicated in an earlier study with Norway spruce (*Picea abies* (L.) Karst.; Heide 1974*b*), but the effects of temperature and duration of short-day treatment during bud set on subsequent bud burst have not been thoroughly investigated in Norway spruce.

Many *Picea* species have no absolute requirement for chilling; their buds can burst without previous chilling in a 16–20 h photoperiod (long days) at normal growth temperatures (Nienstaedt 1967, Worrall and Mergen 1967). However, chilling reduces the time to bud burst after transfer to flushing conditions. Although effects of temperature during bud set on the chilling requirement in Norway spruce have not been studied, such effects can be expected (cf. Heide 2003, Junttila et al. 2003). In some species, long photoperiods can partly substitute for insufficient chilling (Wareing 1956, Heide 1993*a*,

Myking and Heide 1995), and in beech (*Fagus sylvatica* L.), both long days and chilling are required for dormancy release (Heide 1993*b*). Norway spruce cannot break bud in short photoperiods unless the chilling requirement has been met (Nienstaedt 1967, Worrall and Mergen 1967), indicating that the responses of Norway spruce buds to photoperiod are dependent on the state of dormancy. The role of photoperiod in the timing of bud burst under natural conditions may not be significant, but studies on the effects of photoperiod are of interest for understanding the climatic control of bud burst in Norway spruce.

Timing of bud set in northern tree species, including Norway spruce, is under strong genetic control, reflecting different responses to photoperiod (Dormling et al. 1968, Heide 1974*a*). Under similar conditions, bud set occurs earlier in northern provenances than in southern provenances. Timing of bud burst in Norway spruce is also under genetic control (Langlet 1960, Eriksson et al. 1978), and northern provenances generally burst bud earlier than more southern provenances (Worrall and Mergen 1967, Heide 1974*b*, Beuker 1994). These genetic effects highlight the importance of studying a range of provenances when investigating environmental controls of dormancy development and bud burst.

One-year-old Norway spruce seedlings have a free growth pattern, and bud set is induced by short photoperiods. Older seedlings primarily exhibit a determined growth pattern, where timing of bud set is less dependent on environmental conditions. This implies a change in seedling physiology that may affect the responses to environmental factors controlling dormancy. Partanen et al. (2005) observed age-dependent differences in bud burst in twigs from young and old Norway spruce trees but did not identify the underlying mechanism.

To obtain a detailed understanding of environmental control of bud dormancy and bud burst in Norway spruce, we quantified the effects of temperature during short day treatment, duration of the short day treatment, duration of chilling and light regime during forcing on the timing of bud burst. Age-dependent and genetic variations in these responses were assessed by studying 1- and 2-year-old seedlings of nine provenances of Norway spruce, representing latitudinal, altitudinal and maritime-continental gradients. We hypothesized that bud burst in Norway spruce, as in broadleaf species, is affected by temperature during bud set.

Materials and methods

Plant material and growth conditions

Experiments with nine provenances of Norway spruce seedlings (Table 1) were conducted at the University of Tromsø phytotron (69°39′ N, 18°55′ E). Temperature in the phytotron compartments was controlled to ± 0.5 °C, and humidity was adjusted to give a water vapor deficit of 0.5 kPa. Seed was sown in late May in 12-cm pots (volume 0.8 l) in a 7:3 (v/v) mix of fertilized peat and perlite. After germination, seedlings were thinned to a maximum of eight per pot. In all experiments, there were two pots, each with five to eight seedlings per treatment and provenance. During the first year, seedlings were raised at 18 °C in natural light (22–24-h photoperiod) before the experimental treatments. During bud set and dormancy development (dormancy treatment, DT), seedlings were grown in short days (SDs, 12-h photoperiod), in natural daylight (0800–2000 h) supplemented by artificial light (Philips TLD 480 daylight fluorescent tubes) when natural solar irradiance was less than 130–140 µmol m^{-2} s^{-1}. Seedlings were forced in artificial light regimes as specified for each experiment (Table 2). Seedlings were watered daily and fertilized weekly with a complete nutrient solution. Seedlings were chilled in darkness at 4 °C.

Experimental treatments

Three experiments were carried out as summarized in Table 2. Seedlings were first exposed to SDs at temperatures and for periods as indicated, then transferred, either directly or after various chilling periods, to forcing conditions as shown in the Table 2. All provenances were included in all experiments with about 100 seedlings per treatment.

Experiment 1 investigated (a) the effects of different constant temperatures applied during bud set on the time course of dormancy induction, and (b) the interaction between the temperature treatments applied before chilling and the chilling requirement. Because of deviations from the specified growth conditions at 15 °C, we excluded data from this treatment, resulting in 12 treatments for (a) and 15 for (b). Bud burst was observed at 18 °C in a 24-h photoperiod with continuous fluorescent light (130–140 µmol m^{-2} s^{-1}) from Philips TLD 480 daylight fluorescent lamps (CFL).

Experiment 2 assessed the effects of photoperiod and irradi-

Table 1. Origins of Norway spruce (*Picea abies*) provenances selected for study.

Provenance	Name of collection area and country	Latitude (N)	Longitude (E)	Elevation (m)
P1	Rana, Norway	66°25′	14°30′	0–149
M1	Namskogan, Norway	64°50′	13°00′	0–149
N1	Namdalseid/Namsos/Nærøy, Norway	64°40′	11°40′	0–149
BV1	Østre Toten, Norway	60°35′	11°00′	0–149
BV4	Gol, Norway	60°45′	9°00′	350–449
BV8	Etnedal/Nord-Aurdal/Sør-Audal/Gausdal, Norway	60°56′	9°38′	750–849
F1	Arendal/Tvedestrand, Norway	58°35′	8°50′	0–149
Har5	Stadt Osterrode, Germany	51°40′	10°30′	450
HvR	Voloshnirk, Belarus	54°05′	26°31′	200

Table 2. Summary of experimental treatments of nine provenances of Norway spruce (*Picea abies*). During dormancy treatment, seedlings were provided a 12-h photoperiod of daylight augmented with fluorescent light giving a total of 130–140 µmol m^{-2} s^{-1} of photosynthetic photon flux at plant height. In Experiment 3, the seedlings were moved to a phytotron on May 24, 2004. Light regimes: SD, 12 h of daylight supplemented with fluorescent light to obtain 130–140 µmol m^{-2} s^{-1}; CFL, continuous fluorescent light at 130–140 µmol m^{-2} s^{-1}; and DEL, 24-h photoperiod, 12 h of daylight plus fluorescent light (minimum 130–140 µmol m^{-2} s^{-1}) and 12 h of incandescent light (10 µmol m^{-2} s^{-1}).

Expt.	Sowing date	Dormancy treatment			Chilling (weeks at 4 °C)	Forcing	
		Start date	Duration (weeks)	Temperature (°C)		Temperature (°C)	Light regime
One-year-old seedlings							
1a	May 26, 2003	July 28, 2003	4, 6, 8	9, 12, 15*, 18, 21	0	18	CFL
1b	May 26, 2003	July 28, 2003	8	9, 12, 15*, 18, 21	2, 4, 6	18	CFL
2	May 18, 2004	July 12, 2004	8, 12	12, 21	0, 10	12	SD, CFL, DEL
Two-year-old seedlings							
3a	June 10, 2003	June 21, 2004	4, 8, 12	9, 12, 15, 18, 21	0	15	CFL
3b	June 10, 2003	June 21, 2004	12	9, 15, 21	3, 6, 9	15	SD, CFL

* Treatments excluded because of errors in growth conditions.

ance during forcing on dormancy release in 1-year-old seedlings. Because small but significant differences among provenances were found in Experiment 1, the forcing temperature was reduced to 12 °C. To test the effect of photoperiod on timing of bud burst, SD and long day (LD, 24-h photoperiod) conditions were compared. There were two LD treatments: CFL, the same light regime as used in Experiment 1; and day extension light (DEL), 12 h of daylight supplemented with fluorescent light (minimum 130–140 µmol m^{-2} s^{-1}) and 12 h of low irradiance incandescent light (Osram 75W, 10 µmol m^{-2} s^{-1}).

Experiment 3a tested the effect of temperature (15 treatments) on dormancy development of 2-year-old seedlings. In Experiment 3b, we studied interactions between temperature during dormancy treatment, duration of chilling and light regime during forcing (18 treatments) in 2-year-old seedlings. Seedlings were raised at 18 °C in natural daylight to September 1 during their first growth season. They were then exposed to SD for about 8 weeks at 15 °C followed by 2 weeks at 6 °C before being moved to 0.5 °C and then outdoors for the winter. The next spring, seedlings were grown for 5 weeks at 15 °C in a 16-h photoperiod (12 h daylight, 4 h incandescent light at 10 µmol m^{-2} s^{-1}) before being exposed to experimental treatments when shoot elongation ceased.

Bud burst

Bud burst of terminal and lateral buds was monitored twice a week. Buds were recorded as burst when bud scales were separated and green needles were clearly visible. For the lateral buds, time of flushing of the first lateral bud, irrespective of position, was recorded. Monitoring continued until flushing was complete or for a maximum of about 3 months. The day from the start of forcing until 50% of the seedlings had burst their buds (X_{50}) was calculated by linear regression: $X_{50} = (0.5 - b)/a$, where a is the regression coefficient and b is the intercept of regression. For each combination of treatment and provenance, each pot was used as a replicate and two regressions were calculated.

Statistics

An analysis of variance was performed with fixed effects models. Residuals were tested for normality by creating normal probability plots and plots of residuals versus fitted values. All normal probability plots of the residuals were skewed, with tendencies toward a sigmoid shape. However, only small deviations were present in the data from Experiments 1 and 3, and in the data from chilled seedlings in Experiment 2. These moderate departures from normality are of little concern in an analysis of variance with fixed effects (Montgomery 2001). In Experiment 2, the distributions of residuals deviated from normality for the data for unchilled seedlings, the plots showed a non-constant variance, and the design was unbalanced; therefore, we performed a nonparametric analysis of the data. Equality among group means was assessed by the Wilcoxon and the Kruskal-Wallis test. We followed the recommendation in Montgomery (2001) and performed analyses based on both parametric and nonparametric methods and assumed that the analyses of variance assumptions were satisfied reasonably well when both procedures gave similar results. When results of the two methods differed, results of the nonparametric analysis were used to confirm or reject the null hypothesis.

The analyses were based on days to 50% bud burst. Seedlings forced under SD without prior chilling treatment never reached 50% bud burst. For these seedlings, an analysis of variance was run on the percentage of seedlings that burst buds with the same model for both terminal and lateral buds. Before analysis, percentages were arcsine transformed to meet requirements for normality and homogeneity of variances among means (Snedecor and Cochran 1967).

Each experiment was analyzed separately, with treatment effects considered fixed effects. Only significant 2-way interactions between main effects were included, and the random residual variance was used as the error term for all F tests. Relationships between provenance origin and time to 50% bud burst were evaluated by linear regression analysis.

The linear statistical models applied were similar in structure but differed in details, depending on the experiment. For

brevity, we present the basic model structure that was applied to Experiment 1a (see Table 2; seedlings without chilling treatment):

$$Y_{ijkl} = \mu + T_i + D_j + P_k + TD_{ij} + TP_{ik} + DP_{jk} + e_{ijkl}$$

where Y_{ijkl} = date at 50% bud burst for pot l (1 or 2), provenance k (1–9), dormancy treatment j (1–3) and temperature treatment i (1–4), T_i = the fixed effect of temperature applied during treatment i (9,12,18, 21 °C), D_j = the fixed effect of the duration of treatment j (4, 6 and 8 weeks) P_k = the fixed effect of provenance k (P1, M1, N1, BV1, BV4, BV8, F1, Har5 or HvR), TD_{ij} = the fixed interaction between temperature i and the duration of treatment j, TP_{ik} = the fixed interaction between temperature i and provenance k, DP_{jk} = the fixed interaction between the duration of treatment j and provenance k, and e_{ijkl} = the residual error term used as the denominator in all F-tests of the fixed main effects and their interaction (two pots).

In this example, the error and model mean squares had 156 and 59 degrees of freedom, respectively. Because of the large number of observations, the standard error of means were small and the P-values of the F-tests of the main effects were low (large F values) in most experiments.

In Experiment 1b (chilled seedlings; Table 2), we substituted the duration of dormancy treatment term with the duration of chilling term. In Experiment 2, we added a fixed effect of chilling in the model as well as significant two way interactions with chilling and other main effects, and also a fixed effect of light regimes. In Experiment 3, the models were the same as for Experiment 1, except for chilled plants for which the forcing light condition was added as an effect.

Results

Environmental control of bud burst in 1-year-old seedlings

Both duration and temperature of DT influenced the time to bud burst (Figure 1A). In general, a short DT under warm conditions delayed bud burst compared with a long DT at low temperature. The difference between temperatures was greater than between length of DT, but both effects were highly significant ($P < 0.0001$). The interaction between temperature and duration was also significant ($P < 0.001$). In general, chilling hastened bud burst ($P < 0.001$; Figure 1B). Two weeks of chilling hastened the timing of bud burst when plants were exposed to 21 °C during DT, but short duration chilling delayed bud burst in seedlings exposed to 9 and 12 °C during DT. Both 4 and 6 weeks of chilling advanced bud burst compared with the control in each temperature treatment. However, the influence of temperature during DT was significant ($P < 0.001$) even after 6 weeks of chilling (Figure 1B), as was the interaction between DT temperature and duration of chilling ($P < 0.001$).

Bud burst was affected by both photoperiod and irradiance during forcing. Unchilled seedlings forced in SDs never attained 50% bud burst. After 224 SDs, the total mean per cent of unchilled seedlings with burst buds was 17 and 20% for terminal and lateral buds, respectively (Figure 2A). Thus, for unchilled seedlings, long days (24-h photoperiod) during forcing seemed to be obligatory for normal timing of bud burst (compare Figures 2A and 2B). Extending the photoperiod at a low iradiance (DEL) generally delayed bud burst compared with the 24-h high irradiance treatment (CFL), but the effect was small and insignificant except for seedlings in the 12-week DT at 21 °C (Figure 2B). For terminal buds, the effect of light regime was significant only in the parametric analysis ($P = 0.0051$). For lateral buds the difference between light regimes was significant in both the parametric and nonparametric analyses ($P = 0.0025$ and $P = 0.0314$, respectively; data not shown).

In Experiment 2, bud burst was delayed by high (21 °C) compared with low (12 °C) temperature applied during DT, and this was evident for both terminal (Figure 2B) and lateral buds (data not shown; $P < 0.0001$ in both parametric and nonparametric analyses). The interaction between DT temperature and duration was highly significant for both terminal and lateral buds ($P < 0.0001$ ANOVA). By extending the duration of DT from 8 to 12 weeks, terminal bud burst was delayed in seedlings exposed to 21 °C during DT, but hastened in those exposed to 12 °C (Figure 2B). For lateral buds, prolonged DT enhanced bud burst at both temperatures, but the effect was
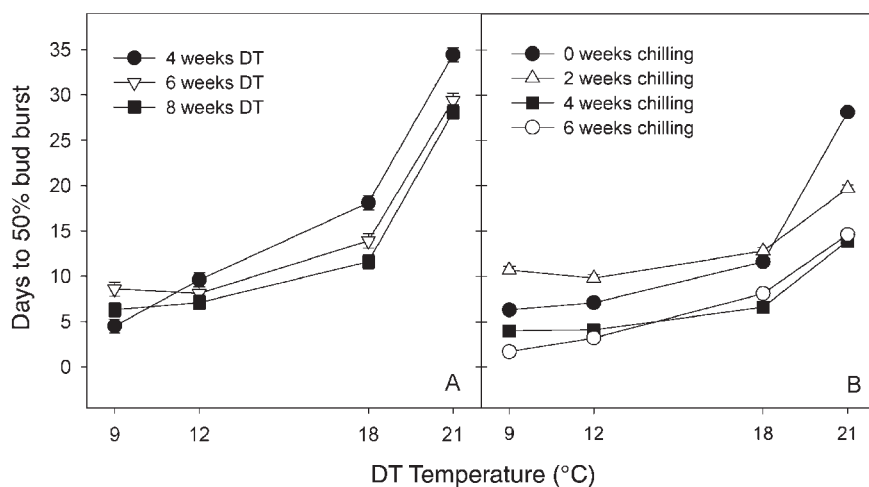

Figure 1. Time to 50% bud burst in 1-year-old seedlings of Norway spruce (*Picea abies*) as affected by (A) temperature and duration of dormancy treatment (DT; Experiment 1a), and (B) DT temperature and duration of chilling (Experiments 1a and 1b). In B, DT was given for 8 weeks. Seedlings were forced at 18 °C under continuous fluorescent light. Results are means (± SE) of nine provenances.
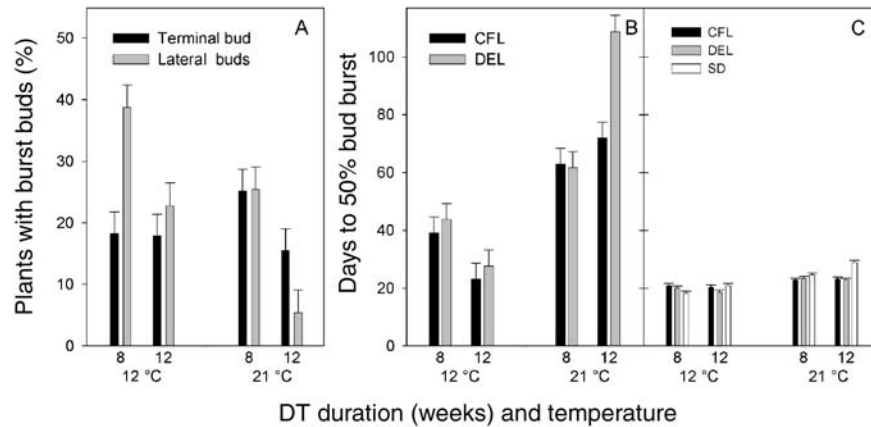
Figure 2. Effects of light regime during forcing on bud burst in 1-year-old seedlings of Norway spruce (*Picea abies*; Experiment 2). (A) Percent bud burst in unchilled seedlings after 224 short days (SDs) as affected by temperature and duration of dormancy treatment (DT). Time to 50% bud burst as affected by temperature and duration of DT and light regime (B) in unchilled seedlings during forcing in 24-h photoperiod and (C) in seedlings chilled for 10 weeks at 12 °C during forcing in 12- and 24-h photoperiods. Results are means (± SE) of nine provenances. Light regimes: SD, 12-h photoperiod at 130–140 µmol $m^{-2} s^{-1}$; CFL, continuous fluorescent light at 130–140 µmol $m^{-2} s^{-1}$; and DEL, 12 h of fluorescent light at 130–140 µmol $m^{-2} s^{-1}$ and 12 h of incandescent light at 10 µmol $m^{-2} s^{-1}$.

more pronounced at 12 °C than at 21 °C (data not shown).

Ten weeks of chilling hastened bud burst (Figure 2C; $P < 0.0001$ in the nonparametric analysis for both terminal and lateral buds). For seedlings in the 21 °C plus 12-week DT, time to terminal bud burst was reduced from 72 to 23 days when forced in CFL and from 109 to 23 days when forced in DEL (cf. Figures 2B and 2C). Chilling also reduced the difference between temperature treatments, but the difference between 12 and 21 °C was still significant ($P < 0.001$ in both the parametric and nonparametric analyses for both terminal and lateral buds). Interactions between light regime during forcing and temperature or duration of DT were highly significant for terminal buds ($P < 0.0001$ for both interactions), mainly because of the effect of forcing in SD compared with CFL and DEL. After chilling, seedlings given 12 weeks DT at 21 °C had delayed bud burst when forced in SD compared with CFL or DEL (Figure 2C). No significant differences between CFL and DEL were found in the chilled seedlings (Figure 2C).

Environmental control of bud burst in 2-year-old seedlings

Temperatures applied during DT at the end of the second growth season affected timing of bud burst in the 2-year-old seedlings (Figure 3). Both terminal and lateral buds burst without chilling, and the response to the duration of DT was similar to that in 1-year-old seedlings; a longer DT hastened bud burst (Figure 3). However, bud burst was strongly delayed in 2-year-old seedlings compared with 1-year-old seedlings. After 4 weeks of DT, it took the 2-year-old seedlings between 66 and 74 days to reach 50% bud burst depending on the DT temperature compared with 5 to 34 days in the 1-year-old seedlings. As in the 1-year-old seedlings, there were significant main effects of both temperature and duration of DT on time to bud burst ($P < 0.0001$ for both). In general, short and warm DT delayed bud burst, but unlike the 1-year-old seedlings, bud burst was not delayed more by DT at 21 than at 18 °C (cf. Figures 1B and 3). The interaction of temperature treatments and duration of DT on time to bud burst was highly significant ($P < 0.0001$).

In chilled seedlings, there was an interaction between the temperature during DT and duration of chilling (Figure 4A; $P < 0.0001$). Warmer DT temperatures delayed bud burst, but chilling reduced the difference between temperatures. The longer the chilling period, the smaller (but still significant, $P < 0.0001$) the response to DT temperature.

Long days during forcing generally hastened bud burst (Figures 4B and 4C). The difference between forcing in DEL and SD slightly increased with increasing DT temperature. The interaction between DT temperature and light regime during forcing was significant ($P = 0.0086$). During forcing, SD delayed bud burst, but the effect was substantially reduced after 9 weeks of chilling (Figure 4C). The interaction between duration of chilling and light regime during forcing was highly significant ($P < 0.0001$).
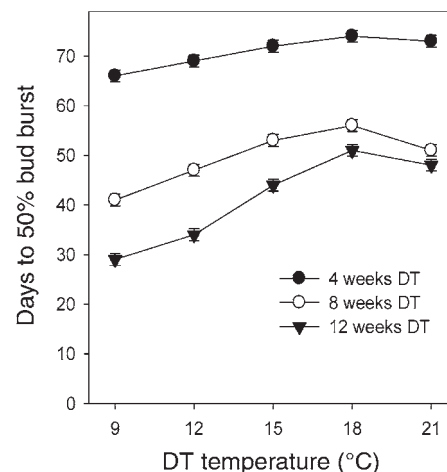

Figure 3. Experiment 3a. Effects of temperature and duration of dormancy treatment (DT) on timing of bud burst in 2-year-old unchilled seedlings of Norway spruce (*Picea abies*). Seedlings were forced at 15 °C in continuous fluorescent light. Results are means (± SE) of nine provenances.
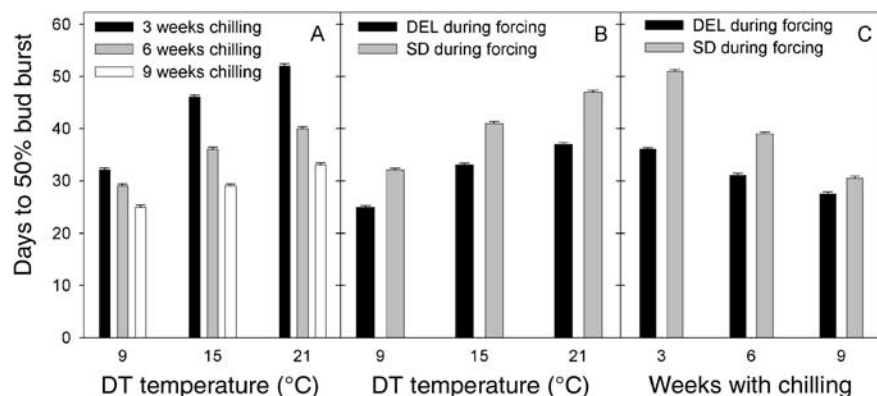
Figure 4. Experiment 3b. Time to 50% bud burst in 2-year-old seedlings of Norway spruce (*Picea abies*) as affected by temperature during dormancy treatment (DT) and (A) duration of chilling or (B) light regime during forcing, and as affected by (C) chilling time and light regime during forcing. Results are means ± SE of nine provenances. In B, results are means of chilling treatments (3, 6 and 9 weeks), and in C, they are means of three temperature treatments during DT (9, 15 and 21 °C).

Differences among provenances in timing of bud burst

In Experiment 1, seedlings of Norwegian provenances exhibited an inverse linear relationship between latitude of origin and time to bud burst (Figure 5). All provenances in the regressions originated from 100–150 m elevation. The regressions were significant for seedlings held at 18 and 21 °C during DT (Figure 5), and the cline was steeper in unchilled seedlings than in chilled seedlings (Figures 5A vs. 5B). Bud burst was earlier in seedlings from higher elevations (data not shown). There was no coastal–inland gradient in the Norwegian provenances, although bud burst was later in the inland German and Belarusian provenances than in the coastal Norwegian provenances (data not shown). These inland provenances were, however, also from different altitudes and longitudes than the Norwegian provenances.

In Experiment 2, a similar, but steeper, clinal variation with latitude among provenances from Norway was found after DT in 21 °C and forcing in 12 °C pattern and CFL (data not shown). However, no significant clinal pattern of variation was found when seedlings were forced in DEL or if they were chilled for 10 weeks (data not shown). In 2-year-old seedlings forced in DEL or SD, provenances differed significantly in time to bud burst, but there were no statistically significant correlations between latitude, longitude or altitude of origin on time of bud flushing (data not shown).

Discussion

State of dormancy depends on temperature and duration of short day conditions

Bud burst in both 1- and 2-year-old seedlings of Norway spruce was significantly delayed by high temperatures during bud set and bud development under SDs (Figures 1 and 3), corroborating previous findings for Norway maple (*Acer platanoides* L.; Westergaard and Eriksen 1997), black alder and birch (Heide 2003). In birch (Junttila et al. 2003), apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.; Jonkers 1979), a more complex pattern has been reported, with more rapid dormancy development and deeper dormancy at intermediate than at lower and higher temperatures. In Nor-

way spruce, delayed bud burst due to high DT temperature was significant even after prolonged chilling, indicating a strong effect of temperature on the state of bud dormancy that is likely an adaptation that reduces premature bud burst in early spring (cf. Heide 2003).

Contrary to data for broadleaf species like birch (Junttila et al. 2003), time to bud burst decreased with DTs in both 1- and 2-year old Norway spruce seedlings (Figure 1 and 3). These contrasting results may indicate that the pattern of induction and release of dormancy differs between coniferous and broadleaf species, or that the effect of DT duration is related to bud development. Norway spruce seedlings exposed to low temperatures during DT all stopped elongation growth, but had not formed visible buds after 4 weeks. However, development of visible buds took place within 2 weeks after transfer to 18 °C and a long photoperiod, and flushing started after the seedlings had formed a macroscopic apical bud (data not shown).
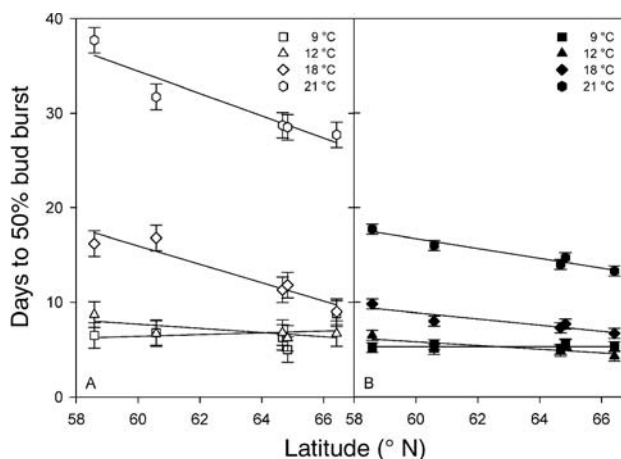

Figure 5. Time to 50% bud burst in 1-year-old seedlings of Norway spruce (*Picea abies*) from provenances of different latitudinal origins given 8 weeks of dormancy treatment (DT) at different temperatures, either (A) without (Experiment 1a) or (B) with (Experiment 1b) chilling. Results in A are means (± SE) of 4, 6 and 8 weeks of DT. Results in B are means (± SE) of 2, 4 and 6 weeks of chilling treatments. Seedlings were forced in continuous fluorescent light at 18 °C.

Norway spruce seedlings need no chilling to release bud dormancy

None of the applied temperatures or durations of DT prevented bud burst completely after transfer to LD without chilling (Figures 1, 2B and 3), corroborating earlier results (Nienstaedt 1967, Worrall and Mergen 1967) and indicating that young Norway spruce seedlings do not develop true bud dormancy. The ability to initiate growth under LD conditions without preceding chilling assures that Norway spruce will start normal growth in geographical areas with relatively warm winters and if the winter temperatures rise. However, chilling significantly advanced bud burst in young Norway spruce seedlings (Figure 1B; cf. Figures 2B, 2C, 4A and 4C), as reported earlier (Nienstaedt 1967, Worrall and Mergen 1967, Hannerz et al. 2003). The only exception in our study was that 2 weeks of chilling, compared with no chilling, delayed bud burst in seedlings exposed to 9 or 12 °C for 4 weeks during DT (Figure 1B). Effects of low temperatures (9 and 12 °C) are, however, difficult to interpret. Although temperatures around 3–5 °C are generally optimal for chilling (Perry 1971, Sarvas 1974, Fuchigami et al. 1982, Hänninen 1990), temperatures of 9 and 12 °C may have a chilling effect (Hänninen and Pelkonen 1988, Hänninen 1990, Heide and Prestrud 2005), but they also contribute to the thermal time for bud burst. Furthermore, bud development itself is strongly delayed at low temperatures.

More importantly, chilling counteracted the effect of light regime during forcing and bud burst took place even in SDs (Figures 2B, 2C, 4B and 4C), and the longer the chilling period, the smaller the DT temperature effects (Figures 1, 2 and 4). Therefore, chilling is not an absolute requirement for bud burst in Norway spruce, but it has a significant quantitative effect on dormancy release. Consistent with this finding, planted Norway spruce grows well in coastal areas in the western part of Norway south of 64° N, where the winters are mild and humid (Magnesen 1992, 2000). Even in SDs, about 20% of unchilled seedlings started growth when forced at 12 °C, but the response was slow (Figure 2A). We did not measure effects of chilling on elongation growth after bud burst.

Forcing conditions

Developmental processes leading to bud burst under forcing are strongly dependent on temperature. Although we did not examine these temperature effects systematically, they are evident when results from Experiments 1 and 2 are compared. For example, in unchilled seedlings exposed for 8 weeks to SDs at 21 °C, time to 50% bud burst was about 27 and 60 days at 18 and 12 °C, respectively. After chilling, the respective times were 12 and 20 days. Under natural conditions, the relationship between temperature and rate of bud burst is linear in some cases (Monteith 1977, Bloomberg 1978) and logarithmic in other cases (Campbell 1974, 1978, Campbell and Sugano 1979). A linear relationship has been used to model time to bud burst based on thermal time (Cannel and Smith 1983). Under natural conditions, temperature fluctuates between day and night. Fluctuating temperatures during forcing

lessen the requirement for forcing units in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco; Campbell and Sugano 1975, van den Driessche 1975), Scots Pine (*Pinus sylvestris* L.) and Norway spruce seedlings (Hänninen 1990), and thus, advance bud burst. The forcing temperatures we tested did not change the relationships among treatments. Similar findings were reported by Heide (2003) who found that, in birch, fall temperature explained more of the variation in time to bud burst under natural conditions over the years than spring temperature. Fall temperature may serve as a modifying component, whereas heat sum accumulation in spring represents the main driving force for timing of bud burst.

A long photoperiod is required for bud burst of unchilled Norway spruce seedlings. We used continuous light as the LD treatment, and although irradiance itself may have some influence (Figure 2B), our results indicate that the observed effect is mainly photoperiodic. There is a strong genetic adaptation of bud set to photoperiod (Dormling et al. 1968, Heide 1974*a*), but there is no published evidence for similar adaptation of bud burst. Photoperiodic regulation involves the phytochrome system, and the role of phytochrome in control of bud set in Norway spruce has been studied (Mølmann et al. 2005); however, its role in control of Norway spruce bud burst remains unknown.

Bud burst in different provenances

Differences among provenances in timing of bud burst were affected by temperature during dormancy induction, chilling, and forcing temperature. Variation among provenances was larger in unchilled plants and after dormancy induction at high temperatures (Figure 5). Norway spruce normally expresses a clinal variation pattern, in which southern or lowland provenances burst bud later than northern and highland provenances (Langlet 1960, Worrall and Mergen 1967, Beuker 1994). As expected, forcing at 12 °C increased differences among provenances compared with forcing at 18 °C. In addition, the clinal gradient became steeper when unchilled plants received continuous fluorescent light during forcing. However, when we applied low irradiance daylength extension (DEL) during forcing, the relative differences among provenances were affected. In Experiment 3, with 2-year-old seedlings, forcing was done in DEL and SD. Because these forcing treatments revealed no clinal patterns of variation in the 1-year-old seedlings, use of the DEL treatment might explain why we found no significant relationship between latitude of origin and day to 50% bud burst in the 2-year-old seedlings. However, clinal patterns of variation have been found under natural light in provenance trials of older Norway spruce trees (Worrall and Mergen 1967, Beuker 1994). Genetic variation in flushing date among families is large in progeny trials (Dietrichson 1969, Yakovlev et al. 2006), and differences in bud flushing among and within provenance crosses were pronounced over three growth seasons in a phytotron study (Eriksson et al. 1978). Partanen et al. (2005) reported that dormancy patterns of Norway spruce are age-specific, with a change in flushing pattern from 15 to 56 years of age. However, the change in pat-

tern between 1- and 2-year-old seedlings in our study may have been due to factors other than age, perhaps related to conditions in the climate chambers.

Although latitudinal patterns of variation have been found in angiosperm trees such as oak (*Quercus* spp.) and birch (Jensen 1993, Myking and Heide 1995), the effect of latitude is often confounded with the coast–inland climatic gradient (Campbell and Sugano 1979, Leinonen 1996). Furthermore, clinal patterns of variation are not always consistent across species and regions. Black alder expresses small differences in timing of bud burst between populations from climatically diverse sites, such as Scotland, Norway and Italy (DeWald and Steiner 1986, Kohmann and Lexerød 2004). In beech and oak from continental Europe, bud burst is longest delayed in trees of northern origin (Liepe 1993, von Wuehlisch et al. 1995), possibly an adaptation to the milder climate caused by the North Sea that could otherwise trigger premature bud burst (cf. Campbell and Sugano 1979). Similarly, southern Italian clones of elm (*Ulmus minor* Miller) flushed earlier than clones from France and northern Italy (Santini et al. 2004, Ghelardini et al. 2006). No coastal–inland variation pattern was evident in our study; however, the eastern continental Norway spruce provenances from Poland and Belarus tend to be late flushing in field trials (Langlet 1960, Skrøppa and Magnussen 1993, Hannerz et al. 2003), a result opposite to that found for angiosperm tree species along distributed maritime–inland climatic gradients (Campbell and Sugano 1979, Leinonen 1996). Norway spruce, however, is not a coastal species compared with, e.g., Sitka spruce (*Picea sitchensis* (Bong.) Carr; Cannell and Smith 1983, Hannerz et al. 2003), and may not have evolved ecotypes specific to coastal areas.

Slaney et al. (2007) estimated that the predicted rise in temperature in this and the next century could advance the time of bud burst by 2 to 3 weeks in Norway spruce growing at 64° N, which could increase the risk of injury by spring frosts and indicates the need to identify late-flushing genotypes for planting in areas where spring frosts frequently occur. An efficient way of doing this is to utilize within-region and within-population variation in bud burst, which is larger than variation among provenances (Worrall and Mergen 1967, Dietrichson 1969). Such genotypes are possible to find within well-adapted provenances of Norway spruce (Yakovlev et al. 2006), and heritability of bud burst is generally high in conifers (Howe et al. 2003), especially in Norway spruce (Hannerz et al. 1999).

In conclusion, we showed that Norway spruce expresses a versatile and sometimes complex response to environmental signals related to dormancy, and that timing of bud burst is affected more by environmental treatments than by provenance. No treatment prevented bud burst or onset of growth, indicating that young Norway spruce seedlings do not undergo true bud dormancy. However, chilling significantly advanced timing of bud burst and removed the requirement for a long photoperiod for flushing. High temperature during bud development significantly delayed bud burst, an effect only partially reversed by chilling. The significance of this high temperature effect under natural conditions is unknown.

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