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Day Extension with Far-Red Light Enhances Growth of Subalpine Fir (*Abies lasiocarpa* (Hooker) Nuttall) Seedlings

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Abstract: Subalpine fir (*Abies lasiocarpa* (Hooker) Nuttall), which is native to western North America, is of considerable interest for Christmas tree production in northern Europe. Seedlings are usually grown from seeds under combined nursery greenhouse/outdoors conditions, but commonly show early growth cessation in the nursery, resulting in small plants for field transplanting. This increases the production time and makes the seedlings vulnerable to stressors at the planting site. Day extension with far-red (FR) light was shown to enhance elongation and delay bud set in seedlings of some woody species, but such information is limited for *Abies*. Here, we investigated the effects of day extension with FR, red (R), different R:FR-ratios or blue (B) light from light emitting diodes on subalpine fir seedlings grown at different temperatures. Day extension with FR or combined R-FR light, in contrast to R or B light, increased shoot elongation significantly as compared to short days without day extension, often with more growth at 18 °C than 24 °C. The FR treatments delayed terminal bud development, although bud set was not completely prevented. These results demonstrate that larger seedlings of subalpine fir seedlings for Christmas tree production can be obtained by employing day extension with FR or combined R:FR light, preferably under cool temperature.

Keywords: *Abies lasiocarpa*; blue light; Christmas trees; far-red light; light quality; red light; shoot elongation; subalpine fir; temperature; terminal bud development

1. Introduction

Due to its ornamental value and good keeping quality as a Christmas tree, a coniferous subalpine fir species (*Abies lasiocarpa* (Hooker) Nuttall), which originates from mountain areas of western North America [1], is subject to considerable interest for Christmas tree production in areas of northern Europe such as Scandinavia. In Norway, currently 60% of the Christmas trees sold are fir, of which 50% are subalpine fir [2]. Seedlings are usually grown from seeds and cultivated in nurseries for two years under combined nursery greenhouse and outdoor conditions. During the greenhouse growing phase, plants of this species commonly show undesirable early growth cessation and terminal bud formation, resulting in small plants. This will increase the planting stress, prolong the period where intensive weed control is needed, and thereby increase costs for the Christmas tree grower [3–5]. To obtain larger, potentially more robust plants during the growing period in the nurseries, delayed growth cessation and bud set as well as enhanced shoot elongation are prerequisites.

It is well known that young individuals of a wide range of tree species of the boreal and temperate zone show sustained growth under photoperiods longer than a critical photoperiod (long days; LD),

and growth cessation and terminal bud development under shorter photoperiods (short days; SD) with the critical photoperiod depending on latitudinal origin [6–9]. In deciduous woody species such as downy birch (*Betula pubescens* Ehrh.), white birch (*Betula pendula* Roth), bay willow (*Salix pentandra* L.) and the conifer Norway spruce (*Picea abies* (L.) H. Karst.), it has been shown that light quality is also important in this respect. Sufficient far-red (FR) light was shown to be required for sustained growth in northern tree provenances with the FR requirement increasing with increasing latitude of origin [10–16].

Furthermore, in a study using light from light emitting diodes (LED) with wavelengths corresponding to the main wavelengths absorbed by the red (R)-FR absorbing phytochrome light receptors, a mixture of R:FR given as day extension (energy level ranging from 1.8–6.6 W m^{-2}) was even more efficient in preventing bud set in northern provenances of Norway spruce than FR light provided separately [15]. Delayed bud set in Norway spruce compared to a reference SD treatment was also reported when plants were exposed to day extension with R or blue (B) light at energy levels of 1.8 W m^{-2} and 3.3 W m^{-2} [15,16]. Bud set was then more delayed for more southern compared to more northern provenances. The wavelengths of the B light used correspond to absorption by known B light receptors such as cryptochromes. Although enhanced growth was obtained with these treatments compared to SD-exposed Norway spruce plants, the R or B light treatments were not able to prevent growth cessation and terminal bud formation as was the case for the FR treatments. The irradiance of the different light qualities apparently also matters. Bud set was more delayed under higher compared to lower irradiance. Furthermore, different latitudinal provenances differed in their irradiance requirement, with northern provenances requiring higher irradiance of the tested light qualities (FR, R, B) for sustained growth or delayed bud set as compared to more southern provenances [15,16].

Differences in response to light quality and photoperiod is an adaptation to the light climate at different latitudes during the growing season [8,9]. In addition to increasing photoperiod with increasing latitude, the relative proportion of FR light varies with latitude, time of the day and year due to differences in solar angle. At northern latitudes, lower solar angles in general and longer twilight periods than at more southern latitudes during the growing season result in the presence of an increased FR proportion and thus lower R:FR ratio [8,9]. Also, the long twilight period at high latitudes results in the presence of more diffuse B light than at more southern latitudes where the twilight period is shorter.

Light quality not only affects bud set in woody species but is also well known to affect shoot elongation with inhibition of shoot elongation by R and B light and stimulation of elongation growth by FR light in a wide range of plant species [17]. However, different species differ in their responses. B light-enhanced elongation growth was demonstrated in species like *Salvia* L., *Tagetes* L. and *Petunia* Juss. [17–21]. Also, enhanced elongation in response to FR light or low R:FR ratios is exhibited in plants classified as sun plants but not in shade plants [22]. Furthermore, in subalpine fir, germination and initial seedling survival increased with increased light under conditions for natural regeneration [23] even if the species is considered shade-tolerant [24].

The effect of the photoperiod is modified by temperature, probably due to a temperature modification of the critical photoperiod for sustained growth [8,9,25–28]. A range of studies under controlled conditions in growth chambers with constant temperature or different day and night temperatures showed accelerated terminal bud development at higher, compared to lower, average daily temperature [29–35]. Furthermore, terminal bud development is delayed when day temperature is lower than night temperature when average daily temperature is kept constant [36]. On the other hand, field studies employing natural temperature gradients or continuous temperature enhancement using heating lamps under natural temperature fluctuations have shown delayed bud development in response to higher temperature [26,37,38]. Thus, response to temperature apparently depends on the actual light or temperature regime. Although interactive temperature-photoperiod effects have

attracted increased attention in recent years, information about interactive effects of temperature and light quality is limited.

Although a number of studies have reported enhanced elongation growth and delayed growth cessation and bud set in woody species in response to day extension with different light qualities, with the largest effect of FR light treatments, such information is very limited for subalpine fir. Aiming at the production of larger, more robust seedlings of subalpine fir for Christmas tree production, the goal of the present study was to investigate whether day extension treatments with FR, R, different R:FR ratios or B light can enhance shoot elongation and delay or prevent growth cessation and bud set, and whether these responses are affected by temperature.

2. Materials and Methods

2.1. Plant Materials and Pre-Growing Conditions

Seeds of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) from the provenance CAN10 from 53.59° N latitude, 122.23° W longitude, 1000–1200 m a.s.l. from the George Mountains in British Columbia, Canada (seed lot B13-106, Skogfrøverket, Hamar, Norway) were used in four different experiments. The seeds were stratified for three weeks in Petri dishes with moist filter paper in darkness at 4 °C. Thereafter, the seeds were sown in a 3:1 mixture of peat and perlite (S-Jord, Hasselfors, Oslo, Norway) in pots of 5.5 × 5.5 × 4.5 cm (Vefi, Drammen, Norway). The pots were placed on 50 × 50 cm trolleys in growth chambers manufactured by Norwegian University of Life Science (Ås, Norway).

During the pre-growing period of 7 weeks at 18 °C (±1 °C), a 24 h photoperiod (LD) was set, ensuring a photoperiod longer than the critical one, using quartz metal halide lamps (HPI) as the principal source of light (Master HPI-T Plus 400 W/645 E40 1SL, Phillips, Amsterdam, The Netherlands). In a pilot experiment (not shown), where pre-growth was done in a greenhouse during late autumn (sowing 30 October at Norwegian University of Life sciences, Ås, Norway; 59°39′47″ N 10°47′38″ E) with supplementary light of a photosynthetic photon flux density (PPFD) of 100 µmol m^{-2} s^{-1} at 400–750 nm (measured with a Li-Cor, model Li-250 quantum sensor, Lincoln, NE, USA), all plants had terminal buds at the end of a 6-week pre-growing period. Therefore, in the first two experiments reported here, a higher PPFD of 200 umol m^{-2} s^{-1} was applied since growth in field-grown *A. lasiocarpa* was previously shown to benefit from increased irradiance despite the species being considered shade-tolerant [24,39]. Since bud set was observed in about 15% of the plants at the end of the pre-growing period, in two subsequent experiments, higher PPFD at 250 umol m^{-2} s^{-1} was applied by reducing the distance between the lamps and the trolleys. A red:far red (R:FR) ratio of 1.8, as measured by an R:FR sensor (Skye instruments, Llandrindod Wells, UK) was achieved using incandescent lamps (Narva 60W, Germany and Philips Electronics, Amsterdam, The Netherlands). The relative air humidity (RH) was adjusted to 76%, corresponding to a water vapour pressure deficit of 0.5 kPa. During the pre-growing and the experimental phases, the plants were watered as required and fertilized twice a week with a complete nutrient solution (mixture of Calcinit and Kristalon Indigo (Yara, Oslo, Norway) containing 14.3 mM N, 1 mM P, 5.1 mM K and the other essential macro- and microelements, at an electric conductance (EC)of 1.5 mS cm^{-1}).

2.2. Experimental Design and Conditions

During the experimental treatments, each trolley, having a perforated platform for plants, was isolated from the others by reflecting plastic curtains with an opening in the top and bottom to allow air circulation. Use of large growth chambers with airflow from beneath ensured similar airflow for all trolleys. High-pressure sodium lamps (HPS; Lucalox 400 W, General electric, New York, NY, USA) were used as the main light source during the 12 h main light phase. In the first two experiments, 160 µmol m^{-2} s^{-1} was applied with the R:FR adjusted to 2 using incandescent lamps. This was the maximum irradiance for each trolley when they were isolated from each other by plastic curtains and the lamps were in their regular positions on the ceiling of the chambers. In the two subsequent

experiments (experiments 3 and 4) a higher irradiance of 260 µmol m^{-2} s^{-1} was used, as measured in the middle of each trolley. The higher irradiance was obtained by reducing the distance between the lamps and the plants. One HPS lamp per trolley was then mounted 1 m above the plants. Since the light sources were closer to the plants in experiments 3 and 4 than in the two first experiments, instead of using heat-generating incandescent lamps, R:FR was adjusted to 2.5 (lower not possible) using four FR (peaking at 730 nm) light emitting diode (LED) panels with 5 LEDs in each, for each trolley.

Different subsets of plants were exposed to extension of the main daily light period with R (peaking at 660 nm), FR (peaking at 730 nm), blue (B; peaking at 460 nm) or different R:FR ratios, provided by LEDs (Philips GreenPower LED research module, Phillips, Amsterdam, The Netherlands), using an equal amount of energy (7 W m^{-2}) in all cases. The LED modules were mounted on the trolleys about 1 m above the plants. The day-extension treatments were applied for 13 h with an overlap of half an hour at the start and end of the main light phase to ensure 24 h of lighting.

In the first two experiments, different subsets of plants were exposed to day extension treatments with B, R or FR. In experiment 3, plants were exposed to FR, R, or R:FR ratios of 0.5, 1 or 2, corresponding to phytochrome photostationary state (PPS) values of 0.24, 0.72, 0.78, 0.81 and 0.88, respectively. In experiment 4, FR, R or R:FR ratios 0.1, 0.2 or 0.5 were tested, corresponding to PPS values of 0.24, 0.4, 0.56, 0.72 and 0.88, respectively. Phytochrome molecules exist in two forms; the R-light absorbing form (P_r) and the FR-absorbing form (P_{fr}), and the phytochrome photostationary state (PPS) or photoequilibrum is defined as the proportion of P_r to the sum of P_r and P_{fr} [40,41].

In all experiments, a control treatment without day extension (SD) was included. The different light quality treatments (on individual trolleys properly isolated with reflecting plastic curtains) were provided under two temperatures in separate growth chambers: 18 °C and 22 °C in the first experiment and 18 °C and 24 °C in the three other experiments. An equal water vapour pressure deficit of 0.5 kPa was used for each temperature, equivalent to 76, 81 and 83% RH at 18 °C, 22 °C and 24 °C, respectively.

At the beginning of the experiments, the plants had already formed buds in close to 15% of the plants with mainly light green buds (stage 1). These were distributed among the different treatments.

In the second experiment, after 50 days of day-extension treatment, in order to assess effect of the light quality treatments on the depth of bud dormancy, plants with terminal buds were re-transferred to the same growing conditions as those during the pre-growth period in this experiment: 18 °C, 24 h photoperiod at an irradiance of 200 umol m^{-2} s^{-1}.

In addition to the four experiments with LEDs, in three experiments, effects of LD obtained by day extension with incandescent lamps (=FR-rich light) and SD without day extension (in separate chambers) were compared. Pre-growth was then done as in experiments 1 and 2 described above. During the experimental periods, light was provided by HPI and incandescent lamps for 12 h. In one experiment, a PPFD of 160 µmol m^{-2} s^{-1} was provided at 18 °C and 22 °C, and in two experiments, 200 µmol m^{-2} s^{-1} was applied at 18 °C. To obtain LD, a 12 h day extension to a 24 h photoperiod was provided using low-intensity light from incandescent lamps only (8–10 µmol m^{-2} s^{-1}).

2.3. Recording of Growth Parameters

In all experiments, the height of 16–19 plants per treatment was measured once a week, as height from the edge of the pot to the shoot apical meristem. The increase in height (cumulative growth) was calculated. The terminal bud development was recorded for all plants three times per week using codes, where growing plants without buds were coded as 0, light green buds as 1 and brownish buds as 2. In the second experiment, bud burst was recorded, with 2 representing the presence of the terminal bud, 1 bud burst and 0 growth.

2.4. Statistical Analysis

To evaluate the effects of the light quality and temperature treatments on shoot elongation and stages of bud development, stages of bud burst and the presence/absence of buds in the time courses, two-way analysis of variance (ANOVA) was performed for each experiment, with the different light

quality and temperature treatments as sources of variation in the ANOVAs. For this, we used R software for statistical computing and graphics (www.r-project.org). In these analyses, effects of the two individual factors as well as the interaction between them were tested. A linear model was used for the results on shoot elongation since these were continuous data, which were normally distributed. A generalized linear model was used for stages of bud development, stages of bud burst and the presence/absence of buds since these results were categorical data and were also inherently not normally distributed. As stated in Section 2.3 above, stages of bud development and bud burst were classified into different categories (0, 1 or 2). Due to violation of the assumption of independence due to repetitive measurements on the same plants in time courses, a source of error was added for the variance using the time for each individual plant as a random effect [42]. The results of these analyses are shown in the Supplementary tables.

In addition to these analyses of overall effects of light quality and temperature treatments for the entire time courses, one-way ANOVAs for the effect of light quality within each temperature were also performed for the final time point only at the end of the experiments. These analyses were performed since the effects on growth and bud development status after long-term growth are of particular interest from the practical perspective of obtaining as seedlings as large as possible for Christmas tree production. For the comparison within and between treatments in each experiment, Tukey's test was used for post hoc analysis. In the figures and supplementary figures, the results of Tukey's test for the final time points at the end of the experiments are shown.

3. Results

3.1. Day Extension with FR Light Enhances Shoot Elongation in Subalpine Fir Seedlings

In experiments applying FR, R or B light from LEDs as day extension to subalpine fir seedlings grown under either 18 °C or 22 °C (first experiment) or 18 °C or 24 °C (second experiment), there was no significant interactive effect between temperature and day extension treatment on shoot elongation when the entire time courses were considered (Figure 1, Table S1; Two-way ANOVAs). Also, for these time courses, there was no significant effect of temperature on shoot elongation, although a trend ($p = 0.068$) was observed in the first experiment. In experiment 2 there was a significant overall effect of the day extension treatment on shoot elongation when all time points were considered, but not in experiment 1.

However, within each temperature, for the final time point at the end of the experiments, the FR light treatment significantly ($p \leq 0.05$) increased the total shoot elongation, compared with SD in both experiments 1 and 2 (Figure 1; One-way ANOVAs). In both of these experiments, at both temperatures, the SD treatment resulted in growth cessation about 15 days after the start of the treatments, with a total average shoot elongation of about 0.5–0.9 cm. Although day extension with FR light was not able to prevent growth cessation, at the end of the first experiment, the FR-treated seedlings had grown on average 217% more at 18 °C and 185% more at 22 °C, as compared to those exposed to SD. At the end of the second experiment, FR exposure at 18 °C and 24 °C had resulted in 173% at and 133% more shoot elongation, respectively, than under SD.

B-light treatment generally did not result in any significant difference in shoot elongation compared to SD-exposed seedlings, only trends of more growth (Figure 1; One-way ANOVAs for the final time points), except for at 18 °C in the second experiment (108% more shoot elongation as compared to SD) (Figure 1B). Although shoot elongation in FR- and B-exposed plants differed significantly in the first experiment at both temperatures, this was not the case in the second experiment. Furthermore, R-treated seedlings did not differ significantly in shoot elongation from SD, except for at 18 °C in the first experiment (93% more shoot elongation as compared to SD (Figure 1A; One-way ANOVAs for the final time points)). The R-exposed seedlings did generally not differ significantly from the FR-treated, except at 22 °C in the first experiment (Figure 1A; One-way ANOVAs for the final time points).

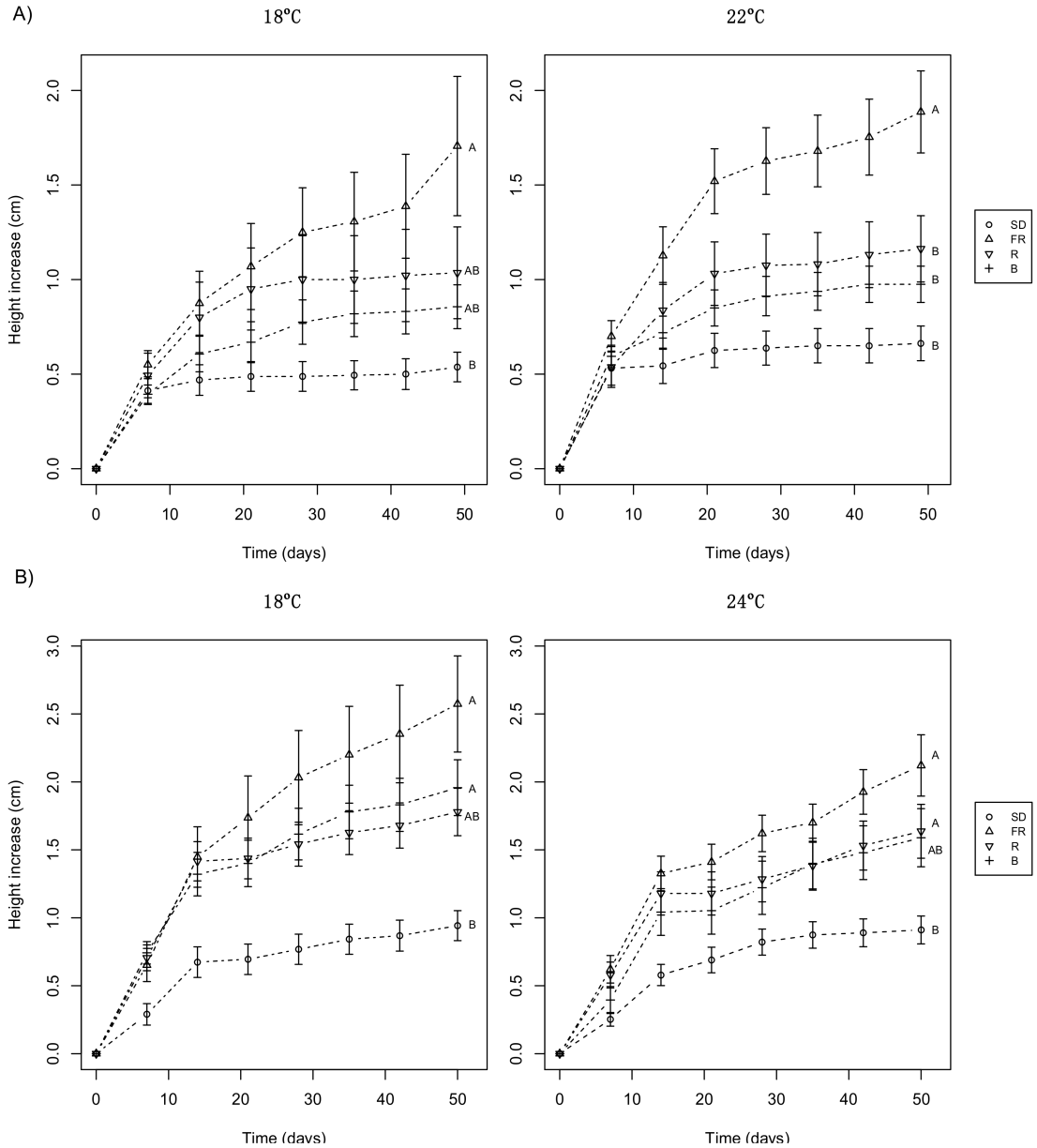

Figure 1. Effect of day extension with red (R), far-red (FR) and blue (B) from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on shoot elongation in *Abies lasiocarpa* grown under (**A**) 18 or 22 °C (experiment 1) and (**B**) 18 or 24 °C (experiment 2). The values represent the average ± SE of (**A**) 16–19 plants and (**B**) 19 plants per treatment. Different letters at the end of the lines indicate a significant difference ($p \leq 0.05$) for the last time point (at the end of the experiment) within each temperature and experiment, based on analysis of variance followed by Tukey's test. Two-way analyses of variance for the entire time courses are shown in Table S1.

In two subsequent experiments (experiments 3 and 4), we tested effect of day extension with different R:FR ratios (corresponding to a gradient in PPS values) under 18 °C and 24 °C. All SD-treated plants showed growth cessation after about 15 days regardless of temperature, with total average shoot elongation of about 0.4–0.6 cm (Figure 2). In these experiments, there was an overall significant effect of light quality and temperature when the entire time courses were considered (Two-way ANOVAs; Table S2). In experiment 3 in which FR, R:FR 0.5, 1, 2 and R day extension treatments were tested (corresponding to PPS values of 0.24, 0.72, 0.78, 0.81 and 0.88, respectively), there was no significant interaction between light quality treatment and temperature (entire time courses; Two-way ANOVAs;

Table S2). However, an interactive light quality-temperature effect was observed in experiment 4 where the effect of FR, R:FR 0.1, 0.2, 0.5 or R was tested (corresponding to PPS values of 0.24, 0.4, 0.56, 0.72 and 0.88, respectively) (time courses; Two-way ANOVAs; Table S2).

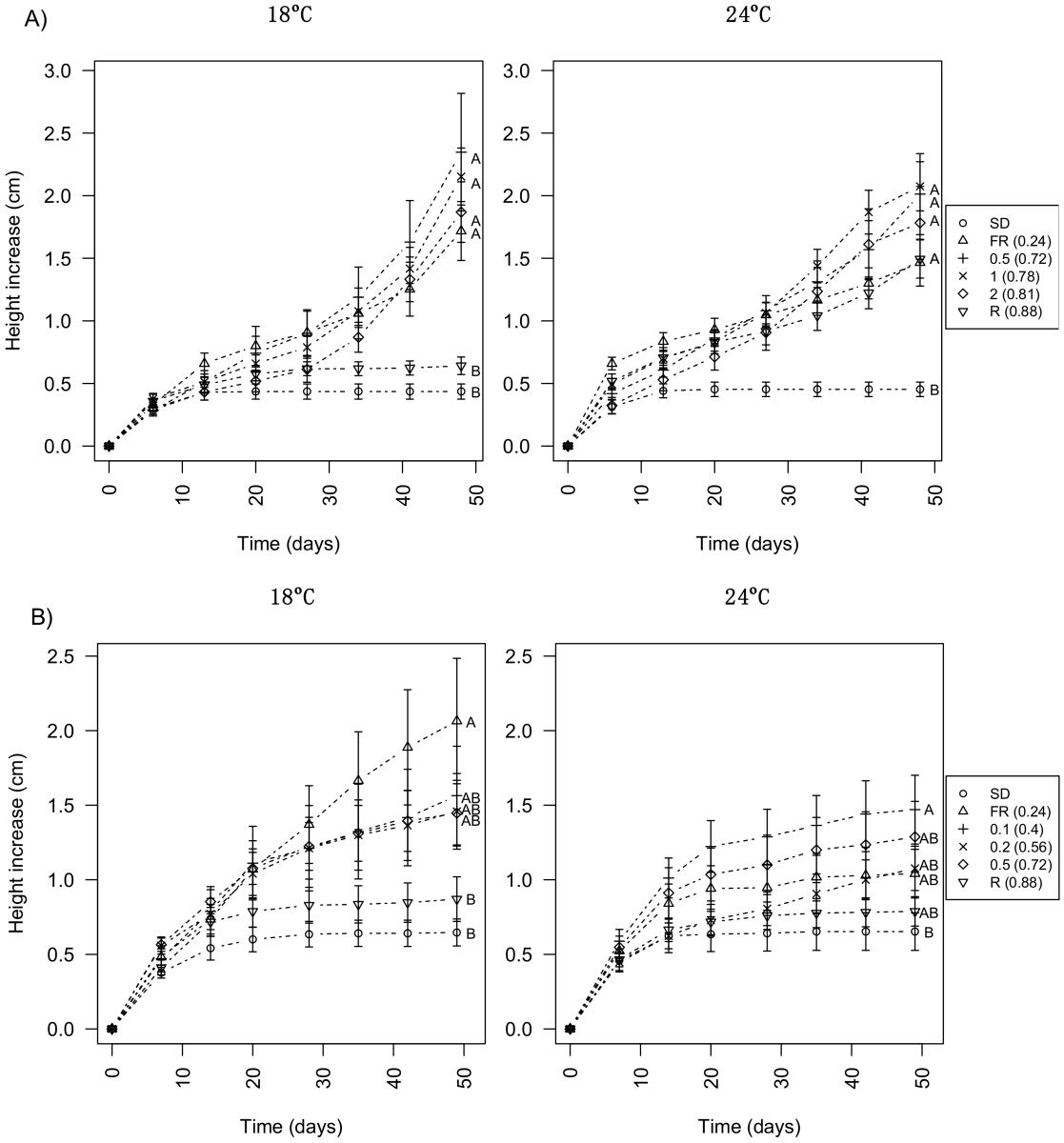

Figure 2. Effect of day extension with different light quality treatments from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on shoot elongation in *Abies lasiocarpa* (Hooker) Nuttall grown under 18 or 24 °C. (**A**) Far red (FR), red (R), R:FR ratio 0.5, 1 or 2 (experiment 3); (**B**) FR, R, R:FR ratio 0.1, 0.2 or 0.5 (experiment 4). The corresponding phytochrome photostationary state values are shown in parentheses. The values represent the average ± SE of 17 plants per treatment. Different letters at the end of the lines indicate a significant difference ($p \leq 0.05$) for the last time point (at the end of the experiment) within each temperature and experiment based on analysis of variance followed by Tukey's test. Two-way analyses of variance for the entire time courses are shown in Table S2.

Regardless of temperature, the day extension with FR only generally induced significantly more shoot elongation than SD in experiments 3 and 4, except at 24 °C in experiment 4 (Figure 2; One-way ANOVAs for the final time points). In experiment 3, the plants showed on average 295% and 223%

more elongation in response to FR compared to SD at 18 °C and 24 °C, respectively (Figure 2A). In experiment 4, 219% more elongation was observed under FR than SD at 18 °C (Figure 2B). Also, in experiment 4, 98% more elongation was observed under FR at 18 °C than at FR 24 °C. In experiment 3, there was a smaller difference between FR 18 °C and FR 24 °C (17%). Furthermore, there was no significant difference in shoot elongation between the different FR day extensions (FR and different R:FR ratios) in any of these experiments (One-way ANOVAs for the final time points). In experiment 3, all the FR treatments resulted in significantly more shoot elongation as compared to SD and R at 18 °C, and as compared to SD but not R, at 22 °C. At 18 °C in experiment 4, with the exception of the FR only treatment, shoot elongation did not differ between any of the FR-containing R:FR treatments and SD and R. At 24 °C in experiment 4, the only FR-containing treatment differing significantly in elongation growth response from SD, was R:FR 0.1.

In two experiments at 18 °C in which day extension to LD was provided by incandescent lamps, 285% ($p \leq 0.0001$) and 160% ($p \leq 0.004$) more elongation was observed under LD as compared to SD (results not shown). In a third experiment comparing effects of such daylength treatments at 18 °C and 22 °C, 485% more elongation was observed under LD 18 °C than SD 18 °C and 330% more elongation under LD 22 °C than SD 22 °C ($p \leq 0.0001$) (results not shown). LD 18 °C resulted in 140% more elongation than LD 22 °C ($p \leq 0.04$).

3.2. Day Extension with FR Light Delays Terminal Bud Development

When the effect of day extension with R, FR or B light on terminal bud development was evaluated under 18 °C and 22 °C (experiment 1) and 18 °C and 24 °C (experiment 2) for the entire time courses, a significant overall effect of light quality was observed in both experiments (Figure 3, Figure S1, Tables S3 and S6; Two-way ANOVAs). An overall effect of temperature ($p = 0.004$) was observed in experiment 2 only. There was no significant overall light quality-temperature interaction in any of these experiments (Two-way ANOVAs for the entire time courses).

The day-extension treatment significantly affected terminal bud development, with the FR-exposure significantly delaying development from green (bud stage 1) towards brown buds (bud stage 2), as compared to SD under both temperatures in both of these experiments (Figure 3, Figure S1; one-way ANOVAs for the final time point at the end of the experiments). In experiment 1, maximum bud set in FR-treated plants was observed after 27 days (18 °C and 22 °C) with terminal buds in 94% of the plants with average bud stage 1.5 (18 °C) and 1.3 (22 °C). In comparison, after 27 days, 94% (18 °C) and 88% (22 °C) of the SD-exposed plants had terminal buds with average bud stages 1.7 (18 °C) and 1.6 (22 °C). Thereafter, due to subsequent bud burst in FR-exposed plants, reduced occurrence of plants with buds was observed after 30 and 36 days for the respective temperatures. At the end of first experiment, 31% (18 °C) and 63% (22 °C) of the FR-exposed plants had terminal buds with average bud stages 0.5 and 0.8 (Figure 3; Figure S3). The corresponding values for SD-exposed plants at the end of the first experiment were the presence of buds in 94% of the plants (18 °C and 22 °C) with average bud stage 1.8.

In the second experiment, a similar pattern was observed for the FR-treated plants with maximum bud set after 37 days with buds in 95% and 100% of the plants at 18 °C and 24 °C, respectively, with average bud stage 1.4 at both temperatures (Figure 3, Figure S3). In comparison, all SD-exposed plants had buds with average bud stages 1.9 at 18 °C and 1.6 at 24 °C. At the end of the second experiment, 53% and 74% of the FR-treated plants had buds at 18 °C and 24 °C, respectively (Figure S1), with average bud stages 1.1 and 1.2 (Figure 3). All SD-exposed had buds with average bud stage 1.9.

Regardless of temperature, neither the R nor the B light treatment delayed bud development significantly compared to SD in any of these experiments (Figure 3, Figure S1; one-way ANOVAs for the final time point at the end of the experiments). Bud development was advanced compared to under FR for both R and B under 18 °C in both experiments and as compared to FR, for R at 22 °C in experiment 1 (Figure 3).

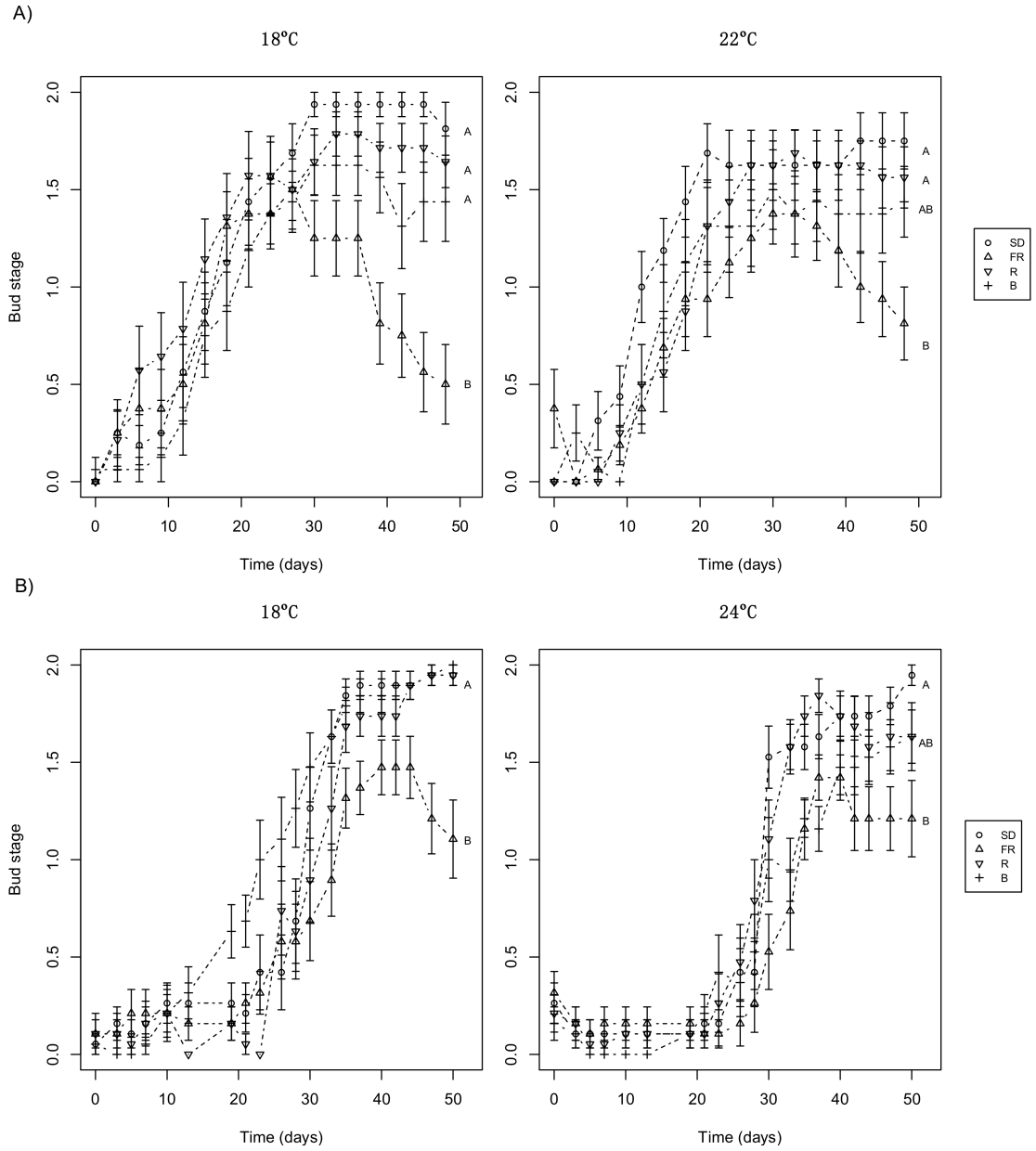

Figure 3. Effect of day extension with red (R), far-red (FR) and blue (B) from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on terminal bud development in *Abies lasiocarpa* grown under (**A**) 18 or 22 °C (experiment 1) and (**B**) 18 or 24 °C (experiment 2). The values represent the average ± SE of (**A**) 16–19 plants and (**B**) 19 plants per treatment, where 0 denotes growing shoot tip (no presence of bud), 1 green bud and 2 brown bud. Different letters at the end of the lines indicate a significant difference ($p \leq 0.05$) for the last time point (at the end of the experiment) within each temperature and experiment based on analysis of variance followed by Tukey's test. Two-way analyses of variance for the entire time courses are shown in Table S3.

3.3. Day Extension with Different R:FR Ratios Delays Bud Development Similarly to FR Only

In the third experiment where effects of day extension with FR, R or R:FR ratios 0.5, 1 or 2 or SD were tested under 18 °C and 24 °C, there was no significant overall effect of temperature on terminal bud development when the entire time courses were considered (Figure 4, Figure S2, Tables S4 and S7; Two-way ANOVAs). However, a significant effect of temperature ($p = 0.004$) was observed in the fourth experiment where the effect of FR, R or R:FR ratio 0.1, 0.2 or 0.5 or SD was tested under these

same temperatures. (Figure 4, Figure S3, Tables S4 and S7; Two-way ANOVAs for the entire time courses). Furthermore, in both experiments, an overall significant interaction between temperature and light quality treatment was observed, and there was an overall significant effect of light quality treatment (Two-way ANOVAs for the entire time courses).

Similar to experiments 1 and 2, in contrast to the situation in SD, in most cases FR-exposed plants in experiment 3 and 4 showed some bud break following maximum bud set (Figure 4). In experiment 3, at 18 °C, maximum bud set occurred after 27 days with 65% of the plants showing terminal buds with average bud stage 1.2. The corresponding values for the SD-18 °C-exposed plants were buds in 88% of the plants with average bud stage 1.6. In this experiment, at 24 °C, maximum bud set was observed after 24 days of FR-exposure with buds in 71% of the plants and average bud stage 1.1. In the corresponding SD-exposed plants, buds were observed in 71% of the plants with average bud stage 1.2. In experiment 4, maximum bud set in response to FR day extension at 18 °C was observed after 35 days with buds in 29% of the plants and average bud stage 0.5. The corresponding SD-exposed plants had buds with average bud stage 1.9. At 24 °C, maximum bud set occurred after 32 days with 88% of the FR-exposed plants with average bud stage 1.1, which was similar to the situation in SD at this time point (88% of the plants had buds with average bud stage 1.2).

At the end of experiment 3 and 4, at 18 °C, the FR- and R:FR-treated plants had significantly lower occurrence of terminal buds and on average less well developed buds in the plants with buds as compared to SD (Figure 4, Figure S2; one-way ANOVAs for the final time point). At this temperature, all FR-treated plants had growing shoot tips in experiment 3 (bud stage 0), and in experiment 4, 29% of the plants had buds with average bud stage 0.6. There was no significant difference between the FR-treatment and different R:FR ratios at 18 °C in any of the two experiments (one-way ANOVAs for the final time point at the end of the experiments). At 24 °C in experiment 3, only the R:FR ratio 0.5 and 1 resulted in a significantly lower average bud stage (bud stage 0.8) at the end of the experiment as compared to SD (bud stage 2) (one-way ANOVAs for the final time point). In experiment 4, none of the treatments differed significantly from SD at 24 °C, only slight trends of lower average bud stage was observed for the R:FR ratios 0.2 and 0.5 (one-way ANOVAs for the final time point at the end of the experiments). In comparison, at the end of experiment 3, all plants exposed to SD had well developed, brown buds with average bud stage 2.0 (brown buds in all plants) at both temperatures. At the end of experiment 4, all SD-exposed plants had brown buds (bud stage 2) at 18 °C, whereas 82% of the plants had buds with average bud stage 1.6 at 24 °C.

In experiment 3, the bud development stage in response to day extension with R light did not differ significantly from the situation in SD at the end of the experiment at 18 °C (one-way ANOVAs for the final time point). In contrast, at this time point, a significant difference between these treatments was observed at 24 °C with average bud stage 0.5 in the R-exposed plants of which 24% had terminal buds (Figure 4A; one-way ANOVAs). At the end of experiment 4, at 18 °C there was a significant difference in terminal bud development stage between the R- and SD-exposed plants with average bud stage 1.6 for the R treatment where 82% of the plants had buds (100% plants with buds and average bud stage 2 in SD) (one-way ANOVAs for the final time point). At 24 °C, there was no significant difference in terminal bud development between the R- and SD-exposed plants at the end of this experiment.

In the two experiments at 18 °C in which day extension to LD was provided by incandescent lamps, SD resulted in well-developed buds (bud stage 2) in all plants at the end of the experiment, whereas 70% and 40% of the plants had buds in LD with average bud stages 0.9 and 0.5, respectively. In the third experiment comparing effects of these daylength treatments, all plants had well-developed buds under SD (bud stage 2), regardless of temperature. Under LD 18 °C and LD 22 °C, 28 and 36% of the plants had buds with average bud stage 0.5.

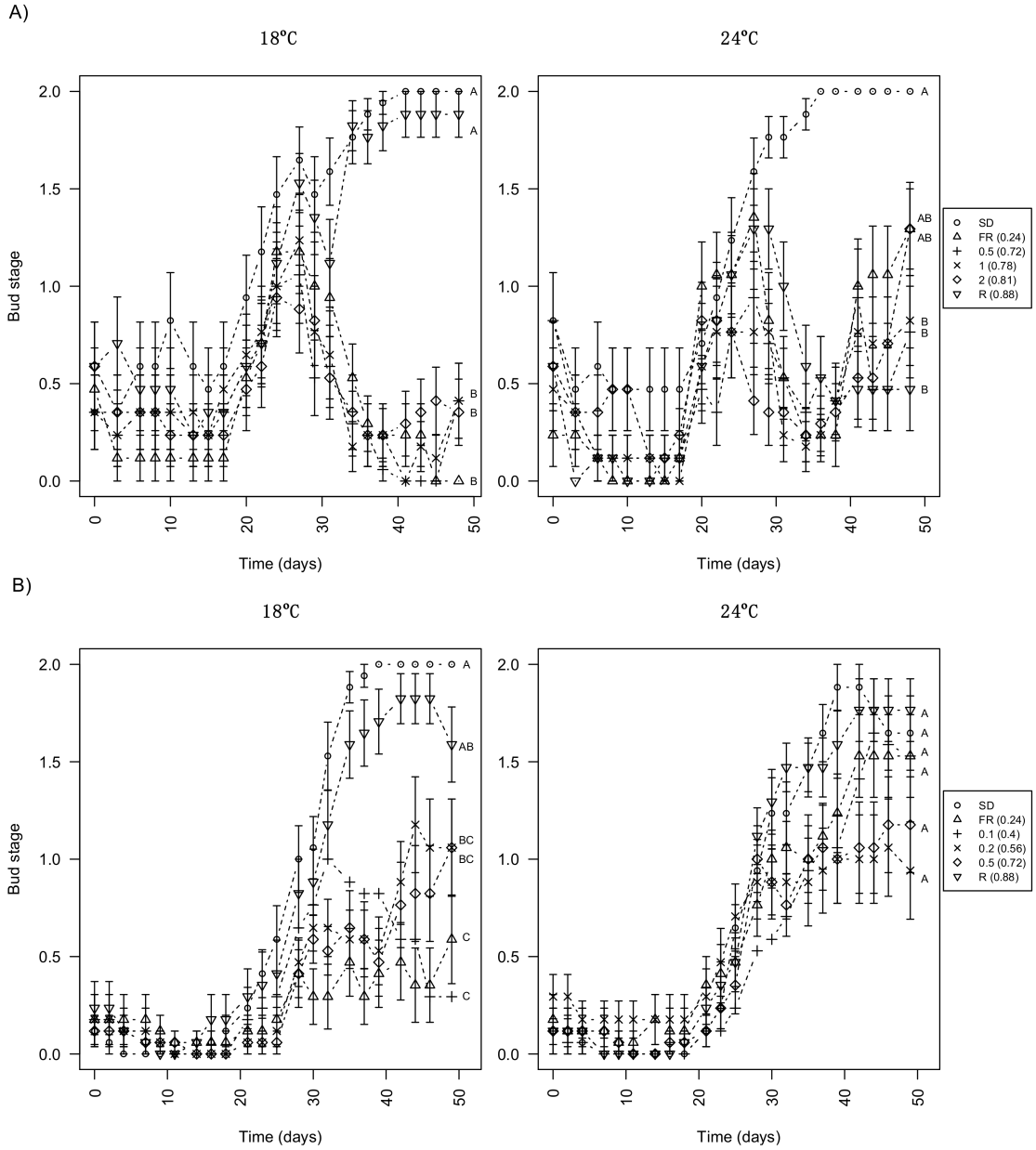

Figure 4. Effect of day extension with different light quality treatments from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on terminal bud development in *Abies lasiocarpa* grown under 18 or 24 °C. (**A**) Far red (FR), red (R), R:FR ratio 0.5, 1 or 2 (experiment 3); (**B**) FR, R, R:FR ratio 0.1, 0.2 or 0.5 (experiment 4). The corresponding phytochrome photostationary state values are shown in parentheses. The values represent the average ± SE of 17 plants, where 0 denotes growing shoot tip (no presence of terminal bud), 1 green bud and 2 brown bud. Different letters at the end of the lines indicate a significant difference ($p \leq 0.05$) for the last time point (at the end of the experiment) within each temperature and experiment based on analysis of variance followed by Tukey's test. Two-way analyses of variance for the entire time courses are shown in Table S4.

3.4. Day Extension Treatment Affects Subsequent Bud Burst

Plants with buds at the end of experiment 2, (day extension with R, FR or B light under 18 °C and 24 °C was tested as compared to SD) were re-transferred to LD at 18 °C and evaluated for timing of bud burst. The plants previously exposed to SD at 18 °C, which resulted in terminal bud formation in all plants, started to show bud burst (68% of the plants; average bud stage 0.4) after 21 days under the

LD-18 °C conditions and all these plants had growing shoot tips between days 30 and 39 (Figure 5, Figure S3). Thereafter, these plants again formed terminal buds, and buds were present in all plants (bud stage 2) at the end of the experiment. Of the plants with buds (all these had brown buds) at the end of the FR day extension treatment at 18 °C, bud burst was observed in 10% of the plants after 12 days under LD-18 °C, and only 20% of the plants showed bud burst (average bud stage 1.6) in total. The plants previously exposed to day extension with R light at 18 °C started to show bud burst after 9 days (6% of the plants; average bud stage 1.7), and after 51 days bud burst was observed in 50% of the plants (average bud stage 1). The B light-18 °C treated plants showed bud burst later than the plants exposed to any of the other light quality treatments. The first occurrence of bud burst was observed after 33 days under LD-18 °C, and 21% of the plants (average bud stage 1.4) showed bud burst at the end of the experiment.

As compared to the SD-18 °C-treated plants, significantly less bud burst and also less new bud set was observed after transfer of the plants to LD-18 °C following exposure to SD at 24 °C (Tables S5 and S8; two-way ANOVAs for the entire time courses). Of these, the first plants with bud burst were also observed after 15 days under LD-18 °C, and bud burst was observed in 68% of the plants (average bud stage 0.6) after 33 days before new bud set again occurred, and at the end of the experiment 52% of the plants had buds (average bud stage 1). For the previously FR-24 °C-treated plants with buds, the first bud burst was observed in 27% of the plants after 27 days, and at the end of the experiment bud burst was observed in 53% of the plants (average bud stage 1.1). The B-24 °C and R-24 °C-exposed plants showed slower and less bud burst. These plants started to show bud burst first after 39 days and with only 11% and 6% of the plants, respectively, showing bud burst within the experimental period of 51 days (average bud stage 1.8).

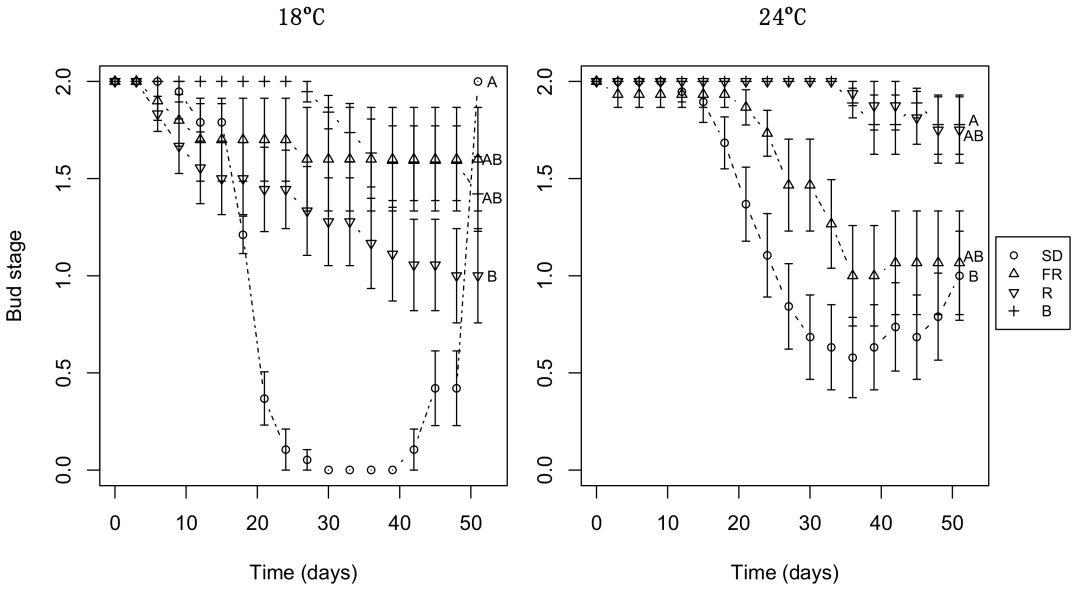

Figure 5. Effect of day extension with red (R), far-red (FR) or blue (B) light from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on bud burst in *Abies lasiocarpa* grown under 18 or 24 °C for 50 days (experiment 2) before transfer to long days at 18 °C. The values represent the average ± SE of 10–19 plants per treatment, where 0 denotes growing shoot tip, 1 bud burst and 2 presence of terminal bud. Different letters at the end of the lines indicate a significant difference ($p \leq 0.05$) for the last time point (at the end of the experiment) within each temperature based on analysis of variance followed by Tukey's test. Two-way analyses of variance for the entire time courses are shown in Table S5.

4. Discussion

During production of subalpine fir seedlings for Christmas tree production in nursery greenhouses in northern European regions like Scandinavia, common early growth cessation results in undesirably small plants. This makes the plants vulnerable to stressors when transplanted to outdoors conditions and increases the production time. Thus, identification of growing conditions making it possible to produce larger plants during greenhouse cultivation is highly desirable. Here, we tested whether day extension with different light qualities from LEDs (FR, R, different R:FR ratios, B) at different temperatures (18, 22 and 24 °C) during the production of subalpine fir plants could increase shoot elongation and delay terminal bud development.

Enhanced elongation growth in subalpine fir seedlings in response to day extension with FR or R:FR light (Figures 1 and 2) is consistent with previous results from the conifer Norway spruce, and deciduous woody species like downy birch, white birch and bay willow [10–16]. On the other hand, whereas day extension with FR light from LEDs or FR light-enriched light in general could prevent growth cessation and terminal bud formation completely in Norway spruce seedlings, this was not generally the case in subalpine fir (Figures 3 and 4, Figures S1 and S2). Nevertheless, taller seedlings were obtained probably due to a delay in bud formation or bud burst and the resumption of growth in many plants showing terminal bud formation during the experimental FR treatments or even during the pre-growth stage before the start of the light quality treatments. Since there was generally no consistent significant differences in terminal bud development or shoot elongation between the different R:FR ratios tested (ranging from 0.1 to 2.0) (Figures 2 and 4, Figure S2), the presence of FR light in the spectrum during the day extension treatment is apparently what is important. This is supported by the enhanced elongation and delay in bud set as well as bud burst and resumption of growth when the day extension was provided by incandescent light, which is rich in FR light in addition to other wavelengths of photosynthetically active radiation.

In seedlings of Norway spruce provenances, day extension with B and R light significantly delayed bud set and thus resulted in taller plants as compared to SD [15]. Although observed here in some cases in subalpine fir, such effects were not consistent among the different experiments. Thus, from a practical point of view, using day extensions with B or R light only to enhance growth or delay bud set during production of subalpine fir seedlings cannot be recommended.

Abies species such as subalpine fire and *Picea* species are well known to be shade-tolerant, i.e., *Abies* somewhat more so than *Picea*, and such species are thus able to survive under low irradiances [24,39]. Nevertheless, *Abies,* including seedlings of subalpine fir, as well as *Picea* species, were shown to react strongly with enhanced elongation growth in response to increased irradiance [24,39]. In Norway spruce provenances originating from latitudes of 59° N, 64° N and 66.5° N, increasing FR irradiance with increasing northern latitude of origin was required to prevent bud set [15]. Given the mentioned physiological similarities of *Abies* and *Picea,* and the relatively high FR irradiance and 24 h photoperiod used in the present study for the subalpine fir originating from 52° N latitude, we initially hypothesised that terminal bud set could be prevented also in this subalpine fir provenance. Since this generally turned out not to be the case, it might be suggested that sustained growth in the mentioned Norway spruce seedlings [15] but not in subalpine fir (Figures 1–4) under similar light regimes, could be ascribed to different general light requirements of seedlings of these species. It could be noted that the existence of altitudinal ecotypes with different light requirements has been demonstrated in woody species [10]. Thus, the difference may also be due to the fact that the subalpine fir provenance studied originates from 1000–1200 m a.s.l., whereas the Norway spruce provenances [15] originates from a low altitude (0–200 m a.s.l.).

It could also be noted that about 15% of the plants had buds at the end of the pre-growing period at 200 μmol m^{-2} s^{-1} in experiment 1 or 250 μmol m^{-2} s^{-1} in experiment 3 and 4. On the other hand, all plants had terminal buds in a pilot experiment (data not shown) with pre-growth in late autumn (November–December) in a greenhouse compartment with supplementary light at 100 μmol m^{-2} s^{-1} (R:FR about 3). This may suggest that a critical irradiance is required for sustained growth. Indeed,

dependence of a certain irradiance for growth was demonstrated in a field study at natural subalpine fir sites [24]. These authors found that very few such seedlings were growing in the understory at 10% of the open-sky light level, and there was a strong relationship between light level and elongation growth. Thus, the relatively low irradiances used in the growth chambers in our experiments as compared to sunny days outdoors in the mountain areas where the subalpine fir provenance originates, may well have contributed to the lack of sustained growth.

Although the effect of temperature was variable, a significant effect of temperature on shoot elongation (experiment 2 and 4, trend in experiment 1 ($p = 0.068$)) and bud formation (experiment 2 and 4) as well as significant interaction between temperature and day extension treatment (experiment 4 for shoot elongation, experiment 3 and 4 for bud development) in two out of four experiments suggests that temperature may modulate the response to light quality. Specifically, day extension with FR apparently delays bud development more and thus results in more growth at 18 °C than at 24 °C. Such a temperature modulation of the response to light quality has to our knowledge not previously been reported in woody species. On the other hand, interactive effects of temperature and photoperiod have been studied more frequently. In previous experiments in woody species grown under controlled conditions, including Norway spruce under SD, a lower temperature delayed the bud development as compared to higher temperature [29–35]. The present results suggest a similar relationship between temperature and light quality.

A relationship between bud development status and subsequent bud burst was suggested on basis of the notion in Norway spruce that more well-developed SD-induced terminal buds that formed at higher temperature (up to 21 °C tested) showed subsequent earlier bud burst than less well-developed buds formed at lower temperature [34]. The overall more comprehensive occurrence of bud burst and resumption of growth (in 100% and 68% of the plants from 18 °C and 24 °C, respectively, after 30 days) following the strongest bud-inducing signal in our study, i.e., SD (brown-well developed buds in all plants) as compared to the day extension treatments (Figure 5, Figure S3, Tables S5 and S8) is consistent with the idea [34] that bud development must pass a certain stage before bud burst occur. Nevertheless, the SD-exposed subalpine fir plants were also the only ones showing comprehensive new bud set after bud burst, i.e., in all plants at 18 °C and 52% at 24 °C.

The impact of spectral composition during bud set on subsequent bud burst in woody species has been studied far less than the effect of photoperiod. The relationship between bud developmental stage and subsequent bud burst was not clear for the FR, R and B- treated plants at the two temperatures, although these showed overall lower bud burst than the SD-exposed plants. For R- and B-treated plants, lower bud burst occurred for the 24 °C- than for the 18 °C-exposed plants, while the opposite situation was observed for the FR-exposed plants. To our knowledge, no comparable studies of such after-effects of light quality on bud burst have been published. However, in Norway spruce provenances, bud burst occurred in populations from 59° N and 64° N, but not 66.5° N under day extension with R light, and in the two northernmost, but not in the southernmost population in FR [15]. In contrast, bud burst did not occur in any of these populations under day extension with B light. Nevertheless, in branches of three deciduous tree species, *Alus glutinosa* (L.) Gaertn., *Betula pendula* and *Quercus robur* L., B light advanced bud burst [43]. Thus, the effect of light quality on bud burst appears to differ between species and sites of origin.

5. Conclusions

Collectively, our results demonstrate that taller first year seedlings of subalpine fir for Christmas tree production can be obtained by the use of FR light as day extension as this delays terminal bud development. Also, the delayed bud development and thus larger plants in response to FR treatment may be enhanced at 18 °C as compared to 24 °C. Such production of larger seedlings of subalpine fir during the first growth season in nurseries is of significant interest for the greenhouse industry raising seedlings for Christmas tree production, since enhanced elongation growth the first year may reduce later production time in addition to potentially providing more robust seedlings.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/9/4/175/s1. Figure S1: Effect of day extension with red (R), far-red (FR) and blue (B) from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on % plants with terminal bud in *Abies lasiocarpa* grown under A) 18 or 22 °C (experiment 1) and B) 18 or 24 °C (experiment 2), Figure S2: Effect of day extension with different light quality treatments from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on % plants with terminal bud in *Abies lasiocarpa* grown under 18 or 24 °C. A) Far red (FR), red (R), R:FR ratio 0.5, 1 or 2 (experiment 3). B) FR, R, R:FR ratio 0.1, 0.2 or 0.5 (experiment 4), Figure S3: Effect of day extension with red (R), far-red (FR) or blue (B) light from light emitting diodes (LEDs) as compared to short days (SD: no day extension) on terminal bud burst in *Abies lasiocarpa* grown under 18 or 24 °C for 50 days (experiment 2), before transfer to long days at 18 °C (conditions as before start of the treatments), Table S1: Two-way ANOVA tables for study of effect of day extension with red, far-red or blue light from light emitting diodes (LEDs) and short days (SD: no day extension) on shoot elongation in a time course in *Abies lasiocarpa* seedlings grown under A) 18 or 22 °C (experiment 1) and B) 18 or 24 °C (experiment 2) (results shown in Figure 1), Table S2: Two-way ANOVA tables for study of effect of day extension with A) far red, red or red: far-red ratio 0.5, 1 or 2 (experiment 3) and B) far-red, red or red: far-red ratio 0.1, 0.2 or 0.5 (experiment 4) from light emitting diodes (LEDs) and short days (SD: no day extension) on shoot elongation in a time course in *Abies lasiocarpa* seedlings grown under 18 or 24 °C (results shown in Figure 2), Table S3: Two-way ANOVA tables for study of effect of day extension with red, far-red or blue light from light emitting diodes and short days (SD: no day extension) on terminal bud stages in a time course in *Abies lasiocarpa* seedlings grown under A) 18 or 22 °C (experiment 1) and B) 18 or 24 °C (experiment 2) (results shown in Figure 3), Table S4: Two-way ANOVA tables for study of effect of day extension with A) far red, red or red: far-red ratio 0.5, 1 or 2 (experiment 3) and B) far-red, red or red: far-red ratio 0.1, 0.2 or 0.5 (experiment 4) from light emitting diodes (LEDs) and short days (SD: no day extension) on terminal bud stages in a time course in *Abies lasiocarpa* seedlings grown under 18 or 24 °C (results shown in Figure 4), Table S5: Two-way ANOVA tables for study of effect of day extension with red, far-red or blue light from light emitting diodes (LEDs) and short days (SD: no day extension) on terminal bud burst in a time course in *Abies lasiocarpa* seedlings grown under 18 or 24 °C (experiment 2) for 50 days before transfer to long days at 18 °C (conditions as before start of the treatments), Table S6: Two-way ANOVA tables for study of effect of day extension with red, far-red or blue light from light emitting diodes and short days (SD: no day extension) on presence/absence of terminal buds in a time course in *Abies lasiocarpa* seedlings grown under A) 18 or 22 °C (experiment 1) and B) 18 or 24 °C (experiment 2) (results shown in Figure S1), Table S7: Two-way ANOVA tables for study of effect of day extension with A) far red, red or red: far-red ratio 0.5, 1 or 2 (experiment 3) and B) far-red, red or red: far-red ratio 0.1, 0.2 or 0.5 (experiment 4) from light emitting diodes (LEDs) and short days (SD: no day extension) on presence/absence of terminal buds in a time course in *Abies lasiocarpa* seedlings grown under 18 or 24 °C (results shown in Figure S2), Table S8: Two-way ANOVA tables for study of effect of day extension with red, far-red or blue light from light emitting diodes (LEDs) and short days (SD: no day extension) on terminal bud burst (presence/absence of buds) in a time course in *Abies lasiocarpa* seedlings grown under 18 or 24 °C (experiment 2) for 50 days before transfer to long days at 18 °C (conditions as before start of the treatments) (results shown in Figure S3).

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