Norwegian University of Life Sciences

Department of Plant Science

Master Thesis 2015
60 credits

Effects of Light Quality and Temperature on Elongation Growth, Dormancy and Bud Burst in Norway spruce (Picea abies) and Subalpine fir (Abies lasiocarpa)

Oda Toresdatter Aas

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Norges miljø- og biovitenskapelige universitet

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Department of Plant Science
The Norwegian University of Life Sciences
P.O. Box 5003, 1432 Ås, Norway

Ås, 2015

Abstract

Elongation growth and bud development in northern perennials is under photoperiodic control. This preparation for a colder season is induced by a shorter day than the critical day for growth and development. Light quality also appears to play a role in this respect and generally affects plant growth and development. In this study we aimed to investigate the effects of different light qualities, i.e. blue (B), far-red (FR) and red (R) as day extension light on elongation growth, bud development and subsequent bud burst in seedlings of Norway spruce and Subalpine fir compared to seedlings exposed to short days conditions (SD). Since photoperiodic responses are known to be modulated by temperature, interactive effect of light quality and temperature on the growth, bud set and bud burst was also investigated. In addition, effects of these light qualities on the expression of the *FLOWERING LOCUS T-TERMINAL FLOWER1-LIKE 2* (*FTL2*-gene) was studied in seedlings of Norway spruce.

FR and B light was the most effective light qualities in maintaining growth relative to R in seedlings of Norway spruce, virtually all plants showed sustained growth under these treatments. There was no clear effect of the tested temperatures (18, 22 and 24°C) on the FR and B responses. However, R-treated seedlings showed higher elongation growth when exposed to elevated temperature, which supports that temperature affects the plant`s capability to detect photoperiod or alternatively signaling downstream of the photoreceptors involved. *FTL2* transcript levels strongly supports that the *FTL2*-gene acts as a growth inhibitor, which is in coincidence with the treatments that ceased growth and developed bud in Norway spruce.

In Subalpine fir, a similar tendency was observed. FR-treated seedlings achieved the highest growth, followed by R- and B treated seedlings. The results indicate that, FR, R and B receptors are involved in the photoperiodic control of elongation growth in seedlings of Subalpine fir as well as Norway spruce. A similar trend as for Norway spruce was observed in response to higher temperature. Many seedlings of Subalpine fir developed buds under pre-growth and in treatments which sustained growth in most plants of Norway spruce, which might indicate that a higher irradiance of light is needed to maintain growth in these seedlings compared to seedlings of Norway spruce.

Sammendrag

Strekningsvekst og knopputvikling i flerårige trær er kontrollert av fotoperiode, dvs. varigheten av lys- eller mørkeperioden. Forberedelsene til en kaldere sesong blir indusert av en kortere dag enn den kritiske daglengden for vekst og utvikling. Lyskvalitet har stor påvirkning på plantevekst og utvikling. I denne studien undersøkte vi effektene av ulike lyskvaliteter, blått (B), mørkerødt (MR) og rødt (R) som dagforlengelseslys på strekningsvekst og knopputvikling og etterfølgende knoppbrytning i norsk gran og fjelledelgran, sammenlignet med hos kortdagseksponerte planter (KD). Et annet viktig aspekt når det gjelder effekt av fotoperiode er temperatur, og samspill mellom lyskvalitet og temperatur ble derfor undersøkt i henhold til prosessene nevnt ovenfor. Videre ble også effektene av lyskvalitet på ekspresjonen av *FLOWERING LOCUS T-TERMINAL FLOWER1-LIKE 2* (*FTL2*-gen) undersøkt, som i tidligere studier er vist å være oppregulert under KD.

MR- og B lys var de mest effektive lyskvalitetene for å opprettholde vekst relativt til R lys i norsk gran. MR- og B responsene viste seg å være sterkere enn en potensiell temperatureffekt. R-behandlede planter viste en høyere grad av strekningsvekst ved høyere temperaturer, noe som indikerer at temperatur har en essensiell rolle i plantenes evne til å detektere fotoperiode eller eventuelt at temperaturen påvirker signaloverføringen nedstrøms for lysreseptorene. Resultatene for *FTL2*-transkript nivået viste seg å støtte hypotesen om at *FTL2* fungerer som en veksthemmer, da høye transkripsjonsnivå gjenspeilte seg i behandlingene som førte til vekstavslutning og knopputvikling i norsk gran.

Når det gjelder fjelledelgranen er tendensen for vekst lik som observert hos norsk gran. MR-behandlede planter oppnådde den største veksten, etterfulgt av R og B-behandlede planter. Resultatene indikerer at MR, R og B-reseptorer er involvert i den fotoperiodiske kontrollen av strekningsvekst også hos fjelledelgran. Trenden var det samme når plantene ble eksponert for en høyere temperatur. Før og i behandling utviklet en del planter av fjelledelgran knopper til forskjell fra norsk gran, noe som indikerer at en høyere lysirradians trengs for å opprettholde vekst hos denne arten.

Acknowledgement

First of all I will like to thank my supervisor, Prof. Jorunn Elisabeth Olsen for all the knowledge, patience and dedication to this research. She has been a continuous help and have encourage me the hold way. I will thank Marit Siira and Yeon Kyeong Lee with the practical help in relation to the plants and cooperation during the harvesting. I will also express my gratitude to Tone Melby for guidance through the laboratory work and Christian Strømme for help with the statically analysis. I will also thank Odd Tore Hegna for willingly share his time to proofread the paper and Marianne Aas for constant support and cheering.

In the end, a special thanks to Marianne Rindedal Jetmundsen.

Abbreviations

FR = far-red

B = blue

R = red

SD = short day

LD = long day

FTL2 = FLOWERING LOCUS T-TERMINAL FLOWER1-LIKE 2

Key-words

Light quality, temperature, elongation growth, bud development, bud burst, Norway spruce, Subalpine fir

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Introduction

Plants are immobile organisms that need to cope with environmental factors, such as photoperiod, light and temperature for survival, growth and reproduction. They have no other choice than to adapt to the climatic changes in the environment. Perennial tree species of temperate and boreal regions exhibit several mechanisms that are crucial for survival and further growth. One of the most important mechanisms is winter dormancy. Winter dormancy is characterized as the inability of a meristem to resume growth under favourable conditions, and is defined as a property of the meristem (Rohde & Bhalerao 2007). Dormancy often begins with cessation of growth and is thereafter followed by bud formation (Olsen 2010; Olsen & Lee 2011). Dormancy is commonly divided into three stages; ecodormancy, which is triggered by limitations in environmental factors, paradormancy, which is characterized by the inhibition of growth by another part of the plant than the dormant structure, and endodormancy, where the inhibition is found in the dormant structure itself (Lang 1987). Earlier experiments have shown that a night length longer than the critical length is a crucial signal for inducing growth cessation and bud development (Nitsch 1957) and that altered light quality can play an important key role in the events that result in bud dormancy (Mølmann et al. 2006). In addition, shifts in genes that are active and modulation of temperature have been shown to be essential in the preparation of trees for colder climate (Asante et al. 2011; Gyllenstrand et al. 2007; Heide 2008; Olsen & Lee 2011).

1.1 Photoperiod

The annual life cycle of woody perennials needs to be synchronized with the environment. Environmental signals will give them clues on when they should start growing, flowering, produce certain kinds of hormones and prepare for colder conditions, which starts with cessation of growth and bud development. A primary factor for controlling elongation growth in first year seedlings of trees was long considered to be photoperiod, i.e. day length (Nitsch 1957). In response to shortening of the photoperiod, conifers starts growth cessation and bud development in late summer, followed by attaining frost tolerance and dormancy (Olsen 2010). This gradual frost tolerance is a response initiated by the photoperiod, followed by

exposure to low temperature (Welling & Palva 2006). The photoperiodic control of bud set have been shown to be achieved through two different processes, i.e. the dark dominant, which relies on critical duration of darkness, and the light dominant, which relies on the duration of day length and light climate during the day (Clapham et al. 1998b).

Previous experiments have shown that there is an increase in critical day length (i.e. actually night length) for growth with increasing latitude and/or altitude (Olsen 2010; Olsen & Lee 2011). Southern populations thus continue to grow later in the growing season compared to northern populations. This difference is believed to be a result of genetic differences among populations and their various response to photoperiods (Gyllenstrand et al. 2007). One other important environmental factor interacting with photoperiod in affecting the ability of trees to synchronize their elongation growth, bud set and bud burst with the environment, is light quality (discussed below).

1.2 Photoreceptors

Phytochrome is a pigment system in plants which sense any changes in photoperiod and light, and is thought to play an important role in perennials in preparation for colder conditions (Olsen et al. 1997). The phytochrome system is photo reversible and have two forms of phytochromes, the active Pr form and the inactive Pfr form, which mainly absorbs R and FR light, respectively (Clapham et al. 1998a). In responses to different light wavelengths the photoreceptors are converted between the inactive and active form, helping the plants to fine-tune development in response to different light environments (Von Der Horst & Hellingwerf 2004). Norway spruce is believed to contain three phytochrome genes, *PHYP*, *PHYN* and *PHYO,* where *PHYP* and *PHYN* resemble *PHYB* and *PHYA,* respectively, found e.g. in the herbaceous model plant *Arabidopsis thaliana* and *Populus* trees. In *Populus* both phyA and phyB are believed to be involved in the photoperiodic control of dormancy (Clapham et al. 1999; Howe et al. 1998; Olsen et al. 1997; Opseth et al. 2015)

Cryptochromes are blue ultraviolet-A receptors that regulate different kinds of light responses, and are found to be involved in photoperiodic control of flowering in plants such as *A.thaliana* (Lin & Shalitin 2003). Both phytochromes and cryptochromes give inputs to components that are involved in circadian rhythms.

These photoreceptors are therefore though to be indirectly involved in sensing of the photoperiod (Olsen 2010).

1.3 Light quality

Light is considered the most fundamental factor to sustain life. Earlier studies indicate that maximum growth in plants is achieved at an irradiance less than full sun out in the nature (Hocker 1979). Irradiance is influenced by solar elevation and atmospheric conditions, e.g. there is an increase cloudiness in north, especially in the coastal areas (Nilsen 1985). The different light qualities in the spectrum play an important role in controlling growth-dormancy cycles (Olsen & Lee 2011). Red (R) and far-red (FR) ratio has been shown to regulate different responses in plants, such as shade avoidance, seed germination, internode elongation, regulation of flowering (Taiz & Zeiger 2010). Although this ratio is little influenced by climatic conditions (Nilsen 1985), it decreases in shade because the leaves absorb most of the R light while the FR light is mostly transmitted (Sarala et al. 2007). Further, this ratio has been reported to vary in response to seasons and different times of the day, e.g. in summer time south of the Arctic Circle there will be both long periods of high R:FR ratio during the day and short periods of lower R:FR ratio during the twilight period (Nilsen 1985).

Previous studies have investigated effects of different light qualities, such as blue (B, 400-500 nm), R (600-700 nm) and FR (700-800 nm) as day extension light. It has been demonstrated that especially FR light have a major influence on elongation growth in certain woody species under short days (SD) (Clapham et al. 1998a; Junttila & Kaurin 1985; Tsegay et al. 2005) Mølmann et al. (2006) analysed the effects of light qualities from light emitting diodes (LED) on growth in three different latitudinal populations of Norway spruce. FR-light was more effective in maintaining growth than R in all three populations. Both R- and B light delayed bud set compared to the SD treatment. In this study the maximum irradiance of each type of LED-light was set to 3.3 Wm^{-2} and effects of higher irradiance on elongation growth and bud set was not investigated. The requirement for FR to maintain elongation growth has been shown to be higher in more northern populations compared to southern (Clapham et al. 1998a; Mølmann et al. 2006). A requirement for FR in sustaining growth has also been suggested for seedlings of Subalpine fir

(Cazarji 2013). However, Cazarji (2013) observed that many of the seedlings developed buds under the pre-growth with low irradiance of supplemental growth light, i.e. 100 μmol m^{-2} s^{-1}.

R light was shown to be less efficient in maintaining growth compared to FR (Mølmann et al. 2006; Olsen 2010). According to Mølmann et al. (2006) the most efficient treatment in sustaining growth in seedlings of Norway spruce was to use a one to one mixture of R and FR, e.g. high irradiance of both light qualities together prevented growth cessation more efficiently in northern perennials than either light quality alone

B light is known to initiate different responses in plants, e.g. phototropism, opening of stomata and slowing of hypocotyl elongation which occurs when seedlings breaks ground (Campbell et al. 2008). Further, B-light is easily scattered in the atmosphere and thus, during the main light period there is no clear difference in B light proportion with latitude. However, at dusk and dawn the presence of diffuse B light is high and varies with latitude since the length of the twilight periods differ with latitude (Kvifte et al. 1983; Olsen & Lee 2011; Taulavuori et al. 2010). In previous studies B light exposure did not prevent but significantly delayed growth cessation in Norway spruce and bay willow (*Salix pentandra)* (Junttila & Kaurin 1985). This delay is believed to be a result of involvement of B light in the process of elongation growth and bud formation (Mølmann et al. 2006; Olsen 2010). B light is well known to inhibit shoot elongation in a range of species such as *Arabidopsis thaliana* (Fankhauser & Chory 1997). Also, depletion of B light from sunlight was reported to enhance stem length in Scots pine, indicating B light inhibition of shoot elongation also in this conifer species (Sarala et al. 2007). On the contrary, B light has been reported to enhance growth in Petunia (Fukuda et al. 2011).

1.4 Temperature

As discussed above, it is commonly believed that growth cessation is mainly induced by SD. In addition, it is well known that temperature play an important role in preparation for colder conditions, i.e. low temperatures for instance induces senescence and abscission of leaves and development of cold hardiness (Olsen 2010; Olsen & Lee 2011; Tanino et al. 2010). In addition, low temperature could also be a requirement for some northern perennials to resume growth after bud dormancy. In

Norway spruce and some other conifers, has been reported to hasten bud break, although it is not strictly required (Nienstaedt 1967; Olsen et al. 2014; Søgaard et al. 2008; Worrall & Mergen 1967)

Warm temperatures combined with SD has been reported to induce accelerated growth cessation and bud development, as well as a delay in subsequent bud burst in seedlings of Norway spruce (Olsen et al. 2014). The effects of temperature on photoperiod have also been reported in different species of *Prunus*. A continuous growth was observed at 21°C regardless of photoperiodic conditions. A lower temperature (9°C) induced growth cessation independent of photoperiod in some species, whereas others ceased growth as a respond to SD (Heide 2008).

1.5 Gene regulation

Modulation of tree growth in response to seasonal shifts in day length, temperature and light quality is controlled genetically (Asante et al. 2011; Gyllenstrand et al. 2007; Olsen 2010; Olsen & Lee 2011). Such growth control share common trait with control of flowering. The flowering pathways in the model plant *A thaliana* are now well understood. Certain environmental clues initiate flowering by activating *CONSTANS*- (*CO*) genes, which then triggers the transcription of other genes, e.g. *FLOWERING LOCUS T* (*FT)*- and *SUPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1)*. This flowering pathway is believed to be similar in *Populus*-species and involve *CO*-and *FT* orthologs; *PtCO* and *PtFT1* (Bohlenius et al. 2006). Bohlenius et al. (2006) showed that these orthologs play a key role in the photoperiodic control of growth and bud development. When there is coincidence between high levels of CO at the end of growth-sustaining long days (LD), *FT* is upregulated, whereas under SD it is downregulated. Although such *CO*-genes are not found in Norway spruce, presence of *CO-LIKE* genes (*COL*), called *PaCOL1* and *PaCOL2,* has been shown (Holefors et al. 2009). Holefors et al. (2009) showed that expression of these genes was higher in the shoot apical meristem than in the needles, and suggested that their down-regulation under SD might cause growth cessation and bud formation, although further studies are needed to verify.

Although the genetic mechanisms behind dormancy in perennial trees are not fully understood, previous studies have reported that the Norway spruce genome lacks *FT* genes (Nystedt et al. 2013). However, Norway spruce contains a gene, which

resembles more the *TFL1* gene encoding a floral inhibitor in *A. thaliana,* which belongs to the same gene family as *FT* in spite of having opposite function. This *TFL1*-*FT*-resembling gene in Norway spruce is denoted *FTL2*-and is upregulated under SD. This specific gene found in *Picea*-species has been renamed three times (*PaFT4* (Gyllenstrand et al. 2007), *TFL1* (Asante et al. 2011) and *FTL2*, respectively (Nystedt et al. 2013)). The up-regulation of *FTL2* under SD indicates a critical involvement in inhibiting growth and induction of bud set, as verified in plants overexpressing the gene (Asante et al. 2011; Gyllenstrand et al. 2007; Karlgren et al. 2013).

1.6 Norway spruce

Norway spruce *(Picea abies)* is one of the main tree species in the boreal and temperate zones of Europe; mountain ranges of central and south-eastern Europe, lowlands in the eastern Europe and in the Scandinavian peninsula (Jansson et al. 2013). This species has a high ecological and economical importance and is used for high quality timber production. In 2013 researchers manage to sequence this tree`s genome, and this has opened a new door of knowledge in physiology and genetics of this species (Nystedt et al. 2013).

Woody species like Norway spruce have a long juvenile phase, and it takes about 20 years before they start develop cones. Seedlings will in late summer start growth cessation and buds will eventually be produced in response to a shortening photoperiod, and frost tolerance will increase in response to the SD and lowered temperature (Gyllenstrand et al. 2007). In spring bud burst will occur as a response to increased temperature and possibly longer photoperiod, and elongation growth will resume. This annual cycle is exposed to various stress factors, e.g. frost, and the correct timing of phenology is critical for surviving the shifting climate in northern part of Europe (Jansson et al. 2013).

1.7 Subalpine fir

There is a wide distribution of different populations of Subalpine fir (*Abies lasiocarpa)* in Western North America, ranging from Arizona and New Mexico in south to Alaska and Yukon in north (Skage & Stavrum 2002). In British Colombia, Subalpine fir is considered a common lumber species. The species is very cold hardy,

and both drought and shade tolerant (Cartwright & Ying 2011). One of the most challenging events is frost damage during the spring (Artsdatabanken 2014)

Although Subalpine fir are not established naturally in Norway, this species is of great interest for Christmas tree production (Skage & Stavrum 2002) and therefore potentially could have a high economical value. When the species is cultivated as Christmas trees, they are sown and grown in nurseries the two first years before they are transported to the producers. Small plants might commonly be vulnerable for infections, e.g. fungi infections (Talgø et al. 2012).

1.8 Christmas tree production

Norway spruce is considered to be the traditional Christmas tree in Norway. 64% of the Norwegian population prefer Norway spruce as Christmas tree according to a survey done of Norsk Gallup Insitutt A/S in 1998, while *Abies*-species is a strong second choice (*Juletrearter*) . However, the cultivation of *Abies*-species such as Subalpine fir is believed to be increase in response to an increased request for this species as Christmas tree. Effects of light quality and temperature on elongation growth may have an important, economic value in greenhouse production of Christmas trees. There have been many studies based on the challenges that the producers face e.g. plant quality, pests, growth, fungi infections etc.

1.9 The aims of the study

In this thesis, the aim was to investigate the effects of light quality (B, FR and R) as day extension and its interaction with temperature on elongation growth, bud set and bud burst in Norway spruce and Subalpine fir. Also, interactive effects of light quality and temperature were investigated, as knowledge on this is very limited. In addition, we aimed to investigate effects of these light qualities on the expression of the *FTL2*–gene in Norway spruce, which was previously shown to be upregulated under SD conditions inducing growth cessation and bud set. An important aim of the study was to learn more about the effects of variables that could possibly result in an increased growth rate and shortening of the production time in Subalpine fir, which could be beneficial in nursery production of seedlings.

(Mølmann et al. 2006) previously showed that B- and R light were less efficient in maintaining growth compared to FR light in Norway spruce. However,

both R and B light delayed bud set compared to SD, and R light often resulted in lower percentage of bud set than SD exposure. Another aim of this study was therefore to investigate if a higher irradiance of especially B- and R light could results in higher growth rate in seedlings of Norway spruce and Subalpine fir.

2.0 Material and methods

2.1 Plant materials and pre-growing conditions

2.1.1 Study species

The seeds of Norway spruce (*Picea abies* (*L.*) H. Karst) were from the provenance CØ1 from 59°N latitude, 0-149 metre above sea level Halden, Østfold, Norway (seed lot 98063, The Norwegian forest seed centre, Hamar, Norway).

The seeds of Subalpine fir (*A.lasiocarpa* (Hook.) Nutt.) were from the provenance CAN10 from 53.39°N latitude, 122.23°W longitude, 1000-1200 metre above sea level, George Mountain in British Columbia, Canada (seed lot B13-106, The Norwegian forest seed centre, Hamar, Norway).

2.1.2 Stratification

Before pre-growth in growth chambers, seeds of Subalpine fir were stratified. The stratification was done by placing the seedlings on moist filter paper in petri dishes in the dark at a temperature of 4°C. Stratification is done to induce germination and took approximately 3-4 weeks. Seeds from Norway spruce do not need any stratification to germinate.

2.1.4 Sowing

Seeds from both species were sown in peat (Degerens Torvstrøfabrikk A/S, Degernes, Norway) that in advance had been added perlite with a ratio of 1:3 (perlite:peat) to enhance the soils humectant capability. The trays measured 596 x 396 x 60 mm with individual pots 5.5 x 5.5 x 4.5 cm (Vefi, Drammen, Norway). Because the seeds of both tree species have about 60% germination rate, two seeds were sown in each individual pot.

2.1.5 Pre-growing

After sowing, plants where cultivated in a growth chambers for 8 weeks. The growth chambers were manufactured by the Center for climatically regulated plant research, Norwegian University of Life Science (Ås, Norway), and held a constant temperature of 18°C during the experiments and the relative air humidity was set to 75%,

corresponding to 0.5 kPa water vapour pressure deficit. The temperature and air humidity was registered and monitored by a Priva climate sensor (Canada, North America). In the first experiment the day length was set to 24 h, with 12 hours main light period provided by High Pressure Sodium lamps (HPS; Gavita 400W HPS, Andebu, Norway) and 24 hours with incandescent lamps (Narva 60W, Germany and Philips Electronics 60W, Netherlands). Many seedlings developed buds under pre-growth in the first experiment; hence many of the seedlings had different starting points at experiment start. The day length was further adjusted to 24 h with main light in the second and third experiment to avoid bud development at experiment start. Unfortunately, because of some misunderstandings the main light was set to 12 h again in the third experiment. Although the light was adjusted to 24 h with main light after approximately 2 weeks, some of the seedlings had already developed buds (tab.X).

Under pre-growth the red:far-red (R:FR) ratio was measured to be 1.7, as measured by a red:far-red sensor (Skye instrument, Llandrindod Wells, UK). A Li-Cor (Quantum/Radiometer/Photometer, Model LI-250 Light Meter produced by LI-COR, Lincoln, Nebraska, USA) was used to measure the photosynthetic photon flux density, and this was measured to be 200 m^{-2} s^{-1} along with the incandescent light bulbs. The same instruments were further used in the growth experiments, and were always used to measure the light in the middle of the trolleys at the top of the plant canopy.

2.2 Experimental conditions

After the 8 weeks of pre-growing, the seedlings were transferred to other growth chambers (Kirkejordet Sør, Ås, Norway) where they were distributed on trolleys (50 cm x 50 cm). Each growth chamber was divided into four sections with white reflecting plastic with one of the following treatments in each: short days (SD), blue (B), far-red (FR) and red (R). The latter three worked as day extension light. In addition, aluminium foil was placed at the sides of the upper part of the trolleys so that the light from the light emitting diodes (LED: Philips, GreenPower Led Module HF, 24Vdc/max 10W, Netherlands) was concentrated down on the plants. The LED-lights were measured by a Li-Cor red sensor (Quantum/Radiometer/Photometer,

model LI-250 Light Meter, Neb., USA). To adjust the R:FR ratio incandescent lamps were used.

Tab.1 Light measurements from the three different experiments measured in the middle of the trolleys before experiment start. The growth light where set on for about 12 hours (day time, 09.00-21.00), while the LED –lights (B, R, FR -light) where set on for 13 hours (night time, 21.30-8.30). The irradiance of day extension light was originally measured in Wm^{-2}, which later was converted to µmol m^{-2} s^{-1}.

Experiment	**Irradiance of day extension light (Wm^{-2})**	**⇔ Irradiance of day extension light (µmol m^{-2} s^{-1})**			**Growth light (µmol m^{-2} s^{-1})**	**R/FR - ratio**
		FR	**B**	**R**		
Exp. 1 (18°C)	about 7	43	27	39	about 160	about 2.0
Exp. 2 (18°C/22°C)	about 7	43	27	39	about 160	about 2.0
Exp. 3 (18°C/24°C)	about 7	43	27	39	about 160	about 2.0

The plants were grown under the following environmental conditions: the HPS – and incandescent lamps where on for 12 hours (day time, 09.00-21.00), while the LED – lights (B, R, FR light) where on for 13 hours (night time, 21.30-8.30). The main light overlapped with the LED-light for about one hour; hence the seedlings did not get any dark periods in the LED treatments.

In the first experiment the air humidity was adjusted to 65%, corresponding to 0.5 kPa water vapour pressure deficit with a constant temperature of 18°C. Two growth chambers with different temperature were used in the second and third growth experiments, i.e. 18/22°C and 18/24°C respectively. This was done to investigate if there were any temperature effect on the light quality responses. In the growth chamber that held a constant temperature of 22°C, the air humidity was adjusted to 73% with a corresponding water vapour pressure deficit of 0.72 kPa. In the growth chamber in the third experiment that held a constant temperature of 24°C, the air humidity was adjusted to 76%, corresponding to 0.72 kPa. The chamber that held a constant temperature of 18°C in the second and third experiment had similar measurements of water vapour pressure deficit and air humidity as the initial one, i.e. 65% air humidity, corresponding to 0.72 kPa. The temperature and air humidity was monitored by an Envic sensor (Envic, Turku, Finland).

Seedlings from the third experiment that had developed buds after exposure to B, FR and R as day extension light as well as SD under 18 and 24°C, were re-transferred to 24 h photoperiod under 18°C in growth chambers (those used under pre-growing). This was done to investigate the effect of temperature on light quality effect on bud burst. The air humidity was adjusted to 65%, corresponding to water vapour pressure deficit of 0.72 kPa.

The plants were watered as needed and fertilized twice per week with a nutrient solution containing calcium nitrate, ammonium nitrate and Kristalon (Yara, Oslo, Norway) with a electrical conductivity number of 1.5. To protect the plants for pests (flies) in the growth chambers, a mixture of nematodes (Nemasys, Becker Underwood, Littlehampton, England).

2.3 Recording of growth parameters

2.3.1 Measuring of elongation growth

Plant height was recorded once per week and was measured from the rim of the pot to the apical meristem with a scale. A negative value was noted if the plants were lower than the rim of the pot. At the end of each experiment cumulative elongation growth was calculated, and growth curves were plotted.

Fig. 1 Seedlings of Norway spruce and Subalpine fir exposed to red (R) as day extension light, at a temperature of 18 or 24°C after approximately 6 weeks in treatment. First and third plant from the left

is Norway spruce at 18 and 24°C, respectively and second and fourth plant from the left is Subalpine fir at 18 and 24°C.

2.3.2 Bud development

Bud development was recorded three times per week. The registrations were based on colours of the buds, i.e. green and brown. These colour codes was later converted to numbers where 1 indicated growth, 0.5; green bud and, 0; brown bud. During recording of bud burst, 0 represented bud, 1; bud burst and, 2; growth. Stages of development and percent bud appearance were later plotted in curves.

Seedlings of Subalpine fir used in these experiments had different starting point since many of these seedlings had already started cessation of elongation growth with development of terminal buds (fig. X). In Norway spruce, no seedlings had developed buds at experiment start.

Tab. 2 Percentage (%) plants with buds in seedlings of Subalpine fir before the start of experiment (Exp.) 1, 2 and 3 where plants were exposed to day extension with different treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) as well as short days (SD) without day extension, at different temperatures depending on the experiment.

Exp. 1	**%**	**Exp. 2**	**%**	**Exp. 2**	**%**	**Exp. 3**	**%**	**Exp. 3**	**%**
SD 18°C	50	SD 18°C	0	SD 22°C	0	SD 18°C	5	SD 24°C	26
B 18°C	58	B 18°C	6	B 22°C	0	B 18°C	5	B 24°C	16
FR 18°C	42	FR 18°C	0	FR 22°C	19	FR 18°C	5	FR 24°C	32
R 18°C	54	R 18°C	0	R 22°C	0	R 18°C	11	R 24°C	21

2.3.3 Harvesting for gene testing

In order to investigate the effect of day extension with the different light qualities as compared to SD on the expression of the *FLOWERING LOCUS T-TERMINAL FLOWER1-LIKE 2* (*FTL2*-gene) in Norway spruce, shoot tip materials from plants were harvested at the end of the first experiment, i.e. approximately after 8 weeks.

The sample harvest was done by putting plants in liquid nitrogen, and then cutting off about 5 mm of the shoot tips and needles at the upper 5 mm. Three samples, each consisting of materials from 4 plants, were harvested from each light quality treatment as well as SD –treatment for further analysis in the laboratory.

2.4 Analysis of FTL2 -expression

2.4.1 RNA isolation

Aiming at analysis of *FTL2* expression, RNA was purified using Small Scale RNA Isolation kit (PureLink Plant RNA reagent, AMBION, Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) by which it is possible to purify RNA from ≥ 0.1 g plant tissue. The RNA isolation and purification where conducted with 4 samples at the time, one from each treatment, i.e. 12 needle samples and 12 shoot tip samples. The plant material was crushed with a Mixer Mill (MM301 by Retsch in Haan, Düsseldorf, Germany) with 0.5 mm. beads in the vials and at the same time cooled down by liquid nitrogen. The speed of the crushing was set at 25 Hz in 1 min.

To each tube (Biosphere plus Safeseal Micro Tubes, Sarstedt, Nümbrecht, Germany) 0.5 ml Plant RNA reagent (Invitrogen, Life Technologies, Thermo Fisher Scientific) was added to the crushed frozen plant tissue. These were mixed thoroughly, and further incubated horizontally for 5 min in room temperature.

After 5 min the samples were centrifuged for 2 min at 12000 g in room temperature, and the supernatant transferred to a 2.0 ml biosphere eppendorf tube in addition to a mixture of 200 µl modified 5 M NaCl and 600 µl modified chloroform ($CHCl_3$) (Merck Millipore, Frankfurt, Germany). The samples were then mixed thoroughly by inversion for 1 min.

After 1 min the samples were centrifuged 4°C for 10 min at 12000 g, and transferred to ice. 800 µl isopropanol (Prima, Arcus Kjemi, Vestby, Norway) had been transferred to RNase free eppendorf tubes in advance. After 10 min the top, around 800 µl, aqueous phase was transferred from the samples to the tubes containing isopropanol. These were further mixed and incubated on ice for 10 min to subsequently centrifuge for 10 more min, at 4 °C and 12000 g. We used biosphere filter tips (Biosphere plus Filter tips, Sarstedt).

Thereafter the supernatant was discarded, and the pellet resuspended in 1 ml 75% EtOH (4°C). The samples were centrifuged at 12000 g for 2 min at 4 °C. The 1 ml 75% EtOH (4°C) was discarded. The pellet was centrifuged, and a pipette was used to collect the residual liquid. To make sure that there was not any remaining liquid with the pellet, a laminar floor bench was used in approximately 2 min. The samples were then added 40 µl of RNase free water and tapped to dissolve the pellet.

The levels of RNA in the samples were than checked by a NanoDrop (ND-1000 Spectrophotometer, NanoDrop Products, Wilmington, USA). Thereafter a DNase treatment was added to degrade any DNA contamination in the RNA samples. This was done by mixing 5 µl 10xDNase buffer with 1.5 µl of the enzyme Turbo DNase (TURBO DNA-*free* Kit, Life Technologies, Thermo Fisher Scientific) in each sample. The samples were further mixed, quickly centrifuged and incubated for 30 min at 37 °C. Further, 5 µl of Ambion DNase STOP solution was added.

The samples were mixed for 2 min. in room temperature and centrifuged at 13000 g at 4 °C for 2 min. After this, the solution from each sample was transferred to a new RNase free tube. The stop solution, which were at the bottom of each tube was not transferred further but discarded. Then the sample was divided in two tubes, approximately 20 µl for RNA purification and the rest was placed in a freezer that held a temperature of -70 °C. Again, a NanoDrop was done to check the level of RNA before starting RNA purification.

2.4.3 Purification of RNA

For purification of RNA a kit called Turbo DNA –free kit was used (Total RNA Purification System, PureLink RNA Mini Kit, Invitrogen, Life Technologies, Thermo Fisher Scientific). A mixture of 125 µl of RNA (20 µl RNA + approximately 105 µl RNase free water, depending of the amount of RNA remaining after the last Nano-drop analysis), 125 µl Lysis buffer (1 ml Lysis buffer + 10 µl 2-ME) and 125 µl 100% EtOH (Ethanol AnalaR NORMAPUR ACS, Radnor, USA) was then made. This was mixed by pipetting up and down 5 times, and further transferred to a RNA Spin Cartridge. The samples were centrifuged at 12000 g at room temperature for about 20 sec.

After 20 sec 500 µl Wash Buffer II with ethanol was added to the spin cartridge and centrifuged for 20 sec at 12000 g at room temperature. The flow – through in the cartridge was discarded, and a new cartridge was re-inserted. The process with Wash Buffer was repeated to make sure that all of the RNA had passed through the cartridge. The spin-cartridge was centrifuged at 12000 g for 1 min at room temperature. The collection tube was discarded and the cartridge inserted into a RNA Recovery Tube. RNA was further eluted by adding 40 µl of RNase free water to

the centre of the spin cartridge, and incubated for 1 min at room temperature and then centrifuged for 2 min at 12000 g at room temperature. Finally, levels of RNA were analysed on the NanoDrop. It was important that the levels were above 70 ng μl^{-1} for further analysis of transcript level since a total of 1000 ng RNA was required for the cDNA synthesis. In addition, RNA quality were tested by a Bioanalyzer (Agilent 2100 Bioanalyzer, Agilent Technologies,, Santa Clara, CA, USA).

2.4.4 cDNA synthesis

After the quantity and quality of the RNA samples had been checked, cDNA was synthesized by reverse transcriptase (Superscript VILO cDNA Synthesis, Life Technologies, Thermo Fisher Scientific). The enzyme uses RNA as a template, and the initial product is a single stranded cDNA sequence, which is complimentary to the RNA.

In addition to the enzyme mix, an optimal amount of RNA and RNase free water is needed to conduct the cDNA synthesis. This was calculated manually in advance, and depends on the quantity of the RNA (ng μl^{-1}) in each sample. Two enzyme mixes were made; one for the 24 samples with reverse transcriptase (rt) and one for the 24 samples without rt.

$$\frac{1000\ ng/\mu l\ \ RNA}{\mu l\ RNA\ concentration} = amount\ of\ RNA\ (\mu l)$$

14 µl in total;

$$14\ -\ amount\ of\ RNA = amount\ of\ RNase\ free\ water$$

For example, the first sample the RNA concentration was measured on the Nanodrop to be 268.9 ng/µl;

$$\frac{1000\ ng}{268.9\ ng/\mu l} = 3.7\ \mu l$$

$$14\ \mu l\ -3.7\ \mu l\ = 10.3\ \mu l\ of\ RNase\ free\ water$$

These measurements were further used when calculating the amount of RNase free water that was needed in the samples without rt (7 µl in total).

14 µl of each rt -sample were then transferred a tray of wells, as well as the 7 µl of –rt -sample. The tray was incubated on a regular PCR machine (DNA Engine Tetrad Pelitier Thermal Cycler, Bio-Rad Laboratories, Hercules, California, USA) with a specific cycling program; 10 min in 25°C, 50 min in 42°C, 5 min in 85°C, and finally "forever" in 4°C. After the cycling program the samples was chilled on ice and diluted with RNase free water.

Thereafter the quality of the newly made cDNA and was checked for any DNA remaining in the samples. A qPCR machine (Applied Biosystem, 7500 Fast Real-Time PCR-system, Life Technologies, Thermo Fisher Scientific) would detect any DNA contamination. All of the newly made cDNA were tested against remaining RNA samples without reverse transcriptase. These RNA samples function as controls, i.e. any gene expression from the control samples is an indication of DNA contamination. In advance, a master mix was made in a PCR cabinet (Biosan VVC/T-M-AR, Life Technologies, Thermo Fisher Scientific):

Tab. 3 The content of the master mix used in the qPCR reaction for 1 sample. The content was added up for 48 samples, i.e. 24 for the α-tubulin samples and 24 for the FTL2-samples.

Master mix	**1x (µl)**
Water	7
SYBR green	10
Primer L	0,5
Primer R	0,5
Total	**18**
Template	2
Total	**20**

In addition to RNase free water and primers, SYBR green (SYBR Select Master Mix, Life Technologies, Thermo Fisher Scientific) was added to the master mix. This is a florescent solution that binds to the gene of interest, e.g. *FTL2*, and a detector in the qPCR detects this florescent light.

In addition to check for DNA contamination, an identification of melting curve was done. These melting curves indicate the melting temperatures of the gene and reveals whether the gene is in the samples or not. The gene of interest, i.e. *FTL2*

should have the same melting curve in the qPCR, i.e. all of the samples should express the same melting curve in the results.

2.4.6 Quantitative polymerase chain reaction (qPCR)

Finally, measuring of levels of the *FTL2* –gene was done as described above, but instead of using RNA samples without reverse transcriptase as controls, a housekeeping gene called *α-tubulin* was used, i.e. a normalization gene. Such housekeeping genes are expressed in all cells in an organism and are essential to maintain basic cellular function (Johnsen et al. 2005). *α-tubulin* is stable under different environmental conditions, such as varying temperatures (Chu et al. 1993; Stavang et al. 2005). Any abnormalities in the *α-tubulin*-expression would indicate that the RNA purification or PCR reactions have been unsuccessful. On the other hand, if the level of *α-tubulin* is expressed at a constant level, this is an indication that the analysis has been successful.

The same master mix as above was made. The first 12 samples (with needles) were transferred to one plate. At the same plate, 12 samples with *α-tubulin* were transferred as the internal control. The second plate held the 12 last samples, i.e. 13-24 with buds. At the same plate, 12 samples with *α-tubulin* were transferred as the control.

2.4.6 Calculating RQ -values

Relative quantification (RQ) was used when calculating the results of the real time PCR (qPCR). In this way it was possible to analyze any changes in the *FTL2* –gene expression to a given sample, and thereafter compare the RQ -levels with the control. This was done through a threshold cycle (CT) -method, where CT values from the *TFL2* is compared with the CT values from the housekeeping gene, i.e. *α-tubulin.* The CT values express how many cycles in the PCR is needed before a detectable amount of *FTL2* is accumulated as a result of the PCR reaction, i.e. low template concentration means more cycles (S.A.Bustin2004, A-Z of quantitative PCR, International University line). The calculations were done using this equation:

$$1.\ Ct\ (GOI\ c) - Ct\ (norm\ c) = \Delta Ct\ (calibrator)$$
$$2.\ Ct\ (GOI\ s) - Ct\ (norm\ s) = \Delta Ct\ (sample)$$
$$3.\ \Delta Ct\ (sample) - \Delta Ct\ (calibrator) = \Delta\Delta Ct$$
$$2 \wedge (-\Delta\Delta Ct) = RQ$$

$s = sample, c = calibrator$ (average)
$norm = housekeeping\ gene\ (\alpha - tubulin)$
$GOI = gene\ of\ interest\ (FTL2)$

2.4.7 Statistical analysis

Shoot elongation, shoot diameter and *FTL2*-transcript levels from the first experiment were analysed statistically using one-way analysis of variance (ANOVA) in the general linear model mode to test for differences ($p \leq 0.05$) between the light quality treatments. A two-way analysis of variance (ANOVA) was used in the second and third experiment to test for differences between the light quality treatments and temperatures. In all cases a Tukey test (MiniTab 16.1.1, State Collage, Pennsylvania, USA) was conducted to investigate which of the treatments were different from each other.

Bud development and bud burst were first converted from colour codes to numbers, where 1 indicated growth, 0.5; green bud and, 0; brown bud. The same was done for bud burst where 0 represented bud, 1; bud burst and, 2; growth. These codes were plotted in curves, and further analysed statistically using cumulative link models in R, which are regression models for original data (Christensen 2013).

Tab. 4 Number of seedlings of Norway spruce and Subalpine fir used in the first, second and third experiment exposed to blue (B), far-red (FR) and red (R) as light quality treatments from light emitting diodes, as compared to short days (SD) without day extension, all at temperature of 18, 22 or 24°C.

	Norway spruce	**Subalpine fir**
Exp. 1 (18°C)	10-20	12-13
Exp. 2 (18/22°C)	19/19	14-16/15-16
Exp. 3 (18/24°C)	20/20	19/19

3.0 Results

3.1 Effect of light quality on elongation growth and bud set in Norway spruce (experiment 1)

3.1.0 Effects of light quality on elongation growth

The statistical analysis for the last registration point showed that elongation growth was significantly greater when exposed to FR and B as day extension light, compared to R and SD-treatment ($p \leq 0.05$) (fig. 2). There was no significant difference between the FR and B treatment ($p \geq 0.05$).

After 56 days, the plants exposed to the FR and B light showed above 70% more elongation growth compared to the seedlings exposed to SD. Seedlings exposed to R showed a trend of about 40% more elongation growth compared to the SD– seedlings although R –seedlings did not differ significantly from SD –seedlings ($p \geq 0.05$).

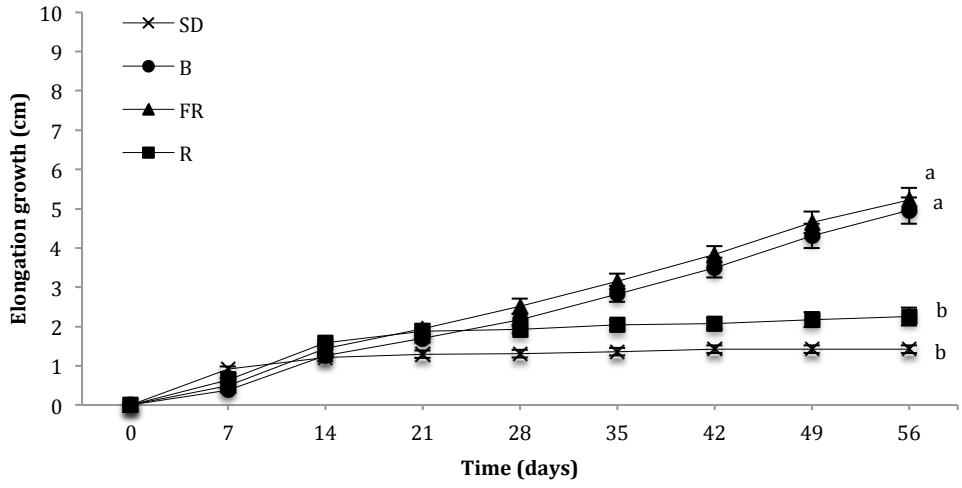


Fig. 2 Effect of light quality on average cumulative elongation growth in seedlings of Norway spruce exposed to day extension with different treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) light (7 W m-^{2}) as compared to short days (SD) without day extension, all at a temperature of 18℃ in the first growth experiment. Treatments that do not share a common letter are significantly different at day 56 based on ANOVA (general linear model) followed by Tukey`s test ($p \leq 0.05$). Results are mean ± SE of 19-20 plants in each treatment.

3.1.1 Effect of light quality on bud set

After 66 days of treatment, none of the seedlings exposed to the growth-sustaining day extensions FR and B had developed buds. Buds appeared after 24 days in R light and 27 days in SD-exposure, where 17% of the R-exposed seedlings had developed buds and 79% of the SD-seedlings had developed buds. After 66 days, 78% of the R-treated seedlings had developed buds whereas all of the seedlings exposed to SD had developed buds (fig. 3).

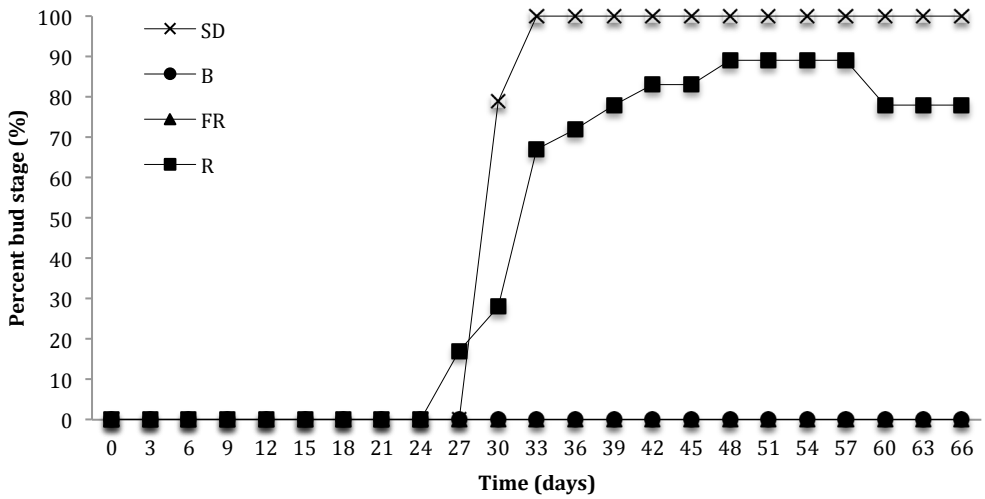


Fig. 3 Percentage buds in seedlings of Norway spruce exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m^{-2}) as compared to short days (SD) without day-extension, all at a temperature of 18°C in the first experiment. Results are mean of 19 plants in each treatment.

The average stage of the bud development reflects the percentage buds in each treatment but provides information also on further bud development after bud set. SD-treated seedlings had fully developed, brown buds, referred as 0 after 66 days in treatment, whereas R-treated seedlings had an average stage of 0.22. An average higher than 0 indicates that not all seedlings had fully developed buds after 66 days (fig. 4). The statistical analysis using the cumulative link model showed that R as a day extension light delayed the bud development in seedlings compared to SD-treatment in seedlings of Norway spruce ($p \leq 0.001$) (fig. 4, tab. 5).

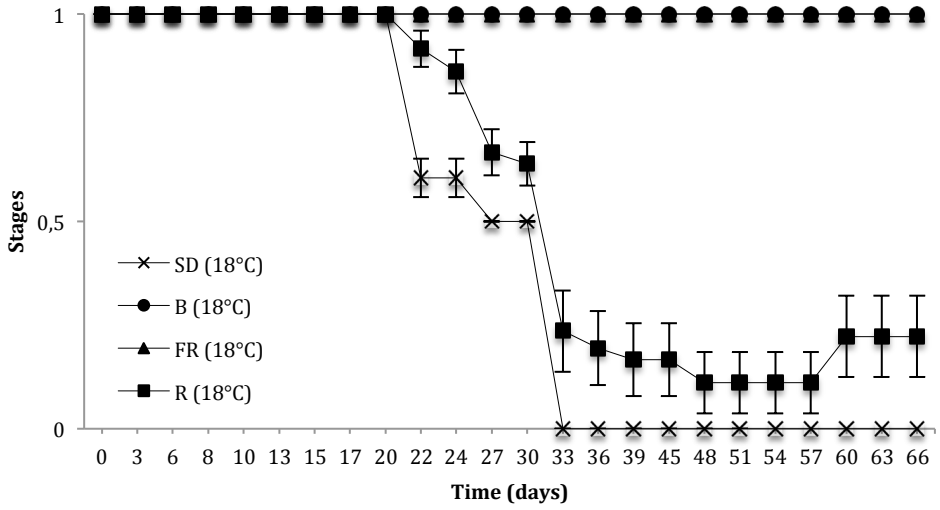


Fig. 4 Stages of bud set in seedlings of Norway spruce exposed to red (R) light (7 W m^{-2}) from light emitting diodes as day extension, as compared to short days (SD) without day extension at a temperature of 18°C in the first growth experiment. 1 = growth, 0.5 = green bud, 0 = brown bud. Results are mean ± SE of 19-20 plants in each treatment.

Tab. 5 Results from cumulative link model in R run to investigate the effect of red (R) light (7 W m-^{2}) as a day extension on bud development in seedlings of Norway spruce compared to short days (SD) in the first experiment. Positive estimated coefficient indicates an increased probability of bud set, while negative estimated coefficient indicates a delay in bud set.

Treatment	Coefficient	SE	Z
R ***	-1.49851	0.23218	-6.454
Day ***	0.31081	0.01881	16.526

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

3.1.3 Effect of light quality on plant diameter

Effect of light quality on average plant diameter at the top of the plants measured after 66 days showed that seedlings exposed to B, R and FR had a slightly wider diameter compared to seedlings in SD treatments. Furthermore, the average plant diameter of B-treated seedlings was significantly different relative to FR and R-treated seedlings ($p \leq 0.05$) (fig. 5).

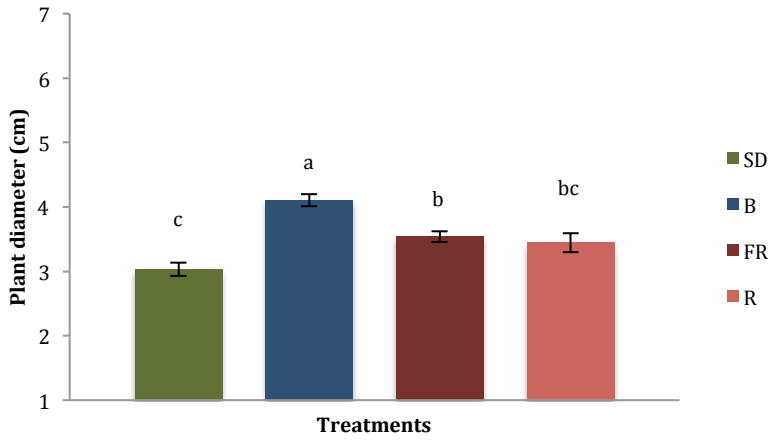


Fig. 5 Effect of light quality on average plant diameter at the top of seedlings of Norway spruce exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m^{-2}) as compared to short days (SD) without day-extension at temperatures of 18°C for a period of 8 weeks in the first growth experiment. Treatments that do not share a common letter are significant different. The different letters are based on ANOVA (general linear model) followed by a Tukey test ($p \leq 0.05$). Results are mean ± SE of 19-20 plants in each treatment.

3.1.4 Effect of light quality on the expression of FTL2 in Norway spruce

To investigate the effect of day extension with the different light qualities on *FTL2*-gene expression, which is known to be strongly upregulated in SD (Gyllenstrand et al., 2007; Asante et al., 2011), the *FTL2* transcript levels were analysed in seedlings of Norway spruce harvested at the end of the first experiment.

As predicted, the SD treatment that gave the lowest average elongation growth and hence earliest bud set showed high levels of *FTL2* transcript (fig. 6). Seedlings exposed to day extension with different light qualities, i.e. B, FR and R showed lower transcript levels of *FTL2* compared to SD-seedlings without day extension. Percentage transcript levels found in B, FR and R-treated seedlings were calculated based on the transcript levels in SD-treated seedlings, which were set to 1. The lowest transcript levels were found in the needles in B- and FR-treated seedlings, with 0.02 and 0.03% of transcript levels, respectively, compared to needles in SD-treated seedlings. The shoot tips in B-treated seedlings had an *FTL2*-transcript level of 0.8%, while the shoot tips in FR-treated seedlings were measured to be almost 7% in comparison with shoot tips in SD-treated seedlings (fig.6).

R-treated seedlings had a slightly higher transcript level of *FTL2* compared to B- and FR-treated seedlings. The needles contained 3% *FTL2* levels compared with the transcript levels found in needles in SD-treated seedlings, while the shoot tips had 32% transcript levels of the levels found in shoot tips in SD-treated seedlings (fig.6).

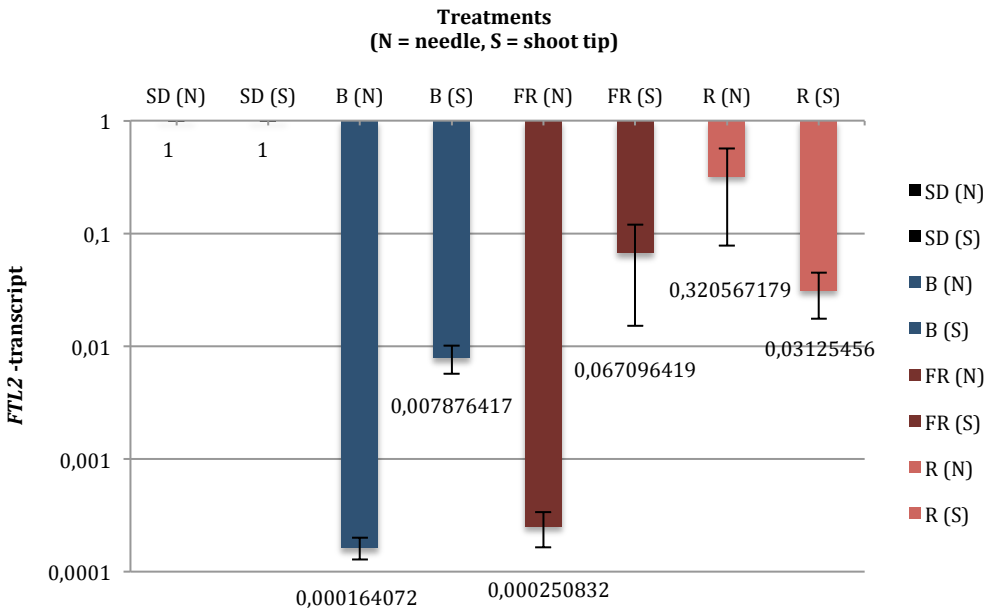


Fig. 6 Effect of light quality on transcript levels of FTL2 in seedlings of Norway spruce after 8 weeks of exposure to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared with short days (SD) without day extension, all at a temperature of 18°C. 3 samples of each treatment, each consisting of plant materials, i.e. needles (N) or shoot tip (S) from 4 plants were used in the gene-expression analysis. The transcript levels were normalized against α-tubulin. The results are based on average RQ-values from mean of 3 plants in each treatment.

3.2 Effect of temperature on the light quality effects on elongation growth, bud set and bud burst of Norway spruce (experiment 2 and 3)

3.2.0 Effect of temperature on the light quality effect on shoot elongation

The elongation growth in FR-and B treated seedlings differed significantly from that of the SD treatment ($p \leq 0.05$) (fig. 7). Also, there was no significant difference between B and FR, which at 18 and 22°C grew on average between 60-70% more compared to SD. However, no significant impacts of temperature on the responses to these light qualities were detected.

In experiment 2, seedlings exposed to day extension with R light at 18 and 22°C showed significantly lower degree of elongation growth compared to B and FR, ($p \leq 0.05$), i.e. like in experiment 1. There was no significant difference in elongation growth between R-treated and SD-treated seedlings. However, there was a trend of slightly more growth in seedlings exposed to R as compared to SD under 22°C compared to 18°C, although not statistically significant (fig.7).

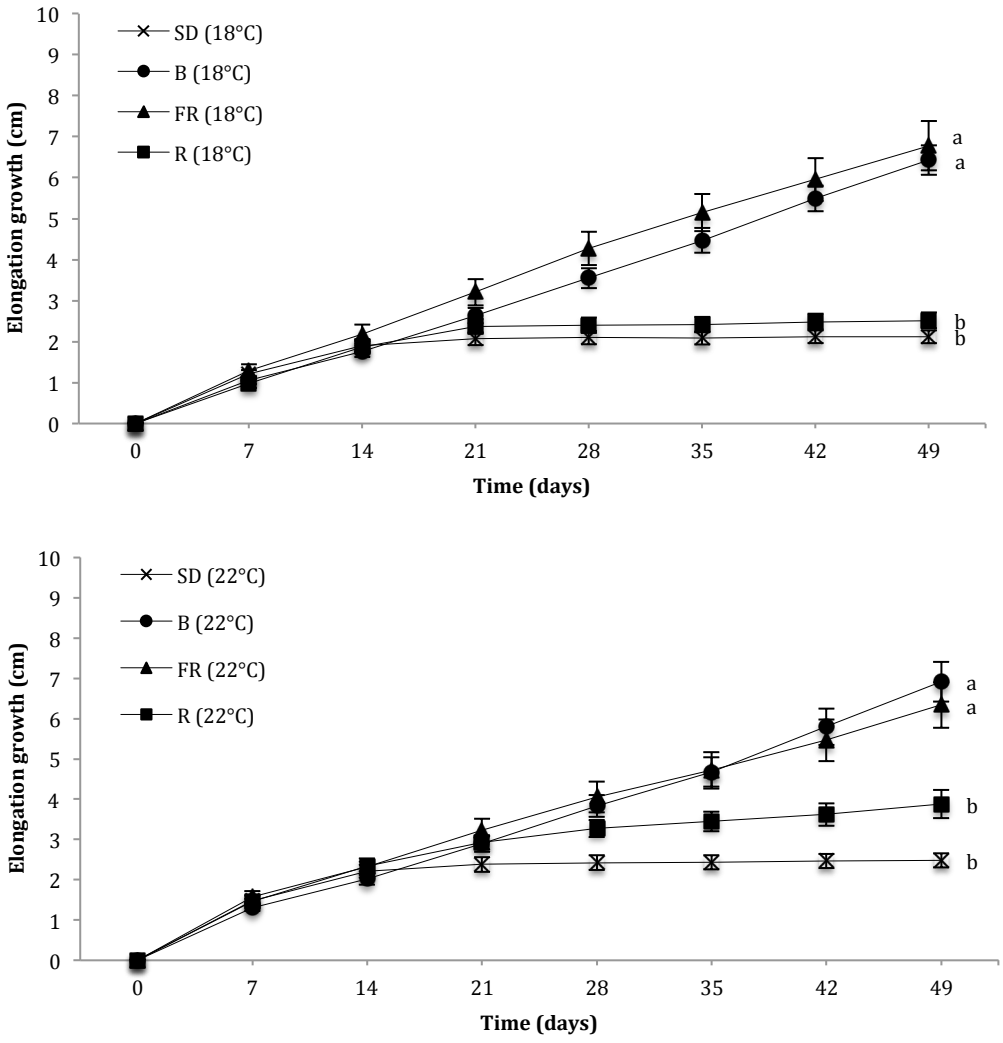


Fig. 7 Effect of temperature on light quality effect on average cumulative elongation growth in seedlings of Norway spruce exposed to day extension with different treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, at a temperature of 18°C or 22°C at the second growth experiment. Treatments that do not share a common letter are significantly different at day 49 based on a two-way analysis of variance (general linear model) followed by a Tukey test ($p \leq 0.05$). Results are mean ± SE of 19 plants in each treatment.

In the third experiment, there was no significant effect of temperature on day-extension with B and FR light on elongation growth ($p \geq 0.05$) (fig. 8). However, there was a slight tendency for higher growth in B-treated seedlings with elevated temperature (24°C versus 18°C). Furthermore, elongation growth in seedlings exposed to day extension with R light and 24°C was significantly different from all the other treatments ($p \leq 0.05$), except for B-treated seedlings exposed to 18°C. While the seedlings exposed to R at 18°C showed 25% elongation growth compared to the SD-seedlings under the same temperature conditions, R-treated seedlings at 24°C had grown 46% more compared to the seedlings exposed to SD under the same temperature condition (fig. 8), i.e. there were a significant effect of elevated temperature on the response to R light.

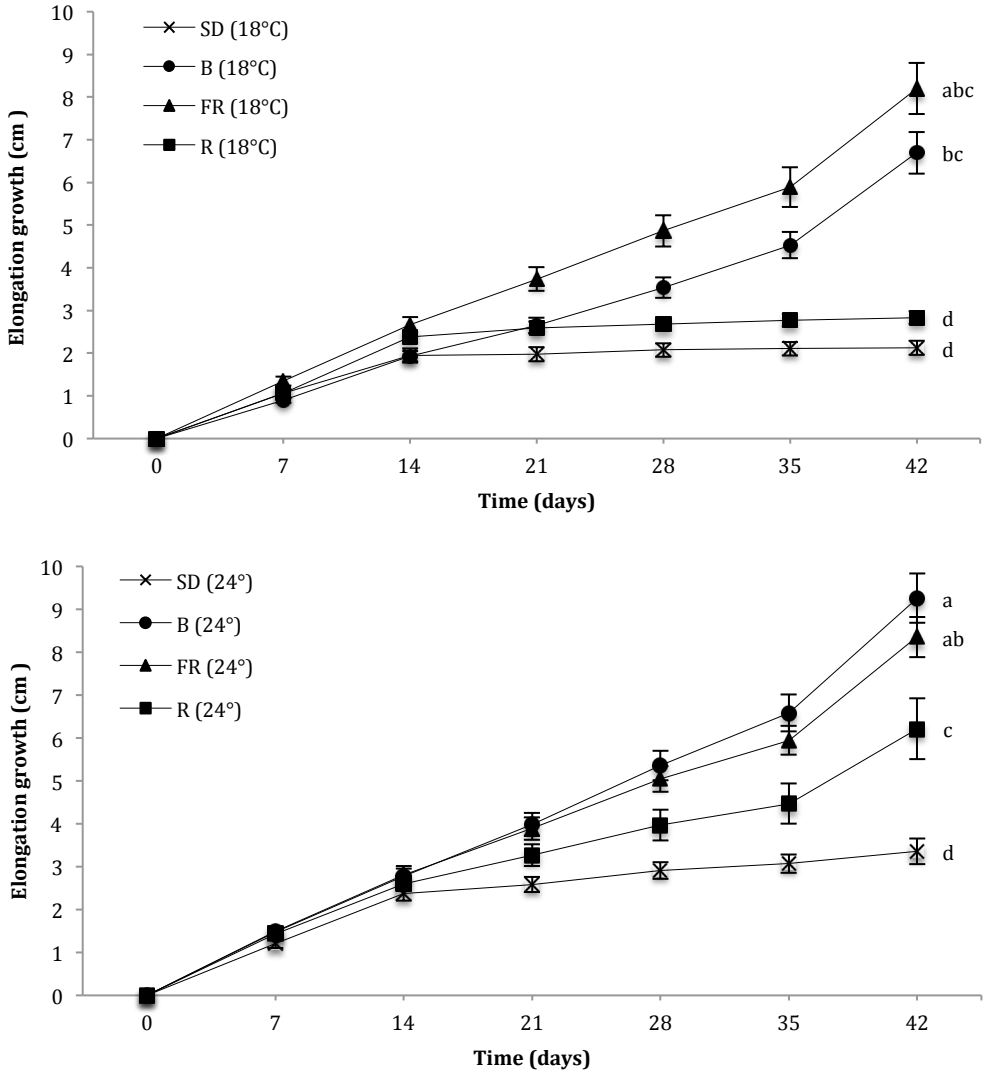


Fig. 8 Effect of light quality on average cumulative elongation growth in seedlings of Norway spruce exposed to day extension with different treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, at a temperature of 18 ℃ and 24 ℃ in the third growth experiment. Treatments that do not share a common letter are significantly different at day 42 based on a two-way analysis of variance (general linear model) followed by a Tukey test ($p \leq 0.05$). Results are mean ± SE of 20 plants in each treatment.

3.2.1 Effects of temperature on the light quality effect on bud set

None of the seedlings exposed to B and FR treatments developed buds under 18°C in the second experiment, which reflects the result from the first experiment (fig.3). However, in 22°C about 5% of the B-treated seedlings developed buds. The first bud appeared after 9 days in this treatment. 16% of the FR-22°C–treated seedlings developed buds, where the first bud appeared after 12 days in treatment. Further analysis of bud development using cumulative link model in R and bud burst from these treatments were not conducted in the second experiment because of the low percentage of seedlings in buds.

In 18°C under SD treatment, all seedlings had developed buds after 9 days (fig. 9). All SD-treated seedlings at 22°C also developed buds, with the first bud appearance already at day 3. This indicated that this seedling had already started bud development under pre-growth. Totally 95% of the 18°C -R-treated seedlings developed buds after 27 days, while 94% of the 22°C –R-treated seedlings developed buds after 27 days in treatment. The first bud appearance was observed after 12 days in both cases (fig.9).

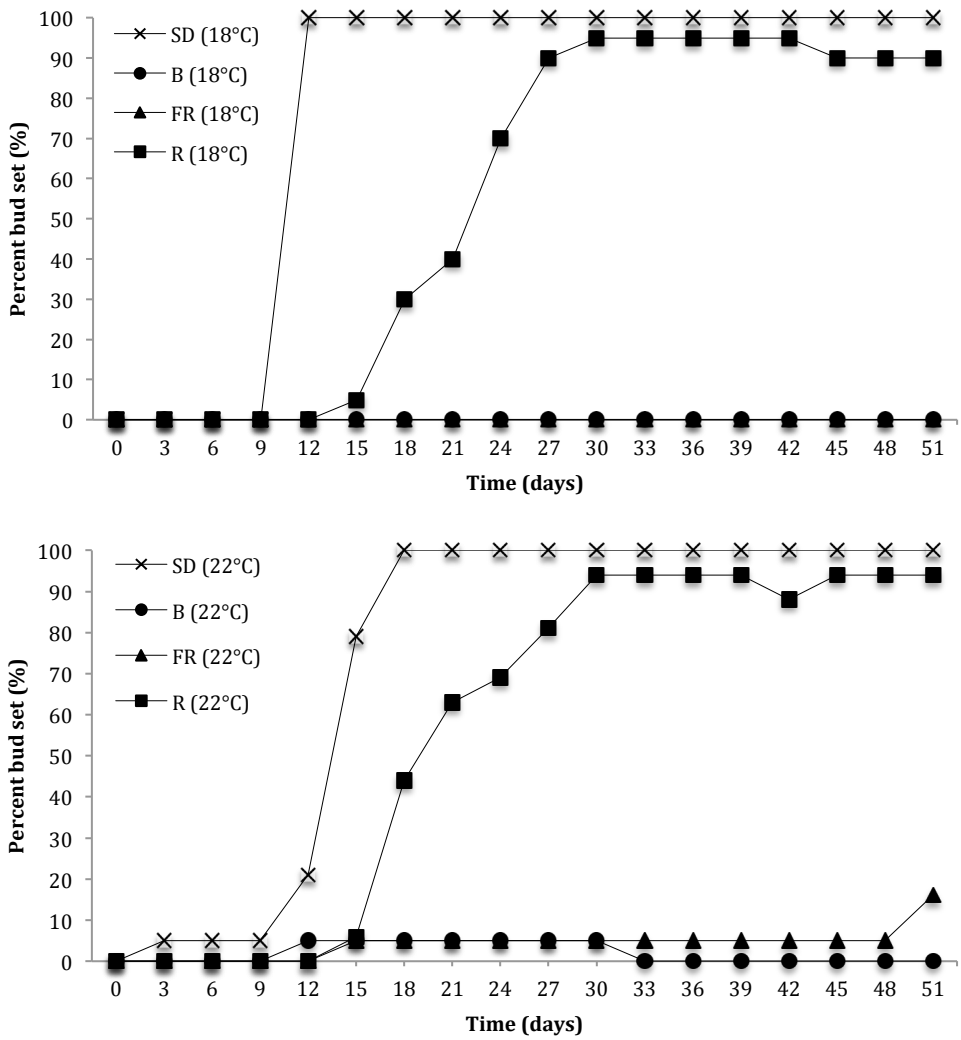


Fig. 9 Percentage buds in seedlings of Norway spruce exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day-extension, at a temperature of 18 or 22°C in the second growth experiment. Results are mean of 19 plants in each treatment.

The average stage of the buds in SD-18°C-treated seedlings resembled the average stage found in SD-22°C-treated seedlings, which after 51 days in treatment had developed brown buds referred as 0. The same trend were found in the R-exposed treatments, although not all of the seedlings had fully developed, brown buds after 51 days (fig. 10). Thus, there was no significant effect of temperature on R-treated seedlings (0.03541) (fig. 10, tab. 6). However, in response to the R light treatment the bud development was significantly delayed compared to SD (tab. 6). Whereas after 27 days of treatment all SD-exposed plants had brown buds (stage 0), while the R-exposed plants had green buds (0.5).

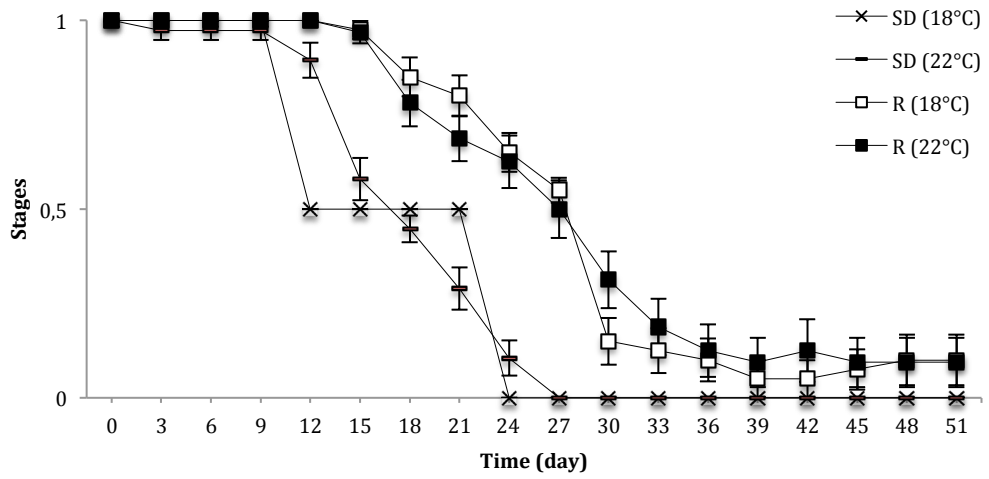


Fig. 10 Stages of bud set in seedlings of Norway spruce exposed to day extension with different light qualities from light emitting diodes, i.e. red (R) (7 W m-^{2}) as compared to short days (SD) without day extension at a temperature of 18 or 22°C in the second growth experiment. 1 = growth, 0.5 = green bud, 0 = brown bud. Results are mean ± SE of 19 plants in each treatment.

Tab. 6 Results from cumulative link model in R run to investigate the effects of temperature (T; 18 and 22°C) and red (R) light (7 W m-^{2}) as day extension on bud development in seedlings of Norway spruce compared to short days (SD) without day extension in the second experiment. Positive estimated coefficient indicates an increase probability of bud set, while negative estimated coefficient indicates a delay in bud set

Treatment	Coefficient	SE	Z
R *	-4.00942	1.73062	-2.317
T	-0.06242	0.05921	-1.054
Day ***	0.42853	0.02008	21.341
R x T	0.03541	0.08626	0.410

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

There was no significant effect of temperature on bud development in seedlings exposed to day extension with B- and FR light in the third experiment ($p \geq 0.05$). A total of 3% developed bud in FR-18°C-treated seedlings, whereas 6% developed bud in B-18°C-treated seedlings (fig. 11). The similar bud percentage was seen at 24°C in both FR and B treatments. All the buds developed in the FR treatments flushes, and no buds are observed after 57 days in treatment (fig. 11).

The first bud appearance in seedlings exposed to SD at 18°C occurred after 15 days of treatment, and all of the seedlings had developed buds after 24 days. The

same was registered for R-18°C-seedlings. SD-24°C-seedlings developed buds after 15 days, and a total of 92% buds were developed in this treatment. After 18 days the first buds appeared in 24°C-R-treated seedlings. At this temperature, not all of these seedlings developed buds, and after 36 days a total of 50% of the seedlings had developed buds and no further bud set was thereafter observed (fig.11).

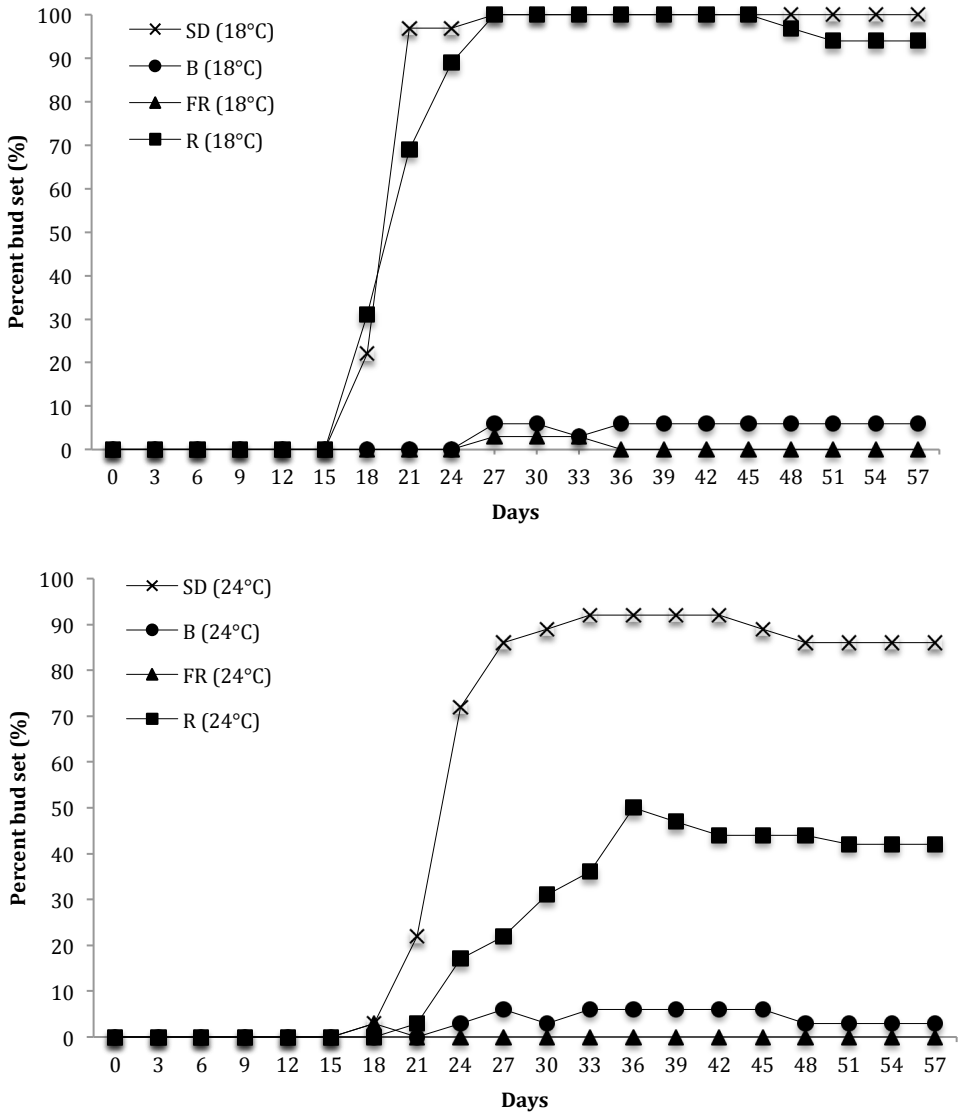


Fig. 11 Percentage buds in seedlings of Norway spruce exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day-extension, at a temperature of 18 or 24°C in the third growth experiment. Results are mean of 36 plants in each treatment.

Although there were no significant effect of temperature on bud development in seedlings exposed to FR and B light, both FR and B –light as day extension light strongly reduced the probability for bud development compared to the SD –treatment ($p \leq 0.001$, $p \leq 0.05$ respectively, tab. 7) with very few plants only showing bud set in B and FR (fig. 12).

The average stage of buds after 57 days in SD-24°C-treatment was slightly different from SD-18°C-treated seedlings in that some seedlings did not develop fully buds in SD-24°C-treatment (fig.12). The difference between the temperatures was far greater for the R than the SD treatment with brown buds in almost all plants (stage 1). After 57 days, R-18°C-treated plants had developed more buds (stage 0.05) compared R-treated seedlings at 24°C (stage 0.58). An average higher than 0 indicates that not all seedlings had fully developed buds after 57 days (fig. 12). Furthermore, there was a significant effect of temperature on bud development under R light ($p \leq 0.001$) (fig. 12, tab. 7), i.e. R light combined with elevated temperature delayed bud development.

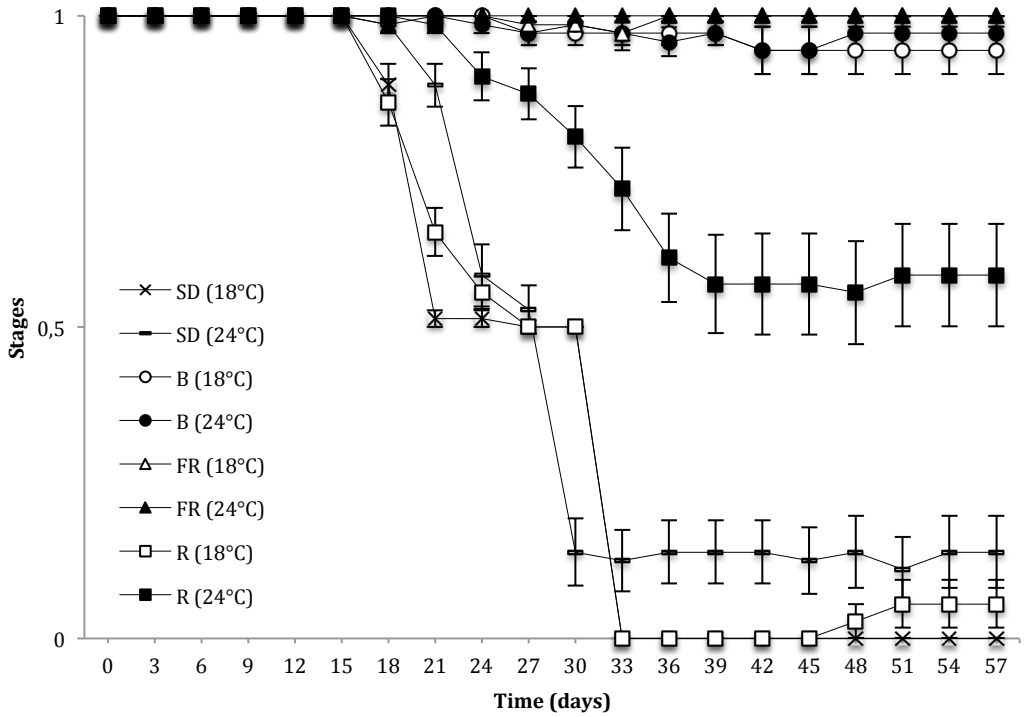


Fig. 12 Stages of bud set in seedlings of Norway spruce exposed to day extension with a light quality from light emitting diodes, i.e. red (R) (7 W m-^{2}) as compared to short days (SD) without day extension at a temperature of 18 and 24°C in the third growth experiment. 1 = growth, 0.5 = green bud, 0 = brown bud. Results are mean ± SE of 36 plants in each treatment.

Tab. 7 Results from cumulative link model in R run to investigate the effects of temperature (T; 18 and 24°C) and blue (B), far-red (FR) and red (R) (7 W m-^{2}) as day extension on bud development in seedlings of Norway spruce compared to short days (SD) without day extension in the third experiment. Positive estimated coefficient indicates an increase probability of bud set, while negative estimated coefficient indicates a delay in bud set

Treatment	Coefficient	SE	Z
B ***	-8.870408	1.296309	-6.843
FR *	-8.057799	3.8422830	-2.097
R ***	8.728735	0.739266	11.807
T ***	-0.101948	0.022770	-4.477
Day ***	0.221409	0.006708	33.006
B x T	0.079326	0.060905	1.302
FR x T	-0.083444	0.194765	-0.428
R x T ***	-0.492020	0.036341	-13.539

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

3.2.3 Effects of temperature on light quality effect on plant diameter

At 18°C, seedlings exposed to B had a slightly wider plant diameter at the end of experiment 2 compared to FR- and R treated seedlings, as well as SD-seedlings ($p \leq 0.05$), which reflected the results from the plant diameter in the first experiment (fig. 13). At 22°C there was no significant temperature effect on plant diameter ($p \geq 0.05$), only a slight trend of wider plant diameter in B-22-°C-treated seedlings compared to the other treatments. Average plant diameter was not measured in experiment 3.

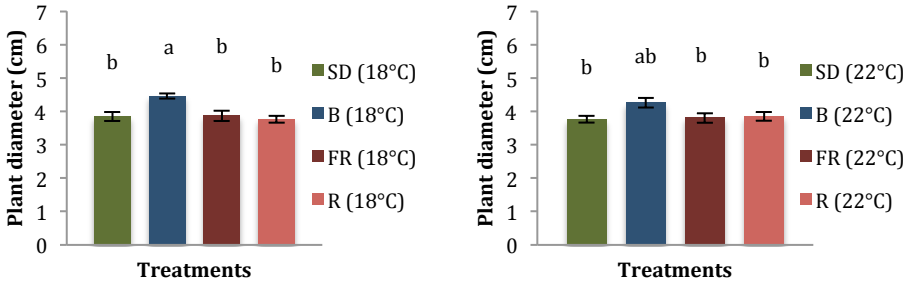


Fig. 13 Effect of temperature on average plant diameter at the top of seedlings of Norway spruce exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) at temperatures of 18 or 22 °C after a period of 7 weeks in the second experiment. Treatments that do not share a common letter are significant different. The different letters are based on ANOVA (general linear model) followed by a Tukey test ($p \leq 0.05$). Results are mean ± SE of 19 plants in each treatment.

3.2.4 After-effects of temperature and light quality during bud set on subsequent bud burst

After 57 days of exposure to day extension light under 18 or 24°C in the third experiment, seedlings of Norway spruce were re-transferred to 24 h photoperiod under 18°C to investigate any effect of those variables on bud burst. Statistical analysis of bud burst of seedlings exposed to B- and FR light was not conducted, since most of these seedlings still grew (fig. 11).

As stated above, overall 94% of the seedlings exposed to R light and 18°C and 42% in the seedlings exposed to R light and 24°C had developed buds at the end of the third growth experiment (fig. 11). 95% and 100% of the seedlings forming buds in response to R light under 18°C and 24°C, respectively, showed bud burst after 51 days. The first bud burst of the R-24°C-treated seedlings appeared after 6 days, while the first appearance of bud burst in R-18°C-treated seedlings occurred after 15 days (fig. 13).

100% of the SD-18°C-treated seedlings and 86% of the SD-24°C-treated seedlings had developed buds after 57 days in the third experiment. The rate of bud burst was faster in seedlings exposed to 18°C compared to those in 24°C. After 51 days, 100% of the SD-18°C-treated seedlings had burst, whereas only 65% of the SD-24°C-treated seedlings had burst (fig.13).

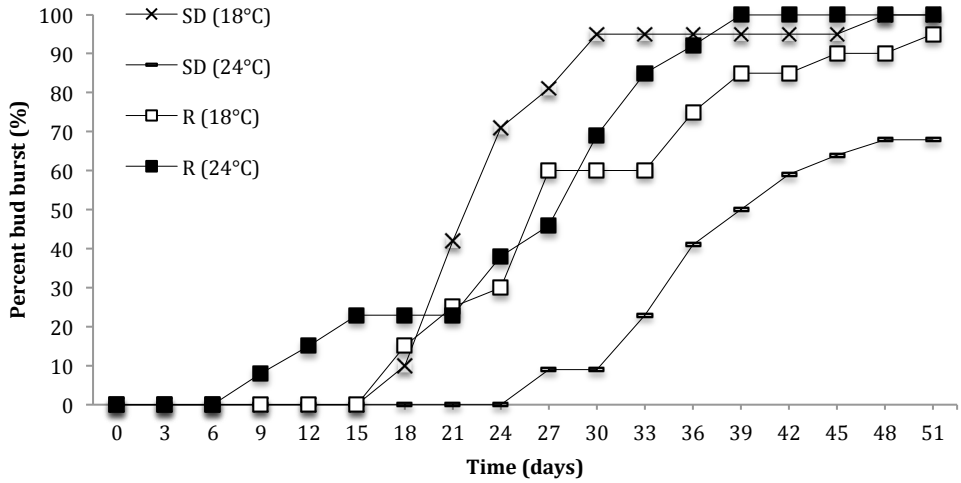


Fig. 13 Percentage bud burst in seedlings of Norway spruce after exposure to red (R) (7 W m-^{2}) as day extension light as compared to short days (SD) under 18°C or 24°C for 57 days before transfer of all plants to 24 h photoperiod under 18°C. Results are mean of 20 and 21 for the R-18°C-treated plants

and SD-18°C-treated plants, respectively and 13 and 22 for the R-24°C-treated plants and SD-24°C-treated plants, respectively.

The average stage of bud burst after 51 days in SD-24°C-treatment was slightly different from SD-18°C-treated seedlings in that some seedlings did not develop fully buds in SD-24°C-treatment (stage 1.36) (fig.14). On the contrary, almost of the R-24°C-treated had burst (stage 1.95), whereas a lower degree of R-18°C-treated seedlings had burst after 51 days in treatment (stage 1.85). An average lower than 2 indicates that not all seedlings had burst after 51 days. The statistical analysis using the cumulative link model showed an effect of temperature on R light, i.e. with an elevated temperature seedlings exposed to R bud burst faster compared to SD ($p \leq 0.001$) (tab. 8).

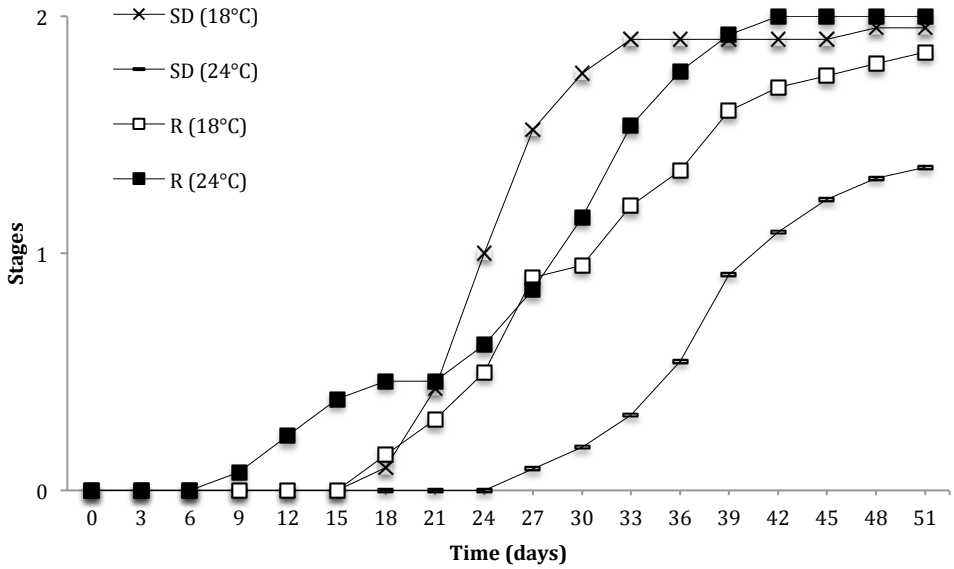


Fig. 14 Stages of bud burst in seedlings of Norway spruce after exposure to red (R) (7 W m-^{2}) as day extension light as compared to short days (SD) under 18°C or 24°C for 57 days before transfer of all plants to 24 h photoperiod under 18°C. . 0 = brown bud, 1 = bud burst, 2 = growth. Results are mean ± SE 20 and 21 for the R-18°C-treated plants and SD-18°C-treated plants, respectively and 13 and 22 for the R-24°C-treated plants and SD-24°C-treated plants, respectively.

Tab. 8 Results from cumulative link model in R run to investigate bud burst in seedlings of Norway spruce, exposed to a temperature (T; 18 or 24°C) and red (R) (7 W m-^{2}) as a light qualities in third experiment before transfer to 24 h photoperiod under 18°C, all compared to short days (SD) under bud set. Positive estimated coefficient indicates an increase probability for bud burst, while negative estimated coefficient indicates a delay in bud burst

Treatment	Coefficient	SE	Z
R ***	-14.55805	1.32946	-10.95
T ***	-0.58471	0.04508	-12.97
Day ***	0.26206	0.01312	19.98
R x T ***	0.74010	0.06482	11.42

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

3.3 Effect of light quality on elongation growth and bud set in Subalpine fir (experiment 1)

3.3.0 Effect of light quality on elongation growth

Elongation growth in seedlings exposed to day extension with FR-light was significantly different from that of seedlings exposed to B, R and SD treatment ($p \leq 0.05$) (fig. 15). Seedlings exposed to FR had grown on average 75% more compared to seedlings exposed to SD. Elongation growth in response to B and R light was not significantly different from the SD-seedlings ($p \geq 0.05$), although there was a slight tendency for higher growth in R-treated seedlings (fig.15).

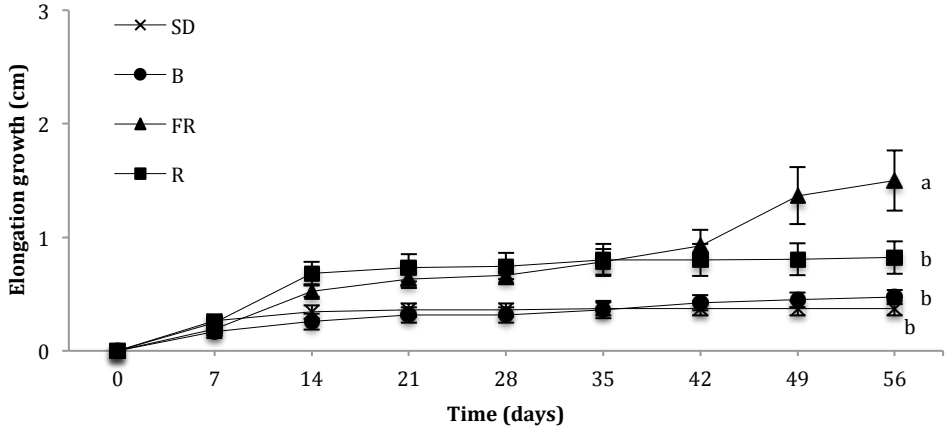


Fig. 15 Effect of light quality on average cumulative elongation growth in seedlings of Subalpine fir exposed to day extension with different treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, all at a temperature of 18°C in the first experiment. Treatments that do not share a common letter are significantly different at

day 56 based on ANOVA (general linear model) followed by a Tukey test (p>0.05). Results are mean ± SE of 12-13 plants in each treatment.

3.3.1 Effects of light quality on bud set

Many of the seedlings had already started developing buds under pre-growth, which explains the disorderly pattern at the start of the first experiment (fig. 16). After 66 days in treatment 100% of the SD-treated seedlings and 92% of the R-treated seedlings had developed buds, while only 75% and 33% of the B and FR-treated seedlings, respectively, had formed buds (fig. 16).

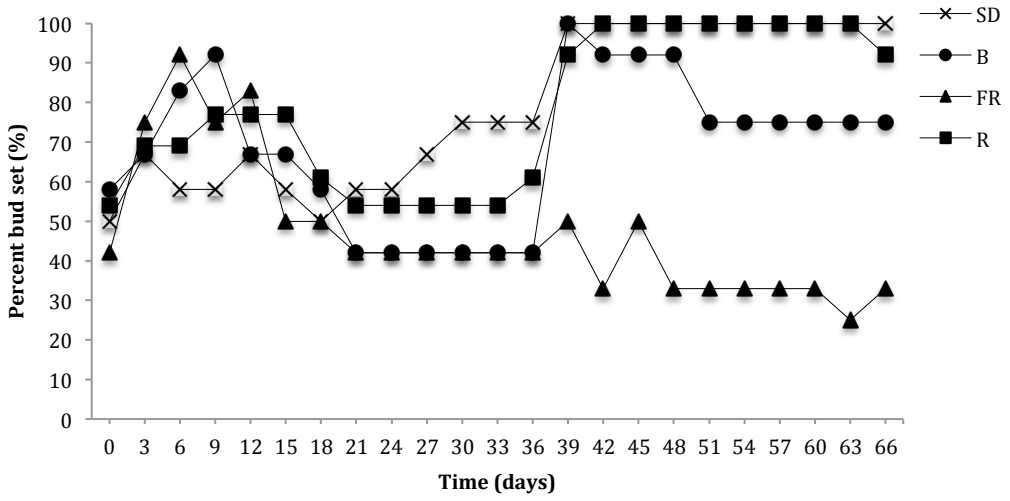


Fig. 16 Percentage buds in seedlings of Subalpine exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day-extension, all at a temperature of 18 °C in the first growth experiment. Results are mean of 12 plants in each treatment.

The disorderly pattern seen in percentage buds was reflected in bud stages. The average stage of bud set of FR-treated seedlings was 0.87, indicating that many of this plants still grew after 66 days (fig. 17). Further analysis showed that exposure to FR as a day extension light delayed bud development in seedlings compared to SD treatment ($p \leq 0.001$). The average stage of B and R-treated seedlings after 66 was also higher than the average stage of SD-treated seedlings (0.37 and 0.11, respectively). Further analysis showed a delay in bud development when exposure to B- and R as day extension as compared to SD treatment ($p \leq 0.01$) (fig. 17, tab. 9).

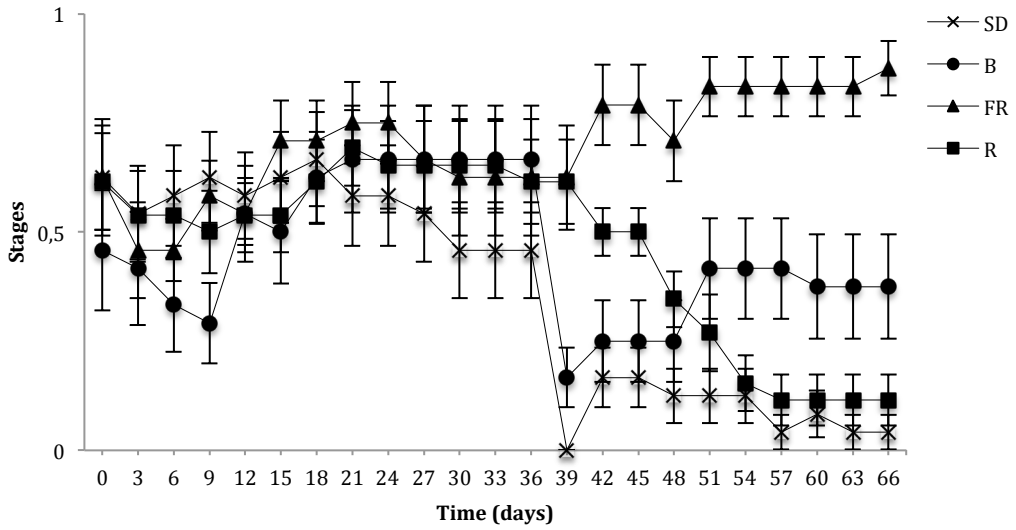


Fig. 17 Stages of bud set in seedlings of Subalpine fir exposed to day extension with a light quality from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, all at a temperature of 18°C in the first growth experiment. 1 = growth, 0.5 = green bud, 0 = brown bud. Results are mean ± SE of 12-13 plants in each treatment.

Tab. 9 Results from cumulative link model in R run to investigate the effects light quality, i.e. blue (b), far-red (FR) and red (R) (7 W m-^{2}) as day extension on bud set in seedlings of Subalpine fir compared to short days (SD) as the dummy variable in the first experiment. Positive estimated coefficient indicates an increase probability for bud set, while negative estimated coefficient indicates a delay in bud set

Treatment	Coefficient	SE	Z
B **	-0.534923	0.163722	-3.267
FR ***	-1,702775	0.168462	-10.108
R **	-0.481408	0.155341	-3.099

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

3.3.2 Effects of light quality on plant diameter

Effect of light quality on plant diameter was significantly different in FR-treated seedlings compared to B-treated seedlings ($p \leq 0.05$), i.e. seedlings exposed to FR had a slightly wider plant diameter (fig. 18). However, there was no significant difference between any other treatments.

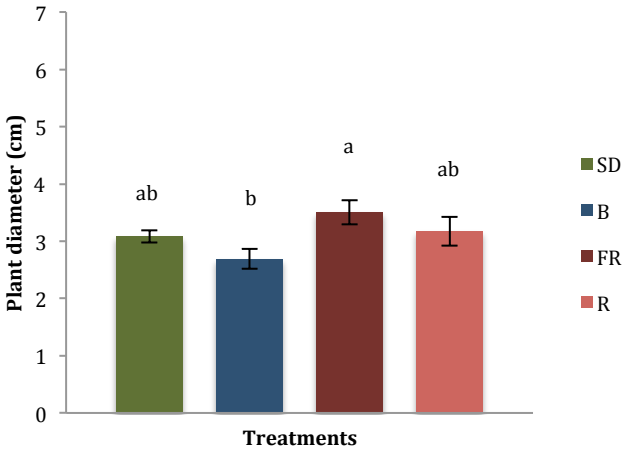


Fig. 18 Effect of light quality on average plant diameter at the top of seedlings of Subalpine fir exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day-extension at temperatures of 18°C for a period of 8 weeks in the first experiment. Treatments that do not share a common letter are significant different. The different letters are based on ANOVA (general linear model) followed by a Tukey test (p>0.05). Results are mean ± SE of 12-13 plants in each treatment.

3.4 Effects of temperature on elongation growth, bud set and bud burst of Subalpine fir (experiment 2 and 3)

3.4.0 Effect of temperature on the light quality impact on shoot elongation

At 18°C in the second experiment, elongation growth in FR-treated seedlings was significantly different from in SD-treated seedlings ($p \leq 0.05$, fig. 19). The FR-treated seedlings had grown 68% more compared to SD-treated seedlings. However, there was no significant effect of temperature on elongation growth in these treatments ($p \geq 0.05$). FR-22°C-treated seedlings had grown 65% more than SD-seedlings under the same temperature.

B and R-treated seedlings showed no significant difference in elongation growth compared to SD-treated seedlings under any of the temperature regimes (18 and 22°C), although there was a slight tendency for higher growth, especially in R-treated seedlings. There was no significant effect of temperature on elongation growth in these seedlings ($p \geq 0.05$, fig. 19).

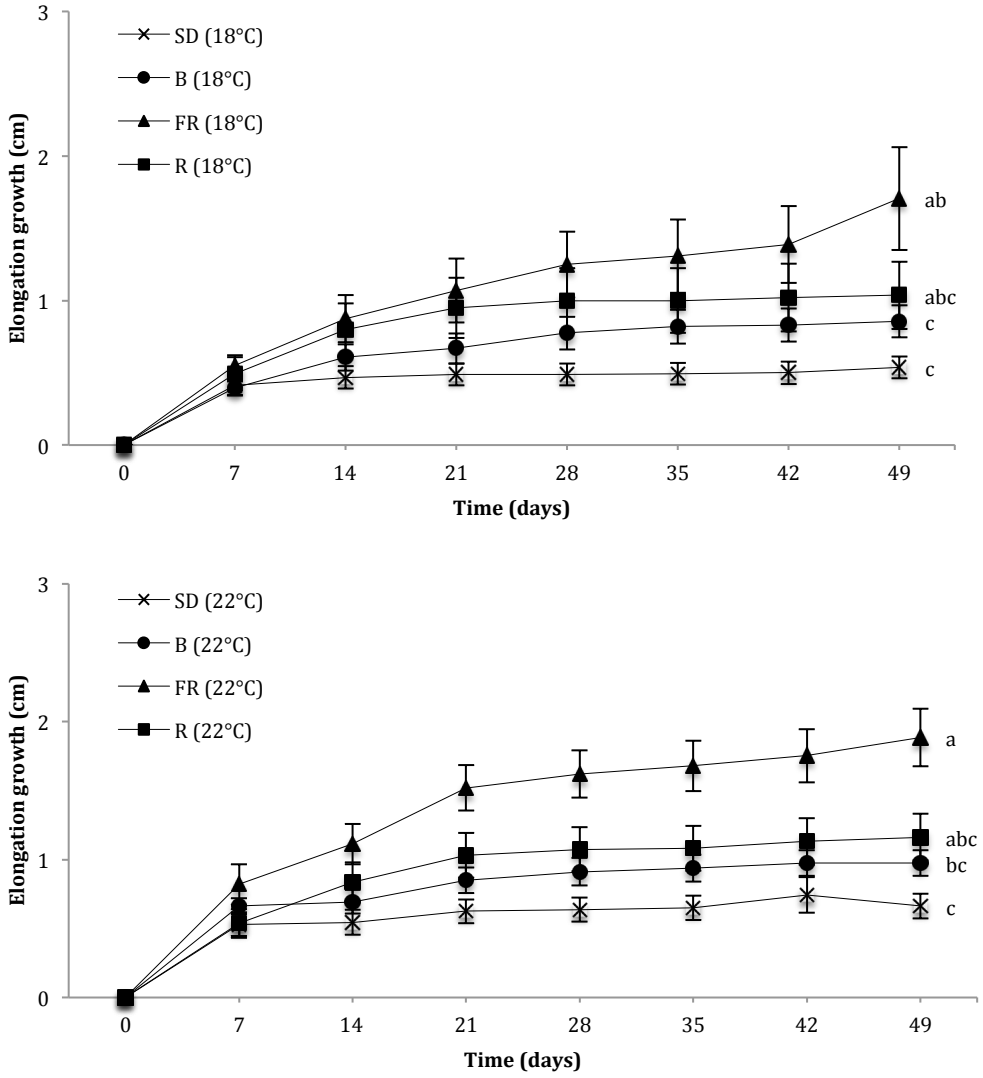


Fig. 19 Effect of light quality on average cumulative elongation growth in seedlings of Subalpine fir exposed to day extension with different treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, at a temperature of 18 ℃ and 22 ℃ in the second experiment. Treatments that do not share a common letter are significantly different at day 49 based on a two-way analysis of variance (general linear model) followed by a Tukey test (p>0.05). Results are mean ± SE of 14-16 plants in each treatment.

At 18°C in the third experiment, elongation growth in the B and FR-treated seedlings was significant different from in SD-treated seedlings ($p \leq 0.05$) (fig. 20). R-treated seedlings did not differ significantly from SD-seedlings, although there was a clear

tendency for higher growth. There was no significant effect of temperature on elongation growth (fig. 20).

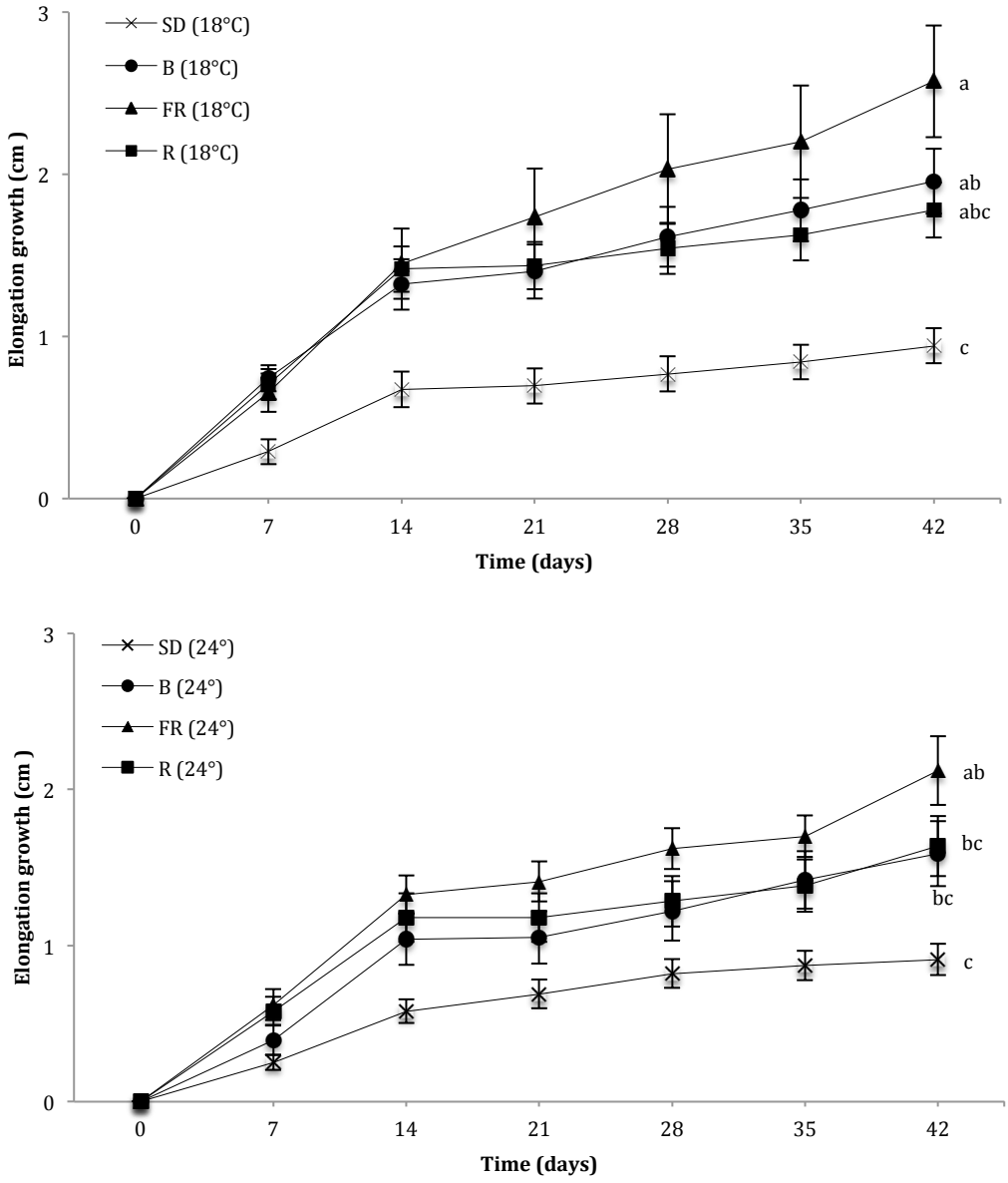


Fig. 20 Effect of light quality on average cumulative elongation growth in seedlings of Subalpine fir exposed to day extension with different treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, at a temperature of 18 °C and 24 °C in the third experiment. Treatments that do not share a common letter are significantly different at day 42 based on a two-way analysis of variance (general linear model) followed by a Tukey test ($p>0.05$). Results are mean ± SE of 19 plants in each treatment.

Fig. 21 Picture illustrating the variation in growth in seedlings of Subalpine fir exposed to FR (7 W m-^{2}) as day extension from light emitting diodes, all at a temperature of 18°C after 6 weeks in treatment in the third experiment.

3.4.1 Effects of temperature on the light quality effects on bud set

In experiment 2, 19% of the FR-22°C-treated seedlings had already developed buds at experiment start. After 3 days of treatment all of these buds had flushed again. However, after 27 days a total of 94% of the FR-exposed plants had terminal buds, but several of these thereafter showed bud burst and after 51 days 63% of the plants had buds (fig. 22). The same tendency is shown at 18°C, which after 27 days 94% of the seedlings had developed buds. Many of these flushed again, and after 51 days 31% of the FR-18°C-treated seedlings had developed terminal buds (fig. 22).

6% of the B-18°C-treated seedlings had developed buds at experiment start. They did not burst under the B treatment, and after 51 days a total of 81% had developed terminal buds. There was a trend of higher percent bud appearance in B-22°C-treated seedlings, which after 51 days had 94% of the seedlings with buds (fig. 22).

After 51 days in treatment, 100% of the R-18°C-treated and 94% of the R-22°C-treated had developed buds. 94% SD-treated seedlings had developed buds after 51 days (fig.22).

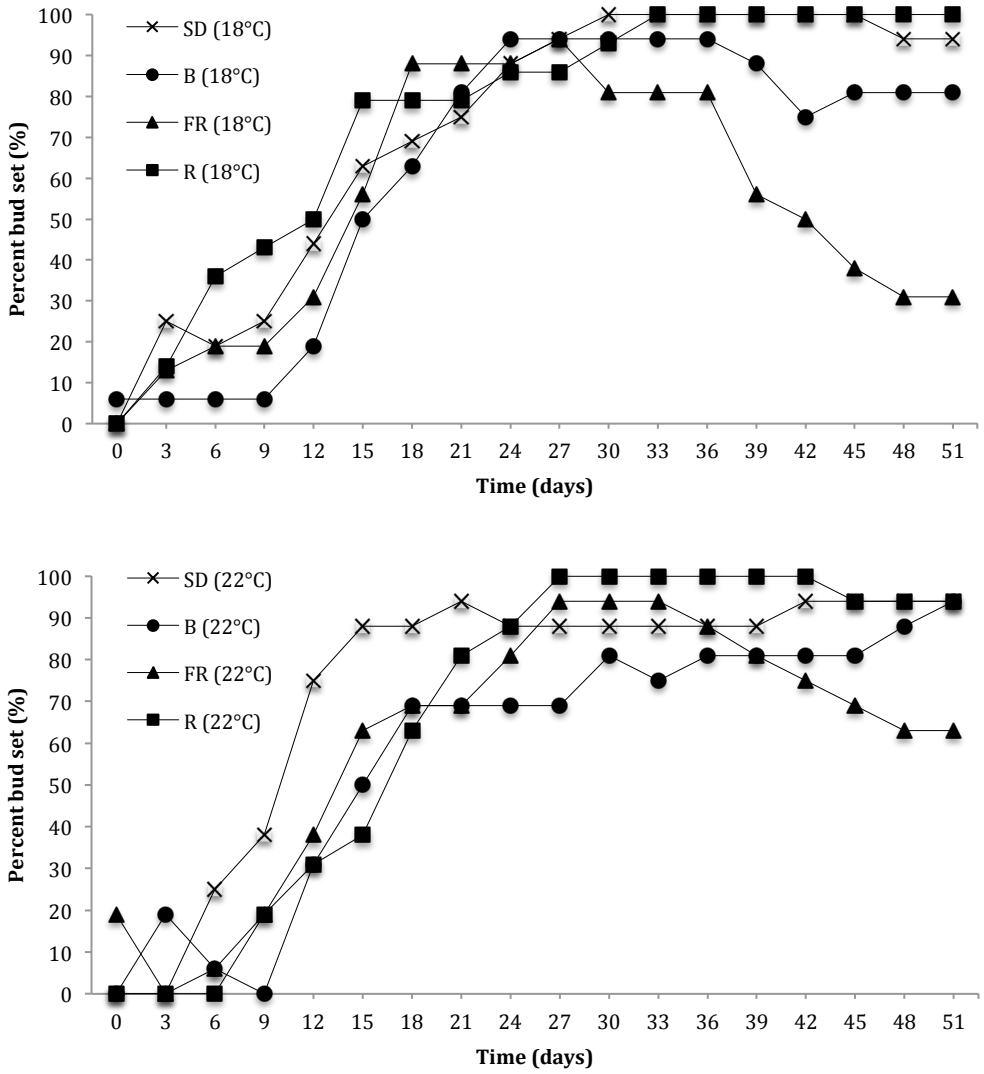


Fig. 22 Percentage buds in seedlings of Subalpine exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day-extension, all at a temperature of 18 or 22°C in the second growth experiment. Results are mean of 16 plants in each treatment.

In the second experiment almost all SD, B and R-treated seedlings seem to have fully developed, brown buds after 51 days in both 18 and 22°C-treatments, whereas many FR-treated seedlings seem to grow (average stage of 0.75) (fig. 23). An average higher then 0 indicates that not all seedlings had fully developed buds after 51 days. Further analysis showed a temperature effect on bud development in seedlings exposed to R light ($p \leq 0.05$), i.e. R light with elevated temperature delayed bud development compared to SD-treatment (tab.10).

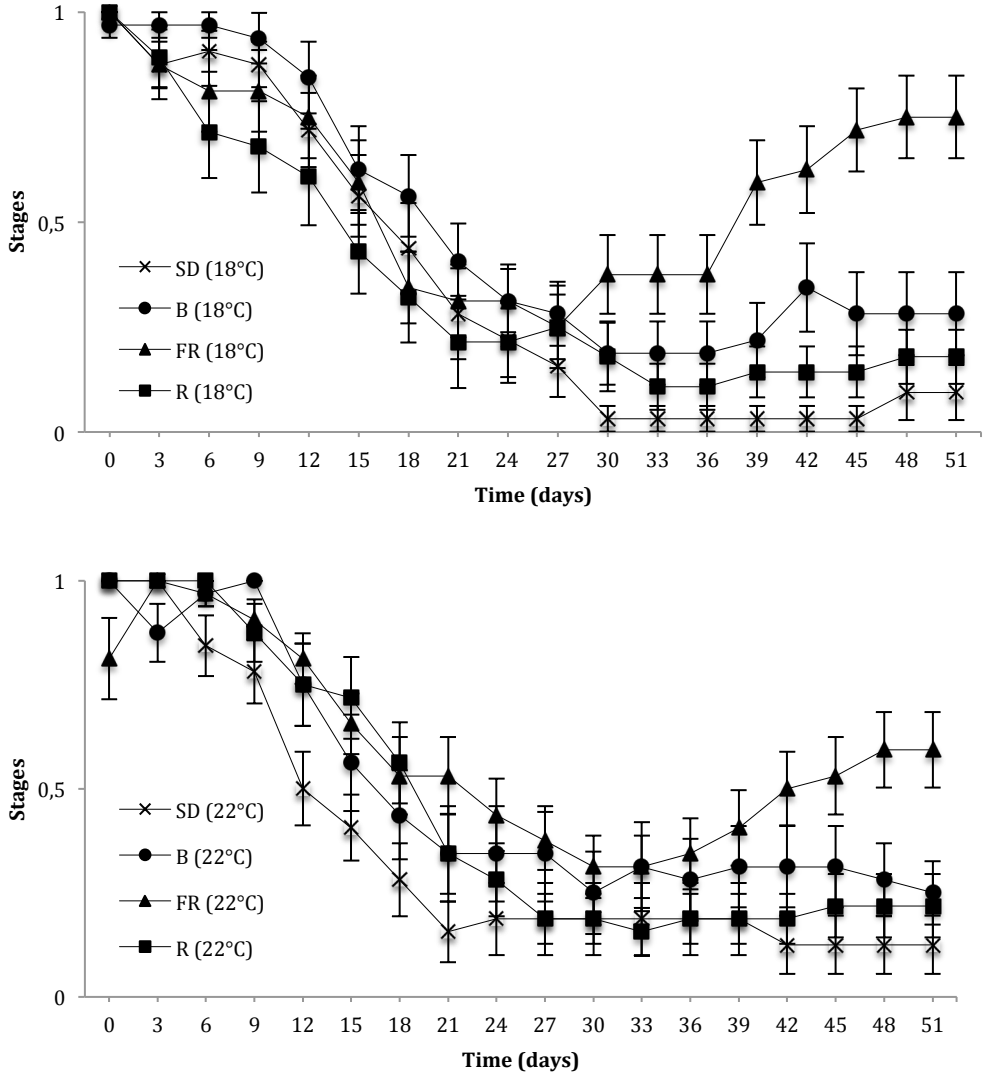


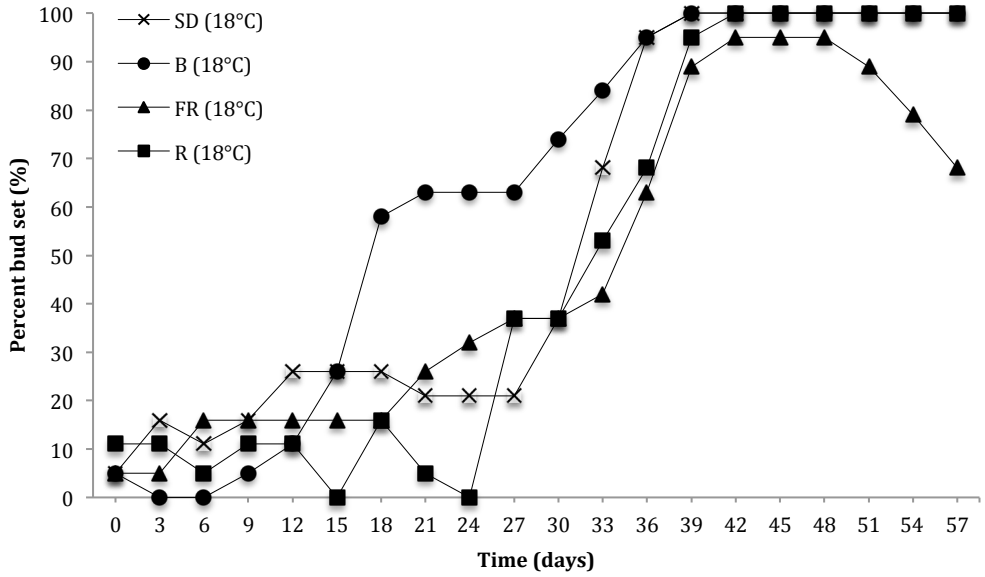
Fig. 23 Stages of bud set in seedlings of Subalpine fir exposed to day extension with a light quality from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, at a temperature of 18 or 22°C in the second growth experiment. 1 = growth, 0.5 = green bud, 0 = brown bud. Results are mean ± SE of 14-16 plants in each treatment.

Tab. 10 Results from cumulative link model in R run to investigate the effects of temperature (T; 18 and 22°C) on the light qualities, i.e. blue (b), far-red (FR) and red (R) (7 W m-^{2}) as day extension on bud set in seedlings of Subalpine fir compared to short days (SD) as the dummy variable in the second experiment. Positive estimated coefficient indicates an increase probability for bud set, while negative estimated coefficient indicates a delay in bud set

Treatment	Coefficient	SE	Z
B	-0.84812	1.22898	-0.690
FR	-1.70741	1.23114	-1.387
R	2.02408	1.25337	1.615
T	-0.01983	0.04363	-0.455
Day ***	0.10002	0.00412	24.275
B x T	0.00178	0.06124	0.029
FR x T	0.01985	0.06098	0.325
R x T *	-0.12158	0.06199	-1.961

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

In experiment 3, many seedlings had already developed buds at experiment start, which explains the disorderly pattern in the beginning (fig. 24). After 39 days 100% of the seedlings exposed to B and SD-treatment at 18°C had developed buds and this was unchanged after 57 days in treatment. 100% of the R-18°C treated seedlings had developed buds after 57 days, with a tendency of less bud development at 24°C in both SD, R and B treatment. At the end of the experiment, after 57 days, the FR-treatment seemed to have a higher degree of bud development in 24°C compared to 18°C, with 68% of the seedlings with buds at 18°C and 74% of seedlings with buds at 24°C (fig. 24).



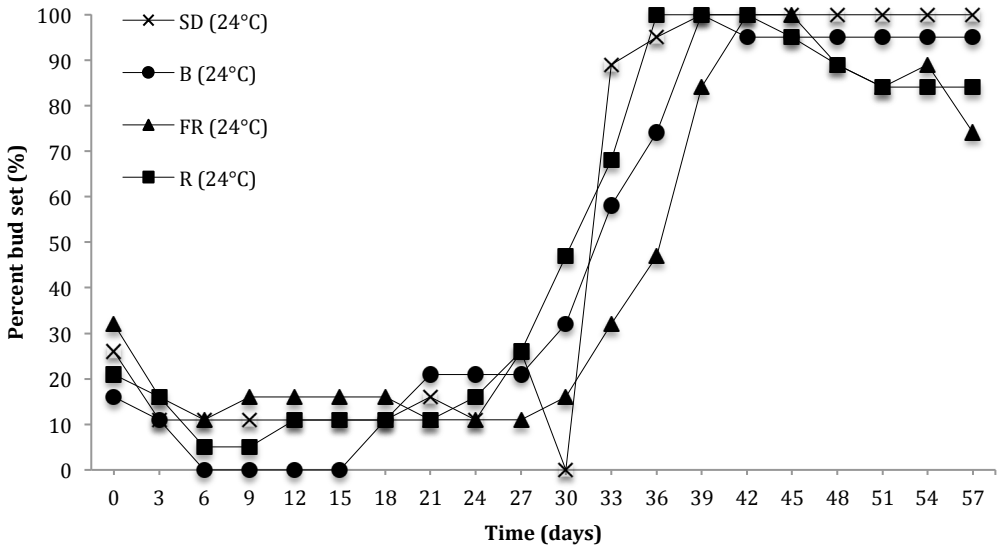


Fig. 24 Percentage buds in seedlings of Subalpine exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day-extension, all at a temperature of 18 or 24°C in the third growth experiment. Results are mean of 19 plants in each treatment.

Almost all SD, B and R-treated seedlings seem to have fully developed, brown buds after 57 days in both the 18 and 24°C-treatments, whereas many FR-treated seedlings still showed growth or delayed bud set (fig. 25). Further analysis showed a significant effect of temperature on bud development in seedlings exposed to B light as day extension, i.e. B light with elevated temperature delayed bud development ($p \leq 0.001$) (tab 11). On the other hand, B light alone accelerated bud development in these seedlings ($p \leq 0.001$) (tab.11). The analyses also show that FR and R light alone delay bud development ($p \leq 0.05$).

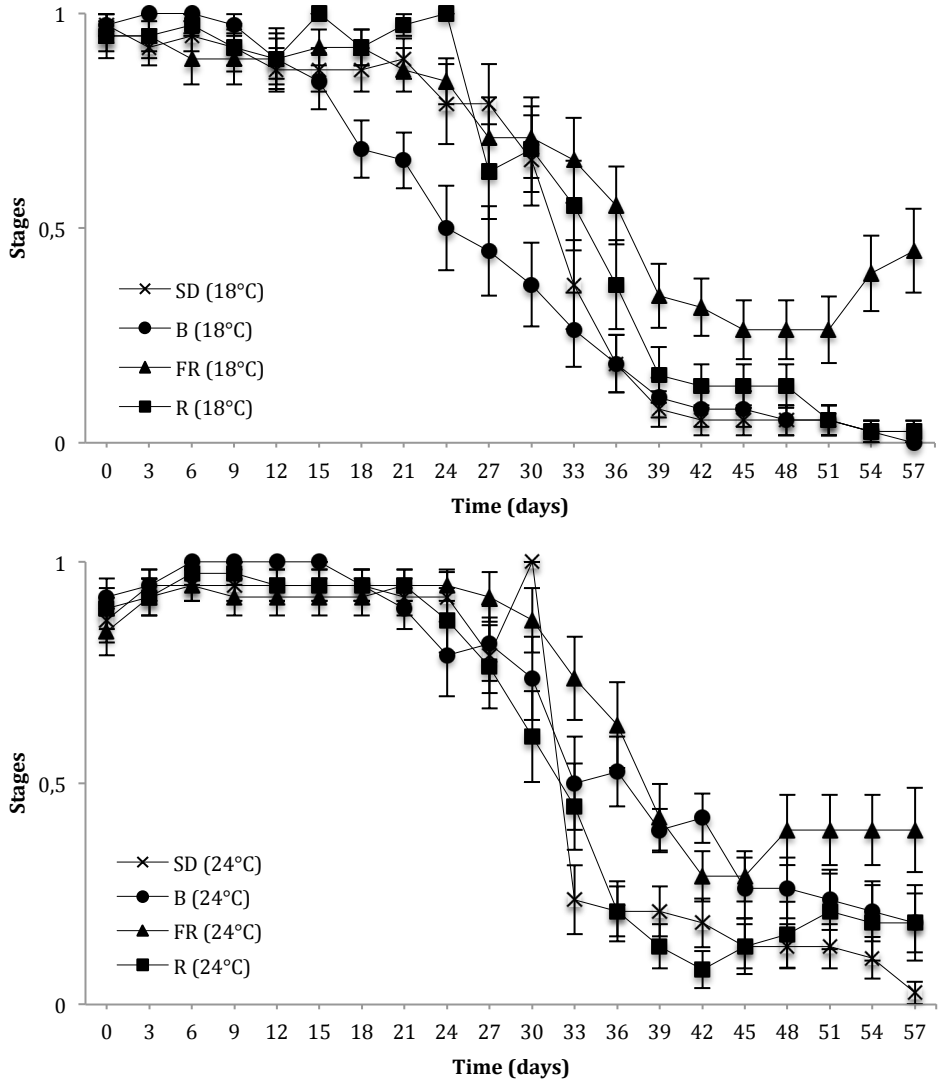


Fig. 25 Stages of bud set in seedlings of Subalpine fir exposed to day extension with a light quality from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day extension, at a temperature of 18 or 24°C in the third growth experiment. 1 = growth, 0.5 = green bud, 0 = brown bud. Results are mean ± SE of 19 plants in each treatment.

Tab. 11 Results from cumulative link model in R run to investigate the effects of temperature (T; 18 and 24°C) on the light qualities, i.e. blue (b), far-red (FR) and red (R) (7 W m-^{2}) as day extension on bud set in seedlings of Subalpine fir compared to short days (SD) as the dummy variable in the third experiment. Positive estimated coefficient indicates an increase probability for bud set, while negative estimated coefficient indicates a delay in bud set

Treatment	Coefficient	SE	Z
B ***	3.546362	0.815143	4.351
FR *	-1.747870	0.820275	-2.131
R *	-1.799596	0.823832	-2.184
T **	-0.079589	0.027240	-2.922
Day ***	0.143216	0.003958	36.180
B x T ***	-0.173747	0.038347	-4.531
FR x T	0.027862	0.038581	0.722
R x T	0.074617	0.038750	1.926

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

3.4.3 Effects of temperature on the light quality effects on plant diameter

The effect of temperature on plant diameter was not significantly different between treatments (p≥0.05) (fig.26).

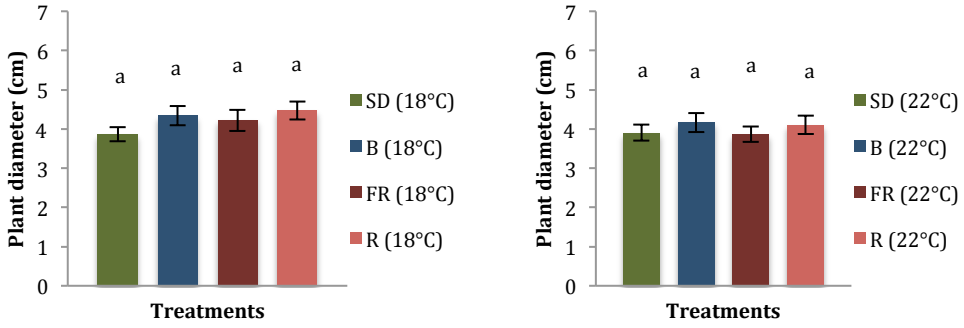


Fig. 26 Effect of light quality on average plant diameter at the top of seedlings of Subalpine fir exposed to day extension with different light quality treatments from light emitting diodes, i.e. blue (B), far-red (FR) and red (R) (7 W m-^{2}) as compared to short days (SD) without day-extension at temperatures of 18 or 22°C for a period of 7 weeks. Treatments that do not share a common letter are significant different. The different letters are based on ANOVA (general linear model) followed by a Tukey test (p≤0.05). Results are mean ± SE of 14-16 plants for the R-18°C-treated plants and 16 for plants for the R-22°C-treated plants.

3.4.4 Effect of light quality and temperature on bud burst

After 57 days of exposure to day extension light with different light qualities under 18 or 24°C in the third experiment, seedlings of Subalpine fir were transferred to 24 h

photoperiod under 18°C to investigate any effect of those variables on subsequent bud burst. All the treatments induced bud burst differently, e.g. while totally 100% of the SD-18°C-treated seedlings showed bud burst at some point in this experiment, only 74% of the SD-24°C-treated seedlings burst (fig. 27). The same trend was observed in B-treated and R-treated seedlings, which indicated a delay in bud burst with higher temperature for those seedlings. However, for FR exposed seedling the situation was opposite. FR-24°C-treated seedlings had a higher percentage of bud burst compared to FR-18°C-treated seedlings (fig. 27).

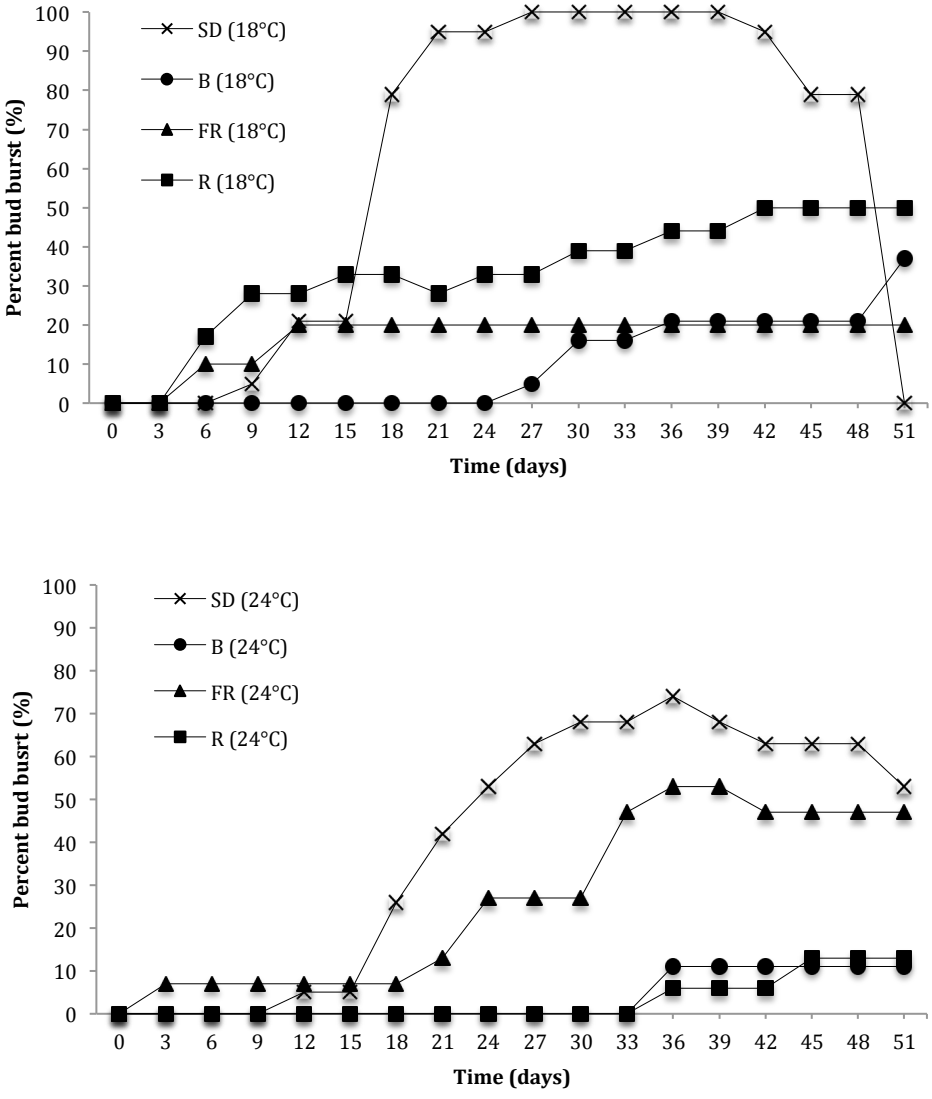
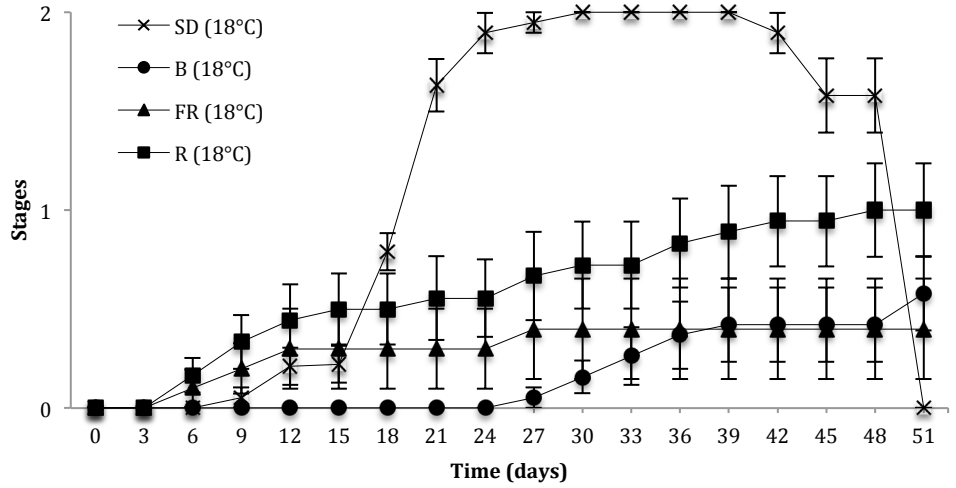


Fig. 27 Percentage bud burst in seedlings of Subalpine fir after exposure to blue (B), far-red (FR) and red (R) (7 W m-^{2}) as day extension light under 18°C or 24°C, for 51 days before transfer of all plants to 24 h photoperiod under 18°C. Results are mean of 19 plants for the 18°C-treated plants and 19 for plants for the 24°C-treated plants.

The average stage of bud burst seemed to be higher at 18°C in SD, B and R treatment than at 24°C, indicating that temperature has an effect on the rate of bud burst (fig. 28). However, the statistical analysis using the cumulative link model showed only a significant effect of temperature on R-treated seedlings, i.e. the effect of temperature on R-treated seedlings delayed bud burst ($p \leq 0.001$) (tab.12). In response to the B light treatment the bud development was significantly delayed compared to SD ($p \leq 0.05$). On the contrary, FR-treated seedlings showed faster development to bud burst at an elevated temperature (24°C) (fig. 28). The cumulative link model showed that FR light combined with elevated temperatures increased the probability of bud burst ($p \leq 0.001$) (tab. 12).



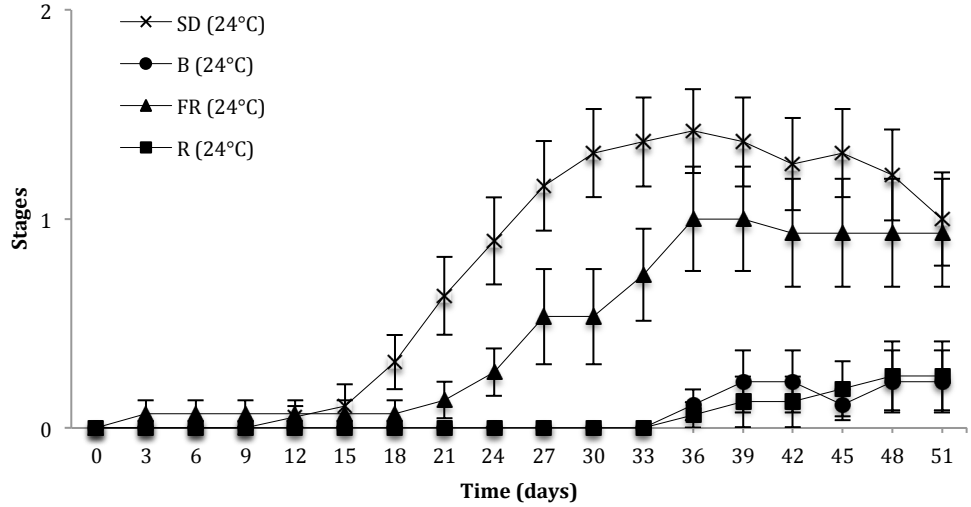


Fig. 28 Stages of bud burst in seedlings of Subalpine fir seedlings showing bud set under exposure to blue (B), far-red (FR) and red (R) (7 W m-^{2}) as day extension light under 18℃ or 24℃, for 51 days before transfer of all plants to 24 h photoperiod under 18℃. 0 = brown bud, 1 = bud burst, 2 = growth. Results are mean ± SE of 19 plants for the 18℃-treated plants and 19 for plants for the 24℃-treated plants.

Tab. 12 Results from cumulative link model in R run to investigate bud burst in seedlings of Subalpine fir, i.e. the same seedlings exposed to a temperature (T; 18 and 24°C) and different light qualities, i.e. blue (b), far-red (FR) and red (R) (7 W m-^{2}) in third experiment before transfer to 24 h photoperiod under 18℃. All compared to short days (SD) as a dummy variable. Positive estimated coefficient indicates an increase probability for bud burst, while negative estimated coefficient indicates a delay in bud burst

Treatment	Coefficient	SE	Z
B *	-2.766853	1.319605	-2.097
FR ***	-7.372927	1.141613	-6.458
R ***	5.109106	1.297285	3.938
T ***	-0.164191	0.028293	-5.803
Day ***	0.095835	0.005498	17.430
B x T	-0.024575	0.065404	-0.376
FR x T ***	0.273307	0.052022	5.254
R x T ***	-0.359980	0.067419	-5.339

*Significance levels: * P<0.05, ** P<0.01, *** P<0,001*

4.0 Discussion

4.1 The effects of light quality on elongation growth and bud set and subsequent bud burst in seedlings of Norway spruce

There was a clear efficiency in using FR light as day extension to maintain elongation growth in seedlings of Norway spruce (fig. 2,7,8). When seedlings were given FR as day extension light almost all of them continued growing, which reflected the observations in bud set, i.e. very few developed buds. The cumulative link model also showed that FR as day extension light delayed bud development (tab.7). Hence, FR seems to maintain elongation growth in seedlings of Norway spruce, i.e. the seedlings perceived FR light as a long day. This requirement for FR to maintain growth is consistent with previous studies of Norway spruce (Clapham et al. 1998a; Mølmann et al. 2006; Olsen 2010; Opseth et al. 2015). In Mølmann et al. (2006) effects of distinct light qualities were studied in three different latitudinal populations of Norway spruce, Halden (59°N), Snåsa (64°N) and Rana (66°). The effect of an increased FR level resulted in a decreased bud development. In addition, FR was shown to be the most effective light quality in stimulating bud burst under long photoperiods, i.e. 24 h.

In addition to sustain elongation growth with the irradiance tested in our study (about 7 Wm^{-2}), the statistical analysis also showed that plant diameter in FR-treated seedlings was slightly wider than SD- and R-treated seedlings (fig. 5). As mention above, FR-light was the most efficient treatment in sustaining elongation growth, but it might be affecting other parts of the plants by enhancing growth, e.g. plant diameter. Another explanation might be that the needles of these FR-treated seedlings had grown more horizontally compared to the seedlings in the other treatments that showed a lower plant diameter, i.e. the angle of the needles that were measured could also affect the average plant diameter.

B-treated seedlings also maintained elongation growth in Norway spruce in all the experiments conducted in this survey (fig. 2,7,8). This was also reflected in bud set, were almost none of the seedlings developed buds. The cumulative link model also showed that effects of B as day extension light delayed bud development (tab.7). Hence, B seems to maintain elongation growth in the same rate as FR-treated seedlings, i.e. the seedlings apparently perceived B light, at least at the tested irradiance, as a long day.

However, previous studies have shown the contrary. Depletion of B-light from sunlight has been reported to increase elongation growth in seedlings of Scots pine. Further, the northernmost (69°N) population then had a higher stem length compared to the southernmost (64°N), which suggest a different B-light response between the populations (Sarala et al. 2007). B-light is not constant and easily scattered in the atmosphere as a consequence of the decreasing solar angle and increased air particles. Hence, B-light is usually diminishing in the evening and towards higher latitudes compared to other light qualities with longer wavelengths (Kvifte et al. 1983; Taulavuori et al. 2005).

On the contrary to Scots pine, there are strong indications that B light is indirectly involved in the process of elongation growth in Norway spruce (Mølmann et al. 2006; Olsen 2010). Mølmann et al (2006) showed that seedlings exposed to B light ($3.3 Wm^{-2}$) delayed bud set, and because of this delay it appears that B light receptors are involved in photoperiodic control of elongation growth. Furthermore, B-light has been reported to increase elongation growth in Petunia and a few other species (Fukuda et al. 2011). An irradiance of about 7 Wm^{-2} used in our experiment sustained growth in Norway spruce, demonstrating that a higher irradiance of B light that was used in the study of Mølmann et al. (2006) (i.e. 3.3 W m-2) is needed to sustain growth in seedlings of Norway spruce, i.e. there is a dose effect of irradiance.

In addition to sustain growth with this irradiance, the statistical analysis also showed that plant diameter in B-treated seedlings were slightly wider than the other treatments (fig. 5). This was also shown in the second experiment at 18°C (fig. 13). Thus, in addition to that the B-light treatment sustained elongation growth, it might be affecting other parts of the plants to by enhanced growth, as shown here for plant diameter.

R-treated seedlings did not differ significantly from SD-exposed seedlings, although there was a tendency of higher growth rate compared to the SD-seedlings (fig. 2,7,8). Nevertheless, many seedlings showed cessation of growth and bud development. However, the cumulative link model showed that R as a day extension light delayed bud development (tab. 5, 6). Overall, the R-treatment was not perceived as a full day, i.e. long day. This is in line with a previous study done in seedlings of Norway spruce from the same provenance used in this study, where R-light reduced elongation growth relative to FR (Mølmann et al. 2006; Opseth et al. 2015). In the

study done by Mølmann et al. (2006) the irradiance given was 3.3 Wm^{-2}, and after 4 weeks in this treatment approximately 40% developed buds (Halden, 59°N). Although a higher irradiance of R-light was used in our experiment (about 7 Wm^{-2}), 89% of the R-treated seedlings had developed buds after 8 weeks in treatment. This might be explained with different light sources. In Mølmann et al. (2006) fluorescent tubes was used as the main light in the 12 h photoperiod, whereas HPS –lamps were used in this study. Fluorescent tubes are naturally higher in R-light compared to HPS –lamps (Islam et al. 2012; Mølmann et al. 2006). On the other hand, the difference might also be due to difference in the duration of the two experiments.

One important aspect to consider is that seedlings that are grown under day lengths near the critical day for growth cessation and bud development, or critical possibly critical conditions with respect to light quality or irradiance, e.g. seedlings exposed to only R-light as day extension (as discussed above), will have unstable pattern. Some will develop buds, and these buds could later on in the treatment burst. Then, furthermore, be developed again. This could be an explanation for the high growth rate in FR and B treated seedlings compared to the R-treated ones.

As expected, SD-treated seedlings induced growth cessation and development of buds (fig. 3). Thus, the seedlings perceived the 12 h of light as a SD, consistent with the earlier notion that woody species originating from 59°N, like the Norway spruce provenance in the present study, has a critical photoperiod of about 16-17 hours (Thomas & Vince-Prue 1997). The response was reflected in the bud development, were all had developed buds after 51 days in treatment. In response to SD, modulations of expression certain genes (*FTL2*, discussed below) and plant hormone content or signaling have been documented. The growth-sustaining hormone, gibberellin (GA) is for example well known to be to be down regulated as a response to SD in *Salix pentandra* (Olsen et al. 1995), whereas an upregulation of abscisic acid (ABA) has been reported under SD in Norway spruce, where it has its role in bud dormancy and cold hardiness (Lee et al. 2014).

4.2 The effect of light quality on FTL2 transcript levels in seedlings of Norway spruce

FTL2 is known to be strongly upregulated under SD (Asante et al. 2011; Gyllenstrand et al. 2007), and as predicted, SD-treated seedlings had the highest *FTL2* transcript

level, whereas the growth-sustaining treatments, FR and B, had the lowest *FTL2* transcript levels (fig. 6). None of the FR- and B-treated seedlings had developed buds when the plant materials were harvested. R-treated seedlings had a slightly higher *FTL2* transcript level compared to the other day extensions, but still very low levels compared to SD-seedlings. *FTL2* transcript levels have also previously been studied in Norway spruce under day-extension with different light qualities at lower irradiance (Opseth et al. 2015). However, in the study by Opseth et al. (2015) SD- and B-treated seedlings had a higher *FTL2* transcript level relative to FR- and R-treated seedlings. The lowest transcript level was then found in FR-treated seedlings. As mentioned above, in our study B-treated seedlings had a low *FTL2* transcript level relative to SD-treated seedlings. In addition, B-light sustained growth in our study (about 7 Wm^{-2}), whereas 100% of B-treated seedlings in the study by Opseth et al. (2015) developed buds after 42 days in treatment (3.3 Wm^{-2}). Furthermore, the absence of bud development in our study apparently explains the low *FTL2* transcript levels found in B-treated seedlings. This demonstrates that B-light is perceived as a long day under higher irradiance. The strong correlation between the *FTL2* transcript level and degree of bud set under day-extension with different light qualities, strongly supports that *FTL2* acts as a growth inhibitor, like suggested earlier (Asante et al. 2011; Gyllenstrand et al. 2007; Karlgren et al. 2013; Opseth et al. 2015).

4.3 The impact of temperature on light quality effects on elongation growth, bud set and subsequent bud burst in Norway spruce

There was no significant effect of elevated temperature (22°C (experiment 2) or 24°C (experiment 3) versus 18°C) on FR-treated seedlings (fig. 7, 8). As stated above, FR-treated seedlings maintained growth and very few developed buds, and the seedlings thus apparently perceived FR as a long day, i.e. an extension of the photoperiod. This FR-response is apparently stronger than a potential effect of temperature, which is in line with long days and a relative warm climate under the growing season. Such long photoperiods and a relatively warm climate sustain growth in trees of southern and northern ecotypes (Olsen & Lee 2011).

There was a tendency for higher growth in B-treated seedlings exposed to elevated temperatures, i.e. 22 and 24°C, which exceeded the growth rate in FR-treated seedlings under the same temperature conditions (fig. 7, 8). However, the effect of

temperature on elongation growth was not significant, but might indicate that B-light at this irradiance overshadows any potential effect of elevated temperature. In our study the B-light was approximately 7 Wm^{-2}. With this irradiance the seedlings managed to maintain growth, which as mentioned above could indicate a dose-effect of B-light.

In contrast to at 18°C, under 22°C plant diameter in B-treated seedlings did not differ from the other light quality treatments, indicating that the differences in plant diameter observed at 18°C in the first and second experiment reduces with elevated temperature (22°C) (fig. X). On the other hand, the average plant diameter measured in the other treatments under 22°C were similar at 18°C, which indicate that these are not affected by an elevated temperature. It might therefore be that this effect is not an effect of temperature, but rather that the needles have grown in a different angles.

There was a slight tendency for higher growth in R-treated seedlings exposed to 22°C compared to 18°C (fig. 7, 8). However, time to bud set was not affected by temperature, nor the total percentage of buds developed between these two treatments. Nevertheless, 24°C-R-treated seedlings differed significantly from SD-24°C-treated seedlings. This significant difference in elongation growth compared to SD-treated seedlings was not observed at 18°C, which indicates that temperature have an essential role in plant´s capability to detect photoperiod. This also explains the tendency for higher growth observed at 22°C. R-light under elevated temperatures seems to be perceived as a longer day than the critical day length for induction of growth cessation and bud development. Hence, the growth was higher. The temperature effect was reflected in bud set (24°C), where totally 50% developed buds with an average stage of 0.5, which denotes green buds (not yet having mature, brown bud scales). In comparison, all R-18°C-treated seedlings developed complete buds with brown bud scales at some point in the treatment. Furthermore, when re-transferred back to 24 h photoperiod at 18°C, a higher percentage of the R-24°C-treated seedlings burst compared to R-18°C-treated seedlings. The cumulative link model also showed that R-treated seedlings under elevated temperatures exhibited faster bud burst, as compared to SD-treated seedlings.

Although not statistically significant at $p \leq 0.05$, there was a slight tendency for higher elongation growth in SD-treated seedlings under 24°C compared to 18°C (fig. 8). Previous studies have reported that a shortening of the photoperiod with warmer climate will induce more rapid growth cessation and a deeper dormancy in conifers and deciduous woody species as compared to colder climate (Tanino et al. 2010). 100% of the seedlings developed buds in the SD-18°C-treatment after 57 days, whereas 86% developed buds in the SD-24°C-treatment, which could indicates the contrary to that stated above. However, to our knowledge, the previous studies of temperature effect on bud set and subsequent bud burst in Norway spruce done under controlled conditions, have investigated the effect of temperatures up to 21°C (Olsen et al. 2014; Søgaard et al. 2008) and not 24°C. There is strong evidence that modulation in temperature could have a significant impact on photoperiodic responses (Olsen & Lee 2011), and long day conditions combined with low temperatures have been reported to completely bypass the SD requirement in northern populations (Mølmann et al. 2005; Tanino et al. 2010). It might be that a temperature of 24°C overrides the SD to a certain extent, leading to the perception as a longer day than at lower temperature. Hence, a lower percentage of buds were observed in the SD-24°C-treated seedlings compared to SD-18°C-treated seedlings. In regards to global warming, more knowledge is needed on interactive effects of light climate (light quality, irradiance and photoperiod) and higher temperature.

Furthermore, not all SD-24°C–treated seedlings had fully developed buds with mature, brown bud scales relative to SD-18°C–treated seedlings. This indicates that many had passed different stages in the bud development process. However, the rate of the bud burst happens faster in seedlings exposed to 18°C compared to 24°C in SD-treatment. This is in line with previous studies stated above, where a shortening of the photoperiod in a warm climate induces deeper dormancy (Olsen et al. 2014; Søgaard et al. 2008; Tanino et al. 2010).

4.4 The effects of light quality on elongation growth, bud set and subsequent bud burst in seedlings of Subalpine fir

Since many of the plants of Subalpine fir had developed buds at the start of the experiments, the results from Subalpine fir are less clear than those of Norway spruce.

The results from elongation growth might have been significantly different between the treatments if seedlings that already had developed buds were removed before the analysis of variance were conducted. Nevertheless, FR-light was the most efficient light quality in achieving higher growth in seedlings of Subalpine fir, which is similar to the observations in Norway spruce discussed above (fig.15, 19, 20). The cumulative link model also showed that FR as a day extension delayed bud set in these seedlings (exp. 1 and 3) (tab. 9,12). However, 33% of the seedlings developed buds after 66 days in treatment in the first experiment, indicating that they did not respond quite similar to seedlings of Norway spruce under the same environmental conditions. FR as a day extension light has also been reported to enhance growth in seedlings of Subalpine fir previously in a preliminary study (Term paper by (Cazanji 2014). After 6 weeks in treatment with 12 h of main light (about 130 μmol m^{-2} s^{-1}) along with 7-7.5 Wm^{-2} of irradiance in FR-day extension, 88% of the seedlings developed buds. The irradiance of the main light was lower than measured in our experiment (about 160 μmol m^{-2} s^{-1}), which could explain the lower percentage of bud development in our study.

In addition to being the most efficient light quality in achieving higher growth, FR-treated seedlings showed a tendency for highest measured plant diameter. Although not statistically significant at $p \leq 0.05$, this growth-enhancing light quality may affect other parts of the plants to by increased growth, as discussed regarding Norway spruce.

Although there was a tendency for higher growth, neither B- or R-treated seedlings differed significantly from SD-seedlings. A similar trend of higher growth in B-and R-treated was observed in seedlings of Norway spruce under the same climatic conditions. Furthermore, R-treated seedlings seemed to have higher growth relative to B-treated seedlings (fig. 15). The cumulative link model showed that both B- and R-light delayed bud set compared to SD, indicating an involvement in the photoperiodic control of elongation growth. Yet, a total of 100% of the R-treated seedlings and totally 75 % of the B-treated seedlings developed buds at some point in the treatment. As stated above, there was a tendency for higher growth in R-treated seedling compared to B-treated seedlings, although the percentage of buds was so high in R-treated seedlings. This also indicates large variations between the seedlings in the individual treatment.

4.5 The impact of temperature on light quality effects on elongation growth, bud set and subsequent bud burst in Subalpine fir

There was no significant effect of elevated temperature (22°C (experiment 2) or 24°C (experiment 3) versus 18°C) on light quality effects on elongation growth, other than a trend of difference between seedlings exposed to day extension light and SD-treatment. A similar trend was observed in bud development, where SD-treated seedlings have the highest percentage of buds developed totally. Results from the cumulative link model showed that R-light together with elevated temperature delayed bud set in the second experiment (22°C) compared to SD, while B-light with elevated temperatures delayed bud set in the third experiment (24°C) compared to SD (tab. 10, 11).

Furthermore, when re-transferred to 24 h photoperiod under 18°C, SD-18°C-treated seedlings exhibited faster bud burst than the SD-24°C-treated seedlings (fig. 27), which indicates that the 24°C-treated seedlings had initiated a deeper dormancy like discussed above for Norway spruce. Furthermore, R-18°C-treated seedlings showed faster bud burst than R-24°C-treated seedlings. In addition, the cumulative link model also showed a delay in bud burst in seedlings exposed to R-light along with higher temperatures (tab.12). This might indicate that R-light at 18°C is not perceived as a longer day than the critical day for cessation of growth and bud development seen in SD-18°C-treated seedlings as discussed above, whereas R-24°C-treated seedlings behave similar to SD-24°C-treated seedlings and initiate a deeper dormancy. A similar tendency was also observed in B-treated seedlings. However, it could also be due to the morphological stage of the buds. It has previously been suggested that, when first initiated, development of bud must be completed or proceed to a certain stage, before bud burst can occur (Olsen et al. 2014). Olsen et al. (2014) suggested this when studying the effect of altering day-and night temperature on SD-induced bud set and subsequent bud burst under LD in seedlings of Norway spruce. When seedlings without visible buds were retransferred to long days, the development of buds continued and buds were eventually observed under these long day conditions, followed by bud break under continued long days.

On the other hand, FR-24°C-treated seedlings exhibited more rapid bud burst compared to FR-18°C-treated seedlings. This is also showed in the cumulative link model, which indicates a temperature effect on bud burst in FR-exposed seedlings. As

stated above, FR was the most efficient day extension light to maintain growth. Although there was no effect of temperature on elongation growth, the results showed an effect of temperature on bud burst, indicating an enhanced growth under elevated temperature.

4.5 Subalpine fir is adapted to a different climatic environment

As mentioned above, many seedlings of Subalpine fir developed buds already under the pre-growing when the main light was 12 h and the day was extended to 24 h with low-intensity light. Such light conditions were used during the entire pre-growing period in the first experiment and due to failure for approximately 2 weeks in the third experiment before being increased to 24 h with daylight. The percentage of bud development was lower after the pre-growing period in experiment 2 where the main light period was 24 h, and lower in experiment 3 than 1 (tab. 2). Nevertheless, in all cases some seedlings still developed buds. Bud development was not observed in seedlings of Norway spruce, neither for the southern ecotype (59°N) used in this study or for a northern ecotype (67°N) studied earlier (Holefors et al. 2009) under pre-growing under the same light conditions. Since bud development was observed in Subalpine fir, it is possible to assume that these seedlings has a different demand for light compared to at least these Norway spruce provenances. The light conditions were apparently perceived as a shorter day than the critical day for growth and development. This was also the case in a previous study done on the effect of different light qualities on growth and bud development in Subalpine fir, where seedlings developed buds under pre-growth (Cazanji 2014). In the term paper by Cazanji (2014) only 100 μmol $m^{-1}s^{-1}$ of supplemental light provided by HPS-lamps was used in addition to the natural light in a greenhouse compartment. Then all seedlings showed bud set before the start of the experiment. In light of this and the reduced bud set when more light was used during pre-growing in our study, it is possible to assume that Subalpine fir requires a higher irradiance of light relative to the studied Norway spruce provenance. The seedlings of Norway spruce came from 0-149 meters above sea level (m.a.s.l.), whereas seedlings of Subalpine fir came from 1000-1200 m.a.s.l., i.e. these seedlings is probably adapted to different climatic conditions. At higher elevations the air gets thinner and sunlight passes through a thinner layer of atmosphere, which makes the solar irradiation stronger. Different

requirement for light has been reported in different latitudinal populations of Norway spruce from Norway (Mølmann et al. 2006). Seedlings were then exposed to 12 h main light with extended photoperiod with LED-lights. When exposed to FR-light at 1.7 Wm^{-2}, none of the seedlings in the southernmost population (59°N; same as in our study) developed buds, whereas 43% of the northernmost population (67°N) developed buds, which indicated an increased, clinal requirement for FR.

4.6 Conclusions

Norway spruce

- FR and B light maintain elongation growth in Norway spruce (about 7 Wm^{-2}).
- R light was less efficient in sustaining growth in Norway spruce relative to FR and B, but showed a delay in bud set, indicating an involvement in the photoperiodic responses.
- FR and B as light responses are stronger than a potential temperature effect in Norway spruce.
- R-light are effected by elevated temperature in Norway spruce, indicating that temperature have an essential role in plants capability to detect photoperiod.
- SD-treated plants are affected by elevated temperature (24°C), which strongly support that modulations in temperature have a significant impact on photoperiodic responses. With respect to global warming, more knowledge is needed about the effects of SD combined with higher temperature.
- Our results on *FTL2* transcript level in Norway spruce in the different treatments strongly supports that *FTL2* acts as a growth inhibitor as suggested in previous studies.

Subalpine Fir

- The trends shows that FR-treated seedlings of Subalpine fir achieve the highest growth
- B-and R-light are less efficient in enhancing growth relative to FR in Subalpine fir, but a delayed bud development indicates that B and R receptors are involved in photoperiodic control of elongation growth.
- There was no significant effect of temperature on elongation growth, except a higher degree of growth in seedlings exposed to the different light qualities compared to SD-treated seedlings.

- Seedlings of Subalpine fir exposed to B and R combined with elevated temperature delayed bud development, which as stated above, indicates that B and R receptors are involved in photoperiodic control of elongation growth.
- Overall, Subalpine fir seems to require a higher irradiance of light to sustain growth, both under pre-growth and in experiments compared to Norway spruce.

4.7 Future perspectives

For Subalpine fir, more knowledge about the precise determination of the amount of light irradiance is needed, i.e. the irradiance of HPS -lights and LED-lights. Furthermore, it could be important to characterize the effects of other light sources, such as fluorescent lamps and metal halide lamps. Interaction between light and temperature is an important aspect in both plant production and with respect to climatic changes in natural environments as our results along with several other studies indicate that temperature can modulate photoperiodic responses.

Many phenolic compounds serve as a defense mechanism against herbivores and pathogens, whereas others function as mechanical support and generally as antioxidants in protection towards oxidative damage (Taiz & Zeiger 2010).
One of the goals in greenhouse productions is to produce more robust seedlings, which are not easily attacked by pathogens. Lignin is a phenolic compound found in all cells, which function as mechanical support by strengthening the cell walls. Furthermore, lignification is a common response to infections, which would block the growth of pathogens (Taiz & Zeiger 2010). Further investigations of the effect of light quality and temperatures in regards such defense mechanisms could be beneficial to the greenhouse production.

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7. Appendix

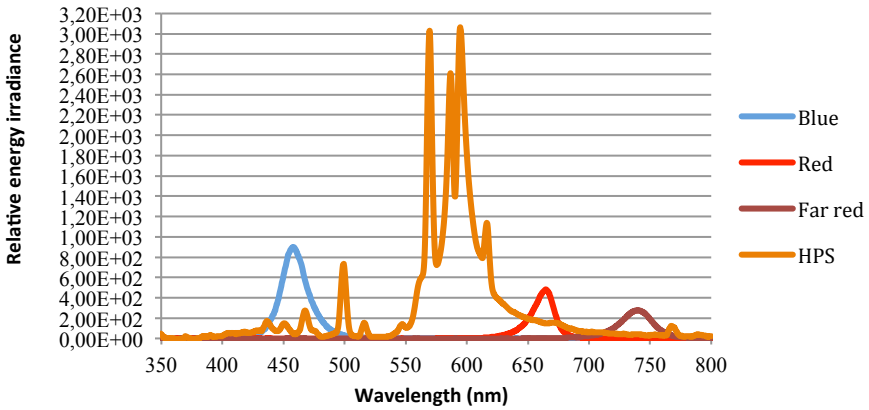
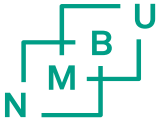


Fig. 29 Spectral distribution of the light emitting diodes, i.e. blue (B), far-red (FR) and red (R) and the High Pressure Sodium (HPS) lamps.



Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no