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Interactive effects of light quality and temperature on bud set and shoot elongation in Norway spruce (*Picea abies*) and Subalpine fir (*Abies lasiocarpa*).

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Interactive effects of light quality and temperature on bud set and shoot elongation in Norway spruce (*Picea abies*) and Subalpine fir (*Abies lasiocarpa*)

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Abstract

In recent studies, temperature was shown to modulate the response to short days (SD) in woody species. This is an important issue for plant production in zones with marked seasons. Furthermore, light quality affects bud set, but information on interactive effects of light quality and temperature is scarce. In the woody conifer *Picea abies*, *FLOWERING LOCUS T-TERMINAL FLOWER1-LIKE2* (*PaFTL2*) was shown to be up regulated during SD compared to growth-sustaining long days (LD) and far-red (FR) treatment, whereas *SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*PaSOC1*) and the *CONSTANS-LIKE* genes (*PaCOL1* and *PaCOL2*) were down regulated. However, there is little information about levels of these transcripts in *Picea abies* grown under different temperature-light quality combinations. In the present study, interactive effects of the red: far red ratio (R:FR) during a day extension and temperature (18ºC and 24ºC) on growth cessation and terminal bud formation in seedlings of *Picea abies* and *Abies lasiocarpa* were investigated.

There was a interaction between the temperature and light quality treatments on shoot elongation and bud set in *P. abies.* R light and SD treated plants showed bud set, with the effect of R light being dependent on temperature. At 18ºC and 24ºC, all plants at SD stopped growing, whereas complete growth cessation in the R-treated plants was observed just at 18ºC. At both temperatures, the largest shoot elongation was achieved with intermediate values of R:FR. There was interaction between the light quality and temperature for *FTL2*, with higher transcript levels under R light and SD treatments as compared to FR. The transcript levels of *PaSOC1*, *PaCOL1* and *PaCOL2* where higher with the presence of FR light, compared to R light and SD.

In *Abies lasiocarpa* there was an interaction between light quality and temperature for bud set and shoot elongation. R light treatment induced more bud set at 24ºC than 18ºC. SD induced complete bud set in both temperatures. The largest shoot elongation at both temperatures was obtained for intermediate R:FR values. The only treatment without any terminal bud set at the end of the experiment was FR light at 18ºC, i.e. in one out of two experiments.

In conclusion, growth cessation and bud set in *Picea abies* and *Abies lasiocarpa* are affected by light quality and temperature, and these factors interact. FR-light can reduce bud set in both species with this being more marked at the lowest temperature. In contrast, R light or SD treatments induced bud set at both temperatures, although bud set under R light was affected by temperature. The largest shoot elongation was achieved in both species at a R:FR 0.5 at 18ºC. Finally, bud stage in *Picea abies* was correlated with the transcript levels of *PaFTL2* (R^2=0.89).

Sammendrag

Nyere studier av noen arter av trær har vist at temperaturen modifiserer responsen på korte dager. Dette er av betydning for planteproduksjon på steder med markert årstidsvariasjon. Det er også vist at lyskvalitet kan påvirke knoppdannelse, men kunnskapen om samspillseffekter av lyskvalitet og temperaturer er begrenset. I bartreet *Picea abies er* det vist at *FLOWERING LOCUS T-TERMINAL FLOWER 1-LIKE2 (PaFTL2)*-genet oppreguleres under kortdagsindusert vekstavslutning sammenlignet med i planter i vekst under lange dager og dagforlengelse med mørkerødt lys. *SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*PaSOC1*) og *CONSTANS-LIKE* genene (*PaCOL1* og *PaCOL2*) ble derimot nedregulert. Det er imidlertid lite tilgjengelig informasjon om hvordan nivåene av disse transkriptene i *Picea abies* påvirkes av forskjellige kombinasjoner av temperatur og lyskvalitet. I denne studien ble samspillseffekter av rødt: mørkerødt-forhold (R:FR) under dagforlengelse og temperatur (18 og 24°C) undersøkt for vekstavslutning og vinterknoppdannelse i unge planter av *P. abies* og *Abies lasiocarpa*.

Det var en tydelig samspillseffekt mellom temperatur -og lyskvalitetsbehandlinger for strekningsvekst og vinterknoppdannelse i *P. abies*. Planter behandler med R lys og korte dager viste knoppsetting, men effekten av R lys var avhengig av temperaturen. Ved både 18 og 24 ºC sluttet alle planter å vokse under korte dager, men fullstendig vekstavslutning i R-behandlede planter ble kun observert med 18ºC. Ved begge temperaturer ble størst strekningsvekst observert ved intermediære R: FR-forhold. Det ble også observert samspillseffekt mellom lyskvalitet og temperatur for transkriptnivåer av *FTL2* i gran, med høyere transkriptnivåer under behandling med R lys og korte dager sammenlignet med FR. Transkriptnivåene av *PaSOC1*, *PaCOL1* og *PaCOL2* økte ved tilstedeværelse av FR lys, mens R lys og korte dager reduserte disse.

I *A. lasiocarpa* var det også samspillseffekt mellom lyskvalitet og temperatur når det gjaldt knoppdanning og strekningsvekst. R-lysbehandling induserte mer knoppdanning ved 24ºC enn ved 18ºC. Korte dager induserte fullstendig knoppsetting ved begge temperaturer. Ved begge temperaturer ble den største strekningsveksten oppnådd ved intermediære R: FR-forhold. Den eneste behandlingen uten endeknopp ved slutten av eksperimentet var FR lys ved 18°C i det først eksperimenter.

Sammenfattet er det tydelige samspillseffekt mellom lyskvalitet og temperatur for vekstavslutning og vinterknoppdanning i *P. abies* og *A. lasiocarpa*. FR-lys kan redusere knoppsetting i begge arter med mest markert effekt ved 18ºC. R-lys og kortdags-behandling induserer imidlertid knoppdanning ved begge temperaturer selv om knoppsettingen under R-lys var påvirket av temperaturen. Den største skuddstrekningen ble oppnådd i begge arter ved R: FR-forholdet 0.5 ved 18ºC. Knoppsettingen i *P. abies* var korrelert med transkriptnivåene av *PaFTL2* (R2 = 0.89)

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During the last 16 months I have live in Norway learning about plants and how these interacts with different factors. Interestingly I have learned much more about people, values, relationships and how this interacts with other human related topics. For this, I would like to say thanks to anyone who has, have and will come into my life even with the smallest interaction possible, helping me to learn how to be a better writer, researcher, lifter, climber, friend, partner or person. Thanks Jorunn Olsen, the people of SKP and Plantecellelaben for your time and dedication to teach and help. Thanks to my friends, old and news, for your friendship, patience and time. Thanks to my family for your support. Thanks to that people in the planes that can change your life. Thanks God for allowing an amazing experience.

Because at the end of the day, our world does not need smarter people, it need better people. Enjoy the lecture!

Abbreviations

COL1	*CONSTANT-LIKE 1*
COL2	*CONSTANT-LIKE 2*
FTL2	*FLOWERING LOCUS T-TERMINAL FLOWER1-LIKE2*
FR	Far red
LD	Long day
LED	Light emitting diode
MASL	Meters above sea level
PAR	Photosynthetic active radiation
R	Red
R:FR	Red: Far red ratio
SD	Short day
SOC1	*SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1*

Key-Words:

Picea abies, Abies lasiocarpa, R:FR ratio, shoot elongation, *FTL2*, *COL1*, *COL2*, *SOC1*.

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1. Introduction

1.1 Preface

Light is the main factor for plant development and the principal source of energy of the planet (Taiz and Zeiger, 2006). Plants, as sessile organism use this energy, to develop different structures that allow their survival and propagation. It is known that the light requirements may differ between species, in quality or quality (Arnott and Mitchell, 1982).

The fact that light is among the major environmental factors affecting the physiology of plants, makes it one of the most interesting variables to study. It is well known that in regions with marked seasons, such as in northern areas like Norway, the principal limitation for plant production is the low temperature and low light availability during large parts of the year (Dorming *et al*. 1968, Arnott and Mitchell 1982).

This is partially solved through the use of greenhouses, like is the case for the production of pot plants, vegetables, cut flowers and nursery plants**.** The increase in the flexibility of the agriculture due the use of partially controlled spaces, allows a better management of the resources. This could help to grow plants even when outdoors temperatures are below 0ºC, enabling an independence of the external weather, although this increases the costs, particularly due to the electricity consumption. It has already been shown that energy costs can be between the 22% and 44% of the total production costs in Norwegian greenhouses (Verheul, 2012)**.** Management of the light can be one of the most important variables for increasing the production, reducing costs and decreasing the environmental impact.

Long production times, like more that 18 months in tree nurseries, can be taken as an interesting study case. Particularly temperature and light quality, light quantity and photoperiod, will affect the final plant phenotype. Thus, optimising these conditions is important for reducing production costs while optimising the production time and plant quality.

1.2 Winter dormancy.

As sessile organisms, plants are forced to adapt to the different seasons. Perennial plants cease their growth before the winter and resume growth in the spring. Such species commonly enter winter dormancy characterised by lack of cell divisions in the meristems, allowing them to overcome harsh weather conditions (Taiz and Zeiger, 2006).

An important process in perennial plants is the sensing of the decrease in temperature and the change in the light conditions at the end of the growing season. Through this, plants are able to prepare for the subsequent months by increasing their dry matter, changing their composition of lipids and several other compounds (Garner and Allard, 1923; Nitsch, 1957;

Lee *et al.,* 2014). Normally different overlapping processes occur: growth cessation, bud set and cold hardening (Holliday *et al*., 2008). Together, these processes are necessary for initiating winter dormancy. The plants then prepare for the winter and attain cold hardiness. Being in an dormant state will allow survival during the cold period, followed by onset of normal growth in the spring. (Nitsch, 1957).

Dormancy can be divided in three principal types, depending of what is causing the dormancy. If the environmental conditions do not allow growth, plants are in a state of ecodormancy. If the lack of growth depends on an internal factor, it is called endodormancy, and if the reason for growth inactivity is due to a factor outside the tissue in question but resides in another plant part, it is called paradormancy (Lang *et al*., 1987).

Several experiments have shown that multiple plants under unfavorable environmental conditions enter winter dormancy with the formation of winter buds. Some species form buds even in the most favorable conditions, indicating that these species have an internal regulation that is independent of the environmental conditions. The behavior of such species is thought to be dependent of endogenous signals (Taiz and Zeiger, 2006; Olsen 2010).

The dormancy is considered to be primarily triggered by the change in the light conditions, i.e. the photoperiod and light quality (Garner and Allard, 1923; Juntilla, 2007; Olsen, 2010). Gradual decrease in the mean temperature is also an important factor which has been shown to modify the responses to the light parameters (Tanino *et al.*, 2010). It has been shown earlier that several species such as Norway spruce (*Picea abies* (L.) Karst), require a certain minimal day length and a certain amount of far-red light in the spectrum as well as a minimal temperature to grow and avoid winter dormancy (Nitsch 1957; Dormling *et al*., 1968; Mølmann et al. 2006).

1.2.1 Temperature sensing

Until now, no specific sensors for the changes in temperature have been found. *i.e.* that could alter growth or start the development of cold hardiness, also called cold acclimation. It is well known that cold hardiness is a result of a complex set of chemical reactions that require a prolonged exposure to low but non-freezing temperatures at the onset of winter to allow a decrease of the water content and a state of hardiness (Levitt, 1980).

This involves multiple reactions starting at the cellular level where the membrane fluidity is strongly influenced by low temperatures. This affects the calcium influx that have been considered one of the principal signals for cold hardening (Welling and Palva, 2006).

In alfalfa, this change in the ion channel activity and movement of calcium to the cytosol increase the expression of certain *CAS* (*COLD ACCLIMATION SPECIFIC*) genes (Monroy and Dhidsa, 1995). At the chemical level there is a decrease in enzyme functionality that finally affects other physiological parameters such as the water uptake, ATP production, CO_2 fixation, stomata opening and photosynthesis. These changes in turn, affect growth and result in several events leading to cold adaptation (Taiz and Zeiger, 2006, Örvar *et al.*, 2000).

1.3 Light

1.3.1 Quality, Quantity and Photoperiod

In 1923 Garner and Allard were among of the first ones to describe effects of photoperiod in plants, with some plants requiring longer days to change from vegetative growth to reproductive growth than others. Later this effect was shown to be very marked also in trees, with many species forming buds and getting ready for the winter under short days (SD; Nitsch, 1957). It has also been shown that higher irradiances produce shorter plants and that lower irradiances also induce bud formation with each species having its own requirements of light (Arnott and Mitchell, 1982).

A wide range of studies have shown how photoperiod, irradiance and light quality affect the growth of plants. The introduction of low cost technologies *i.e*. light emitting diodes (LED) that allow the use of monochromatic lights, have increased the light quality studies in recent years (Bourget, 2008).

One of the most studied light quality parameters is the proportion of red (R) and far red (FR), also called the R:FR ratio (R:FR), present in the spectrum, which in a range of plant species affects elongation of hypocotyls and stems (Morgan and Smith, 1979).

Morgan and Smith (1979) showed, that sun adapted plants increase elongation exponentially in environments with high levels of FR, whereas shade adapted plants will not react to changes in the R: FR. It is important to mention that although Morgan and Smith (1979) did all their experiments with herbaceous plants, this has also been shown in angiosperms and gymnosperms (Håbjørg 1972, Clapham *et al.* 2002). Also, combinations of R and FR sustained shoot elongation and prevented bud set better than just FR (Mølmann *et al.*, 2006). It has been shown that the requirement for R and FR light changes along a latitudinal gradient with northern populations requiring higher levels of FR (Clapham *et al*., 2002; Mølmann *et al*. 2006; Opseth *et al.* 2016). These adaptations appear to work as a strategy to prevent growth during winter (Olsen, 2010)

1.3.2 Phytochromes and Cryptochromes

The light is sensed in plants by several light sensors that include among others phytochromes and cryptochromes, that can sense the R:FR and the amount of blue light, respectively (Bae, 2008).

Phytochromes are proteins consisting of two apoproteins linked by covalent bounds and a phytochromobilin, where two forms have been described: The P_r form that absorbs R light with peak absorption at 660 nm, by which it is transformed to the P_{fr} form which is regarded the active form. P_{fr} absorbs FR light with peak absorption at 730 nm, by which it is converted back to its original form. This can also happen in darkness and is called dark reversion (Bae, 2008).

It has been shown that P_{fr} can also be phosphorylated which results in inhibition of P_{fr} action without changing its form (Kim *et al.* 2004). The phosphorylated P_{fr} could be used for subsequent dark reversion, degradation or dephosphorylation for light responses (Bae, 2008).

An estimation of the phytochrome photoequlibrium (PPS), *i.e.* the proportion of the P_r to the P_{fr} form, is possible to calculate using the light spectrum and the absorbance of both phytochrome forms. The proportion of P_r of total P_{r+fr} in a plant under normal solar radiation is usually at a value of 0.6 (Sager *et al*., 1988).

This change from one phytochrome form to another is result of a photoisomerazation where functional groups at the double bond between C15 and C16 changes configuration of the protein from a C15-Z, *anti* to C15-E, *anti* (also called cis and trans; Bae, 2008). Transition of the P_r form to P_{fr} allows the passage of P_{fr} from the cytoplasm to the nucleus where it can interact with other genes. In *Arabidopsis thaliana* five phytochromes has been described called PHYA, PHYB, PHYC, PHYD and PHYE, whereas it has been found that some trees such as Norway spruce (*Picea abies*) have just three (PHYP, PHYN and PHYO) (Bae, 2008; Opseth *et al.* 2016).

The phytochromes can be divided into two groups: PHYA that is light labile and present in relatively high amounts in etiolated plants and the other phytochromes that are light stable. It is known that PHYB is the predominant form that can sense the R:FR and that this phytochrome plays overlapping roles with the other phytochromes. This have several ecological implications, and allows plants to know *e.g.* when the seeds can germinate under favorable conditions or makes plants able to regulate their different physiological processes in synchrony with environmental conditions (Taiz and Zeiger, 2006).

Phytochrome can interact with more than 20 proteins that allow the movement of phytochromes to the nucleus or its interaction with other genes in the nucleus. *e.g.* FAR-RED

ELONGATED HYPOCOTYL 1 (FHY1) and FHY-like (FHL). This promotes the translocation of PHYA to the nucleus, allowing the expression of different genes (Genoud *et al.,* 2008).

In *Arabidopsis thaliana* it is known that the P_r: P_{fr} cycle is regulated principally by 3 proteins. The reversion from P_{fr} to P_r is regulated by ARABIDOPSIS RESPONSE REGULATOR 4 (ARR4), the degradation of P_{fr} by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and the dephosphorylation of P_{fr} to the more active form of P_{fr} by PHYTOCHROME ASSOCIATED PHOSPHATASE 5 (PAPP5) (Bae, 2008).

It has been shown in *A. thaliana* that in darkness phytochromes can interact also with proteins inside the nucleus, called phytochrome-interacting factors (PIFs), that can inhibit the light response (Bae, 2008). Other proteins that activate the light response are degraded by COP1. These include LONG HYPOCOTYL IN FAR-RED 1 (HFR1), LONG HYPOCOTYL 5 (HY5) and LONG AFTER FAR-RED LIGHT (LAF1) (Bae, 2008).

In light the P_{fr} interacts with other proteins such as PHYTOCHROME KINASE SUBSTRATE (PKS1), which upon entering the nucleus with (PHYA) or without (PHYB) P_{fr} allows the degradation of PIFs and COP1. By this, accumulation and expression of HFR1, HY5 and LAF1 are allowed and a light response occur (Bae, 2008).

Cryptochromes are involved in a wide range of developmental processes, including photoperiod control, flowering, stomata opening and phototropism (Taiz and Zeiger, 2006). It has also have been suggested that cryptochromes work as sensors of irradiance (Mølmann *et al.,* 2006). In *A. thaliana* three *CRY* genes have been found (*CRY1*, *CRY2* and *CRY3*, also called *CRY-DASH*) (Brudler *et al.,* 2003), whereas in the conifer species Norway spruce, two orthologous genes have been reported: *PaCRY1* and *PaCRY2* (Opseth *et al.*, 2016).

Overlapping functions between phytochromes and cryptochromes have been reported, mainly due to the similarity of the wavelength sensitivity between these light sensors. Different experiments have demonstrated similar effects on the flowering or elongation, through application of R and blue light in different species (Ahmad *et al.,* 2002, Heo *et al,* 2003). Mølmman *et al.* (2006) and Opseth *et al.* (2016) showed that the blue light can delay bud set in Norway spruce, which is in line with other works that the increase in blue light has similar effects as higher irradiance or high R:FR in other species (Terfa *et al.* 2013).

1.3.3 Genetic, hormone and metabolic cascade.

Cooler and shorter days during fall will trigger a cascade of genetic events resulting in dormancy of the plants. In Norway spruce, this can start immediately after transfer to such

conditions where it can take between 2-4 weeks to develop a visible closed bud, depending on latitudinal ecotype (Olsen, 2010).

In *A. thaliana* 6 phosphatidylethanolamine binding proteins (PEBP) are known to play a crucial role in the control of flower induction by affecting several other proteins *i.e.*: FLOWERING LOCUS T (FT), TERMINAL FLOWER1 (TFL1), ARABIDOPSIS CENTRORADIALIS HOMOLOG, TWIN SISTER OF FT, BROTHER OF FT AND FTL1 and MOTHER OF FT AND TFL1 (Kobayashi *et al.*, 1999). Furthermore, high levels of CONSTANS (CO) at the end of the day stimulates the expression of the *FT* gene, through GIGANTEA (G1) (Suarez-Lopez *et al*., 2001).

In *Populus* the *FT* levels are reduced with reduced *CO* levels at the end of the day when bud set occurs under SD (Böhlenius *et al.* 2006). This suggests a link between the light receptors, the circadian clock and FT as a growth stimulator in this species (Olsen, 2010).

Gyllenstrand *et al*. (2007) studied 4 *FT*-like genes in Norway spruce (*PaFT1-4*) and discovered that bud set and bud burst are associated with an increase and decrease of the expression of *PaFT4* under SDs and long days (LDs), respectively. *PaFT4* have a daily pattern where its level increases during night and decreases during day and *PaFT3* works in the opposite way, where SDs allow the expression of *PaFT4* and inhibit *PaFT3* (Gyllenstrand *et al*., 2007). This work also suggests that *PaFT1*, *PaFT2* or *PaFT4* could participate in the control of vegetative bud set. Olsen (2010) classified this more as a *TFL1*-like gene due that it apparently acts as a growth inhibitor, which was later demonstrated in Norway spruce plants expressing the gene under control of an inducible promoter (Karlgren *et al.* 2013). Asante *et al.* (2011) found that *PaTFL1* expression increased during the first 6 days of SD exposure and there was also an increase during 20 days of SD exposure, of a *CCH-TYPE FINGER* (*PaCCCH*) and *C- REPEAT BINDING FACTOR 2 and 3* (*PaCBF2 & 3).*

Nystedt *et al*. (2013) showed that in Norway spruce there is a lack of an *FT*-like gene like the one present in *Populus* and the *PaFT4* and *PaTFL1* correspond to the same gene, later called *PaFTL2* (Karlgren *et al.,* 2011).

Similar results have been found for *Pinus sylvestris* where expression of *PsFTL2*, an homolog of *PaFTL2* in Norway spruce, increased during bud set and decreased during bud burst (Avia *et al.*, 2014). No such gene has been found in *Populus*, where higher levels of *FT* correlate with active shoot elongation (Olsen, 2010).

Two *CO-LIKE* (*COL*) genes has been found in Norway spruce that might interact with the *FT*/ TFL genes: *PaCOL1* and *PaCOL2* (Holefors *et al*. 2009), but this has not been demonstrated. These genes show decreased transcript levels under SD-induced growth

cessation, as compared to under LD. They also show daily fluctuations with increase and decrease during the day and night, respectively.

Opseth *et al*. (2016) reported an accumulation of *PaFTL2* and a reduction of *PaCOL1*, *PaCOL2* and *PaSOC1* levels under SD, R or blue light exposure, compared to LD or FR light exposure. This occur before visible morphological changes associated with bud set.

SD induce a reduction in gibberellin levels and increase of abscisic acid together with the bud formation. However, it is not known how the the different proteins mentioned above trigger these processes. In seeds it is known that PIFs/ PILs can also inhibit the biosynthesis of GA, by inhibiting two specific GA biosynthesis genes (*GA3ox1* and *GA3ox2*) and activating a catabolic gene inactivating the active form of GA (*GA2ox2*) (Oh *et al.,* 2007). PILs can also activate the expression of the GA signaling-related *DELLA* genes that are down-regulated in response to GA (Oh *et al.,* 2007).

In higher plants such as Poinsettia (*Euphorbia pulcherrima*) application of R light at the end of the day was shown to reduce the levels GA by 29% and ABA by 19%, correlating with reduced height compared to under FR light (Islam *et al.,* 2014). In hybrid aspen (*Populus tremula x tremuloides*) Olsen and Junttila (2002) showed that FR light applied at the end of the day was able to increase the plant height as compared to R light. The application of FR light at the end of the day did not significantly stimulate the level of GA, but appeared to affect the tissue responsiveness to GA. Inhibitors of the GA biosynthesis also resulted in bud set even in LD, when the night temperature was low (Mølmann *et al.,* 2005).

In populus, DELLA factors increase their expression under SD, *i.e.* the two genes: *GA-INSENSITIVE* (*GAI*) and *REPRESSOR OF GA l-3* (*RGA*) (Ruttink *et al*. 2007, Druart *et al*. 2007).

The metabolic changes that occur during bud formation in Norway spruce has been studied by Lee *et al.* (2014). One of the most notable metabolic changes after transfer of the plants from LD to SD was a reduction of ascorbate levels and increased levels of some energy- related components. Ascorbate was reduced quickly after a LD to SD transition, where this is used as an antioxidant resulting in H_2O_2 degradation. The energy-related compounds started to increase together with the amino acids. Lee *et al.* (2014) reported growth cessation under SD after about two weeks and terminal winter bud after 21 days. After 8 weeks there was a high pool of ABA, antioxidants, flavonoids, sugar, lipids in the well developed winter buds.

1.4 The selected species

1.4.1 Abies lasiocarpa

Abies lasiocarpa (Hook.) Nutt. also known as subalpine fir, is a conifer tree from the *Pinaceae* family originating from the west cost in North America. It is distributed from Alaska (64º N) to New Mexico (32ºN), and normally found from 600 to 3600 meters above sea level (MASL) (Foiles *et al*., 1990).

Being a mountain species, subalpine fir is adapted to a wide range of temperature conditions that can change between the -45ºC in winter to 35ºC in summer (Foiles *et al*., 1990). It has been found that subalpine fir can grow under high levels of light. During the first year growth could be close to 2.5 cm, with a similar growing rate during natural establishment (Alexander *et al.* 1984). Light-saturated net photosynthesis (A_{max}.) was shown to be close to 0.5 umol CO_2 m^{-2} s^{-1} at 240 μmol of photosynthetic active radiation (PAR) for the same year (Cui and Smith, 1990).

The slow growth rate of subalpine fir forces the nurseries to grow these plants for at least two years before selling them, normally with a phase of dormancy during this period. Also, the provenance will affect the growth rate (Jensen *et al.* 2013).

During the last 100 years, subalpine fir has been introduced to Norway with an increasing interest as a Christmas tree due to its shape and color. Thus, there is increasing economical importance of this species (Kvaalen *et al*. 2005).

Previous studies have shown bud formation under irradiances of 100- 200 umol m^{-2} s^{-1} and different light qualities as extension of the photoperiod during the night in *Abies lasiocarpa*. These studies have failed to avoid the bud formation, but have shown that this is dependent of the light quality and might interact with the temperature (Aas 2015, Rindedal 2015).

1.4.2 Picea abies

Norway spruces is one of the main species in the boreal forest in Europe and is found from the west coasts of Scandinavian countries, across some of the eastern countries of Europe and entire Russia, ranging from latitude 72ºN to 41ºN and from sea level to 2300 MASL (Jansson *et al.,* 2013). In addition to its great ecological significance, its principal use is for construction timber and in the paper industry but it is also used as a decorative tree in parks and gardens and as a Christmas tree.

As an important tree in Europe, Norway spruce is part of breeding programs and its genome has been already sequenced (Nytedt *et al*., 2013). For a more efficient management thorough genetic and physiological understanding is required (Klápště et al., 2007).

It has been shown that under SD this species will cease its growth, form dormant buds and increase the frost tolerance (Olsen, 2010). Mølmann *et al.* (2006) showed that day extensions with FR or R:FR 1 can keep up the growing phase in seedlings of Norway spruces. This could allow a faster production of such trees. In addition, the temperature plays an important role in the bud formation (Aas 20015). Although effects of R and FR on elongation growth has already been shown in Norway spruce (Mølmann *et al.* 2006, Opseth et al. 2016; Aas 2015), the effect of a mixture of different proportions of these light qualities is still unclear.

1.5 Aims

The principal aim of this study was to understand the effect of the temperature and light quality on growth and development of the two selected species, subalpine fir and Norway spruce

The specific aims were to:

- Understand the effect of the R:FR provided by LEDs as day extension and PPS on shoot elongation of Norway spruce and subalpine fir.
- Understand the interactive effect of the temperature and light quality on shoot elongation and bud formation.
- Explore if formation of buds could be avoided in subalpine fir.
- Increase the elongation in Norway spruce and subalpine fir through the use of light quality treatments and temperature.
- Evaluate the correlation in Norway spruce between the plant response and expression of *FTL2*, *COL1*, *COL2* and *SOC1*, thought to be involved in control of growth and/or bud set.
- Explore the use of FR LEDs as replacement of incandescent lamps to modify the R:FR during the light phase without affecting the normal behavior of the plants.

2. Materials and methods.

2.1. Plant materials and pre-growing conditions

2.1.1 Plant materials

Seeds of Norway spruce (*Picea Abies (L.)* H. Karst) from the provenance CØ1 59ºN latitude, 0-140 metre above sea level (MASL) Halden, Østfold, Norway (Skogfrøverket, Hamar, Norway) and Subalpine fir *(Abies Lasiocarpa (Hook)* Nutt.) from the provenance CAN10 from 53.39ºN latitude and 122.23 ºW longitude, 1000-1200 MASL (Skogfrøverket, Hammar, Norway) were used in the experiments.

2.1.2 Pre-growing

Two seeds were sown in each pot of 5.5 x 5.5 x 4.5 cm (Vefi, Drammen, Norway) to ensure the presence of at least one plant per pot due the germination rate of 60% and 70% for Subalpine fir and Norway spruce, respectively. These were sown in a 3:1 medium of peat and perlite (S-Jord, Hasselfors, Oslo, Norway).

Before sowing, the seeds of Subalpine fir were stratified by placing the seeds for three weeks in petri dishes with moist filter paper in darkness at 4ºC, whereas the seeds of Norway spruce do not require stratification.

The pots were placed at 12 trolleys of 50 x 50 cm under controlled conditions in a growth chamber at the Norwegian University of Life Science (NMBU, Ås, Norway).

2.1.3 Pre-growing conditions

During the pre-growing period of 7 weeks, a photoperiod of 24 hours was set (LD). This ensures a photoperiod longer that the critical that allows continued growth. A photosynthetic photon flux density (PPFD) of 250 umol m^{-2} s^{-1}, as measured by a Li-Cor (Quantum/Radiometer/Photometer, Model LI-250 light meter, LI-COR, Lincoln, Nebraska, USA), was applied with a quartz metal halide lamps (HPI) as the principal source of light (Master HPI-T Plus 400W/645 E40 1SL, Phillips, Amsterdam, Nederland) at 1 m of distance. A red to far red ratio (R:FR) of 1.8 was achieved using incandescent lamps (Narva 60W, Germany and Philips Electronics, Amsterdam, Netherlands). This was measured by a R:FR sensor (Skye instrument, Llandrindod Wells, UK).

The seedlings were grown in a constant temperature of 18ºC (± 1ºC). The leaf temperature was measured to 23ºC by an infrared camera-pistol (InfraCAM, Flir systems, Nashua, USA). The relative air humidity (RH) was adjusted to 76%, corresponding to a water vapour pressure deficit of 0.5 kPa.

The plants were watered as required and fertilized twice a week during the pre-growing and during the experimental phases with a nutrient solution containing calcium nitrate, ammonium nitrate and Kristalon (Yara, Oslo, Norway) with an electrical conductivity of 1.5. Due to a suspected presence of fungus during the first experiment in the third week of the pre-growing, a fungicide was applied (Rovral, BASF, Manheim, Tyskland).

2.2 Experimental design and conditions

2.2.1 Light phase conditions.

During the two experiments performed high pressure sodium lamps (HPS; Lucalox 400W, General electric, New York, USA) were used as a source of light during the light phase (from 9 AM to 9 PM), at an irradiance of 260 umol m^{-2} s^{-1}, as measured in the middle of each trolley. One HPS was mounted above each trolly. To ensure sufficient FR light in the spectrum of the main light phase, the R:FR was modified to a R:FR of 2.5 using five FR LEDs for each trolley, with a bandwidth between 680 nm and 750 nm and a maximum at 725 nm.

2.2.2 Temperature and day-extension treatments

In the two experiments five different subsets of plants were exposed to five different R:FR in growth chambers with controlled conditions. The different R:FR were given on individual trolleys separated by plastic curtains and under two temperatures: 18ºC and 24ºC in separate growth chambers. During the first experiment the actual temperatures (± standard deviation (SD)) of the rooms were on average 17.86 (±0.04) and 23.83 (±0.07) ºC. In the second experiment the temperature (± SD) were on average 17.95 (±0.09) and 23.9 (±0.08) ºC

A short day treatment (SD) was also established as a control treatment. During the first experiment different R:FR were tested, where as for the second experiment different R:FR were used to achieved uniformly distributed phytochrome photostationary state ratios (PPS) (Table 1). The same amount of energy (7 W m^{-2}) was applied for 13 h as an extension of the 12 h main daily light period (described below). An overlap of half an hour during the morning and half an hour during the night with the main light phase was used to ensure 24 hours of lighting. The energy of the day-extension light was based on previous experiments where this energy level was shown to prevent bud formation in latitudinal ecotypes of Norway spruce and white birch (*Betula pubescens*) originating from 59-66°N (Tsegay *et al.,* 2005; Mølman *et al.*, 2006; Aas, 2015).

Equal water vapour pressure deficit of 0.5 kPa for both temperature treatments was the target RH applied, equivalent to 0.76 and 0.83 RH in the 18ºC and 24ºC treatments,

respectively. The RH (±SD) was 0.63 (±0.13) and 0.69 (±0.023) respectively in the first experiment and 0.72 (±0.01) and 0.79 (±0.023) in the second experiment. In both cases the RH was lower than expected and statistically different ($p<0.05$). In the first experiment the RH was not constant along the experiment (Appendix 4). In contrast to the first experiment there were not such fluctuation of RH in both rooms for the second experiment (Appendix 5).

Phillips LED (Philips GreenPower LED research module, Phillips, Amsterdam, Netherlands) where used as a source of the R and FR radiation during the day extension period (Figure 1). The wavelength of these LEDs were between 620 and 690 nm with a peak at 660 nm for the R light, and between 680 and 750 with a peak at 725 nm for the FR light, as shown in Figure 1. The irradiance and ratio between these light qualities where adjusted changing the height of the lamps and covering some of the R and FR LEDs in each lamp with aluminium foil.

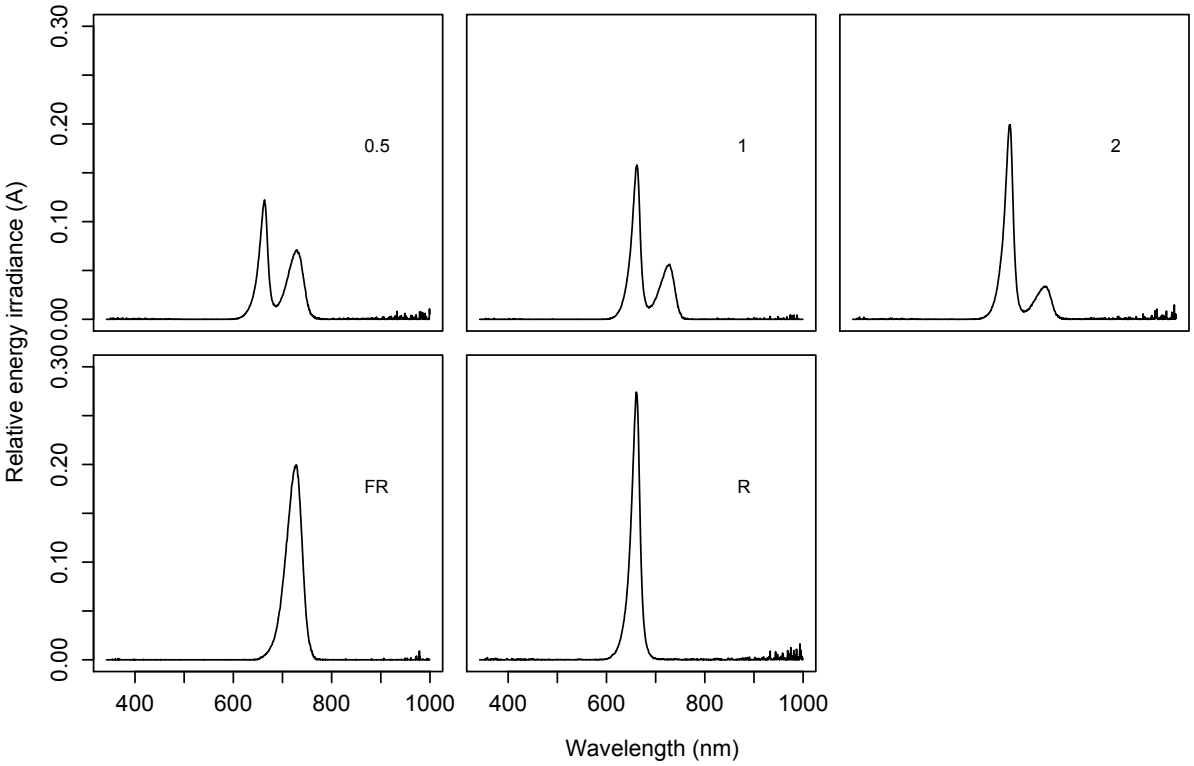


Figure 1: Relative energy irradiance for each wavelength (nm) of the different light treatments provided by light emitting diodes during the photoperiod extension of 13 hours in the first experiment with Norway spruce and subalpine fir. The treatments correspond to different ratios of far red (FR) and red (R) light. All treatments had the same amount of energy (7 W m^{-2})

The different combinations of R:FR, were applied under the two mentioned constant temperatures, resulting in a total of 12 treatments in each experiment, as shown in table 1. The leaf temperature during the day was also measured by an infrared camera (InfraCAM, Flir systems, Nashua, USA) and was 20ºC and 25ºC for the two treatments, respectively. The increase in the leaf temperature during the pre-growing and the experimental treatment, were mainly due the high competence of the plants to save heat. The leaf temperature variation between the pre-growing and the experimental periods was mainly due to the different light sources used (HPI plus incandescent lamps vs HPS), which in all the cases were mounted at a distance of approximately 70 cm from the plants.

Tab 1. Treatments applied during each experiment investigating effect of day extension with different red far-red ratios (R:FR) or phytochrome photostationary state (PPS) and temperature on growth and bud formation in Norway spruce and subalpine fir, as measured in the middle of the trolleys at the beginning of first and second experiments.

		Light quality treatment: R:FR (PPS)	
Treatment code	Temperature	First experiment	Second experiment
T1-1	18ºC	Short day (SD)	Short day (SD)
T2-1	24ºC		
T1-2	18ºC	Far red (FR) (0.24)	Far red (FR) (0.24)
T2-2	24ºC		
T1-3	18ºC	0.5 (0.72)	0.1 (0.4)
T2-3	24ºC		
T1-4	18ºC	1 (0.78)	0.23 (0.56)
T2-4	24ºC		
T1-5	18ºC	2 (0.81)	0.5 (0.72)
T2-5	24ºC		
T1-6	18ºC	Red(R) (0.88)	Red(R) (0.88)
T2-6	24ºC		

For each treatment the PPS was calculated using the photoconversions proposed by Sager *et al*. (1988), using the following formula:

$$\phi(PSS) = \frac{\sum_{300}^{800} N\lambda * \sigma_{r\lambda}}{\sum_{300}^{800} N\lambda * \sigma_{r\lambda} + \sum_{300}^{800} N\lambda * \sigma_{fr\lambda}}$$

Nλ corresponds to the wavelength of the light, $\sigma_{r\lambda}$ to the photoconversion of P_r to P_{fr} and $\sigma_{fr\lambda}$ to the photoconversion of P_{fr} to P_r.

2.3 Measured parameters

2.3.1. Morphological parameters.

In both experiments the height of 18 plants for each treatment was measured once a week, as total height from the edge of the pot to the apical meristem. The increase in height (cumulative growth) was calculated. At the end of the experiments, 5 randomly selected plants were taken from each treatment for dry weight (DW) measurements. For this, the plants were dried for two days at room temperature before removing them from the pots at room temperature. After this the plants were divided in two: shoot and roots. The roots where washed in cool water to remove any soil remaining. Both parts of the plants were placed separately in paper bags and dried at 72ºC during 1 week. Finally, the dry weight was measured using a scale (PG503-S, d=0.001 g; Metter Toledo, Columbus, USA) and the shoot: root ratio was calculated.

The bud development was registered three times per week using codes where growing plants without buds were coded as 0, light green buds was recorded as 1, green bud as 2 and brown buds as 3.

At the beginning of both experiments subalpine fir had already formed buds in close to 25% and 18% of the plants, respectively, with mainly light green buds (stage 1). These were equally distributed along the different treatments.

2.3.2. Studies of gene expression.

2.3.2.1 Sample collection

Norway spruce shoot tip tissues were sampled from different treatments to assess the effect of light quality and temperature treatments on the expression of *FLOWERING LOCUS T-TERMINAL FLOWERI-LIKE 2* (*PaFTL2*), *SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*PaSOC1*) and two *CONSTANS-LIKE* genes (*PaCOL1* and *PaCOL2*). Four samples per treatment from four different treatments (SD, R, FR and R:FR 1) were collected in a time course at three different time points, corresponding to the beginning of the experiment (Day 0), after seven days (Day 7) and at the end of the experiment (Day 48). Each sample consisted of 3 shoot tips from different plants. The upper 5 mm of the shoot tip and needles were splitted in two samples: needles and shoot that were saved in Eppendorf tubes, which were placed in liquid nitrogen. The samples were kept at -80ºC for later analysis in the laboratory.

2.3.2.2 RNA extraction and purification

RNA was extracted and purified using the the Masterpure™ Plant RNA Purification Kit (Epicentre, Madison, USA) and the PureLink™ RNA Mini kit (Thermo Fisher Scientific, Massachusetts, USA), according to the product datasheets as follows.

2.3.2.2.1 Lysis of the tissue samples.

In order to perform RNA extraction from the tissues, the shoot tip tissues were crushed in a mixer mill (MM301, Retsh in Haan, Düsseldorf, Germany) at 25 Hz during 1 min with beads of 5 mm, cooling the collecting vials with liquid nitrogen.

Thereafter, 600 μL of Plant Tissue and Cell Lysis Solution, 6 μL 100 mM DTT and 1 μL proteinase K were added. These were mixed during 1 min at room temperature and incubated at 56ºC during 15 min, with mix every 5 min during 30 sec. The vials were centrifuged for 5 min at 12000 x g at room temperature. The supernatant of each sample was transferred to a 2.0 ml Eppendorf tube and placed at ice for 3-5 min.

2.3.2.2.2 Precipitation of Nucleic Acids.

250 μL MPC protein precipitation reagent were added to the sample and mixed vigorously for 10 sec. The samples were centrifuged at 4ºC during 10 min at 12000 x g and the supernatant extracted and the pellet discarded. 500 μL isopropanol were added and mixed with the supernatant and the vials centrifuged for 10 min at room temperature and 12000 x g and then the supernatant was discarded.

2.3.2.2.3 RNA quantification and removal of contaminating DNA from RNA preparations

RNA levels were checked by a NanoDrop (ND-1000 Spectrophotometer, NanoDrop Products, Wilmington, USA), before application of a DNase treatment to remove any DNA contamination in the samples.

The DNase treatment was done by mixing the nucleic acid from the previous step with 200 μL DNase I solution (173 μL RNase-Free water, 20 μL 10X DNase Buffer, 5 μL RNase-Free DNase I and 2 μL RiboGuard RNase inhibitor).

Thereafter the samples were incubated for 10 min at 37ºC with 200 μL 2X T and C lysis solution that was added by vortexing for 5 sec. Then 200 μL MPC protein precipitation reagent were added by vortexing 10 sec and left on ice for 4 min. This solution was centrifuged at 10 min at 4ºC at 12000 x g and the supernatant was transferred to a clean micro centrifuge tube

with 500 μL isopropanol and mixed by inverting the tubes 20 times. The samples were then centrifuged for 10 min at 4ºC at 12000 x g. The isopropanol was removed and the remaining pellet was washed twice with 70% ethanol, removing the residual ethanol after quick centrifugation.

The RNA was resuspended in 20 μL RNase-Free water, and 1 μL RiboGuard RNase inhibitor added.

2.3.2.2.4 DNase treatment and purification of the samples

An aditional DNase treatment was done to remove remaining DNA, adding 5 μL 10X TURBO DNase buffer, 1 μL TURBO DNase and 4 μL H_2O to the previous 20 μL. These were incubated at 37ºC during 30 min, mixing every 10 min. After this, the solution was incubated for 5 min with 5 μL DNase inactivation reagent, mixing occasionally. The samples were then centrifuged at 13200 rpm for 1.5 min and the supernatant transferred to new Eppendorf tubes and diluted again in 40 μL RNa-Free water. 125 μL RNA (40 μL RNA plus 85 μL water from the kit), 125 μL Lysis buffer (from 1ml Lysis Buffer + 10 μL 2-ME) and 125 μL 100% EtOH were then added. Then solution was transfered to a RNA Spin cartridge and centrifuged at 12000 x g at room temperature for 20 sec. Discarding the flow solution, the filter's tube was rinsed with 500 μL Wash Buffer II with ethanol by centrifuging this at 12000 x g for 20 sec at room temperature. Again the flow through was discarded and the process repeated. After this the spin cartridge was centrifuged at 12000 x g for 1 min at rom temperature. The flow through and the tube were discarded, inserting a new collector tube (RNA Recovery tube). The RNA was eluded with 40 μL DEPC- treated water after 1 min of incubation, followed by centrifuging this for 2 min at 12000 x g at room temperature. The samples were then stored at -80ºC, divided in two tubes, 20 μL for RNA purification and the remaining as a backup. The RNA levels were checked again, using the NanoDrop spectrophotometer.

2.3.2.2.5 cDNA synthesis.

cDNA was synthesized from the isolated RNA using a mix of reverse transcriptase (Superscript VILO cDNA Synthesis, Life Technologies, Thermo Fisher Scientific) and RNase free water. The amount of RNA (ng ul-1) for each sample was calculated using the following equation:

$$\frac{1000\ ng\ ul^{-1}\ RNA}{ul\ RNA\ concentration} = \ amount\ of\ RNA\ (ul)$$

A total volume of 20 μl was used for reverse transcriptase (rt) samples and 10 μl for the negatives controls without rt (-rt). For the rt samples, 6 μl of reverse transcriptase mix was mixed with the required amount of RNA for each sample, using the previous formula, and water was added up to 20 μl. For –rt, 4 μl of the reverse transcriptase mix was incubated at 65ºC for 10 min to denature the rt. After this, half of the required amount of RNA for the rt samples was added to the –rt samples and RNase free water was added, up to 10 μl.

The samples were incubated in a PCR machine (DNA Engine Tetrad Pelitier Thermal Cycler, Bio-Rad Laboratories, Hercules, California, USA) with the following program: 10 min at 25ºC, 50 min at 42ºC and 5 min at 85ºC. After this 80 and 40 μl of RNase free water were added to the rt and negative control samples.

Thereafter, the presence of any remaining DNA in the samples was checked used a real time PCR (qPCR) machine (Applied Biosystem, 7500 Fast Real-Time PCR-system, Life Technologies, Thermo Fisher Scientific) where –rt was used in each sample as a control. 2 μL of the rt and –rt templates were mixed with 7 μL of RNa-Free water, 10 μL SYBR green (SYBR Selected master mix, Life technologies, Thermo Fisher Scientific) and primers of a housekeeping gene (0.5 μL of α-tubulin L and 0.5 μL α-tubulin R). Then the plate with the samples was subjected to 2 steps cycling: a first step of 50ºC for 2 minutes followed by 95ºC for 2 minutes and a second step of 40 cycles of 95ºC for 15 seconds and 60ºC for 1 minute. The florescence of the samples was measured along the different steps and cycles.

2.3.2.2.6 Quantitative polymerase chain reaction (qPCR)

The levels of *PaFTL-2*, *PaSOC1*, *PaCOL1* and *PaCOL2* gene were measured through qPCR using the same protocol that for the check of remaining DNA, but without using the –rt samples. Three different housekeeping genes were used as internal reference genes *α-tubulin, actin* (*PaAct*) and *elongation factor 1 alpha* (*PaEF1*). Four technical replicates were made for each gene.

2.3.2.2.7 RQ-values calculated

With the threshold cycle (CT) from the results of qPCR the relative quantification (RQ) was calculated. The average of transcript levels of the housekeeping genes was used to normalise transcript levels of the target genes for each sample. The RQ was calculated using the following equation:

$$2^{-\left(\left(Ct(IntG\ s)-Ct(normal\ s)\right)-\left(Ct(IntG\ c)-Ct(normal\ c)\right)\right)} = RQ\text{intG}$$

where:

- IntG is the gene of interest
- Normal correspond to the housekeeping genes.
- S, corresponds to the sample of interest
- C, corresponds to the control samples. These were taken during LD, before the start of the experiment.
- RQintG corresponds to the transcript level of the gene of interest.

2.4 Statistical analysis.

2.4.1 General analysis.

To evaluate the effect of the light quality and temperature treatments on shoot elongation and bud set a two-way analysis of variance (ANOVA) was performed using a linear model and a generalized linear model, respectively. For the linear model, due to that the measurements were performed on the same plants in a time course which violate the assumption of independence, plant and time where treated as random effects. Crawley (2007) was used as a reference guide. The fitted models were the following for each respective model:

$$Y_{ijkl} = (\mu+\alpha_i) + \beta_i * X_{ijkl} + \gamma_j + \varphi_l + \lambda_{jl} + \varepsilon_{ijkl} \quad (1)$$

$$Y_{ijkl} = \exp((\mu+\alpha_i) + \beta_i * X_{ijkl} + \gamma_j + \varphi_l + \lambda_{jl} + \varepsilon_{ijkl}) \quad (2)$$

Where:

- μ corresponds to the general mean
- α_i is a **plant** specific constant where $\alpha_I \sim NID(0,\sigma^2_\alpha)$
- β_i is a plant specific random effect of the **week** where $\beta_i \sim NID(0, \sigma^2_\beta)$
- X_{ijkl} is the week number for observation ijkl
- γ_j is the general effect (fixed) of the light quality treatment, where j=1,2,3,4,5 and 6
- φ_l is the general effect (fixed) of the temperature treatment, where l= 1 and 2
- λ_{jl} is the interaction between the temperature and the light treatments for every j and l
- ε_{ijkl} is the non-predictable residuals or error for every plant-day-treatment combination.

- Y_{ijkl} is the response dependent of the treatment, temperature, plant and time where Y_{ijkl} $\sim NID(\mu+\gamma_{j+}\varphi_l+\lambda_{jl,},\sigma^2)$

For the model 2 the following parameter differ:

- Y_{ijkl} is the response dependent of the treatment, temperature, plant and time where Y_{ijkl} $\sim$ binomial (p_i, n_i), where $p_i = \exp(\mu+\gamma_{j+}\varphi_l+\lambda_{jl,})/(1+\exp(\mu+\gamma_{j+}\varphi_l+\lambda_{jl,})$

Following Crawley (2007) and Schwarz (2015), an analysis per date was performed. The following linear model and generalized linear model were fitted for each date point for the shoot elongation and bud set, respectively.

$$Y_{jkl} = \mu + \gamma_j + \varphi_l + \lambda_{jl} + \varepsilon_{jkl} \quad (3)$$

$$Y_{jkl} = \exp(\mu + \gamma_j + \varphi_l + \lambda_{jl} + \varepsilon_{jkl}) \quad (4)$$

The used symbols of the models are the same as mentioned above for model 1 and 2, respectively. When the interaction in model 3 and 4 was not significant, the model was evaluated without interaction.

For the general analysis and analysis by date of bud set, the analysis was also made as absence or presence of bud. The same models were used with a binomial distribution (Crawley, 2007).

2.4.2 Final state analysis.

The effect of the experimental treatments (light quality and temperature) on shoot elongation, dry weight and gene expression (Pa*FTL2, PaSOC1, PaCOL1 and PaCOL2*-transcript level) at the end of each experiment was compared using a two-way ANOVA using model 3. Also, an exponential version of model 3 was used to fit regression curves between the bud category or shoot elongation at the end of the experiment and the gene expression. For analysis of the bud status, branches and shoot: root ratio at the end of the experiment, a generalized linear model (model 4) was used. For the comparison within and between treatments and experiments Tukey's test was used as a post hoc analysis.

The statistical analyses were all made with the R (version 3.2.3; CRAN project) statistical software with a significance level of 95% ($p \leq 0.05$) set in all the analyses evaluating effect of the different variables. A post hoc Tukey test was done ($p \leq 0.05$) when required.

3. Results.

3.1 *Picea abies*.

3.1.1 First experiment.

3.1.1.1 Shoot elongation.

The effect of the temperature and light quality treatments on shoot elongation in a time course is shown in figure 2 A. The SD treatment resulted in growth cessation of all plants in both temperatures. On the other hand, the R-treated plants showed different response depending of the temperature. At 18ºC shoot elongation ceased after 40 days whereas as 24ºC this took close to 52 days. The light quality treatment resulting in the highest increase in shoot elongation depended on the temperature. At 18ºC and 24ºC, an average elongation growth of 9.1 and 11.8 cm was observed at R:FR 1 and 0.5, respectively. Overall, the R:FR 0.5 treatment at 24ºC showed the greatest shoot elongation. Shoot elongation at the end of the experiment shows the relative effects of the light treatments more clearly (figure 2 B). At 18ºC the difference between the treatments with FR light was generally smaller than at 24º C.

An ANOVA of the shoot elongation showed a significant interaction between light quality and temperature (table 2). Also, for comparison, an ANOVA for the final shoot elongation at the end of the experiment was performed and showed similar results to the analysis shown in table 2, with all factors and interactions being significant (appendix 6).

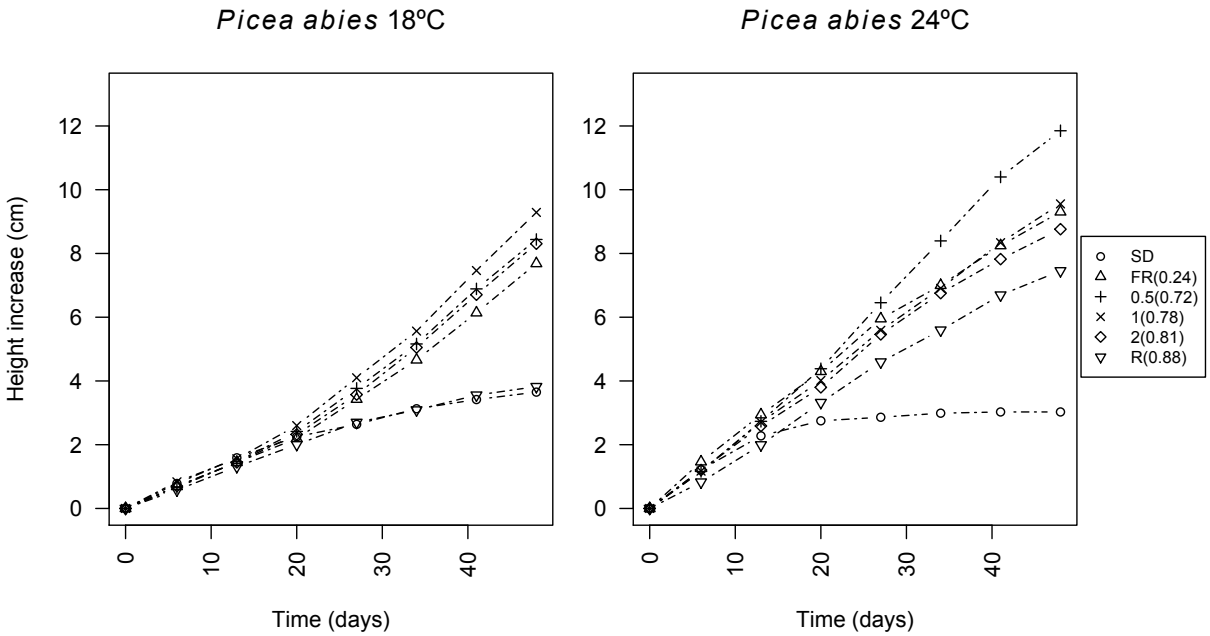
The p value of the interaction between the light quality and temperature treatments for each time point is presented in figure 3. After 27 days the interaction was significant and stayed stable until the end of the experiment.

3.1.1.2 Terminal bud set.

The effect of temperature and light quality treatments on bud stage development in a time course in the first experiment is shown in figure 4 A.

At both temperatures the first terminal buds in the SD treated plants were observed after 16 days. The only treatments that resulted in buds during the experiment were the SD and day extension with just R light. At 24ºC, the SD treated plants showed more rapid development of mature (brown) terminal buds than at 18ºC (3 vs 2.2). The R light treatment induced less formation of buds at 24ºC, compared to 18ºC (0.5 vs 1.8). Presence of buds independently of bud stage is shown in Appendix 7 and 8, where the effect of the treatments was similar as the results of the categorical analysis.

A)



B)

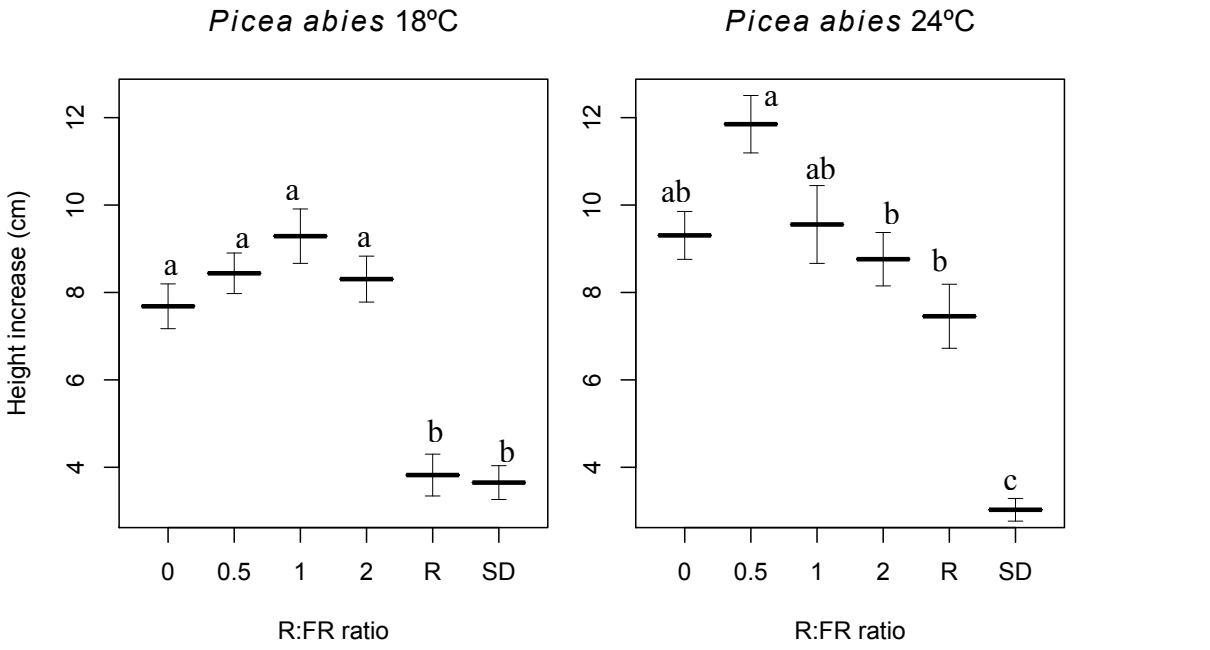


Figure 2: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) average height increase (cm) in a time course and B) final shoot elongation in *Picea abies* in the first experiment. The values represent the average ± SE of 18 plants. SD = short days without day extension, 0.5, 1 and 2 refer to R to FR ratios (R:FR) with their respective phytochrome photostationary state (PPS) in brackets, during the day extension. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey test.

Table 2: ANOVA for the linear model of the shoot elongation using the time and plant as random variables for the first experiment *Picea abies*.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	76.385	1	2.20E-16	***
Temperature	97.027	1	2.20E-16	***
Light treatment	39.507	5	1.88E-07	***
Temperature: Light treatment	35.563	5	1.16E-06	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

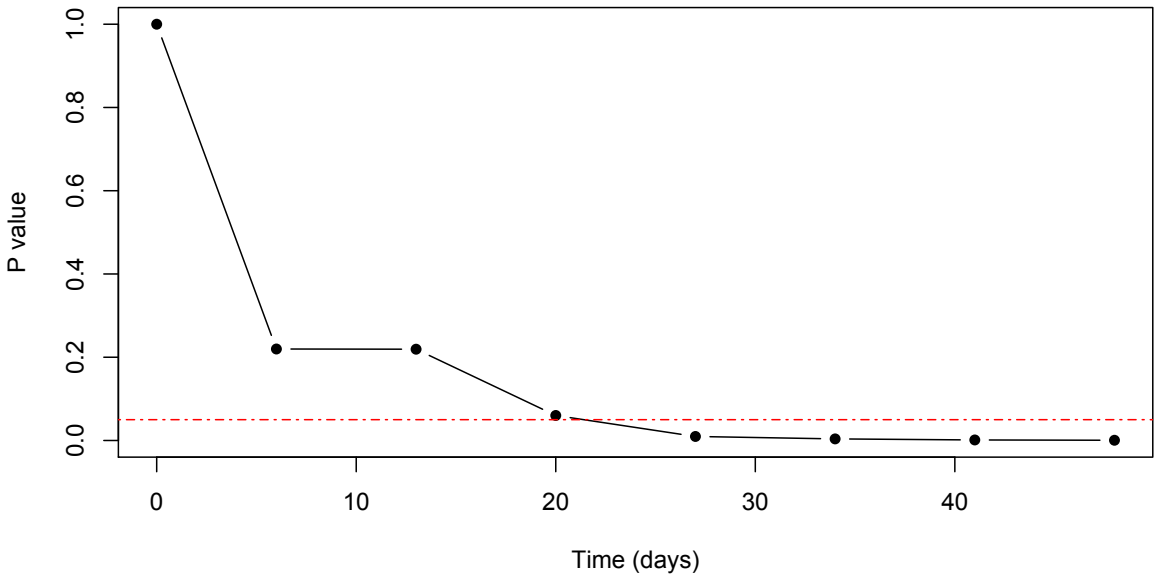
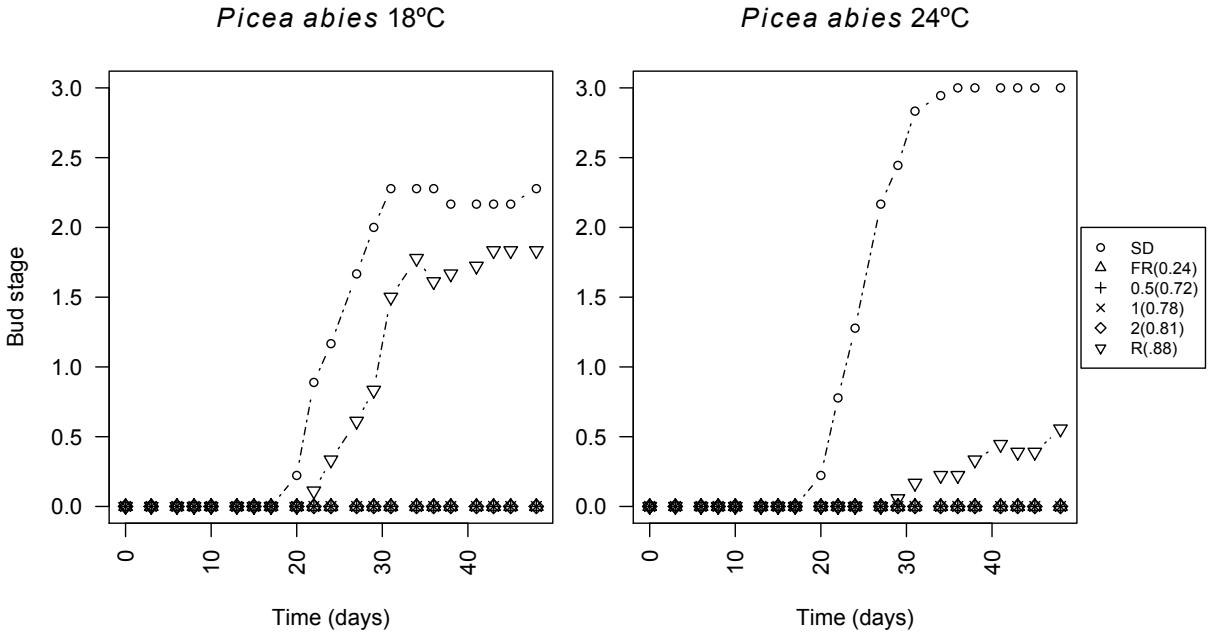


Figure 3: P values of the interactive effect of the light quality and temperature treatments on shoot elongation in *Picea abies* in a time course in the first experiment.

ANOVA of the bud classification is presented in table 3. Like for shoot elongation, there was an interaction between the temperature and light quality treatments. In contrast, an ANOVA for the presence of buds or not, without using categories, showed no significant interaction between the temperature and light quality treatments (appendix 9). When this interaction was removed the light treatments showed a trend of significance ($p = 0.059$) (appendix 10).

A)



B)

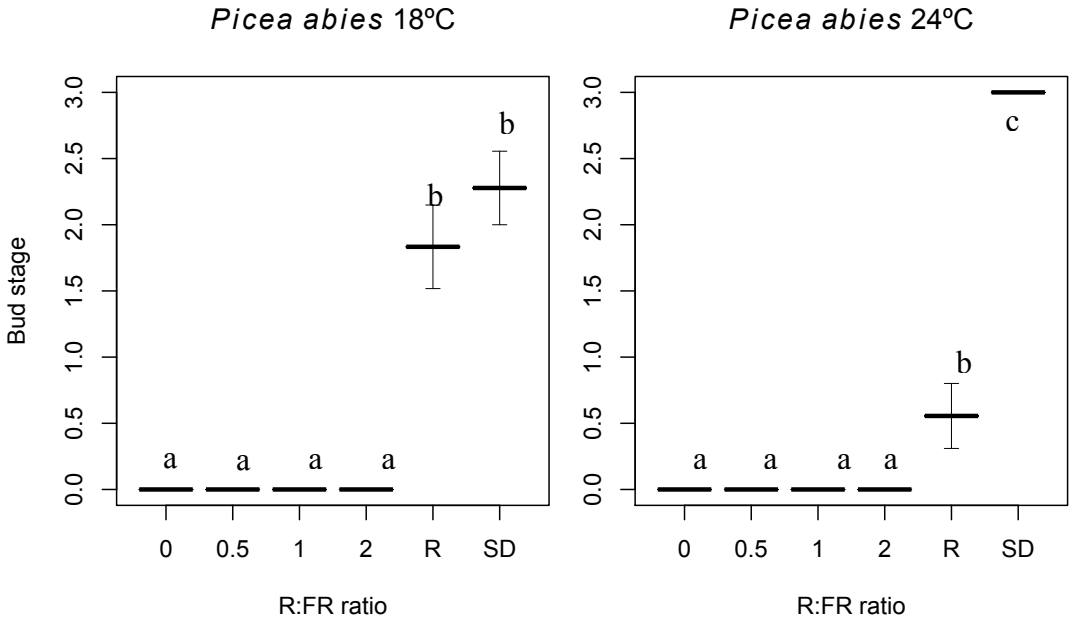


Figure 4: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) bud stage in a time course and B) final bud stage classification at day 48 in *Picea abies* in the first experiment. The values represent the average ± SE of 18 plants, where 0 denotes no presence of bud, 1 green bud, 2 brownish bud and 3 brown bud. SD = short days without day extension, 0.5, 1 and 2 refer to R to FR ratios (R:FR) with their respective phytochrome photostationary state (PPS) in brackets, during the day extension. Different letter indicates significant difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

Table 3: ANOVA for the generalized linear model of the bud stage classification in the first experiment with *Picea abies*. For this the plant and time where used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
(Intercept)	0.3081	1	5.79E-01	
Temperature	0.0002	1	9.90E-01	
Light treatment	43.282	5	3.24E-08	***
Temperature: Light Treatment	12.7265	5	0.02608	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVAs for the final bud category and presence of terminal bud after 48 days of treatments are shown in appendix 11 and 12. Similar to the analysis in table 3, these ANOVAs showed significant p values for the interaction of the temperature and light quality and for the light quality treatment only.

The p value of the interaction between light quality and temperature for the analysis of bud stage in a time course is presented in figure 5. Also a plot of the interaction analysis, for the presence or absence of buds was done (appendix 13).

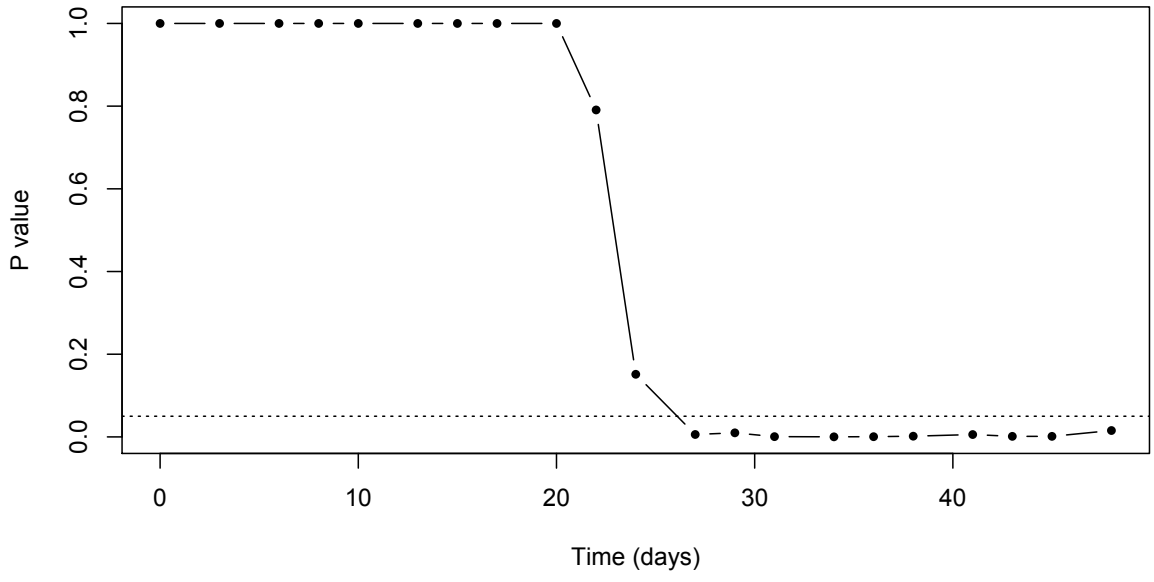


Figure 5: P values of the interactive effect of the light quality and temperature treatments for development of bud stages in *Picea abies* in the first experiment.

Similar to the situation for shoot elongation (figure 2), both bud stage and presence/absence of buds (figure 5 and appendix 13) showed significant interaction after 26 days (figure 2). The bud category analysis had more stable p values than the presence/ absence of buds, where the p values of the last showed more variation and values higher that 0.05, even after 26 days.

3.1.1.3 Biomass.

The final total dry biomass and the shoot: root DW ratio for 5 plants of each treatment of *Picea abies* in the first experiment is shown in figure 6. The overall highest average biomass production was observed at 24ºC with the R:FR 0.5 (0.90 g) meanwhile the overall lowest average one was at SD at 24ºC (0.24 g). The dry shoot: root ratio at 18ºC seemed to be clearly affected by the light quality, whereas at 24ºC there was a lower difference between the light treatments. The highest average shoot: root ratio was observed at the R:FR 0.5 at 18ºC (5.8) and the lowest one was in the SD treatment at 24ºC (1.38).

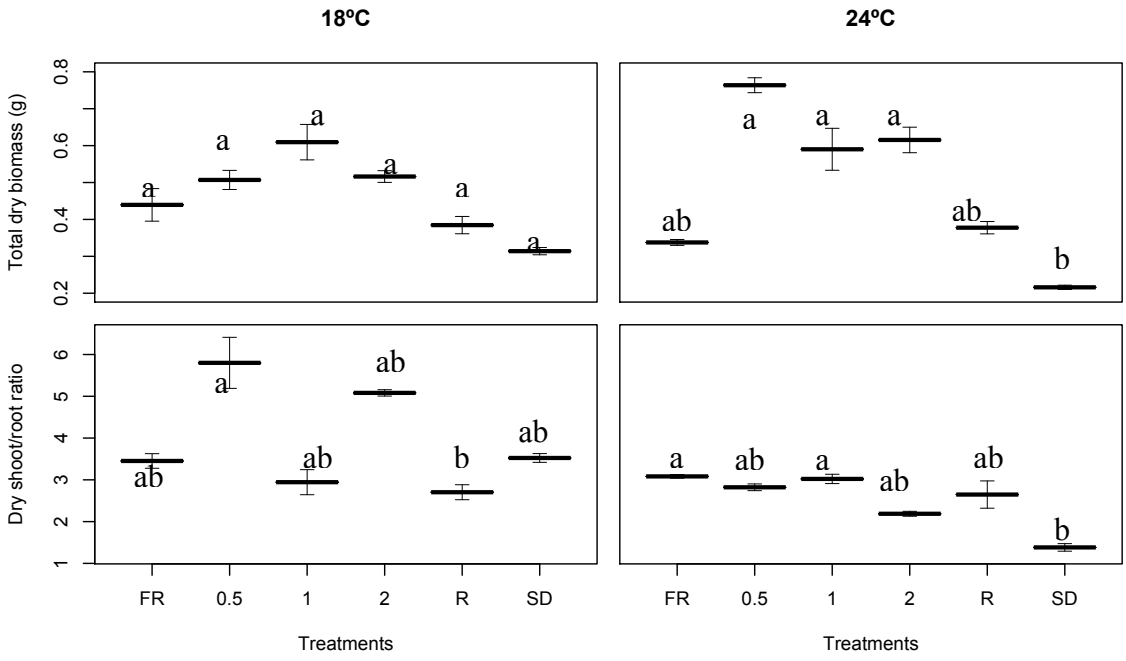


Figure 6: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on total dry biomass and dry shoot/ root ratio in *Picea abies* in the first experiment. The values represent the average ± SE of 5 plants. The x-axis corresponds to: FR: Far red, R: red and 0.5, 1 and 2 refer to R to FR ratios (R:FR) and SD = short days without day extension. Different letter indicates significant difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

There was no significant interaction between the temperature and light quality treatments and no significant effect of any of the treatments on the total DW and in the shoot: root DW ratio, as shown in appendix 14 and 15. Once the interaction term was removed, the light quality treatment was significant in both measured parameters: total DW (Table 4) and shoot: root DW ratio (Table 5).

Table 4: ANOVA for the linear model of the final total DW of *Picea abies* in the the first experiment, whiteout including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.02248	1	0.6018	0.4420375	
Temperature	0.13671	1	3.6603	0.0622437	.
Light treatment	1.18742	5	6.3586	1.59E-04	***
Residuals	1.64333	44			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 5: ANOVA for the generalized linear model of the final shoot: roots DW ratio of *Picea abies* in the first experiment, whiteout including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	2.7002	1	0.1003331	
Light treatment	24.4327	5	0.0001792	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

3.1.1.4 Branches

The final average number of branches is shown in figure 7. At both temperatures the R:FR 0.5 resulted in the highest average number of branches, 5 and 6.6 for 18ºC and 24ºC respectively. The effect of light quality on the number of branches appeared to depend on temperature. This was verified through an ANOVA, which showed a significant interaction between the temperature and light quality treatments (Table 6).

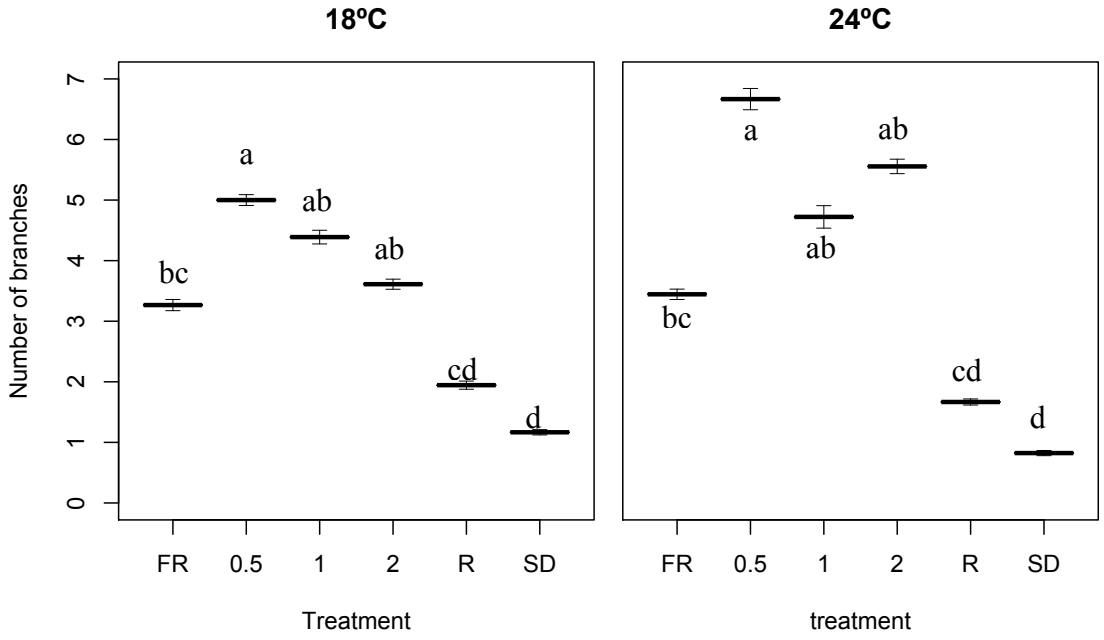


Figure 7: Final average number of branches in *Picea abies* in the different treatments of temperature and light quality as extension of the photoperiod in the first experiment. The values represent the average ± SE of 18 plants. SD = short days without day extension, 0.5, 1 and 2 refer to R to FR ratios (R:FR). Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey test.

Table 6: ANOVA for the linear model of the number of branches of *Picea abies* in the the first experiment, including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	5.0287	1	0.02493	*
Light treatment	4.6495	5	0.46014	
Temperature: Light treatment	12.101	5	0.03343	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.1.1.5 Transcript levels

3.1.1.5.1 Gene transcription levels

FTL2, *COL1, COL2* and *SOC1* transcript levels are shown in the figure 8. The SD treatment increased the expression of *FTL2* compared with the other light treatments, but this was not significantly different between 24ºC compared with 18ºC (p value=0.15). The other light treatments showed lower values, with the R:FR 1 at 18ºC showing the lowest. At 24ºC the

lowest value was achieved with the FR treatment. At both temperatures the R treatment resulted in higher *FTL2* expression than FR or R:FR 1. There was a significant interaction between the temperature and light treatments in the *FTL2* transcript level (Table 7).

The transcript levels of *COL1* at 24ºC showed high standard error (SE) compared with the other genes at both temperatures. The FR treatment induced higher values than the R or SD treatment in both temperatures. At 24ºC the transcript level at R:FR 1 did not differ from that the FR treatment (p value=0.95). There were no significant effects of the treatments on the *COL1* transcript level, even after removing a possible interaction (Appendix 16).

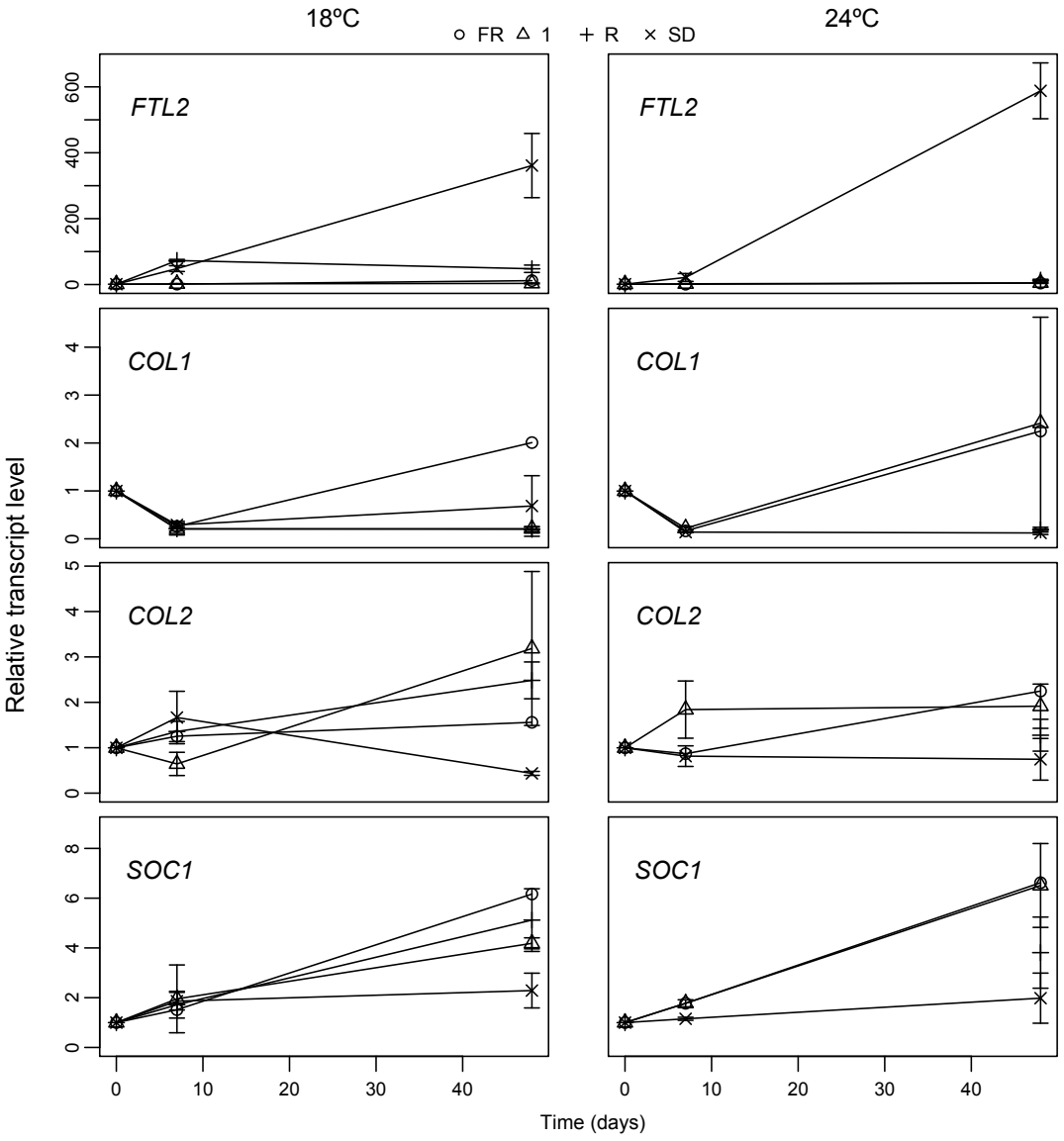


Figure 8: Effect of day extension with different red (R) -far red (FR) ratios and temperature on the transcript levels of *FTL2*, *COL1*, *COL2* and *SOC2*. The values represent the average ± SE of 3 samples, each consisting of 3 plants. SD = short days without day extension, 1 refer to the R to FR ratio (R:FR) 1.

Table 7: ANOVA for the linear model of the *FTL2* transcription level of *Picea abies* in the first experiment, including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	Sum Sq	DF	F value	Pr(>F)	
(Intercept)	401270	1	63.3106	5.95E-07	***
Light treatment	955981	3	50.2768	2.29E-08	***
Temperature	13031	1	2.0559	0.17087	
Light treatment: Temperature	66090	3	3.4758	0.04087	*
Residuals	101410	16			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

At 24ºC, *COL2* also showed a reduction in the transcript level under SD, R and R:FR 1, compared to FR. At 18ºC this tendency was less marked and the maximum was at R:FR 1. The SD treatment resulted in lower values than the FR and R treatment. The R treatment was not significantly lower that the FR (p value=0.14). There was no significant interactive effect between the treatments and no significant effect of any of the treatments. Once the interaction was removed; the light treatments showed a trend of effect (p value=0.07) (appendix 17).

The expression of *SOC1* was reduced in both temperatures under the SD treatment, compared with the FR treatment. The R treatment resulted in lower transcript levels than the FR, but higher that the SD treatment. An ANOVA of the transcript levels showed that there was a significant effect of the light treatments, with or without removing the interaction (appendix 18).

3.1.1.5.2 Bud set and shoot elongation relationship with gene transcription

The transcript levels of the different genes were correlated with the shoot elongation and the bud stage at the end of the experiment (Figure 9).

The genes correlated better with the bud formation and shoot elongation with an exponential that with a linear relationship. The highest correlation for both parameters, bud classification and shoot elongation, were achieved by *FTL2* (R^2= 0.86 and 0.79), followed by *SOC1* (R^2=0.41 and 0.3) and *COL2* (R^2=0.34 and 0.25). In contrast, the lowest correlation for both parameters was *COL1* (R^2=0.16 and 0.12).

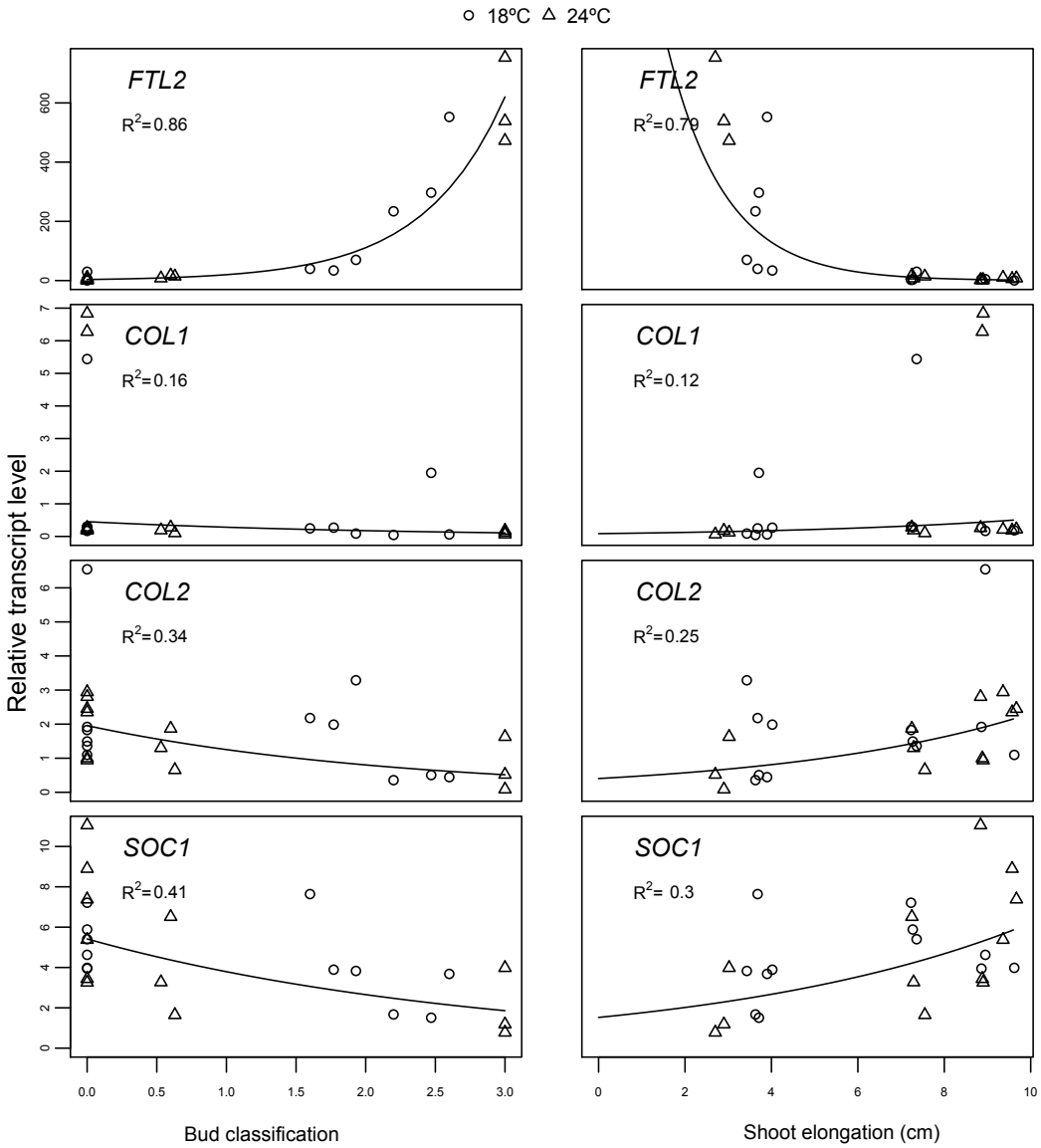


Figure 9: Correlation of bud classification and shoot elongation with the transcript levels of *FTL2*, *COL1*, *COL2* and *SOC2,* at two different temperatures of 18 and 24º C. Each point correspond to the average of 3 samples.

3.1.2 Second experiment

3.1.2.1 Shoot elongation

The effect of the temperature and light treatments in a time course is shown in figure 10. Similar to the first experiment, the SD treatment stopped the growth in both temperatures with similar average shoot elongation corresponding to 2.7 and 3 cm at 18ºC and 24ºC respectively. The highest PPS (0.88), obtained just with R light, resulted in total cessation of the shoot elongation with 18ºC at equal that in the first experiment. At 24ºC there was a reduction of the shoot elongation but the cessation of this one was not complete at the end of the experiment.

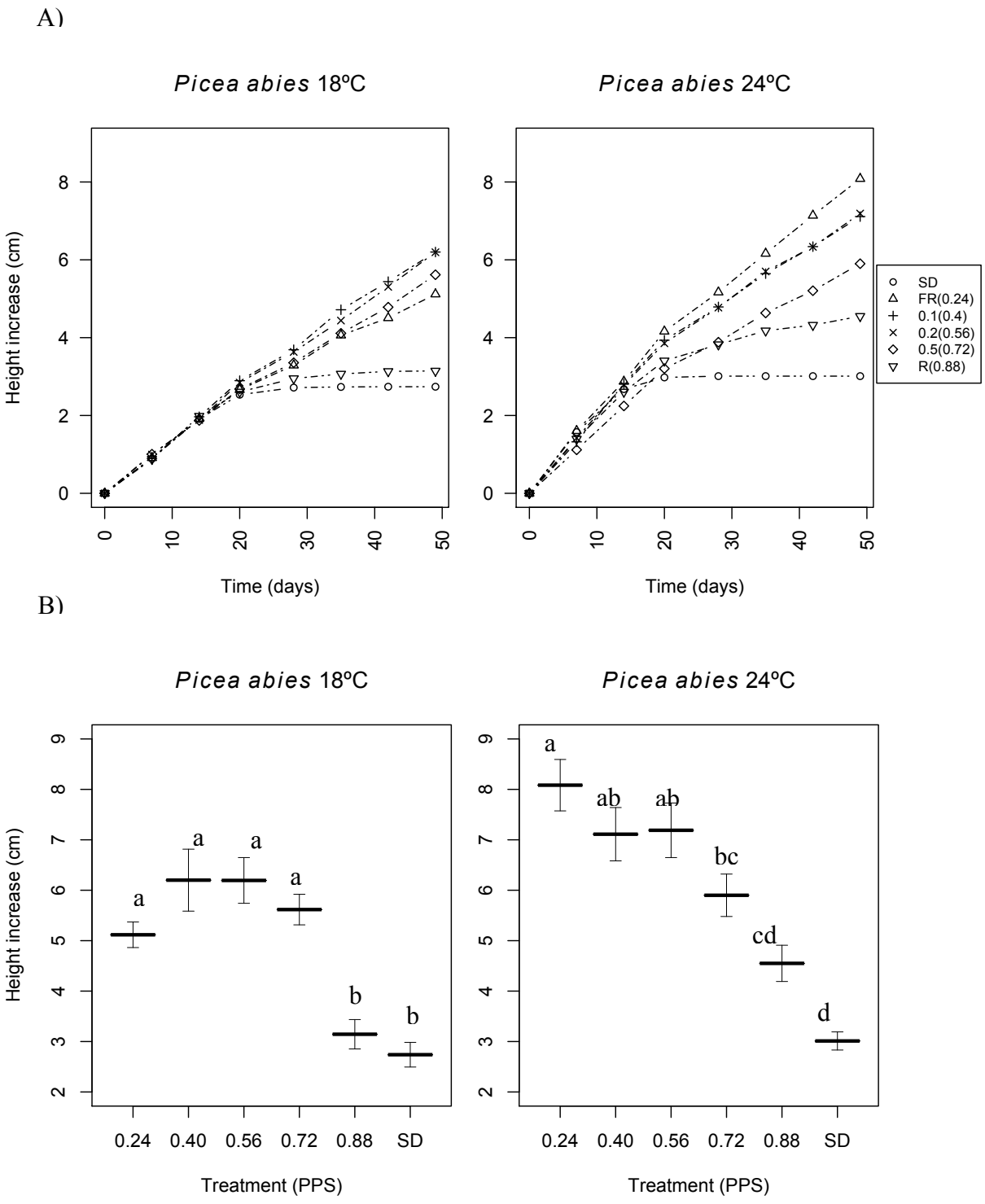


Figure 10: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) average height increase (cm) and B) final shoot elongation in *Picea abies* in the second experiment. The values represent the average ± SE of 18 plants. SD = short days without day extension, 0.1, 0.2 and 0.5 refer to R to FR ratios (R:FR) with their respective phytochrome photostationary state (PPS) in brackets. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

The average shoot elongation at the end of the experiment is shown (Figure 10 B). At 18ºC the treatments adjusted better to a quadratic model, where intermediate PPS values allow higher shoot elongation than extreme values. For 24ºC a linear tendency is more evident, with the lowest PPS (PPS from 0.24 to 0.56) producing the highest elongation that was between 7.1 and 8 cm. At 18ºC the average maximum elongation was between 5.1 and 6.2 cm achieved with any PPS lower than 0.88. In the second experiment shorter final average elongation in both temperatures were obtained compared to the first experiment, at exception of the SD-treated plants.

ANOVA of the shoot elongation is presented in table 8. In this analysis the interaction was removed due to non-significance, in contrast to in experiment 1 (Appendix 19). The effect of light quality and temperature treatments were significant.

The p value of the effect of the temperature – light quality interaction on shoot elongation in a time course is presented in figure 11. The interaction was significant after one week but thereafter there was no significant interaction until the end of the experiment, where the two last evaluations were significant. An ANOVA for shoot elongation at the end of the experiment identified both factors and their interaction as significant (Appendix 20).

Table 8: ANOVA for the linear model without interaction of the shoot elongation using the time and plant as random variables in the second experiment of *Picea abies*.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	9.4416	1	0.002121	**
Temperature	45.2414	1	1.74E-11	***
Light treatment	114.9197	5	2.20E-16	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

3.1.2.2 Terminal bud set.

The effect of the temperature and light quality treatments on stages during bud formation and at the last evaluation using bud stage classification in the second experiment is shown in figure 12. The 18ºC and 24ºC temperature treatments resulted in the first buds in the SD treatment 16 days after the start of the treatment. At the end of the experiment only the 0.88 PPS and SD treated plants had buds (appendix 21 and 22). The effect of the treatments was similar, especially for the SD treated plants independently of temperature, but less bud set was achieved at PPS 0.88 at 24ºC that at 18ºC. The results are similar to the results of the first experiment.

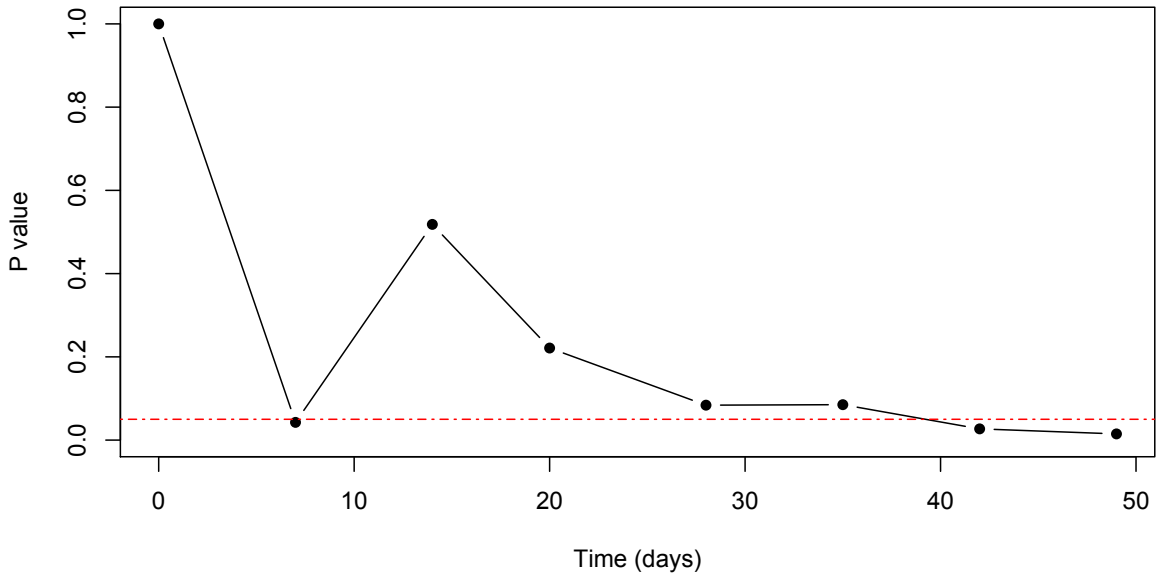


Figure 11: P values of the interactive effect of the light and temperature treatments in a time course for the shoot elongation in *Picea abies* in the second experiment.

An ANOVA for the bud stages is presented in table 9. The interaction between the temperature and light quality treatment was removed due to non significant (Appendix 23). Before and after this, just the light treatments showed a significant effect which is in line with the situation for the shoot elongation, where the effect of PPS was also not dependent on the temperature. An was performed also for presence/ absence of buds, and showed no significant interaction between the temperature and light treatments. Similar to the categorical analysis, the effect of the light treatment was significant before and after removing the interaction (appendix 24 and 25).

Table 9: ANOVA for the generalized linear model without interaction of the bud set classification using categories in the second experiment of *Picea abies*. For this, the plant and time where used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
(Intercept)	0.0001	1	0.994	
Temperature	1.3814	1	0.2399	
Light treatment	54.6266	5	1.56E-10	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

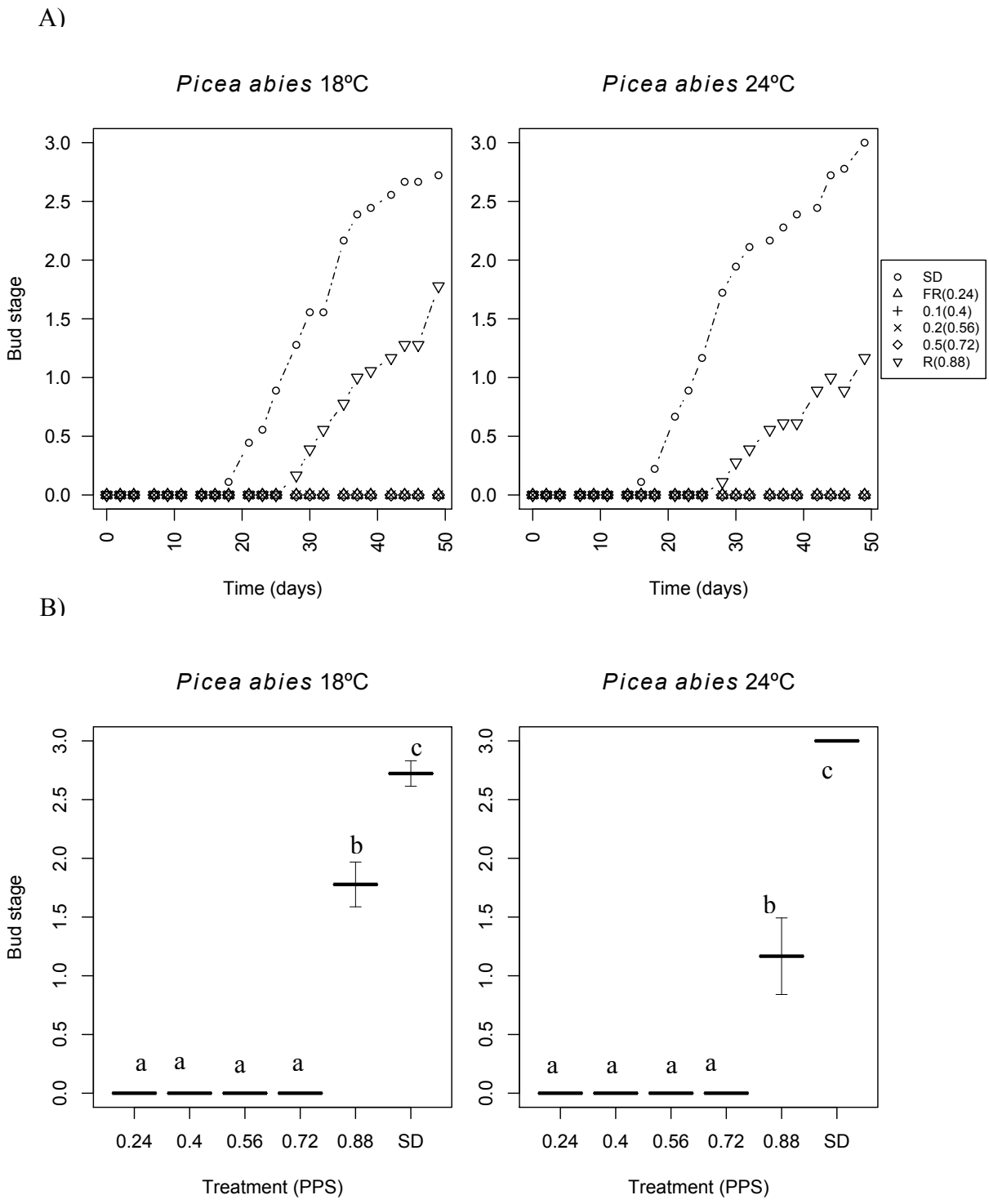


Figure 12: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) bud stage and B) final bud stage in *Picea abies* in the second experiment. The values represent the average ± SE of 18 plants, where 0 denotes no presence of bud, 1 green bud, 2 brownish bud and 3 brown bud. SD = short days without day extension, 0.1, 0.2 and 0.5 refer to R to FR ratios (R:FR) with their respective PPS in brackets. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

The final average bud set classification (figure 12 B) showed clearly that all the plants after 48 days at 18ºC and 24ºC treatments with presence of FR (PPS equal or lower than 0.72) did not form buds. At 24ºC, the SD treated plants had significantly more mature terminal buds than at 18ºC (p value = 0.02), but this was not the case for the PPS 0.88 (R light) (p value = 0.11).

ANOVA of the bud stage at the the end of the experiment is shown in the appendix 26 and 27. These analyses showed similar results as the complete analysis, but just after removing the non significant interaction, the light treatment was significant. Similar results were obtained for the analysis of presence/ absence of buds (appendix 28 and 29).

The p value of the interaction for the analysis of bud stage in a time course is presented in figure 13. This was also made for the analysis of presence/ absence of buds (appendix 30). Both figures (figure 13 and appendix 30) showed similar tendencies and there was no significant interaction. This is differed from the first experiment, where the interaction was significant after 26 days.

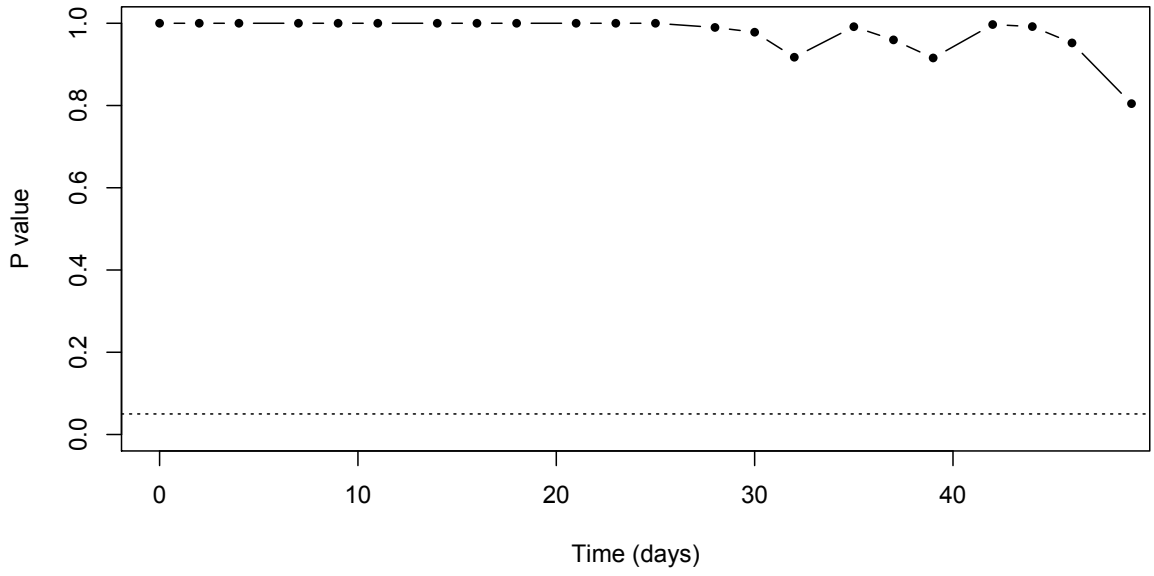


Figure 13: P values of the interactive effect of the light and temperature treatments in a time course for the bud set in *Picea abies* as categories in the second experiment.

3.1.2.3 Biomass.

The final total dry biomass and the shoot: root DW ratio for 5 plants of each treatment of *Picea abies* in the second experiment is shown in figure 14.

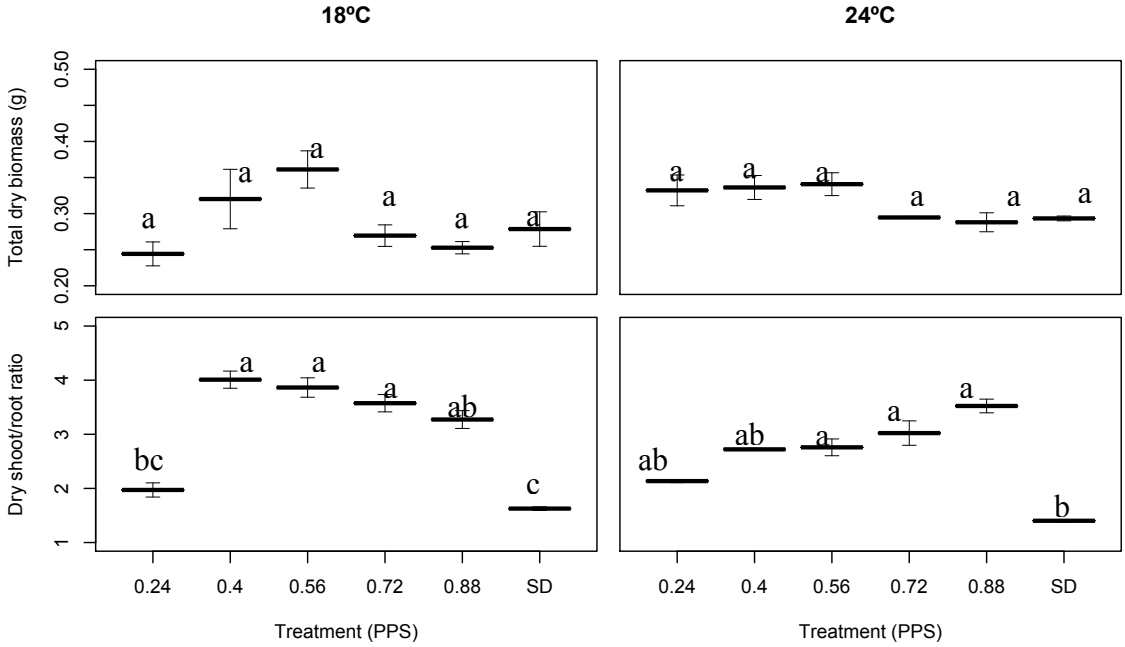


Figure 14: Average total biomass (gr) and shoot: root DW ratio of *Picea abies* in the different treatments of temperature and light quality as extension of the photoperiod in the second experiment. The values represent the average of 5 plants and the bars plus/minus the SE. The x-axis corresponds to the different PPS and SD = short days without day extension. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

ANOVAs of the total DW or for the shoot: root DW ratio showed that in both cases there was no significant effect of the different treatments, before or after removing the non significant interaction. This is shown in appendix 31, 32, 33 and 34.

There was no significant difference within each temperature for the total dry biomass. For the shoot: root DW ratio the highest value was for all the PPS higher than 0.24 at 18ºC with values between 3.8 and 4.0. At 24ºC there was not difference between all lighted plants, with values between 2.2 and 3.4. Lower weight and number of branches were obtained in the second experiment, as compared with the first experiment.

3.1.2.4 Branches

The final average number of branches is shown in figure 15. Higher temperature appeared to increase the number of branches in both of the light treatments very markedly at intermediate PPS at 24ºC. At 18ºC this was not the case, where all light treatments produce similar average number of branches, excluding the SD. The maximum number of branches was between 3.8 and 3.7 in the PPS 0.4 and 0.56 of the treated plants at 24ºC.

An ANOVA was made for the number of branches. There was a significant interaction between the temperature and light treatments (Table 10).

Table 10: ANOVA for the linear model of the number of branches of *Picea abies* in the second experiment, including the interaction between the temperature and light treatment. n=18.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	13.2166	1	0.0002775	***
Treatment	8.8586	5	0.1148405	
Temperature: Treatment	15.2052	5	0.0095208	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

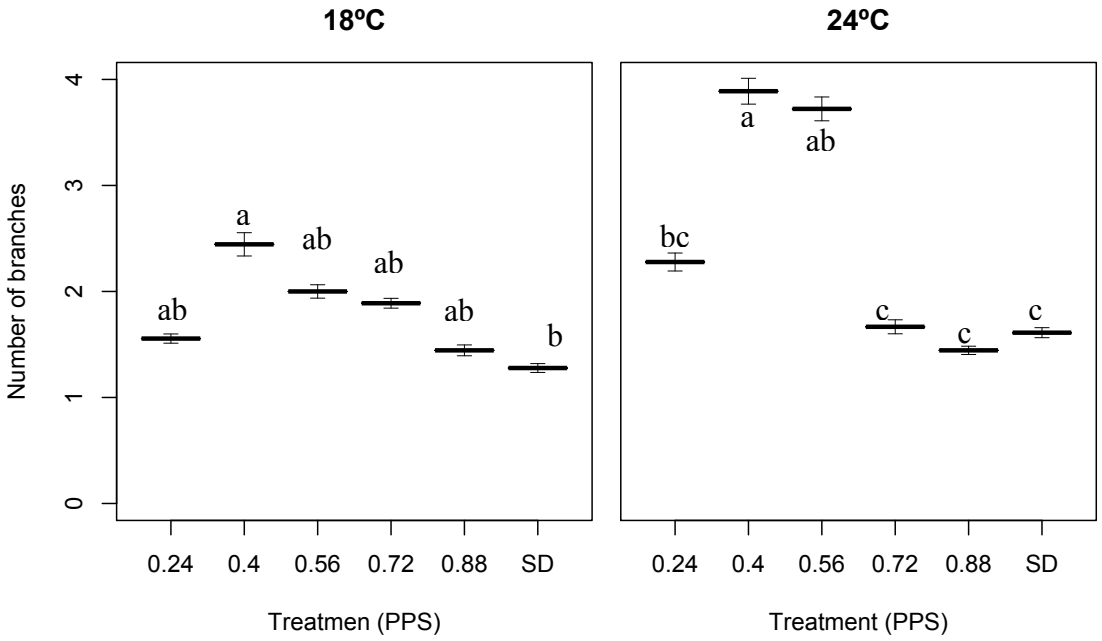


Figure 15: Average final number of branches of *Picea abies* in the different treatments of temperature and light quality as extension of the photoperiod in the second experiment. The values represent the average of 18 plants and the bars plus/minus the SE. Different letter indicates significant difference ($p \leq 0.05$) within each temperature treatment using Tukey test.

Photos of the 5 selected plants for biomass analysis in each treatment of both experiments are shown in figure 16.

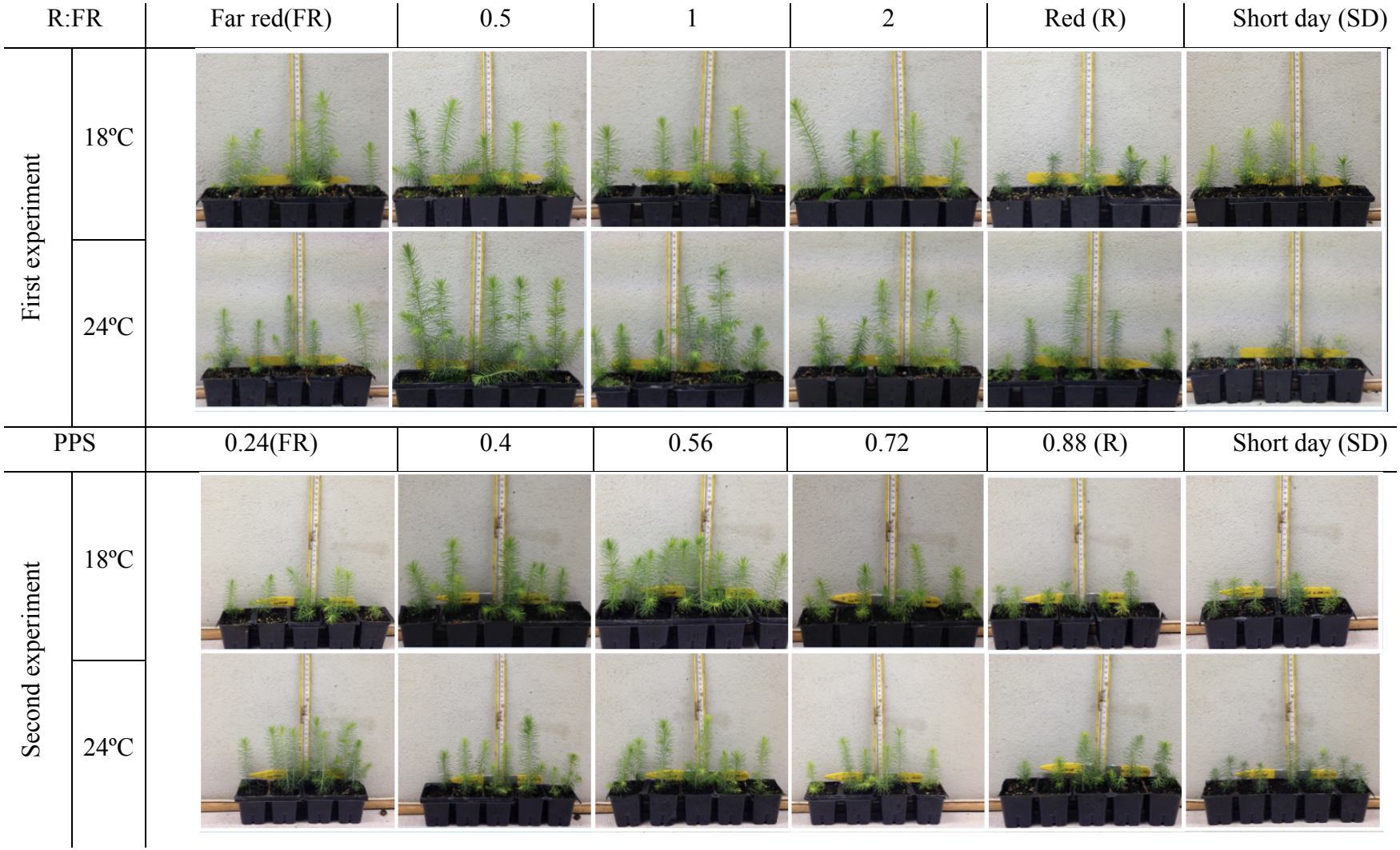


Figure 16: Photos of 5 randomly selected plants for biomass analysis in both experiments in *Picea abies*. The treatment corresponds to R:FR and PPS in the first and second experiment, respectively.

3.2 *Abies lasiocarpa*.

3.2.1 First experiment.

3.2.1.1 Shoot elongation.

The shoot elongation of *A. lasiocarpa* in different temperatures and light quality treatments is shown in figure 17. After 20 days the SD treated plants ceased their shoot elongation in both temperatures. Also, the R light-treated plants showed growth cessation at 18ºC, similar to the results of *P. abies*. The average shoot elongation of the SD-treated plants was 0.4 cm in both temperatures and 0.6 cm for the R-treated plants at 18ºC. It is possible to observe a double sigmoid curve in both treatments but that is more clear at 18ºC. The final shoot elongation is presented in figure 15 B, where the maximum average shoot elongation was at R:FR 0.5 and 1 for 18 ºC and 24 ºC with 2.3 and 2 cm, respectively. Contrary to the results of *P. abies*, greatest shoot elongation was achieved under the lowest temperature.

An ANOVA for the shoot elongation is presented in table 11. The interaction between temperature and light quality treatments was not significant and was thus removed (appendix 35). The temperature and light quality treatments resulted in a significant effect on shoot elongation. This was also the case when the final shoot elongation was analyzed separately (appendix 36 and 37).

The p value of the interaction in a time course is shown in figure 18. This is showing a p value smaller that 0.05 after 5 days after the start of the treatments but the p value then increased until day 21 before decreasing again.

Table 11: ANOVA of the shoot elongation in *Abies lasiocarpa* using the time and plant as random variables in the first experiment without interaction between the factors.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	3.2164	1	0.0729	.
Temperature	23.7351	1	1.11E-06	***
Light treatments	49.9031	5	1.45E-09	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

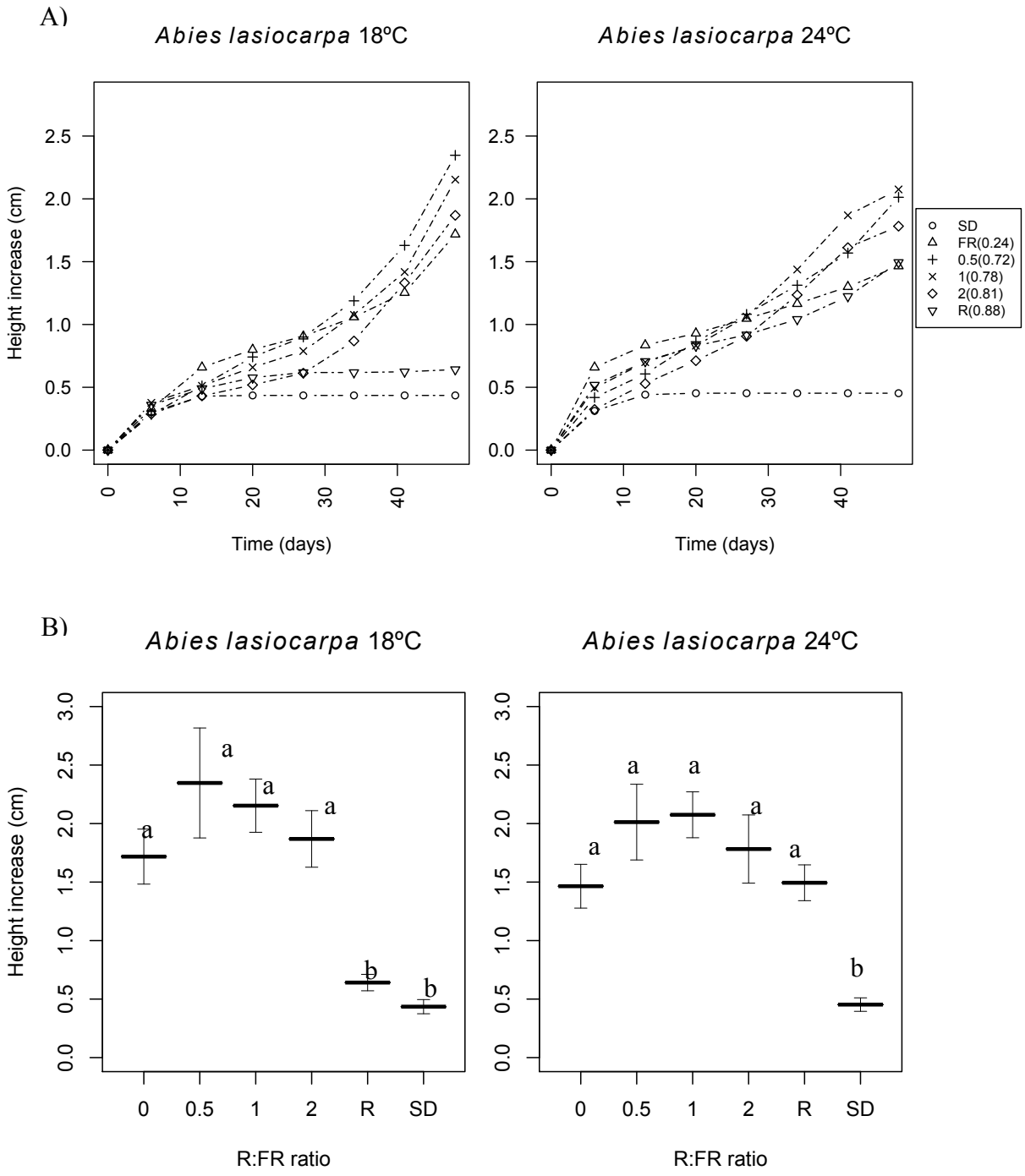


Figure 17: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) average height increase (cm) in a time course and B) final shoot elongation in *Abies lasiocarpa* in the first experiment. The values represent the average ± SE of 18 plants. SD = short days without day extension, 0.5, 1 and 2 refer to R to FR ratios (R:FR) with their respective PPS in brackets. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

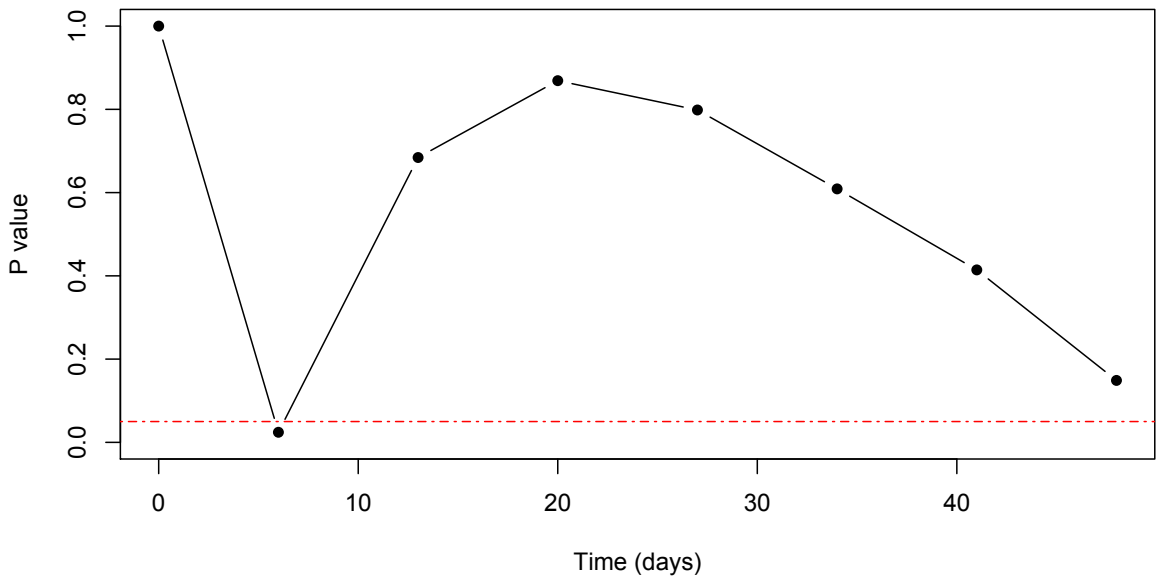


Figure 18: P values of the interactive effect of the light and temperature treatments in a time course for the shoot elongation in *Abies lasiocarpa* in the first experiment.

3.2.1.2 Terminal bud set

The effect of the different treatments on terminal bud formation as categories is shown in figure 19. Many of the *A. lasiocarpa* plants showed presence of terminal bud at the start of the experiment. The occurrence of buds then decreased after the start of the treatments. This was followed by an increase in bud set in all treatments with maximum value close to day 25, with the highest values at 18ºC.Thereafter all treatments with presence of FR light decreased their presence or maturity of buds. At 18ºC this tendency stayed until the end of the experiment but at 24ºC several treatments again increased the maturity or presence of buds. The lowest mean bud stage value was observed close to day 40 and 35 for 18ºC and 24ºC, respectively. A similar situation was observed for the analysis of presence or absence of buds (Appendix 38).

At the end of the experiment (Figure 19 B) the R light treatment resulted in brown buds in all the plants at 18 ºC, but this was occurred at a lower degree at 24ºC (2.8 vs 0.64). Also, the FR-treated plants at 18ºC were the only plants that did not show presence of terminal buds at the end of the experiment. At 24ºC the average bud stage for this time point was 1.8. Finally, the SD treatment induced the formation of mature and buds in almost all plants at 18ºC and in all plants at 24ºC. The results for percent of plants with buds are similar and presented in the appendix 38 and 39.

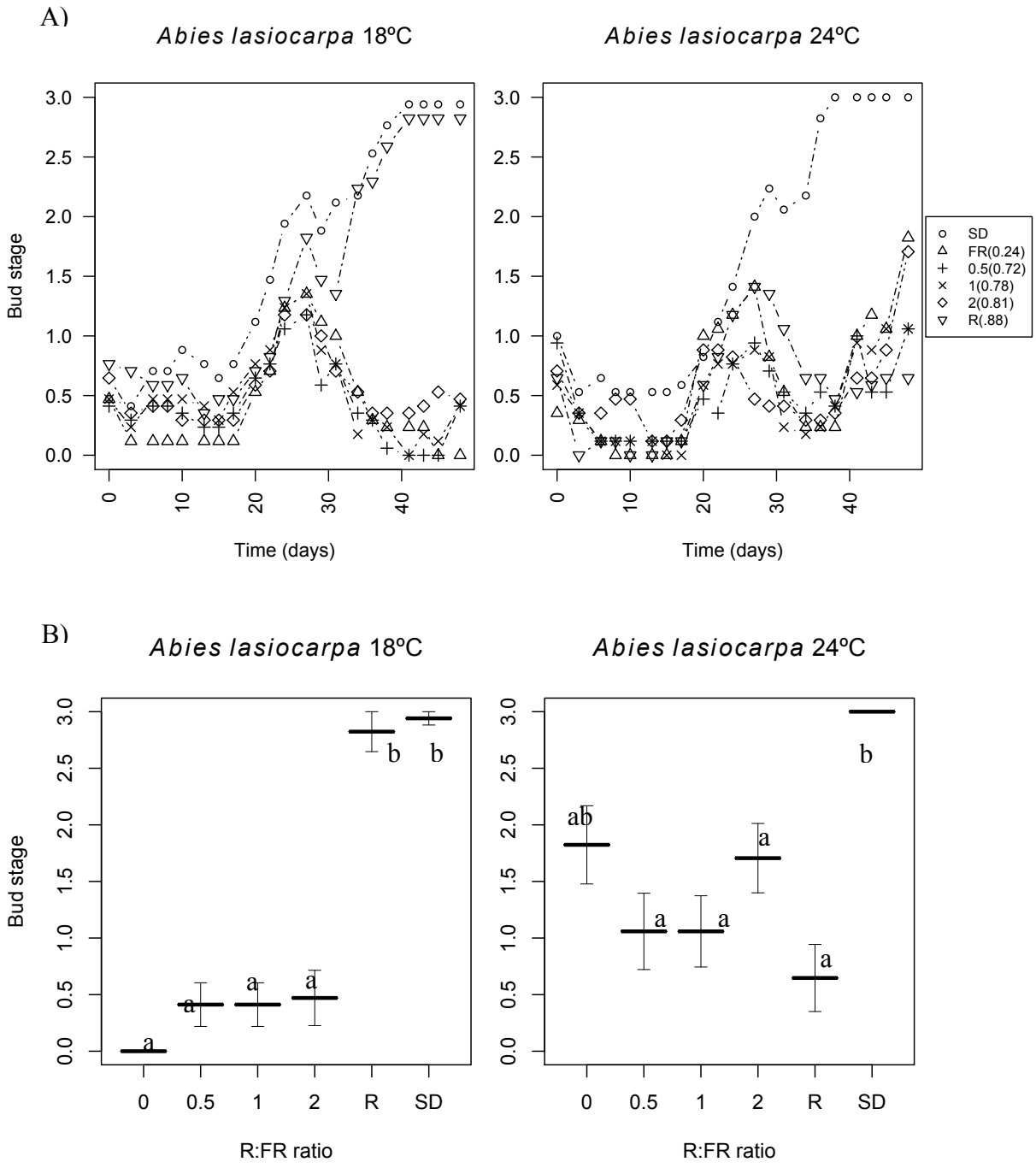


Figure 19: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) bud stage in a time course and B) final bud stage of *Abies lasiocarpa* in the first experiment. The values represent the average ± SE of 18 plants, where 0 denotes no presence of bud, 1 green bud, 2 brownish bud and 3 brown bud. SD = short days without day extension, 0.5, 1 and 2 refer to R to FR ratios (R:FR) with their respective PPS in brackets. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

The ANOVA for the bud stage development is shown in table 12. There was a significant interaction between the temperature and light quality treatments. The analysis of percent of plants with terminal buds also showed a significant interaction between the temperature and light quality treatment, with significant effect also of light treatments (appendix 40)

Table 12: ANOVA for the generalized linear model of the bud stage classification in the first experiment in *Abies lasiocarpa*. For this the plant and time where used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	84.0203	1	2.20E-16	***
Temperature	0.0905	1	7.64E-01	
Light treatment	116.9677	5	2.20E-16	***
Temperature: Light treatment	21.9227	5	0.0005416	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVAs of bud stage and presence of bud set at the end of the experiment showed similar results as the analysis in table 12 (appendix 41 and 42). The interaction between the temperature and light treatment was significant.

The p value for the interaction between the temperature and light treatments in a time course for the bud stage is shown in figure 20. Similar results were obtained for percentage of plants with buds (appendix 43). In both cases the p value showed an unstable response with smother changes for the categorical analysis. The highest value was close to day 25 of the treatments, where after day the p value stabilised at values lower that 0.05 in both analyses.

3.2.1.3 Biomass.

The final total dry biomass and shoot: root DW ratio under the different treatments are shown in figure 21 for *A. lasiocarpa* in the first experiment.

There was no significant difference in total biomass in any of the temperatures, except at 24ºC where R:FR 2 resulted in higher biomass than SD, where R:FR 2 showed the maximum average dry biomass (0.32 g) and SD the minimum (0.16 g)

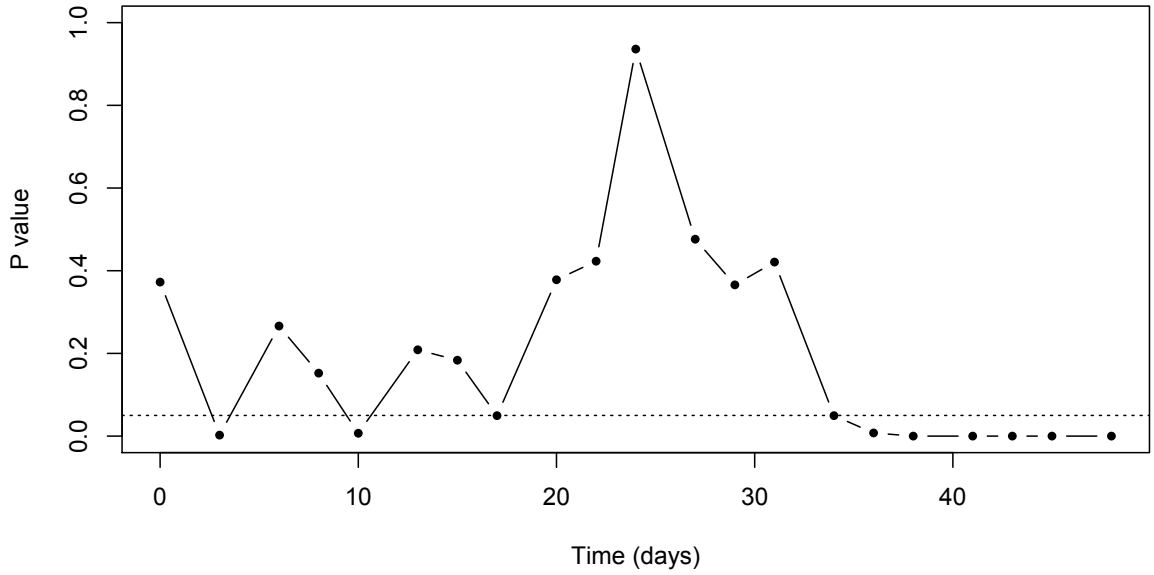


Figure 20: P values of the interactive effect of the light quality and temperature treatments in a time curse for the bud set in *Abies lasiocarpa* as categories in the first experiment.

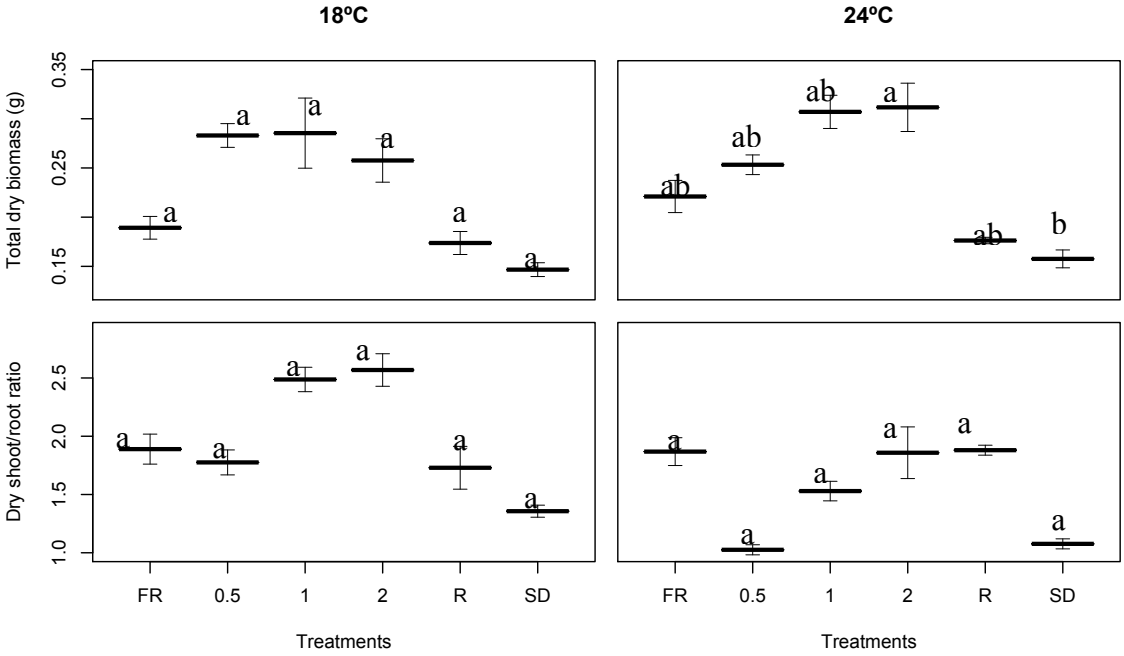


Figure 21: Average total biomass and shoot: root DW ratio of *Abies lasiocarpa* exposed to the different treatments of temperature and light quality as extension of the photoperiod in the first experiment. The values represent the average ± SE of 5 plants. The x-axis corresponds to: FR: Far red, R: red and 0.5, 1 and 2 refer to R to FR ratios (R:FR) and SD = short days without day extension. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

The shoot: root DW ratio did not differ significantly between the light treatments in any of the temperatures. This present a more erratic response where is not clear a effect trend of the light treatments where no significant difference within each temperature was detected.

An ANOVA for the total dry biomass and the shoot: root dry ratio is shown in table 13 and 14. In both cases the interaction was removed due non significance (appendix 44 and 45).

Table 13: ANOVA for the linear model of the final total DW of *Abies lasiocarpa* in the first experiment, without including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.03009	1	4.1444	0.047432	*
Temperature	0.00411	1	0.5662	0.455529	
Light treatment	0.164	5	4.517	0.001918	**
Residuals	0.34129	47			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 14: ANOVA for the generalized linear model of the shoot: root dry ratio DW of *Abies lasiocarpa* in the first experiment, without including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0.6465	1	0.42137	
Light treatment	19.3865	5	0.001628	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.2.1.4. Branches

No branches were observed in any of the *A. lasiocarpa* plants in the first experiment.

3.2.2 Second experiment.

3.2.2.1 Shoot elongation.

The effect of the different temperatures and light quality treatments on the shoot elongation in a time course of *A. lasiocarpa* in the second experiment, is shown in figure 20 A.

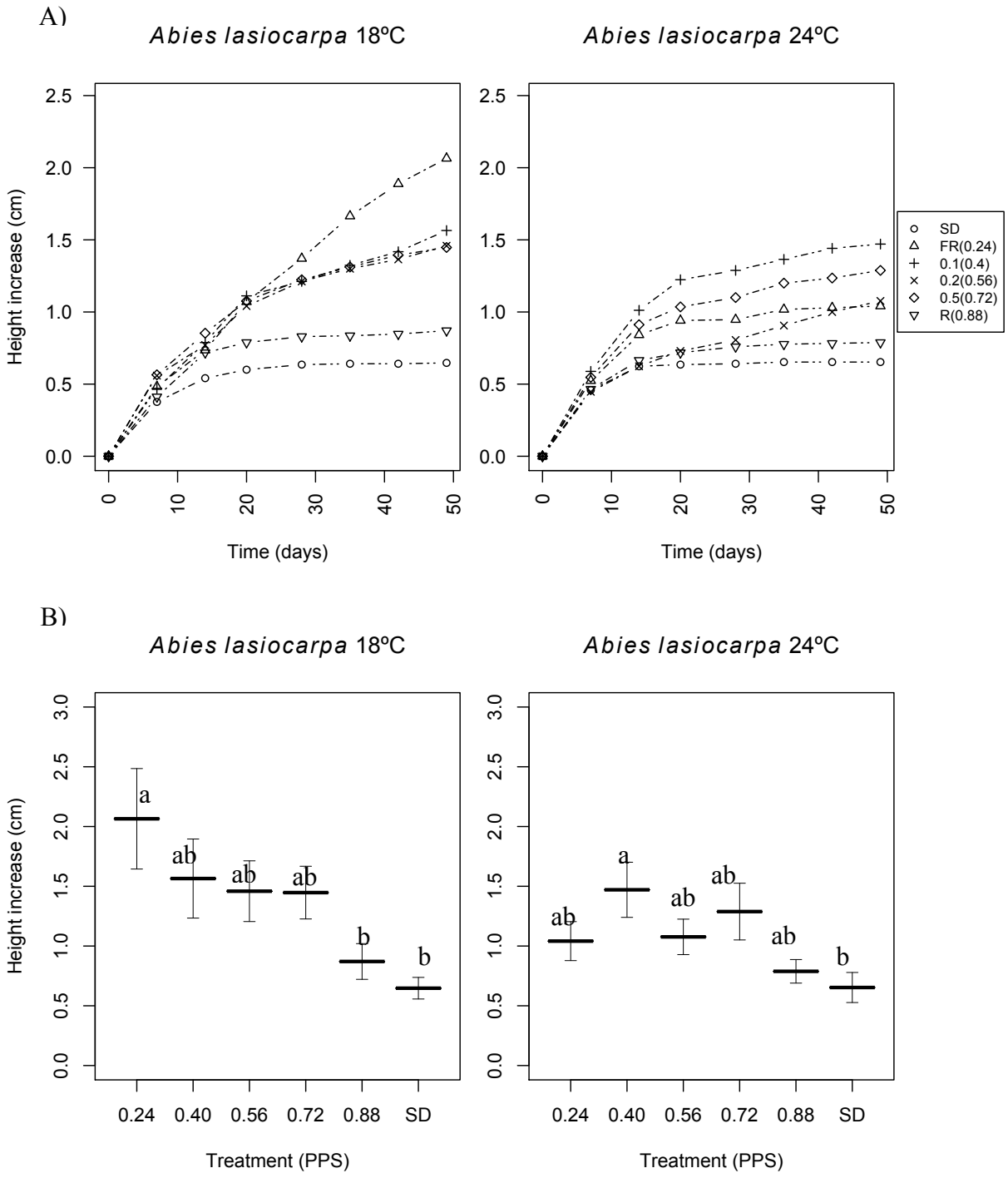


Figure 22: Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) shoot elongation in a time course and B) final shoot elongation of *Abies lasiocarpa* in the second experiment. The values represent the average ± SE of 18 plants. SD = short days without day extension, 0.1, 0.2 and 0.5 refer to R to FR ratios (R:FR) with their respective PPS in brackets. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

Like in the first experiment, the SD treatment resulted in growth cessation after 15 days in both temperatures and also the R light was able to stop shoot elongation at 18ºC. Like in the first experiment, the lower temperature promoted higher elongation. All the treatments at 24ªC resulted in a decreased elongation growth. In contrast to the first experiment several light treatments did not stop to grow. *A. lasiocarpa* shoot elongation was lower compared with the first experiment in both temperature treatments with a maximum average of 2.0 cm. At the end of the experiment (figure 22 B) the PPS 0.24-treated plants at 18ºC were the highest ones with an average shoot elongation of 2 cm. For 24ºC the highest plants were observed at PPS 0.4 with an average increase of 1.5 cm. In both temperatures the lowest increase in shoot elongation occurred in the SD-treated plants with an average increase of 0.65 cm. For 18ºC a linear relationship was visible meanwhile at 24ºC the same relationship tended to have a lower slope (figure 22 B).

An ANOVA for the the shoot elongation is shown in table 15.

Table 15: ANOVA for the linear model of the shoot elongation using the time and plant as random variables in the second experiment of *Abies lasiocarpa*.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	2.1219	1	0.145205	
temperature	6.8736	1	8.75E-03	**
Light treatment	16.5878	5	0.005352	**
Temperature: Ligh treatment	16.656	5	0.005201	**

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

The interaction between the factors was significant and there was also a significant effect of the individual factors, temperature and light quality. An ANOVA of the final shoot elongation (appendix 46) showed a non significant-interaction. After removing the interaction, the effect of light quality treatments was significant (appendix 47). The p value of the interaction in a time course is shown in figure 23. There were no significant p values, at any point during the experiment, but the values trended to decrease during the experiment.

3.2.1.2 Terminal bud set

The effect of the different treatments on the bud stages is shown in figure 24 A. Similar to experiment 1, *A. lasiocarpa* had terminal buds at the beginning of the experiment.

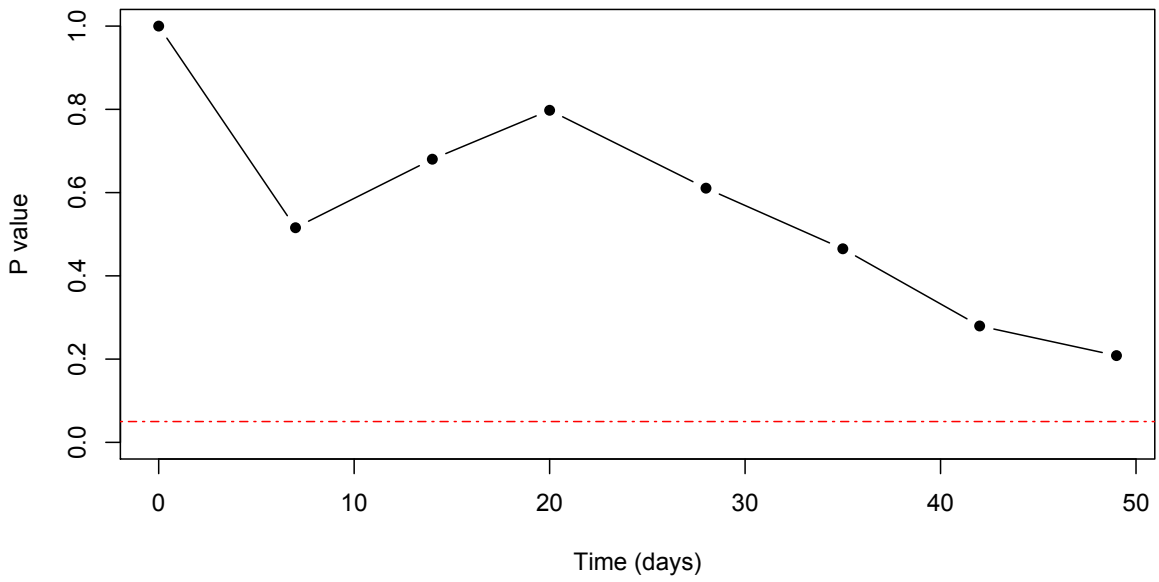


Figure 23: P values of the interactive effect of the light and temperature treatments in a time course for the shoot elongation in *Abies lasiocarpa* for the second experiment.

The maturity of these buds decreased during the first days. After this, bud set and bud maturity increased in all treatments, where this was more constant at 18ºC for the SD and PPS 0.88-treated plants, compared with the other treatments at the same temperature. Similar results were obtained for the analysis of bud presence (appendix 48). Even before the end of the experiment all the SD-treated plants at 18ºC had terminal buds and at their maximum maturity. At the end of the experiment (Figure 24 B) this was the only treatment that showed 100% bud set (appendix 49) with average bud stage 2.8. In contrast to in the first experiment, the PPS 0.24 (FR) treated plants at 18ºC, were not able to avoid the formation of buds in *A. lasiocarpa.*

Similar to the final shoot elongation (figure 22 B), the relationship between the light treatments and the final bud stage (figure 24 B), at 18ºC had a higher slope in a linear relationship, whereas a 24ºC this relationship showed a lower slope. This was also the case for the analysis aa a presence of bud (appendix 49).

An ANOVA for bud stages is presented in table 16. Like in the first experiment, there was a significant interaction between the temperature and light quality treatments. The analysis of presence of terminal buds also showed a significant interaction between the temperature and light quality treatments (appendix 50).

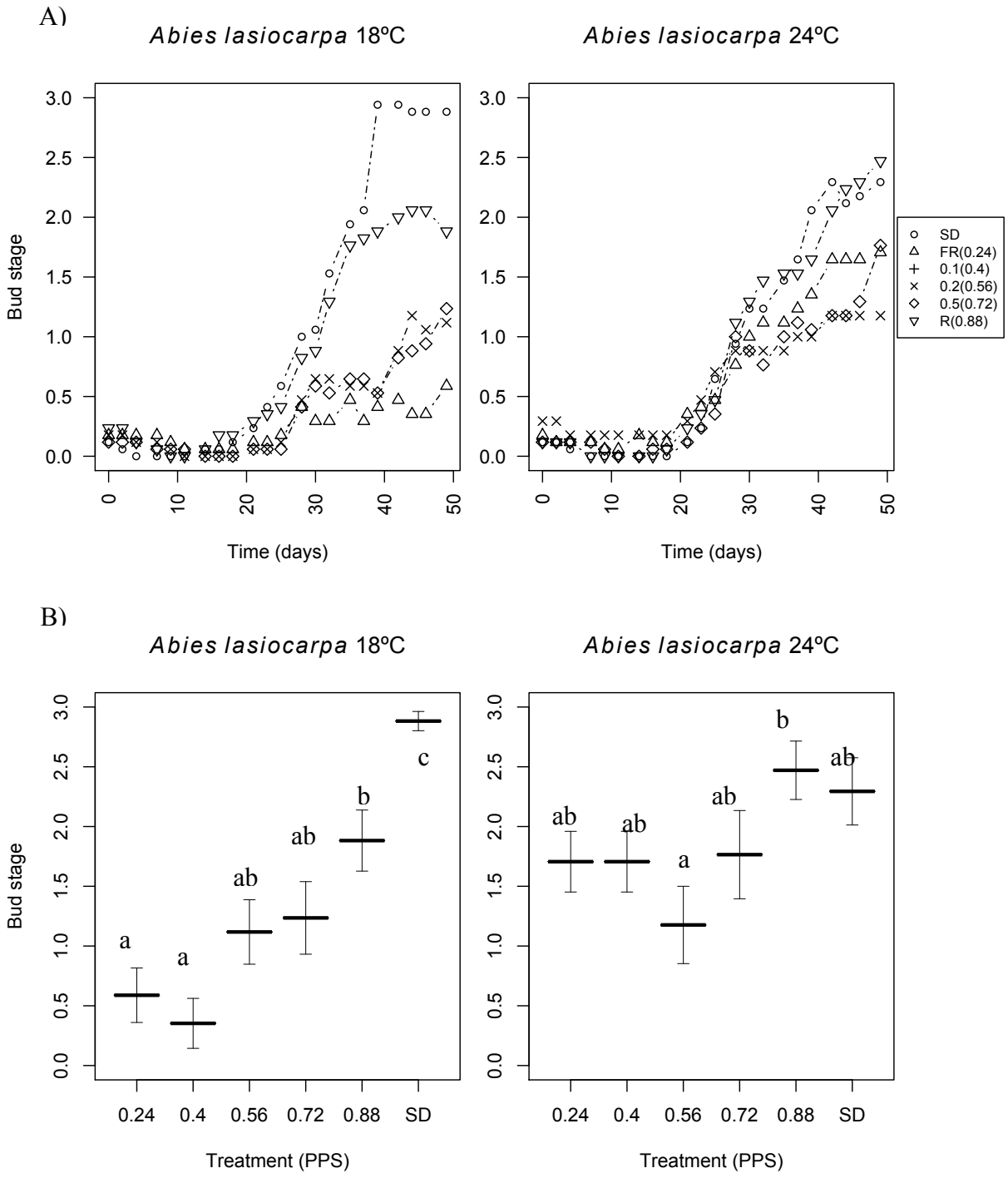


Figure 24. Effect of day extension with different red (R) -far red (FR) ratios and temperatures on A) bud stage in a time course and B) final bud stage of *Abies lasiocarpa* in the second experiment. The values represent the average ± SE of 18 plants, where 0 denotes no presence of bud, 1 green bud, 2 brownish bud and 3 brown bud. SD = short days without day extension, 0.1, 0.2 and 0.5 refer to R to FR ratios (R:FR) with their respective PPS in brackets. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test..

Table 16: ANOVA for the generalized linear model of the bud stages in the second experiment of *Abies lasiocarpa*. For this the plant and time where used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	146.9595	1	2.20E-16	***
Temperature	8.7013	1	3.18E-03	**
Light treatment	31.9825	5	5.99E-06	***
Temperature: Light treatment	17.9033	5	0.00307	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVA for the bud stages or percentage of plants with buds at the end of the experiment showed a significant interaction but no significant effect of the individual factors (appendix 51 and 52).

The p value for the interaction between the temperature and light quality treatments in a time course is shown in figure 25. The same was made for the presence of buds with similar results (appendix 53). In both analyses, as bud stages or percentage of plants with buds, the p value showed an unstable response. For the bud stages theses values were significant (smaller that 0.05) after day 36, where as this was the case for the presence of bud set after 42 days.

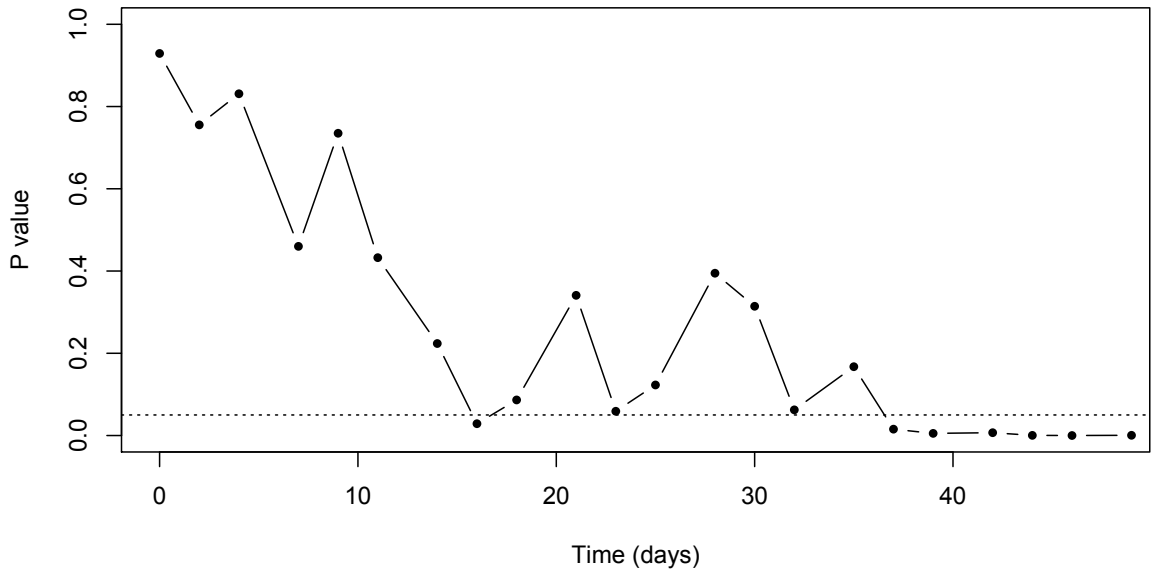


Figure 25: P values of the interactive effect of the light and temperature treatments in a time course for the bud set in *Abies lasiocarpa* as categories for the second experiment.

3.2.1.3 Biomass.

The final total dry biomass and shoot: root DW ratio in the different treatments is shown in figure 26 for *A. lasiocarpa* in the second experiment.

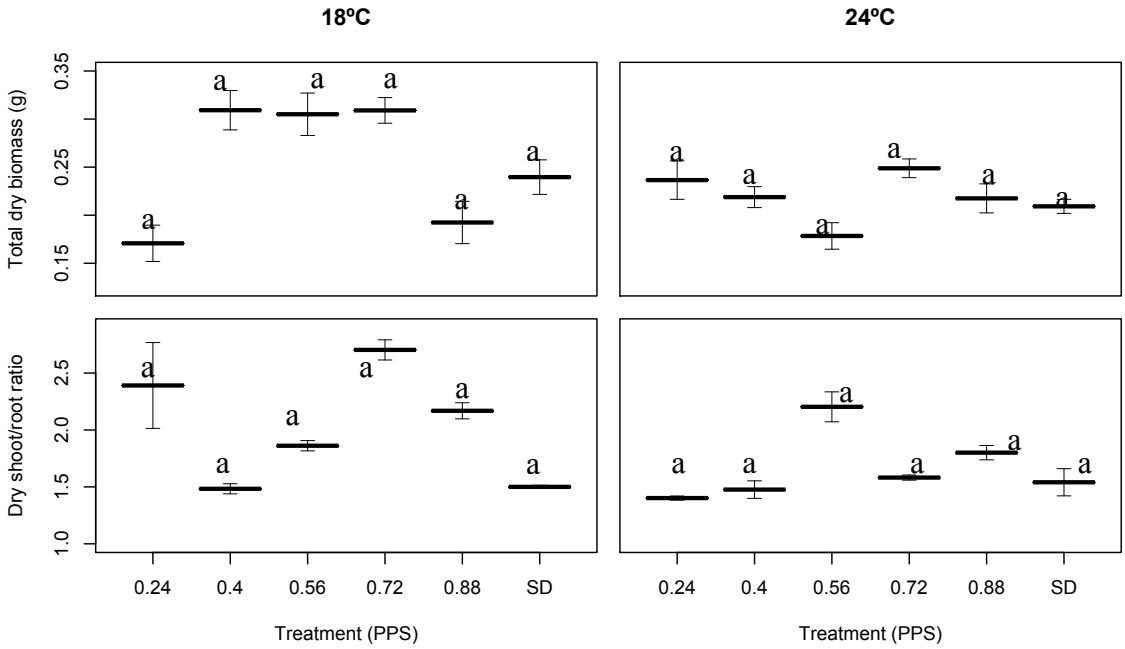


Figure 26: Average total biomass and shoot: root DW ratio of *Abies lasiocarpa* in the different treatments of temperature and light quality as extension of the photoperiod in the second experiment. The values represent the average ± SE of 5 plants. The x-axis corresponds to the different PPS and SD = short days without day extension. Different letter indicates statistical difference ($p \leq 0.05$) within each temperature treatment using Tukey's test.

At 18º C there was a trend only that intermediate PPS values resulted in highest biomass, but no significant difference was detected within any of the temperatures. Similar results were obtained in the first experiment. Similar to the first experiment the shoot: root ratio did not differ significantly within each temperature

ANOVA for the total dry biomass and the shoot: root dry ratio is shown in tables 17 and 18.In both cases the interaction was removed due no significance (appendix 54 and 55), similar of the results obtained for the first experiment.

3.2.1.4. Branches

No branches were observed in any of the *A. lasiocarpa* plants neither in the second experiment.

Table 17: ANOVA for the linear model of the final total DW of *Abies lasiocarpa* in the second experiment, without including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.1641	1	24.1159	1.01E-05	***
Temperature	0.02379	1	3.4967	0.06735	.
Light treatment	0.04702	5	1.3819	0.24704	
Residuals	0.34023	50			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 18: ANOVA for the linear model of the shoot: roots dry ratio DW of *Abies lasiocarpa* in the second experiment, without including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	5.6699	1	0.01726	*
Light treatment	7.34	5	0.19656	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Photos of the 5 selected plants of *A. lasiocarpa* used for biomass analysis for each treatment in both experiments are shown in figure 27.

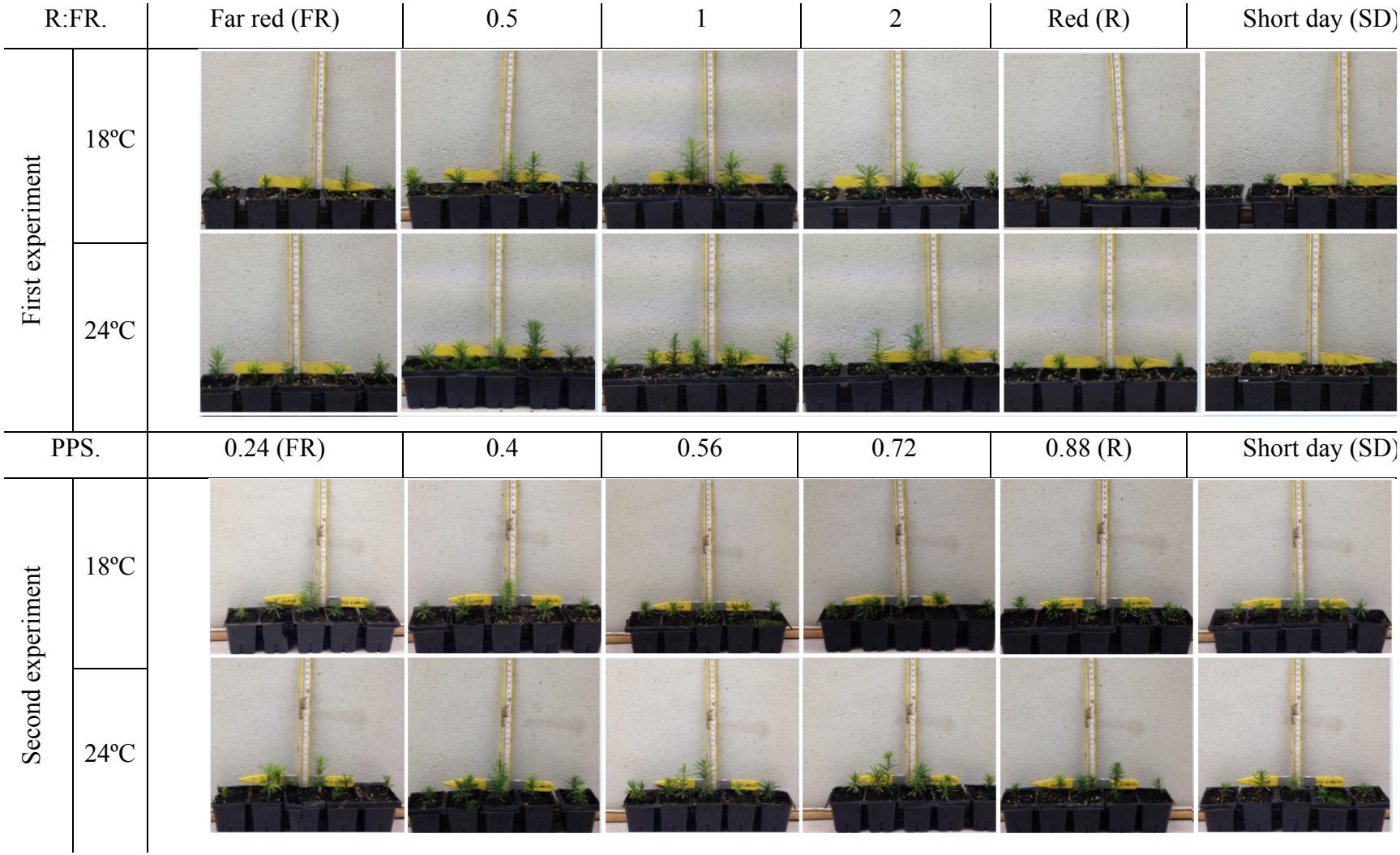


Figure 27: Photos of 5 randomly selected plants for biomass analysis in both experiments in *A. lasiocarpa*. The treatment corresponds to R:FR and PPS in the first and second experiment respectively.

4. Discussion.

4.1. Effect of the light quality and temperature on shoot elongation.

The shoot elongation in both species was significantly affected by the temperature, the light treatments, and the interaction between these in the different experiments (table 2, 8, 11 and 15). As is shown in figures 2, 10, 17 and 22, lower growth rates resulted in lower final shoot elongation. The light quality and temperature treatments appeared to have a constant effect during the time course for *P. abies*, whereas for *A. lasiocarpa* this was not the case (figure 17 and 22). This is consistent with previous experiments in *P. abies* (Mølmann *et al.,* 2006; Lee *et al.,* 2014; Olsen *et al.,* 2014;) and *A. lasiocarpa* (Aas, 2015). No constant effect of FR light in *A. lasiocarpa* plants has been previously reported and at 24º C the growth rate was reduced faster that at 18ºC (Aas, 2015). This could suggest that 18ºC induced larger effect of the light quality on shoot elongation in *A. lasiocarpa*, compared to 24º C.

In the first experiment with *P. abies,* there was an interactive effect and effect of the light quality and temperature treatments separately (table 2). In contrast, the second experiment did not show a significant interaction between the treatments (table 8), but both individual factors affected shoot elongation significantly. In both experiments with *A. lasiocarpa* the light quality and temperature treatments affected the shoot elongation significantly (Table 11 and 15), but only in the second experiment there was a significant interaction between them. This difference between experiments and species could have been due to different effect of the light treatments, where uniform R:FR distribution gave more contrasting effect than a uniform PPS distribution. In contrast, *A. lasiocarpa* was the opposite. Previous work has suggested an interaction between this light quality and temperature in woody species (Olsen *et al.*,2014; Aas, 2015; Opseth *et al.,* 2016). This interaction was shown in seeds at the beginning of the study of phytochromes, where Borthwick *et al.* (1952), showed that the effectiveness of R light treatment was temperature dependent. Later, other authors have shown that there is an increase in the phytochrome dark reversion rate under higher temperatures (Hennig and Schafer, 2001).

In *P. abies*, the FR light treated plants had a significantly higher shoot elongation than the R light treated plants and the SD treated plants, at 18ºC and 24ºC in the first and second experiment respectively (figure 2 and 10). In *A. lasiocarpa* at 18ºC, in both experiments, FR treated plants were significantly higher at the end of the experiment, compared with the R treated plants (figure 17 and 22). Previous studies have shown that FR light increases the shoot elongation compared with R light in *P. abies* (Mølmann et al., 2016; Olsen *et al.,* 2014; Aas, 2015; Opseth *et al.,* 2016), but higher shoot elongation in response to a combination of R and FR is less often reported (Mølmann *et al.,* 2006).

In the first experiment combinations of R:FR produced higher shoot elongation in both temperatures and both species, compared to R or FR given separately (figure 2 and 17). In *P. abies* the maximum average shoot elongation at 18ºC was observed with higher R:FR compared to 24ºC (R:FR 1 and 0.5 at 18ºC and 24ºC, respectively; Figure 2). In *A. lasiocarpa* the maximum average shoot elongation at 18ºC was observed with lower R:FR compared to 24ºC (R:FR 0.5 and 1 at 18ºC and 24ºC, respectively). Mølmann *et al.* (2006), found that in *P. abies* at 18ºC, R:FR of 1 could induce higher shoot elongation than R:FR of 2.2 or 3.7. The results of both experiments in the present study are consistent with the results of Mølmann *et al.,* (2006). Mølman *et al,* (2006) attributes this to a possible effect of the R:FR in maintaining the growth in *P. abies*, which should be reflected also in the biomass. In the second experiment with *P. abies*, at 18ºC there was no significant difference between the R:FR light quality treatments. At 24ºC, PPS values lower than 0.72 produced significantly higher shoot elongation, compared with PPS values higher than 0.56 and the SD treatment. In *A. lasiocarpa* at 18ºC, the lowest PPS (0.24, FR) resulted in significantly higher shoot elongation than the R and SD treatment, whereas at 24ºC there was no difference between the different PPS values. It has been shown that the morphology of *Arabidopsis* under different light qualities is temperature dependent. At 16ºC *Arabidopsis* exposed to low R:FR, showed higher leaf area and thickness, as compared to under high R:FR. However, there was no difference in the leaf shape and thickness under higher temperatures (22ºC) (Patel *et al.,* 2013). This difference in morphology and sensitivity to the light quality under lower temperatures is similar to the results obtained in the present experiments. This supports that in *P. abies* and *A. lasiocarpa* there was an interaction between the tested factors. In *A. lasiocarpa* combinations of R:FR under 18ºC induced a higher shoot elongation that FR light only.

In *P. abies* at 18ºC the R light was able to stop the shoot elongation, but this was not the case at 24ºC in both experiments (figure 2 and 10). In the first experiment with *A. lasiocarpa,* similar results as in *P. abies* were obtained. Aas (2015) and Mølmann *et al.* (2006), showed similar effect in these species. Aas (2015) found that the effect of R light on the reduction of shoot elongation of *P. abies* under 22ºC was lower than at 18ºC and even lower at 24ºC.

In *P. abies* it took 28 and 42 days to detect an interactive effect between the light and the temperature treatments in the first and second experiment respectively, when the interaction was analysed in a time course (figure 3 and 11). This suggest that the difference between treatments was easier to detect in the first experiment. In *A. lasiocarpa* the interaction between temperature and light quality treatments in a time course, was never significant (figure 18 and

23). The decrease in the p values suggests that longer exposure times to the different treatments would have shown an interaction between the factors.

The SD treatment did not result in a difference in the final shoot elongation between the temperatures within experiments for *P. abies* (p value = 0.19 and 0.37) or *A. lasiocarpa* (p value = 0.83 and 0.97). In each experiment similar values were achieved, close to 3 cm and 0.7 cm for *P. abies* and *A. lasiocarpa*, respectively (figure 2,10, 17 and 22). Similar results were obtained for both species previously under the same photoperiod and temperatures (Aas, 2015). The SD treated plants of *P. abies* in the first experiment took shorter time to stop growing under 24ºC compared with 18ºC. It took about 20 days to stop growing at 24ºC compared with almost 48 days at 18ºC. This suggests the SD effect on shoot elongation in *P. abies* was promoted for the higher temperature. In the second experiment it took close to 20 days to stop growth in both temperatures. The SD treated plants of *A. lasiocarpa* at both temperatures and in both experiments, started the reduction of the growth rate at similar times. In the first experiment it took shorter time to achieve a total stop in the growth that in the second experiment (14 vs 21 days). These differences between experiments might be attributed to the RH or the water availability. Ewers *et al.,* (2001) showed in *P. abies* and *Pinus taeda*, that a change in the water balance or nutrient availability can affect the stomatal conductance and the leaf area, and thus the growth of the plants.

P. abies showed overall maximum final shoot elongation in both experiments at 24º C (figure 2 and 10), whereas this was the case at 18º C in *A. lasiocarpa* (figure 17 and 22). It is known that an increase in temperature, in the right range, enhances the growth rate if there are no other limiting conditions, with the temperature for maximum growth being species dependent (Franklin *et al.,* 2014). It has been shown in *P. abie*s that an increase in the temperature, from 18ºC to 24º C, can increase the shoot elongation (Aas, 2015).

In the first experiment shoot elongation of *A. lasiocarpa* showed a double sigmoidal shape, except for the R and SD treated plants (Figure 17). The inflexion point, that corresponds to a stop in the growth, matches with the maximum bud set for each treatment (figure 17 and 19). It is well known that bud set follows a decrease in shoot elongation. In *P. abies* Lee *et al.* (2014) showed that growth cessation and subsequent bud set to be correlated with an increase and decrease of several energy-related metabolites, respectively (Lee *et al.,* 2014). Rindedal (2015) and Aas (2015) have shown that in *A. lasiocarpa* the bud set is correlated with a decrease in the growth rate where the bud set can take between 25 and 30 days under SD conditions. This is also visible in the second experiment (figure 20), where the lack of reduction in the growth rate correlates with the absence of bud set in the middle of the experiment. The

difference between the first and second experiment in bud set could has been due to the water management previous and after started the experiments (Appendix 4 and 5).

4.2 Effect of the light quality and temperature on bud formation.

P. abies showed bud set in both experiments, under R (PPS 0.88) and SD treatments. The effect of the R light was dependent of the temperature. Under 24ºC the maturity of the buds was significantly lower in both experiments compared with the R and SD at 18º C (figure 4 and 12). The R treated plants also took longer time to show initial bud set at 24ºC, compared with 18ºC in both experiments, *i.e.* almost 8 days more. The R light induced similar results as in Mølmann *et al.* (2006) where R light induced bud set in *P. abies* of different provenances, but the presence of bud was later and slower compared with the SD. Bud set in *P. abies* due to application of SD-treatments is well known and has been reported by a range of authors (Olsen *et al.,* 2014; Aas, 2015; Opseth *et al.,* 2016). The dependence of temperature in the R light treated plants, demonstrates that there is an interactive effect of the temperature and light quality on the bud set, similar to what was shown by Aas (2015). ANOVA for the bud set showed a significant interaction and significant effect of the light quality in the first experiment (table 3), whereas in the second experiment just the light quality treatment was significant (table 9). The tested light quality treatments had a higher effect on the variation of the bud set than the range of tested temperatures in *P. abies*.

A. lasiocarpa showed bud set in all the light quality treatments at 24ºC in both experiments. At this temperature the SD treated plants showed lower average bud development stage compared with 18º C (figure 19 and 24). This shows that the effect of the tested light treatment is even more dependent on the tested temperature than in *P. abies*. Aas (2015) obtained similar results at 18ºC where the FR light significantly reduced the average mean bud set stage. At 22ºC the effect of FR light was much lower in the reduction of bud set stage than at 18ºC. ANOVA of the bud set for *A. lasiocarpa s*howed (table 12 and 16), that in contrast to *P. abies*, in both experiments there was a significant interaction between the temperatures and light quality treatments. This confirms the interaction between the tested temperatures and light qualities in *A. lasiocarpa* and a higher dependence on the temperature for the light quality effects than in *P. abies.*

When the interaction between light quality and temperature was analysed for bud stage in a time course for *P. abies*, this was significant after 28 days in the first experiment, similar to the results for shoot elongation, whereas no significant interaction was observed in the second experiment. In the second experiment, like was the case for the shoot elongation, the p values

continued to decrease, suggesting that longer experiments would have shown a significant interaction. In *A. lasiocarpa* the interaction between light quality and temperature took 35 and 46 days to be significant in the first and second experiment.

The SD treatment of *P. abies* increased the bud development significantly under 24ºC compared with 18ºC (figure 4 and 12), whereas for *A. lasiocarpa* there were no significant difference in bud development between the two temperature treatments (figure 19 and 24). It has been hypothesized that the effect of temperature on bud set would depend and whether it has already been triggered by other limiting factors (Strømme *et al.,* 2015), such as the day length in SD treated plants. Thus, an increase in temperature could accelerate or decrease the bud set depending on whether it has been triggered or not. Several studies under indoor conditions have shown that an increase in temperature can accelerate the bud set, as was shown in *P. abies* and *Picea glauca* (Hamilton *et al.*,2016; Olsen *et al.,* 2014; Aas, 2015; Opseth *et al.,* 2016). In contrast, under outdoor conditions an increase of the temperature tend to delay the bud set. This has been shown in *Populus tremula,* where an increase in temperature by 2ºC delayed bud set, allowing a longer growth period and enhanced the subsequent bud break (Strømme *et al.,* 2015).

The use of categories for the bud development stage allows the detection of interactions that recording of the percentage of plants with buds only was not able to detect. The recording of detailed bud stages gives more information that can be correlated with physiological effects and internal processes (Lee *et al.,* 2014).

4.3 Effect of the light quality and temperature on gene expression of *Picea abies*.

There was a strong significant increase in *PaFTL2* transcript levels at the end of the experiment under SD compared to the LD treated plants. There was a trend of increase in *PaFTL2* expression also under the R treatments although this was not statistically significant. The increase of *PaFTL2* under SD after 7 days shown a trend of been higher at 18ºC than at 24ºC (48 vs 16.45; p value= 0.15), but at the end of the experiment this trend was higher at 24ºC than at 18ºC (588 vs 361; p value =0.15). This suggest that the dynamics of *PaFTL2* during the time course was regulated by these factors (figure 8). Several authors have shown that *PaFTL2* increases its expression under SD (Asante *et al.,* 2011; Gyllenstrand et al., 2007; Karlgren et al., 2013; Aas, 2015; Opseth *et al.,* 2016). It has been also shown that the expression of *PaFTL2* increases after transferring the plants from LD to FR, R and SD light with *PaFTL2* transcript levels following the same order (Aas, 2015). This could explain the registered bud set and reduction in the shoot elongation, which is confirmed by the high correlation of the *PaFTL2*

transcript level and the bud set and shoot elongation (R^2= 0.86 and 0.79 respectively; figure 9). This is consistent with the previous hypothesis that higher transcripts levels of *PaFTL2* could be acting to reduce growth. It has been shown that other genes related to PEBP proteins, are independent of the temperature. In *Arabidopsis* wild type plants, the floral bud formation is accelerated by an increase of temperature through an increase of the transcript abundance of *FT* (Halliday *et al.,* 2003).

PaCOL1 showed a trend of increase under FR light, compared with the initial LD, but the final change was not significant in any of the temperatures. Under 24ºC, SD and R light treatments significantly decreased *PaCOL1* (p value = 0.02 and 0.05 respectively) and at 18ºC there was a trend of decrease (p value = 0.06 and 0.06; figure 8). The transcript level under R:FR 1 showed a trend of being dependent of the temperature but there was no interaction between the temperature and light quality treatments in the final levels of *PaCOL1* and there was no significant effect of any of the two factors (appendix 16). This might be due to the large SE and the small number of replicates (n=3). Holefors *et al.* (2009) showed an increase in the expression of *PaCOL1* in *P. abies* when the plants were moved from darkness to light. They also showed a diurnal variation in *PaCOL1* with lower transcript levels under SD that LD. Opseth *et al.* (2016) showed higher expression of *PaCOL1* under FR light, compared with R and SD treatments. The correlation of *PaCOL1* with the bud set and shoot elongation was among the lowest ones (figure 9) compared with the other genes (R^2=0.16 and 0.12 respectively).

PaCOL2 transcript levels showed a trend of lower values in the SD treatment with significantly lower values at the end of the experiment at 18ºC compared to at the start. Under SD, the transcript levels of *PaCOL2* were lower than in other treatments, but there was no significant difference between the temperatures. Similar to *PaCOL1* there was also no significant interaction between the experimental factors. After removing the interaction, only the light quality treatment showed a trend of significance (p value=0.07; appendix 17). Similar results were obtained for Holefors *et al.* (2009) and Opseth et al. (2016), where SD treatments reduced the transcript levels of *PaCOL2*. *PaCOL2* showed lower correlation with the bud stage and with the shoot elongation at the end of the experiment than *PaFTL2,* but higher than *PaCOL1* (R^2=0.34 and 0.25 for bud set and shoot elongation respectively; figure 9).

PaSOC1 showed a trend of increase in its expression in all the treatments compared with LD, but this increase was significantly higher just for the R:FR 1 and FR treated plants at 18ºC. There was no significant interaction between the factors but the light quality treatment was significantly affecting *PaSOC1* expression (p value= 0.02; appendix 18). *PaSOC1*

transcript levels was also well correlated with the bud stage and shoot elongation at the end of the experiment (R^2 = 0.41 and 0.3 respectively, figure 9). Opseth *et al.,* (2016) also found that the transcript levels of *PaSOC1* were reduced under SD and that the expression was inversely correlated with the bud set. They also showed that *PaSOC1* had lower transcripts levels under the R treatment than the FR treated plants, what is consistent with the results of this experiment, shown in figure 8.

4.4 Effect of the light quality and temperature on biomass, shoot: root ratio and branching.

In *P. abies* and *A. lasiocarpa*, the total biomass was not significantly affected by the interaction of the temperature and light treatment or by the temperature in any of the experiments (table 4, 13 and 17).

In the first experiment, only the effect of light quality treatment on total biomass was significant in both species (table 4 and 13), but when the light treatments were analysed within temperatures, there was non significant difference between theses, at exception of the SD of both species in the first experiment. Lack of difference between the light treatments within temperatures could be explained by the application of the same amount of energy (7 W m^{-2}) during the extension of the photoperiod. This suggests that the applied amount of energy should not induce a significant increase in biomass.

In both experiments intermediate R:FR resulted in a trend of inducing higher biomass. The trend of increased biomass in response to combinations of R:FR, or intermediate PPS, observed in both species and both experiments is similar to the situation reported earlier in other species like tomato and cucumber (Hogewoning *et al.,* 2012). In these species, the leaf angle was shown to be different under different combinations of R and FR light and this was suggested to improve the light perception during the light period, without affecting the photosynthetic activity. A difference in the angle of the needles of *P. abies* under FR and R-treated plants for *P. abies* was reported by Aas (2015), with FR light promoting more horizontal needles compared with R light.

In *P. abies* the effect of the temperature showed a trend of significance during the first experiment (p value= 0.06). This trend was also present in *A. lasiocarpa* but in the second experiment only (p value = 0.067). There was a trend of a higher difference between the treatments at 18ºC compared to 24ºC in both species in the second experiment, combinations of R:FR showing a trend of higher biomass.

The dry shoot: root ratio of *P. abies* in the first experiment at 18ºC was significantly higher at R:FR 0.5 compared with the R treated plants. However, this was not the case for the FR treated plants as FR did not result in significantly higher shoot: root ratios than the R treatment. In *A. lasiocarpa* there was no significant difference between the treatments within each temperature in any of the experiments. In the second experiment with *P. abies* at 18ºC, all combinations of R:FR produced significantly higher shoot: root ratios than the FR treatment (0.24 PPS), but not than the R treatment (0.88 PPS) (figure 6 and 12). ANOVA of the shoot/root ratio in the first experiment with *P. abies* and *A. lasiocarpa* showed that only the light treatments were significant. In the second experiment the ANOVA of *A. lasiocarpa* showed a significant effect of the temperature on the shoot: root ratio. This is consistent with the effect of FR and R light in shade avoidance plants and confirms a higher effect of the tested temperature in *A. lasiocarpa* than in *P. abies*. In a range of species higher amount of FR is known to increase the shoot elongation and promote shoot biomass production whereas R light produces shorter plants that will improve the allocation of carbon to the undersoil organs (Gommers *et al.,* 2013). The present results in both species indicate that a higher range of temperature would be required to detect an effect of the temperature on the total biomass.

In *P. abies*, there was a significantly higher number of branches in the first experiment compared with the second one at both temperatures (p value <0.01) (figure 7 and 15). The water availability, RH and the variability between experiments could be main factors affecting this. In the first experiment 24ºC treated plants showed a trend of higher number of branches than the 18ºC treatment, whereas in the second experiment 24ºC showed significantly higher number of branches (p value = 0.08 and 0.001 in the first and second experiment respectively). It has been shown in other species, such as *Euphorbia pulcherrima,* that an increase in temperature promotes lateral branching (Hagen and Moe, 1981). At 24ºC, significantly higher number of branches was obtained with any ratio of R:FR light or intermediate PPS in both experiments, compared with the application of just R, FR and SD. This contradicts previous experiments, which have shown that R light induces the formation of branches or lateral bud formation (Demotes-Mainard *et al.,* 2015; Gautam *et al.,* 2015). Reddy and Finlayson (2014) found in *Arabidopsis* that the effect of the R and FR light on growth of lateral buds at intermediate position will depend of the exposure to previous R:FR, where the lateral buds can be promoted if the plants have been exposed to low R:FR before. The use of FR LEDs during the day to decrease the R:FR and the change in R:FR from 1.8 to 2.5 in the pre-growing phase and the experiment respectively, could have affected the lateral branching. Finally, in both experiments the SD exposed plants produce the lowest number of branches at both temperatures.

5. Conclusions

From the experiments it is possible to conclude:

- There is a clear interactive effect between the temperature and light quality as a day extension on the shoot elongation and bud set on *Picea abies* and *Abies lasiocarpa*.
- In *P. abies* there was also an interactive effect of these factors on the transcripts level of *PaFTL2*, which showed a temperature-dependent increase, under the SD and R treatments. Larger increase was observed at 24 than 18°C.
- An increase in *PaFTL2* in *P. abies* correlates with cessation of shoot elongation and bud set (R^2= 0.86 and 0.79), suggesting an action as a growth inhibitor.
- *PaCOL2* and *PaSOC1* might be involved in the control of shoot elongation, but no significant interaction between the tested combinations of light quality and temperature was found. *PaCOL1* appears to be less reactive to the tested light qualities and temperatures.
- In both species, the effect of light quality on shoot elongation was larger under 18ºC compared to 24ºC. This effect was more visible for *A. lasiocarpa* than *P. abies*. In *A. lasiocarpa* lower temperatures are suggested to improve the effect of the day extensions.
- In both species, FR light was able to prevent the formation of buds in at least one of the experiments, whereas R light induced the formation of buds and the maturity level was dependent of the temperature.
- Finally, the use of FR LEDs to regulate the R:FR during the day did not result in any visible effect or source of variation on the shoot elongation, bud set or total biomass as compared to previous studies where incandescent lamps were used for this purpose (Aas 2015).

6. Suggestions for further research

As a general suggestion, it could be important to measure the angle of the needles under the different R:FR to understand better how this affects the morphology of the plants. Also, an increase in number of samples is recommended for the gene expression and biomass analysis due to the large variability in these. An increase in the number of time points in the gene expression analysis would also help to understand how the light quality affect the final level and dynamic of the genes under different light qualities and temperatures. Testing the effect of lower temperatures or higher irradiances in *A. lasiocarpa* is also recommended, for a possible increase of the light quality effect.

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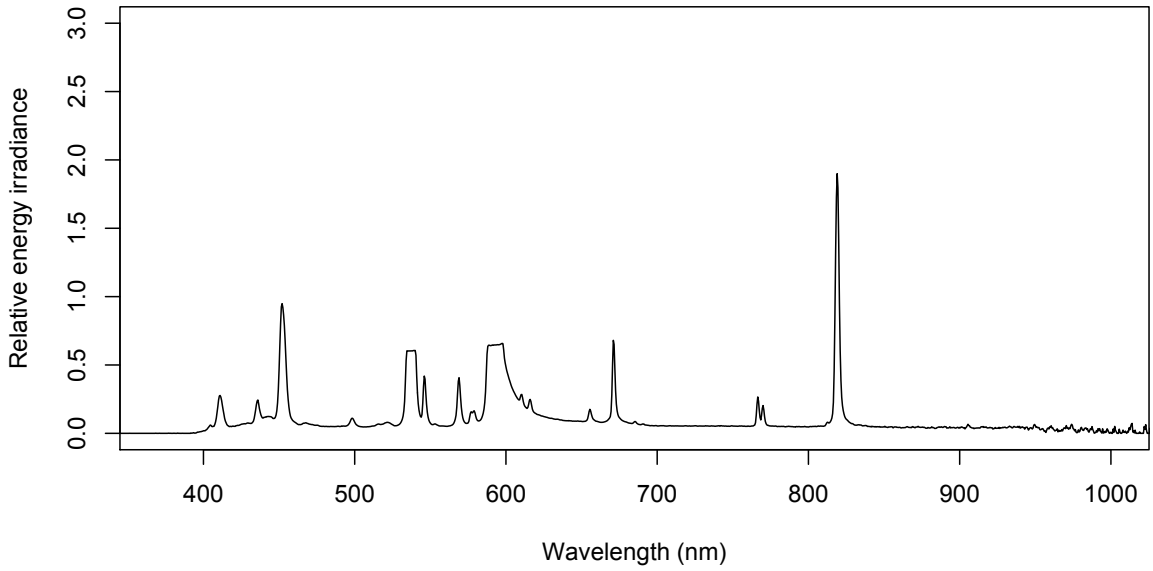
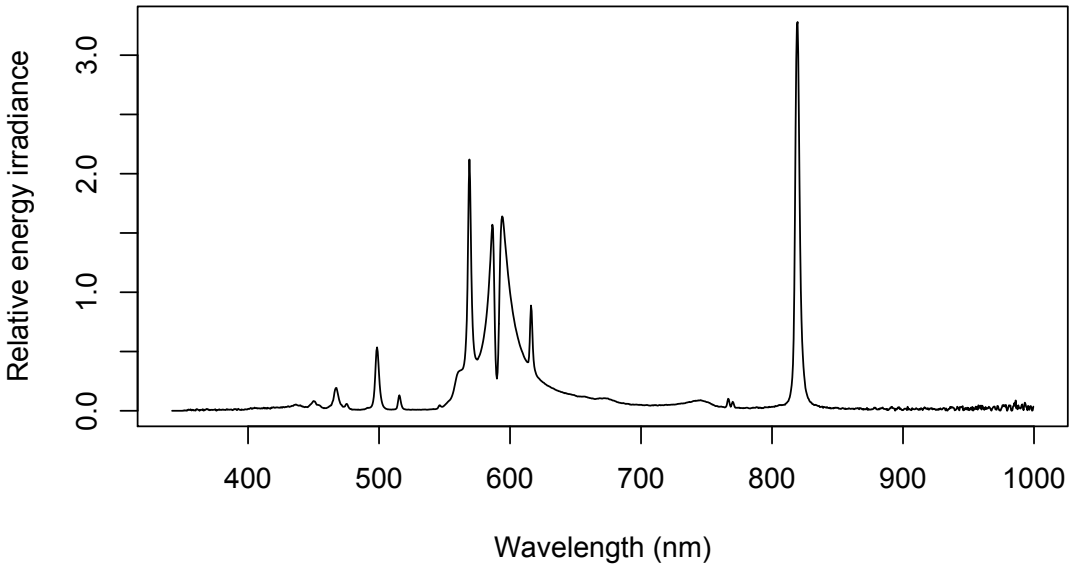
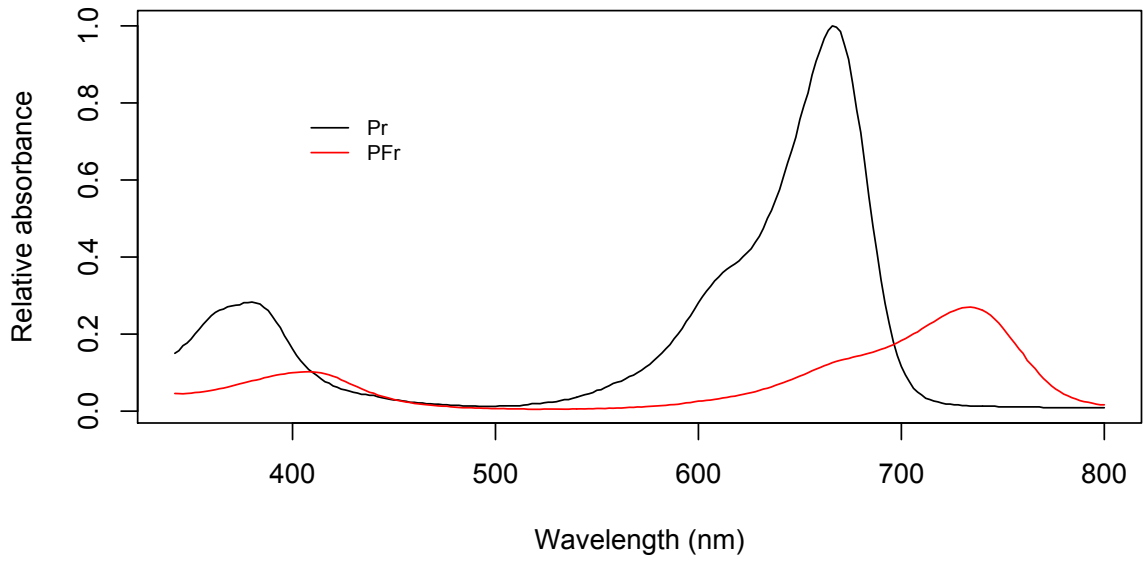
8. Appendix



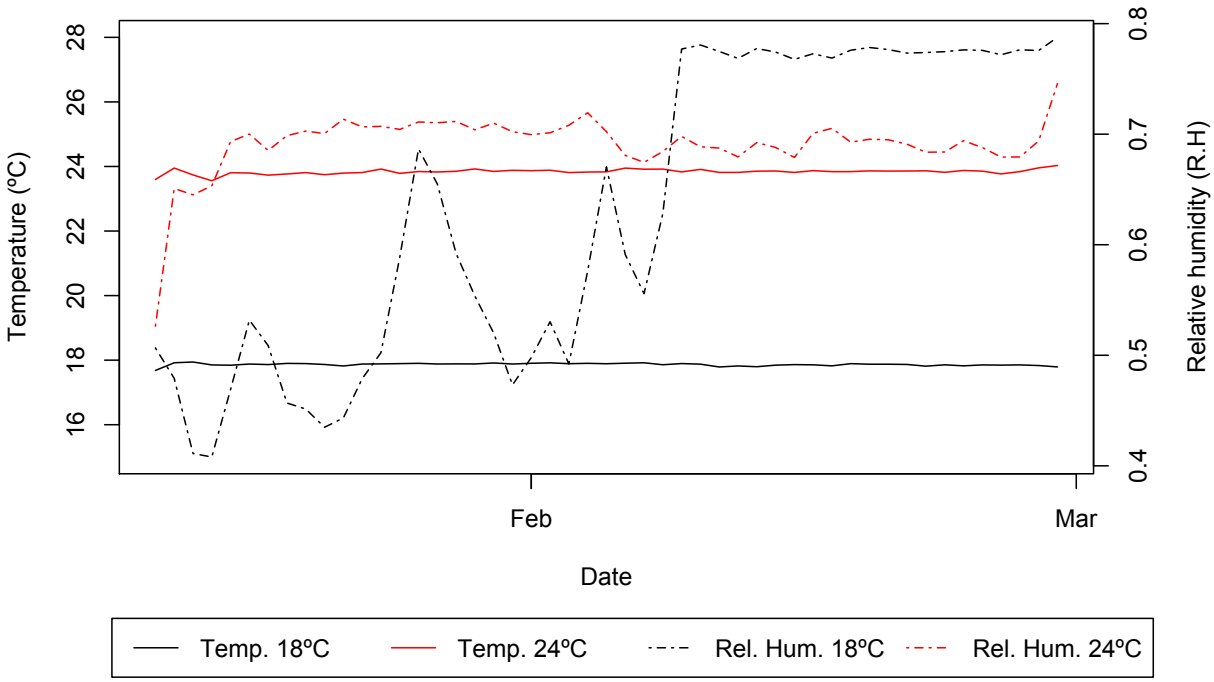
1. Light spectrum during pre-growing with HQI lamps plus 60 W incandescent lamps.



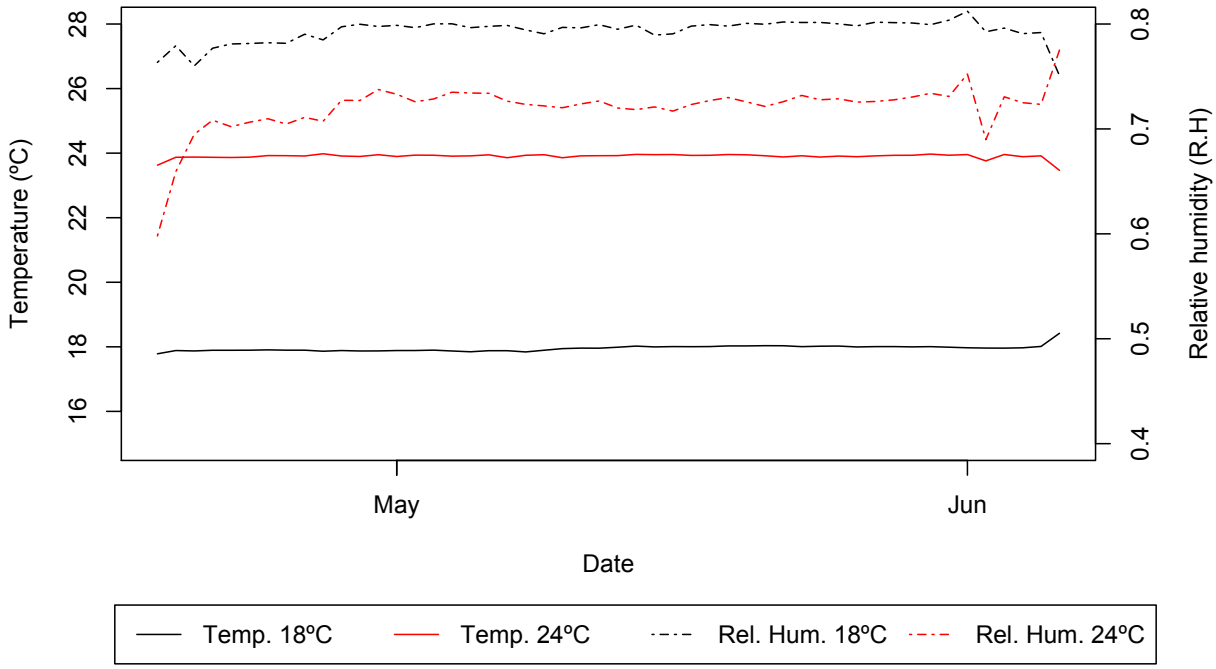
2. Light spectrum during the day in the experiments using HPS and 5 FR LEDs (720-740 nm) at 70 cm of height.



3. Phytochrome photoconversion (data from Sager *et al.*, 1988) used to calculate the PSS.



4. Temperature and Humidity flux during the first experiment.



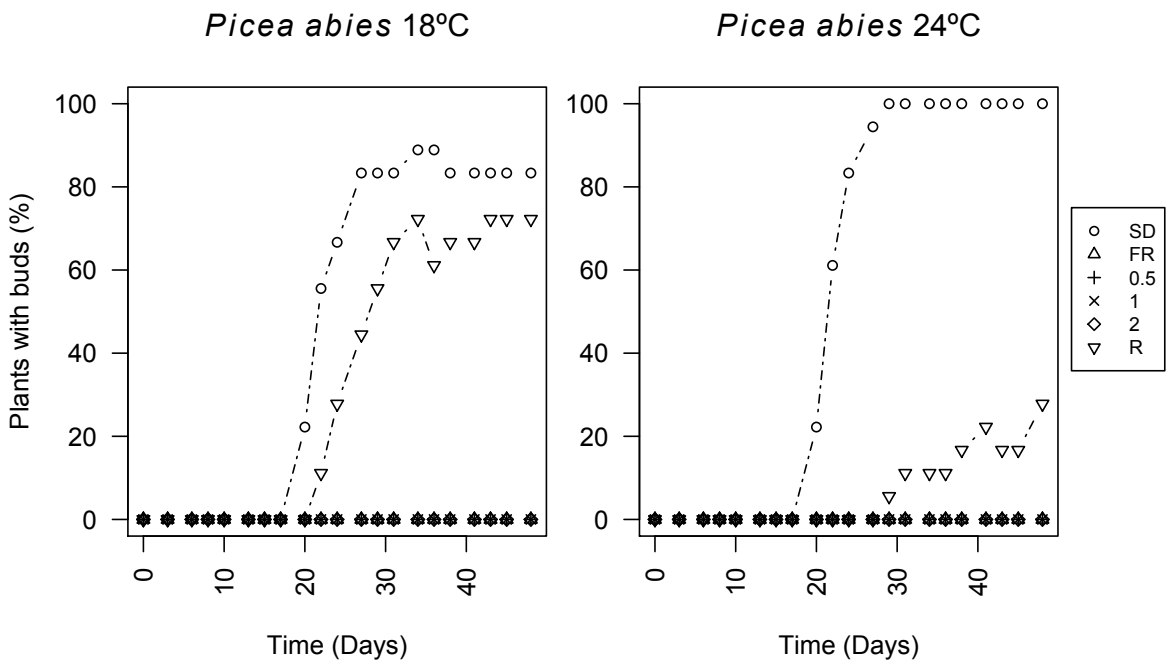
5. Temperature and Humidity flux during the second experiment.

6. ANOVA for the linear model of the final shoot elongation in the first experiment of *Picea abies*.

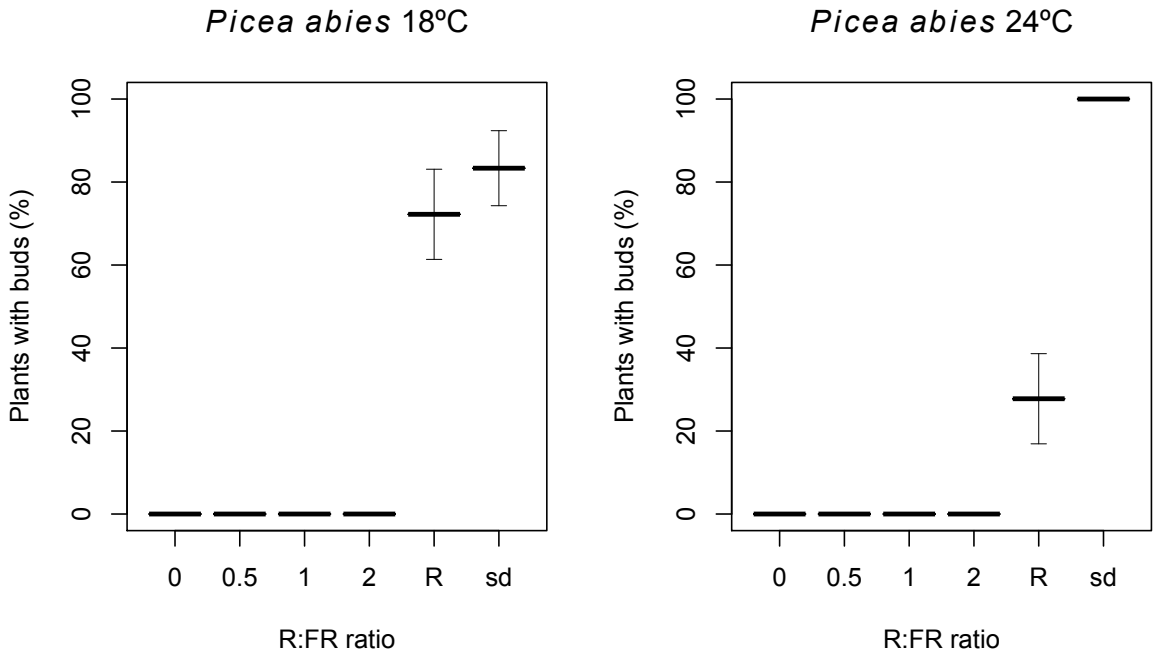
Analysis of Deviance Table (Type III Wald chisquare tests)

	Sum Sq	Df	F Value	Pr(>F)	
(Intercept)	26.34	1	4.4069	0.0370355	*
Temperature	114.17	1	19.1034	1.98E-05	***
Light treatment	124.99	5	4.1826	0.0012252	**
Temperature: Light treatment	137.54	5	4.6025	0.0005344	***
Residuals	1207.27	202			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1



7. Percentage of plants with bud in a time course for *Picea abies* in the different treatments of temperature and light quality as extension of the photoperiod in the first experiment. 0 is no presence and 1 presence of bud. The values represent the average of 18 plants.



8. Final mean bud classification for the different treatments of *Picea abies* during the first experiment as binomial response with plus/minus SE. Where 0 is no presence and 1 presence of buds.

9. ANOVA table for the bud classification as binomial response for *Picea abies* during the first experiment including the interaction between factors.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	8.4524	1	0.003646	**
Temperature	1.1412	1	0.285407	
Light treatment	29.0392	5	2.28E-05	***
Temperature: Light treatment	6.6199	5	2.50E-01	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

10. ANOVA table for the bud classification as binomial response for *Picea abies* during the first experiment excluding the interaction between factors.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	0	1	0.9995	
Temperature	0.4089	1	0.52253	
Light treatment	10.6326	5	0.05917	.

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

11. ANOVA for the generalized linear model of the final bud set classification using categories in the first experiment of *Picea abies*

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0	1	1.00E+00	
Light treatment	178.63	5	2.00E-16	***
Temperature: Light treatment	17.554	5	0.00356	**

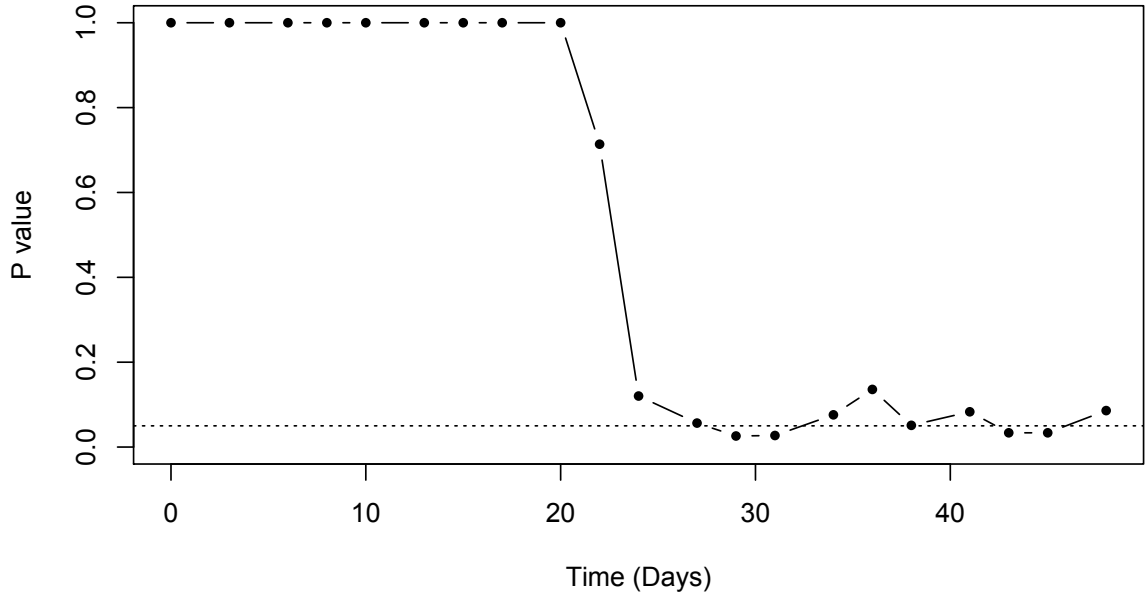
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

12. ANOVA table for the final bud classification as binomial response for *Picea abies* during the first experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
temperature	0	1	0.99998	
Light treatment	308.041	5	2.00E-16	***
Temperature: Light treatment	14.028	5	0.01543	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



13. P value in a time course for the interaction of the temperature and light treatments in the *Picea abies* bud set as a presence or absence for the first experiment.

14. ANOVA for the linear model of the final total DW of *Picea abies* during the first experiment, including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.03346	1	1.007	0.32181	
Temperature	0.10432	1	3.1394	0.08423	.
Light treatment	0.21082	5	1.2689	0.29676	
Temperature: Light treatment	0.34738	5	2.0908	0.0872	.
Residuals	1.29594	39			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

15. ANOVA for the generalized linear model of the shoot: root DW ratio of *Picea abies* during the the first experiment, including the interaction between the temperature and light treatments.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
temperature	3.0179	1	0.08235	.
treatment	5.7968	5	0.32649	
temperature: treatment	9.8018	5	0.08105	.

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

16. ANOVA for the transcription level of *COL1* of *Picea abies* without interaction between the treatments during the first experiment.

Anova Table (Type III tests)

	Sum Sq	DF	F value	Pr(>F)	
(Intercept)	24.492	1	5.7948	2.64E-02	*
Light treatment	14.244	3	1.1234	0.3645	
Temperature	1.313	1	0.3108	0.5837	
Residuals	80.303	1			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

17. ANOVA for the transcription level of *COL2* of *Picea abies* without interaction between the treatments during the first experiment.

Anova Table (Type III tests)

	Sum Sq	DF	F value	Pr(>F)	
(Intercept)	71.912	1	47.4802	1.43E-06	***
Light treatment	12.151	3	2.6743	0.0765	.
Temperature	0.823	1	0.5433	0.4701	
Residuals	28.777	19			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

18. ANOVA for the transcription level of *SOC1* of *Picea abies* without interaction between the treatments during the first experiment.

Anova Table (Type III tests)

	Sum Sq	DF	F value	Pr(>F)	
(Intercept)	504.52	1	104.3137	3.74E-09	***
Light treatment	59.25	3	4.0837	0.02141	*
Temperature	0.51	1	0.106	0.74833	
Residuals	91.89	19			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

19. ANOVA for the shoot elongation for *Picea abies* including interaction in the second experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	9.7395	1	1.80E-03	**
temperature	44.149	1	3.04E-11	***
Light treatment	6.0376	5	3.03E-01	
temperature: Light treatment	5.5065	5	3.57E-01	

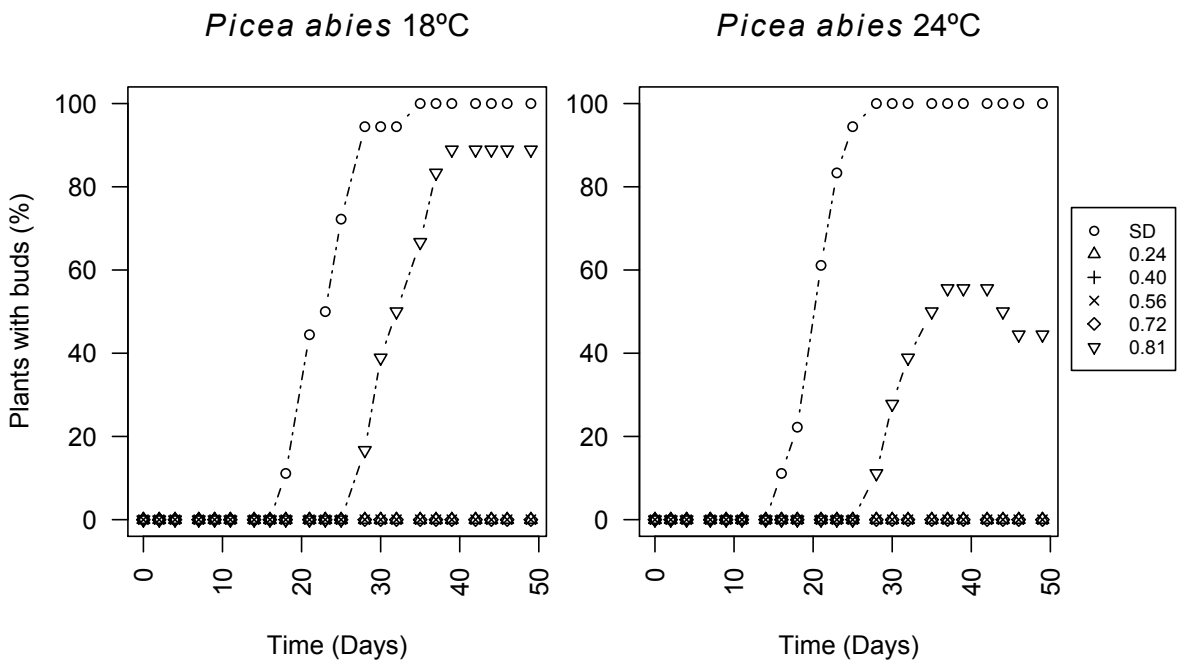
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

20. ANOVA for the final shoot elongation for *Picea abies* including interaction in the second experiment.

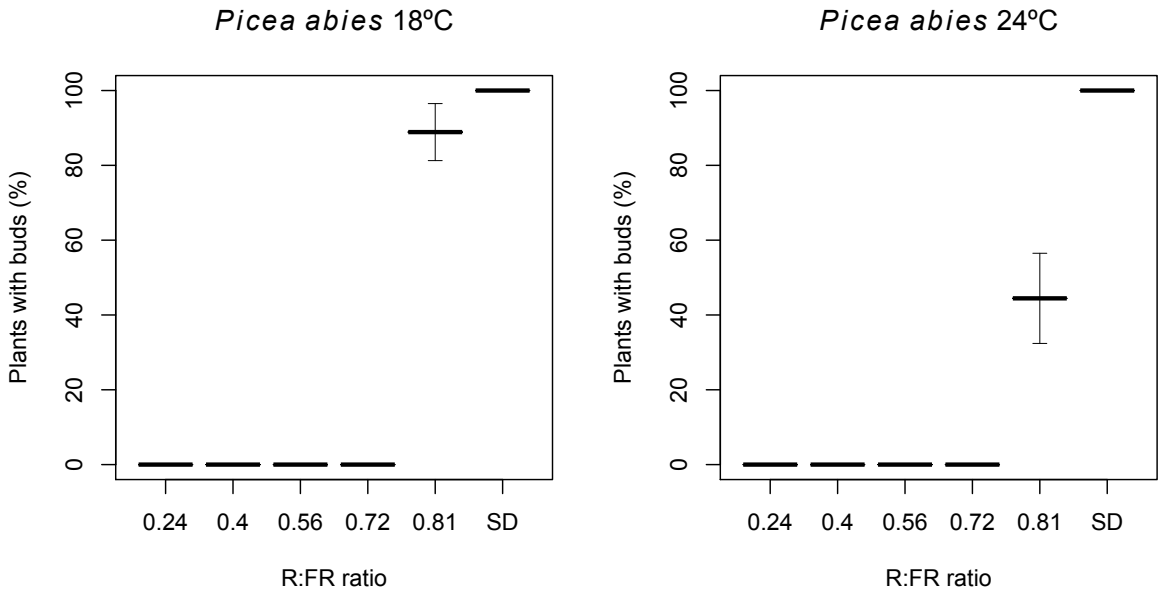
Analysis of Deviance Table (Type III Wald chisquare tests)

	Sum Sq	Df	F Value	Pr(>F)	
(Intercept)	8.69	1	2.8216	0.09453	.
Temperature	70.04	1	22.7354	3.53E-06	***
Light treatment	37.54	5	2.4369	0.03589	*
Temperature: Light treatment	44.71	5	2.9025	0.01485	*
Residuals	628.47	204			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



21. Percentage of plants with bud in a time course for *Picea abies* in the different treatments of temperature and light quality as extension of the photoperiod in the second experiment. Where 0 is no presence and 1 presence of bud. The values represent the average of 18 plants.



22. Final mean bud classification for the different treatments of *Picea abies* during the second experiment as binomial response with plus/minus SE. 0 is no presence and 1 presence of buds.

23. ANOVA for the generalized linear model of the bud set classification using categories in the second experiment of *Picea abies* with interaction. For this the plant and time where used as random variables.

	LR Chisq	Df	Pr(>Chisq)	
(Intercept)	0.0001	1	0.9941	
Temperature	0	1	1	
Light treatment	148.4672	5	2.00E-16	***
Temperature: Light treatment	7.9729	5	0.1577	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

24. ANOVA for the generalized linear model of the bud set classification as presence or absence of buds in the second experiment of *Picea abies* with interaction. For this the plant and time were used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	0	1	1	
Temperature	0	1	1	
Light treatment	71.84	5	4.24E-14	***
Temperature: Light treatment	2.435	5	7.86E-01	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

25. ANOVA for the generalized linear model of the bud set classification as presence or absence of buds in the second experiment of *Picea abies* without interaction. For this the plant and time were used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	3259.5748	1	2.00E-16	***
Temperature	1.3523	1	0.2449	
Light treatment	9061.2461	5	2.00E-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

26. ANOVA for the generalized linear model of the last bud set classification using categories in the second experiment of *Picea abies* with interaction. For this the plant and time were used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)
Temperature	0	1	1.00E+00
Light treatment	0.89802	5	9.70E-01
Temperature: Light treatment	2.31169	5	0.8045

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

27. ANOVA for the generalized linear model of the last bud set classification using categories in the second experiment of *Picea abies* without interaction. For this the plant and time were used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0.23	1	6.31E-01	
Light treatment	359.08	5	2.00E-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

28. ANOVA for the generalized linear model of the bud set classification as presence or absence response in the second experiment of *Picea abies* with interaction. For this the plant and time were used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
temperature	1.81E-09	1	1	
treatment	-6.80E-08	5	1	
temperature:treatment	1.85E-08	5	1	

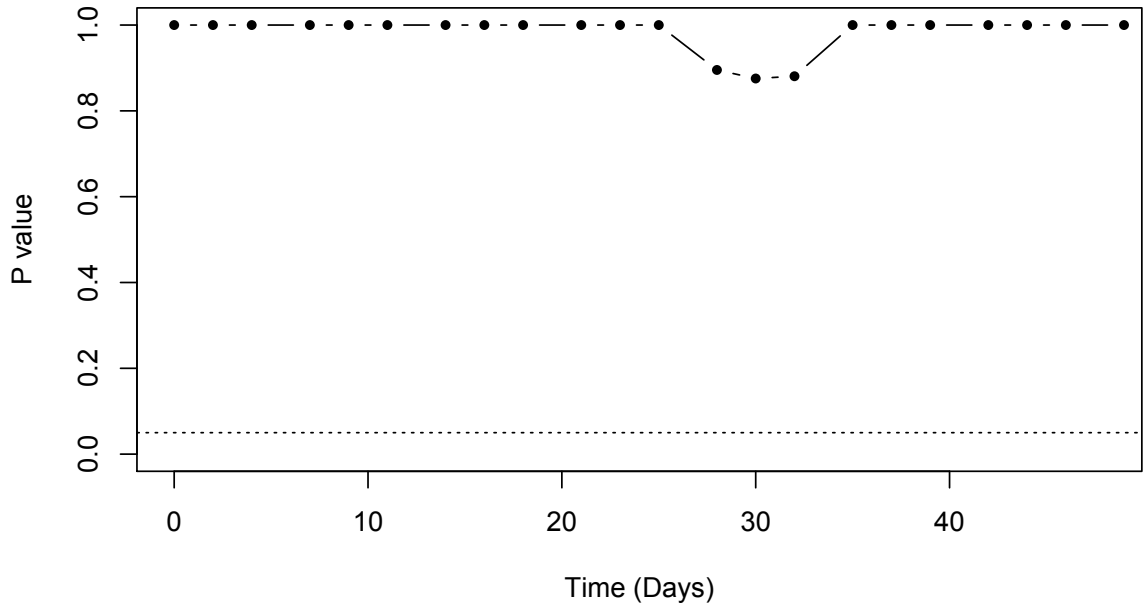
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

29. ANOVA for the generalized linear model of the bud set classification as presence or absence response in the second experiment of *Picea abies* without interaction. For this the plant and time were used as random variables.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
temperature	8.54E+00	1	0.003473	**
treatment	2.16E+02	5	2.20E-16	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1



30. P value in a time course for the interaction of the temperature and light treatments in the *Picea abies* bud set as a presence or absence response in the second experiment.

31. ANOVA for the linear model of the final total DW of *Picea abies* during the second experiment, including the interaction between the temperature and light treatments using n = 5.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.04536	1	4.2632	0.04531	*
temperature	0.00895	1	0.8415	0.36434	
treatment	0.02111	5	0.3967	0.8482	
temperature: treatment	0.01562	5	0.2937	0.91367	
Residuals	0.43623	41			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

32. ANOVA for the linear model of the final total DW of *Picea abies* during the second experiment without interaction between the factors. n = 5.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.04526	1	4.6078	0.03713	*
temperature	0.0096	1	0.9775	0.32798	
treatment	0.04549	5	0.9263	4.73E-01	
Residuals	0.45185	46			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

33. ANOVA for the generalized linear model of the shoot: roots DW ratio of *Picea abies* during the second experiment, including the interaction between the temperature and light treatments using n = 5.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)
temperature	1.759	1	0.1847
treatment	1.3728	5	0.9273
temperature: treatment	1.4258	5	0.9215

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

34. ANOVA for the generalized linear model of the shoot: roots DW ratio of *Picea abies* during the second experiment without interaction between the factors. n = 5.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)
temperature	1.7154	1	0.1903
treatment	8.2914	5	0.1409

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

35. ANOVA for the shoot elongation of *Abies lasiocarpa* during the first experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	3.509	1	0.06104	.
Temperature	24.1729	1	8.81E-07	***
Light treatment	9.2267	5	0.10036	
Temperature: Light treatment	8.7803	5	0.11816	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

36. ANOVA for the final shoot elongation for *Abies lasiocarpa* including interaction.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0.0207	1	0.88564	
Light treatment	13.1347	5	0.02215	*
Temperature: Light treatment	8.138	5	0.14879	

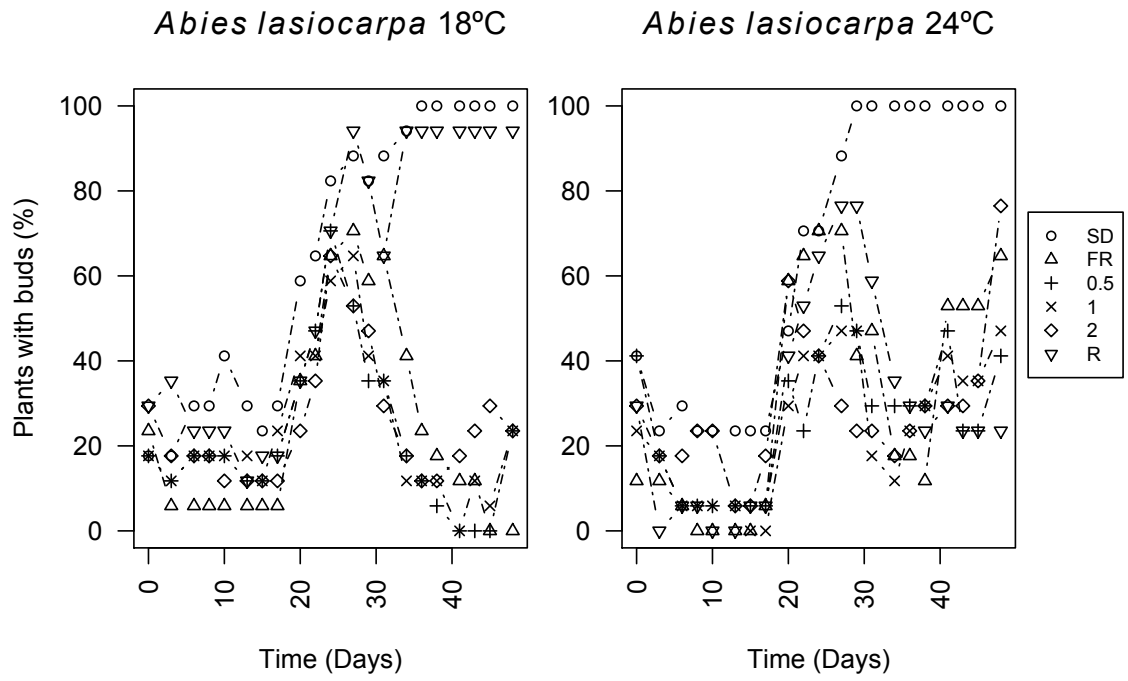
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

37. ANOVA for the linear model of the final shoot elongation in the first experiment of *Abies lasiocarpa* without interaction between the factors.

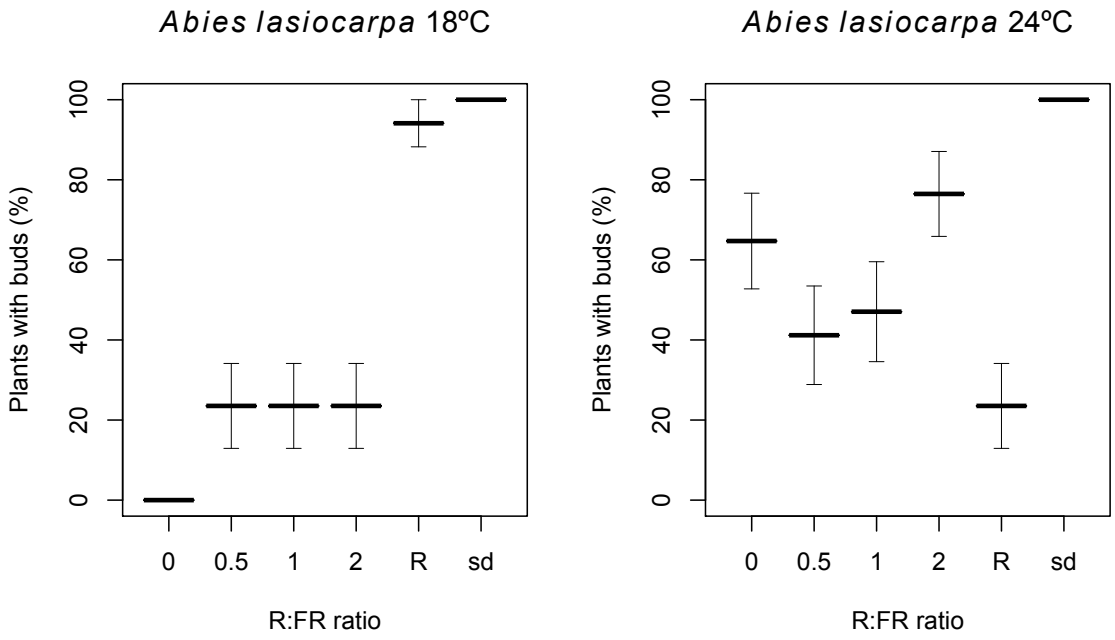
Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0.027	1	0.8703	
Light treatment	78.453	5	1.77E-15	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



38: Percentage of plants with buds in a time course for *Abies lasiocarpa* in the different treatments of temperature and light quality as extension of the photoperiod in the first experiment. 0 is no presence and 1 presence of bud. The values represent the average of 18 plants.



39. Final mean bud classification for the different treatments of *Abies lasiocarpa* during the first experiment as presence response with plus/minus the SE.

40. ANOVA table for the bud classification as presence or absence for *Abies lasiocarpa* during the first experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	49.8432	1	1.67E-12	***
Temperature	1.2648	1	0.260745	
Light treatment	87.6739	5	2.20E-16	***
Temperature: Light treatment	20.1017	5	1.20E-03	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

41. ANOVA for the generalized linear model of the final bud set classification using categories for the first experiment of *Abies lasiocarpa.*

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	5.732	1	0.01666	*
Light treatment	96.053	5	2.20E-16	***
Temperature: Light treatment	62.514	5	3.67E-12	***

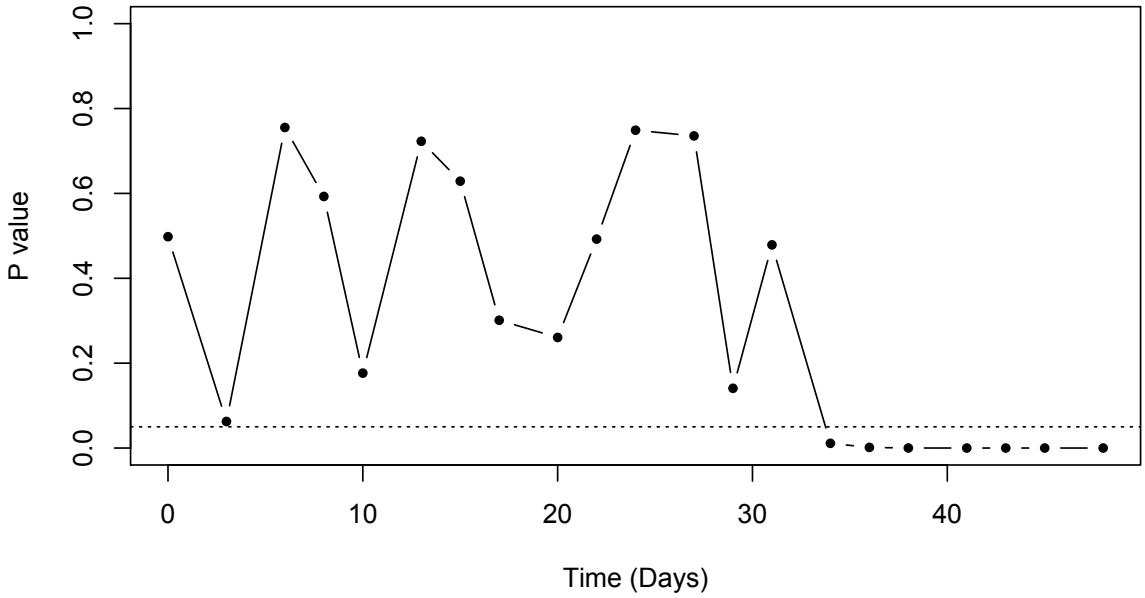
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

42. ANOVA table for the final bud classification as presence response for *Abies lasiocarpa* during the first experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0	1	1	
Light treatment	51.095	5	8.27E-10	***
Temperature: Light treatment	48.234	5	3.18E-09	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



43. P value in a time course for the interaction of the temperature and light treatments in the *Abies lasiocarpa* bud set as a presence or absence response in the first experiment.

44. ANOVA for the final dry biomass for *abies lasiocarpa* including interaction.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.03259	1	4.1174	0.04881	*
Temperature	0.00304	1	0.3835	0.53908	
Light treatment	0.00975	5	0.2465	0.93924	
Temperature: Light treatment	0.00888	5	0.2244	0.94994	
Residuals	0.33241	42			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

45. ANOVA for the shoot: root dry ratio for *Abies lasiocarpa* including interaction during the first experiment.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0.60106	1	0.4382	
Treatment	3.12249	5	0.6811	
Temperature: Treatment	2.94422	5	0.7086	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

46. ANOVA for the final shoot elongation for *abies lasiocarpa* including interaction during the second experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
temperature	0.0207	1	0.88564	
treatment	13.1347	5	0.02215	*
temperature:treatment	8.138	5	0.14879	

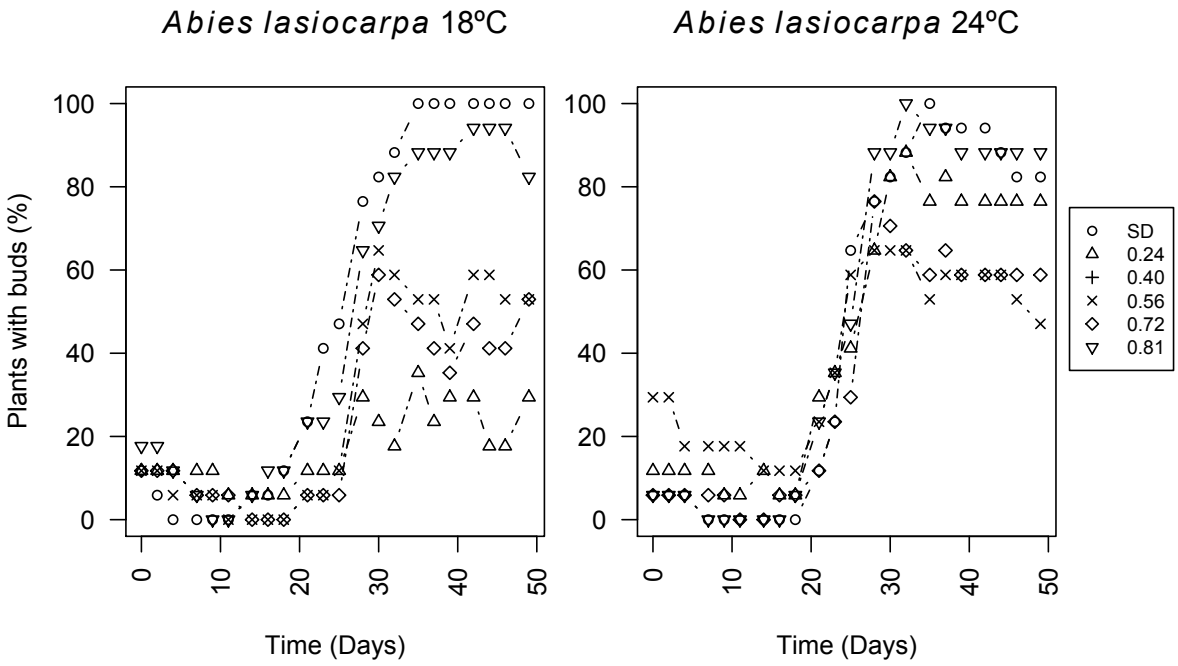
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

47. ANOVA for the linear model of the final shoot elongation in the second experiment of *Abies lasiocarpa* without interaction between the factors.

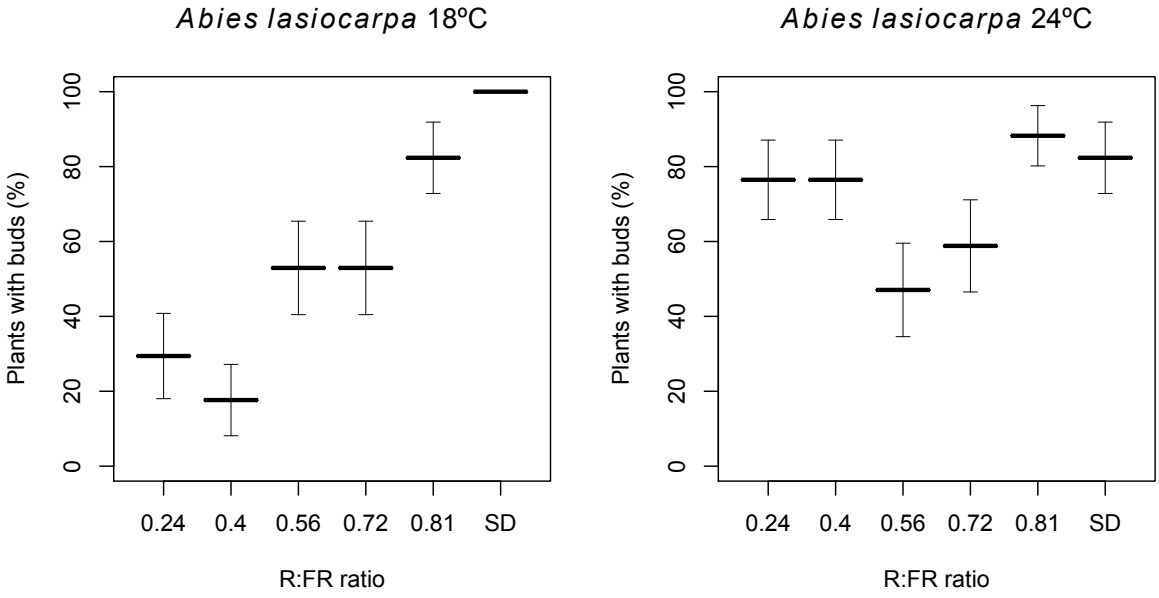
Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0.027	1	0.8703	
Light treatment	78.453	5	1.77E-15	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



48. Percentage of plants with buds in a time course for *Abies lasiocarpa* in the different treatments of temperature and light quality as extension of the photoperiod in the second experiment. 0 is no presence and 1 presence of bud. The values represent the average of 18 plants.



49. Final mean bud classification for the different treatments of *Abies lasiocarpa* during the second experiment as presence analysis with plus/minus SE.

50. ANOVA table for the bud classification as presence of bud set for *Abies lasiocarpa* during the second experiment including the plant and time as random effects

Analysis of Deviance Table (Type III Wald chisquare tests)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	48.9856	1	2.58E-12	***
temperature	6.4365	1	0.01118	*
treatment	15.3265	5	9.06E-03	**
temperature:treatment	14.84	5	1.11E-02	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

51. ANOVA table for the final bud classification using categories for the response of *Abies lasiocarpa* during the second experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	16.633	1	4.54E-05	***
Treatment	28.429	5	3.00E-05	***
Temperature: Treatment	21.899	5	5.47E-04	***

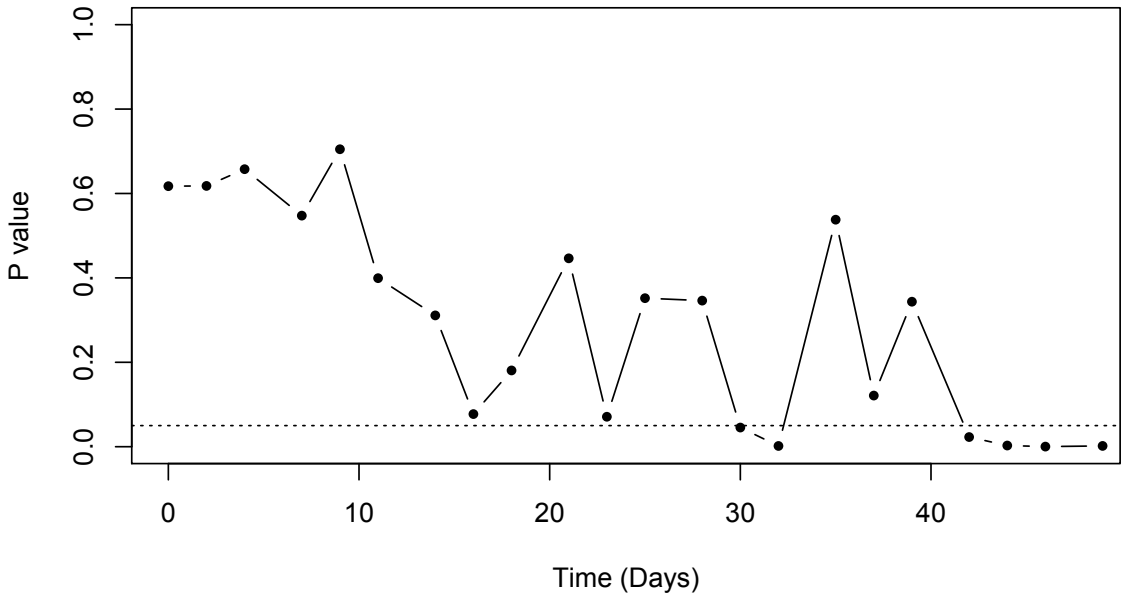
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

52. ANOVA table for the final bud classification as presence of buds for *Abies lasiocarpa* during the second experiment.

Analysis of Deviance Table (Type III Wald chisquare tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	0.0369	1	0.8476268	
Treatment	22.6258	5	3.98E-04	***
Temperature: Treatment	19.0894	5	1.85E-03	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



53. P value in a time course for the interaction of the temperature and light treatments in the *Abies lasiocarpa* bud set as a presence or absence response in the second experiment.

54. ANOVA for the final dry biomass for *abies lasiocarpa* including interaction in the second experiment.

Anova Table (Type III tests)

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.149396	1	23.8305	1.36E-05	***
Temperature	0.018424	1	2.9388	0.09335	.
Treatment	0.06862	5	2.1891	0.07207	.
Temperature: Treatment	0.058122	5	1.8542	0.12151	
Residuals	0.28211	45			

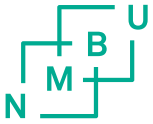
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

55. ANOVA for the final shoot: root ratio of *abies lasiocarpa* including interaction in the second experiment.

Anova Table (Type III tests)

	LR Chisq	Df	Pr(>Chisq)	
Temperature	5.4068	1	0.02006	*
Treatment	3.5913	5	0.60962	
Temperature: Treatment	2.8429	5	0.7242	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1



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