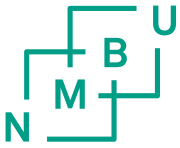
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The Norwegian University of Life Sciences

Norges miljø- og biovitenskapelige universitet

Master Thesis

The effect of UV-B and temperature on photoperiodic control of growth and bud set in Subalpine Fir *(Abies lasiocarpa)* and Norway Spruce *(Picea abies)*

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Abstract

Perennial plants, such as Norway Spruce and Subalpine Fir synchronize their growth and dormancy to different environmental cues, mainly photoperiod, temperature and light quality. In several plant species the UV-B radiation is known to induce changes in plant morphology, such as a decrease in stem elongation, thicker leaves, and shorter petioles. Therefor, the effect of UV-B and temperature on photoperiodic control is an economically and ecologically important question.

The aim of the study was to investigate the effects of the interaction between UV-B and day length on growth and winter bud development in Norway Spruce and Subalpine Fir. Earlier experiments indicate that temperature can modify the effects of day length, however no correlation between UV-B radiation and temperature has been studied under controlled conditions. Therefore the modifying responses to UV-B and day length by temperature were of interest in this present study, such as bud development, elongation growth and the depth of dormancy in Norway Spruce and Subalpine Fir. Previously studies in Norway Spruce have also discovered an up regulation of the gene *FTL2* during short day. This up regulation leads to growth cessation, and later winter bud formation, and studied . The effects of UV-B on the up regulation of *FTL2* were therefore investigated in Norway Spruce.

In conclusion, no consistent effects of UV-B or temperature were discovered in this study on elongation growth in short day (SD) either Norway Spruce or Subalpine Fir. However. UV-B exposure in higher temperatures during SD delayed the bud set in both Norway Spruce and Subalpine Fir. In Subalpine Fir, seedlings exposed to higher temperatures during bud set had a delayed bud burst, i. e a deeper dormancy state. In Norway Spruce a three-way correlation effect was discovered where UV-B in higher temperatures during SD delayed the bud burst. The *FTL2* gene was up regulated during SD in Norway Spruce, but there was no significant effect of UV-B on its expression. Finally, more studies on the effects of UV-B and temperature are necessary in both Subalpine Fir and Norway Spruce.

Sammendrag

Flerårige planter, slik som Norsk gran og Fjelledelgran synkroniserer deres vekst og vinterhvile til ulike signaler i naturen, hovedsakelig daglengde, temperatur og lyskvalitet. UV-B er kjent for å indusere endringer i plantens morfologi, for eksempel reduksjon i strekningsvekst, tykke blader og kortere petioler. Derfor er effekten av UV -B og temperatur på kontroll av fotoperiode et økonomisk og økologisk viktig spørsmål.

Målet med dette studiet var å undersøke effekten av samspillet mellom UV -B og daglengde på vekst og knopputvikling i norsk gran og fjelledelgran. Tidligere forsøk har vist at temperatur kan endre effekten av daglengde, men ingen sammenheng mellom UV -B -stråling og temperatur har imidlertidig blitt undersøkt under kontrollerte forhold. Derfor var de modifiserende reaksjoner på UV - B og daglengde ved temperatur av interesse i dette studiet, for eksempel knopputvikling , lengdevekst og dybden av vinterhvile i norsk gran og fjelledelgran. Tidligere forsøk i norsk gran har også oppdaget en oppregulering av genet *FTL2* under eksponering av kort dag . Denne oppreguleringen fører til veksthemning, og en forsinkelse i dannelsen av vinterknopp. Effektene av UV -B på oppregulering av *FTL2* ble derfor undersøkt i norsk gran.

Til konklusjon ble ingen konsistente effekter av UV -B eller temperatur oppdaget i denne studien i forhold til lengdevekst i kort dag (KD), verken i norsk gran eller fjelledelgran. Imidlertidig førte eksponering av UV -B i høyere temperaturer under KD til en forsinket knopputvikling i både norsk gran og fjelledelgran. I fjelledelgranen hadde frøplanter som var utsatt for høyere temperaturer under knoppdannelse, en forsinket knoppbrytning, dvs en dypere dvale. I norsk gran ble en en treveiskorrelasjon oppdaget, hvor effekten av UV -B i høyere temperaturer i SD forsinket knoppbrytningen . *FTL2*-genet ble oppregulert i SD i norsk gran, men det var ingen signifikant effekt av UV -B på i denne ekspresjonen. Avslutningsvis trengs det flere studier på effekten av UV -B og temperatur er nødvendig både på fjelledelgran og Norge Gran.

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Abbreviations

UV-B	Ultraviolet light-B
SD	Short day
LD	Long day
FTL2	FLOWERING LOCUS T-TERMINAL FLOWER1-LIKE 2

Key words

UV-B, temperature, elongation growth, bud development, bud burst, Norway Spruce, Subalpine Fir.

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1.0 Introduction

1.1 Climate and the role of UV-B and temperature

At Northern/high latitudes the seasonal shifts are pronounced; the temperature drops and the day length shortens significantly before and during the winter compared to the summer season. Perennial plants, such as trees, growing at these latitudes therefore synchronize their growth and dormancy to different environmental cues, and are therefore able to time these mechanisms to the correct season, which is an important adaptive trait for survival. The environmental cues, or factors are mainly photoperiod, temperature and light quality (Mølmann et al. 2006). In addition to the visible light, an important part of the light spectrum in nature is ultraviolet light, which is divided into three wavelength areas; UV-C (220-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). The ozone layer filters away the shortest UV-wavelengths in the spectrum, such as UV-C and part of the UV-B (Caldwel et al. 2003; Rozema et al. 1997).

The climate is changing, especially with increasing temperatures. This will involve changes in seasons, diurnal temperatures, air humidity, cloud cover, storms, weather, etc. Trees of the boreal forest are highly dependent on their growth-dormancy cycle for survival during the winter, and as mentioned above, this is synchronized with environmental cues such as light, photoperiod and temperature. It has long been a fact that the stratospheric O_3 is decreasing, resulting in an increase of the amount of UV-B reaching the surface of the earth (Rozema et al. 1997). However, due to measures implemented following the Montreal Protocol (an international agreement signed in 1989 to reduce ozone depleting substances (ODS) and contribute to decrease some of the greenhouse gases), the stratospheric ozone is recovering (Bornman et al. 2014; Williamson et al. 2014). Independently of the concentration of ODS in the atmosphere, the climate system is warming, and this may have an important effect on future stratospheric ozone, including increasing atmospheric water content; water vapor. This may influence

stratospheric temperatures and winds (Bornman et al. 2014). Ozone depletion still occurs over Antarctica, and this has caused an increased precipitation in the subtropics by a pole wards shift in the Southern Hemisphere (Kang et al. 2011), resulting in shifts in cloud cover. An increase in cloud cover decreases the amount of UV reaching the surface of the Earth, as the UV-light is reflected.

If and how these changes in climatic conditions may affect woody species, their morphology and the very important dormancy cycle, are crucial questions for future conservation.

1.2 Photoperiod, winter buds and dormancy

The photoperiod is the period of daylight, i.e. the duration of an organism`s exposure of light. Photoperiodism is the ability of an organism to detect day length, and the ratio between light and dark hours changes during the year, making it possible for organisms such as plants to respond to daily, seasonally and/or yearly cycles. Appropriate perception of seasonal changes or shifts is thus crucial to avoid miss timing in for example flowering. A specific length of the photoperiod is required to trigger these mechanisms, i.e. a critical day length, which varies between species and/or latitudes. Only at the equator there is an even and constant distribution of light and dark hours, with a ratio of approximately 12:12 light/dark hours. This distribution changes as one moves along the latitudes, and this has through evolution resulted in different adaptations and abilities to detect these seasonal changes in plant species (Clapham et al. 1998a; Garner 1923; Olsen 2010; Tiaz & Zeiger 2010).

The different effects of long days (LD) and short days (SD) have been extensively studied in flowering in plants. In *Arabidopsis thaliana*, which is a model organism in plant biology subject to several studies over the years, a gene called *CONSTANS* (*CO*) play an important role in induction of flowering in long days and acts downstream of light receptors such as phytochromes. Phytochromes are proteins located in the leaf but also other parts of the plant, and these are important in detection of the night length. In *A. thaliana* there are five phytochrome genes, *PHYA* to *PHYE*, which encode the apoproteins of PHYA to PHYE. Each phytochrome can exist in two forms,

a far-red-light-absorbing form (Pfr) and a red-light-absorbing form (Pr). It is well known that in addition to day length, light quality has a big influence on flowering. CO mRNA is translated to CO protein, and long day exposure causes an accumulation of the CO protein. This promotes transcription of another gene, called *FLOWERING LOCUS T* (*FT*); the FT protein is produced and transported to the apical meristem through the phloem. The *FT* gene can be triggered by many pathways, not just long days and an accumulation of CO. Number of leaves, vernalization, relatively high ambient temperature, energy or amount of sucrose, gibberellin, certain stress conditions and light quality, can all induce the FT protein (Clapham et al. 1999; Gyllenstrand et al. 2007; Lee & Lee 2010; Lin 2000). After being transported to the shoot apical meristem, a FT/FD protein complex is formed. This activates other genes such as *SOC1* (a meristem identity gene), which then trigger the induction of flowering.

In relation to woody species a short photoperiod (i.e. when the night period is longer than the critical period) acting through the phytochrome system, induces growth cessation, and formation of terminal winter buds in first-year seedlings of most tree species (Garner 1923; Lee et al. 2014; Mølmann et al. 2006). In relation to Norway Spuce (*Picea abies*) and a few other woody species a requirement for far-red light in the spectrum to prevent terminal bud formation has also been demonstrated (Clapham et al. 1998b; Junttila & Kaurin 1985; Mølmann et al. 2006; Tsegay et al. 2005)

Winter bud dormancy is defined as ”the inability of a meristem to resume growth under favorable conditions” (Rohde & Bhalerao 2007) and as “a temporary suspension of visible growth of any plant structure containing a meristem*”* (Lang 1987). In other words, in the winter buds cell division in the shoot apical meristem is inhibited and the plant stops growing. Growth cessation in Northern tree species, such as Norway Spruce, normally occurs within one to three weeks of exposure to SD, while it takes two-three times as long for the same plant to enter winter dormancy, and develop cold hardiness in time for winter and harsh conditions (Gyllenstrand et al. 2007). The first-year seedlings of this species will in late summer cease its growth due to a response to shortening photoperiod, and thus increase its frost tolerance. The

buds will burst in the springtime when a certain temperature sum is reached. The extension growth of first-year seedlings consists of the expansion of stem units formed in the current season, i.e. the seedlings exhibit a free growth (Nitsch 1957; Olsen 2010).

In previous studies of Norway Spruce, a gene resembling both the *FT*- gene and the floral inhibitor *TERMINAL FLOWER 1* (*TFL1*) in *A. thaliana*, i.e. *FTL2* (earlier referred to as *FT4* or *TFL1*) is strongly up regulated during exposure to SD. This up regulation leads to growth cessation, and later winter bud formation (Asante et al. 2011; Gyllenstrand et al. 2007; Karlgren et al. 2013). Three other genes were also identified, were a down regulation of gene expression during SD exposure correlated with growth cessation and winter bud formation. Two of them are *CO*-like genes (*COL*) called *COL1* and *COL2*, while the third is *MADS1* (Asante et al. 2011; Holefors et al. 2009; Opseth et al. 2015). The *MADS1*–gene contains a nucleotide sequence, which is coding for a protein sequence called MADS, and this occurs in several transcription factors. A transcription factor is a protein, which affects the activity of other genes. The *COL*-genes are similar to *CO* and so-called *CONSTANS-LIKE* genes which affects/stimulate the flowering in *A. thaliana*. The *MADS1*-gene in Norway Spruce is very similar to the flower inducing gene *SOC1* in *A. thaliana*, and these *A. thaliana*-like genes are then connected to day length regulation of flowering. It is believed that these similar genes in the Norway Spruce are affecting the control of day length regulated growth cessation since the expression of these genes is affected by day length (Holefors et al. 2009). It is yet to be known how these genes work, but there are clear correlations between their expression and environmental conditions, as all conditions which give growth cessation is reducing expression of *PaCOL1*, *PaCOL2*, *PaMADS* and increasing expression of *FTL2* (pers. com. Olsen ; Opseth et al. 2015). This may suggest that the proteins encoded by the *COL* genes and the *MADS* gene are associated with stimulation of growth. On the contrary, the *FTL2* gene apparently code for a growth inhibitor or a promoter of growth cessation and bud set (Karlgren et al. 2013). Because of a large genome and a long generation time in Norway Spruce it is difficult to determine the gene function and hard to create mutants and to do gene modification. Norway Spruce has, as most other conifers, a long juvenile period of approximately 20 years. If expression of a gene is similarly

affected by different environmental conditions which leads to bud development, this might suggest that this particular gene plays a part in the process.

In this MSc thesis Norway Spruce and Subalpine fir (*Abies lasiocarpa*) have been studied. The genome in Subalpine Fir (*Abies lasiocarpa*) is not characterized, whereas in Norway Spruce and other species of *Picea* the genome have been characterized (Nystedt et al. 2013). Because of this Norway Spruce was used in the gene expression study in this MSc thesis.

1.3 UV-B, light spectrum and temperature

Ultraviolet-B (UV-B) radiation has an important role in the environment, by affecting individual organisms and larger ecosystems. The ozone (O_3) layer around the earth reduces the total flux transmitted from the sun, and as mentioned above, atmospheric gases absorb all of the short waved UV-light (Rozema et al. 1997). The remaining UV-light naturally varies with cloud cover as mentioned, as well as latitude, altitude, season and time of the day, since these factors affect the solar angle and hence the thickness of the atmosphere which the UV-B must penetrate. The amount of UV-light is also dependent on the surface as the UV is reflected or scattered to a varying degree by different ground surfaces, i.e. snow and sea (Aucamp et al. 2005). There is variation in UV exposure levels as the thickness in the ozone layer has a geographical variation (Frohnmeyer & Staiger 2003; Jansen & Bornman 2012; Jenkins 2009; Rozema et al. 1997).

Earlier studies have focused on the damage and stress caused by high levels of UV-B radiation, such as damaging the DNA, generation of reactive oxygen species (ROS) and impairing cellular processes (Jansen & Bornman 2012; Jenkins 2009; Rozema et al. 1997). After the beginning of the 21st century, there has been a change in the perspective of UV-B as an environmental factor; rather than being a damaging stressor, UV-B acts as a specific regulator triggering events by very low doses of UV-B through a UV-B photoreceptor; UVR8 (UV RESISTANCE LOCUS 8) (Rizzini et

al. 2011). Studies have shown that damage caused by UV-B stress is unlikely to happen at the UV levels in a natural environment (Robson et al. 2014).

The UV-B photoreceptor UVR8 induces changes in the plant morphology when exposed to UV-B radiation by stimulating a rapid translocation of the UVR8-protein to the nucleus (Jenkins 2009). The UVR8-protein then binds to the ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC (COP1) This induces developmental changes such as thicker leaves, shorter petioles, leaf curling, alterations in leaf shape and width, decrease in stem elongation, increased axillary branching and altered root: shoot ratio (Robson et al. 2014). UV-B also stimulates protective responses in plants such as the biosynthesis of flavonoids and other UV-B-absorbing phenolic components (Jansen 2002; Jansen & Bornman 2012; Jenkins 2014; Robson et al. 2014). These work as antioxidants and sunscreens, which reduce the transmittance of the high energy to the cells below the surface, thus prohibiting damage to the molecules (Jansen 2002; Jansen & Bornman 2012; Jenkins 2014; Robson et al. 2014). Recent Studies have shown that the content of certain flavonoids and a range of other antioxidants are up regulated in Norway Spruce during short days (Lee et al. 2014). In other words, the effects of UV-light on plant physiology have been extensively studied, especially in herbaceous plants. Effects of how UV-B affects growth and winter bud development in trees however, have not been studied systematically, except for one recent study. In this study, using UV-B fluorescent lamps, aspen (*Populus temula*) plants were exposed in the field to extra UV-B light, corresponding to a 20 % reduction of the ozone layer. The plants, which were exposed to additional UV-B, started developing winter buds earlier than plants under normal UV-conditions (Stømme et al. 2014).

Other experiments indicate that temperature can modify the effect of day length. Plants in higher temperature during exposure to short days start forming winter buds earlier, show better development of the buds and gives a deeper dormancy than those plants in lower temperatures (Junttila et al. 2003; Olsen et al. 2014). In poplar plants growth cessation and induction of dormancy is highly affected by temperature (Kalcits et al. 2009) as well as in seedlings of Norway Spruce where low temperatures delays bud set, and these effects are modified by chilling and environmental

conditions (Fløistad & Patil 2002; Olsen 2010; Olsen & Lee 2011; Olsen et al. 2014; Søgaard et al. 2007).

The effect of temperature and other environmental cues on day length responses may explain the annual variation in the timing of winter bud development. This has been studied to a relatively limited degree only compared to the effect of day length.

In the UV-B-related study with aspen mentioned earlier, the temperature was increased with 2°C in addition to the UV-B exposure (Stømme et al. 2014). These results indicate that the increase in temperature leads to a later bud development, and that an increase in temperature may reverse the effect of UV-B. However, this was not verified in more field seasons. No other studies so far have examined the correlation between UV-B and temperature under controlled conditions, and the effect on economically and ecologically important conifers has not been studied at all. A study investigating the effect of UV-B radiation on morphology on pot and bedding plants, showed a reduction in internode length under low levels of UV-B radiation (Torre et a. 2012). There was also an indication that a reduction in temperature might affect UV effects on stem elongation (Torre et al. 2012).

1.4 Norway spruce and Subalpine Fir as Christmas trees

The Christmas tree production in Norway is of high economical value; it is increasing not only at a national level – Norwegian Christmas trees are now demanded on the European market, therefore also the export of Christmas trees is increasing. In Norway, a survey done by Norsk Gallup Institutt A/S in 1998 showed that 64 % preferred Norway Spruce (*Picea abies*) as a Christmas tree, while 10 % chose Subalpine Fir (*Abies lasiocarpa*) (the remaining chose pine, other wooden species, or plastic). Subalpine Fir is expected to increase in popularity in Norway (*Juletrearter*). Therefore improved growth conditions, plant quality, regulation of plant morphology and elongation, hardiness and increased resistance to diseases and pests are an economically important aim for Norwegian agriculture (Sæbø et al. 2008).

Norway Spruce grows naturally in the mountain ranges as well as in the lowlands, and it is one of the main species of trees in the boreal and temperate zones of Europe. Due to its wide distribution, the Norway Spruce plays an important role in Europe, both ecologically and economically (Jansson et al. 2013). The Subalpine Fir (*Abies lasiocarpa)* is a small to medium sized coniferous species, and it`s natural habitat stretches from Alaska (USA) and Yukon (Canada) in the North, to Arizona and New Mexico (USA) in the South. It is a mountain species common in mountain slopes; in the North the Subalpine Fir grows from sea level to 900 m.a.s.l., in the South from 2400-3600 m.a.s.l. (Hulten 1968; Nyeggen et al. 2010).

1.5 Aims of the study

The aim of the study was to investigate

- The effects of interaction between UV-B and day length on growth and winter bud development in Norway Spruce and Subalpine Fir.
- How the temperature modifies the responses to day length and UV-B in terms of growth and winter bud development in Norway Spruce and Subalpine Fir.
- The underlying mechanisms for responses, i.e. the activity of a gene we know is affected by day length (*FTL2)*.
- The depth of dormancy after the different treatments.

2.0 Materials and methods

2.1 Plant materials and pre-growing conditions

The seeds of Norway Spruce (*Picea abies* (L). H. Karst) came from the provenance CØ1 from 59°N latitude, 0-149 meters above sea level, in Halden, Østfold, Norway (seed lot 98063, The Norwegian forest seed center, Hamar, Norway). The seeds of Subalpine Fir (*A. lasiocarpa* (Hook.) Nutt.) were from the provenance CAN10 from 53.39°N latitude, 122.23°W longitude, 1000-1200 meters above sea level. The seeds

came from George Mountain in British Columbia, Canada (seed lot B13-106, The Norwegian forest seed center, Hamar, Norway).

Seeds of Norway Spruce do not require any treatment before sowing, but the seeds of the Supalpine Fir need to be stratified. This is a pretreating process to simulate winter conditions, as some species enters embryonic dormancy and will not germinate before chilling breaks this dormancy. The embryo is then triggered by an exposure of cold and moist conditions, and the growth and expansion through the seed coat will start. In this study the seeds of the Subalpine Fir were therefore stratified. The seeds were put on moist filter paper in a petri dish, covered with a lid, and put in a cold room/refrigerator at 4-5°C. Here they stayed for 3-4 weeks in darkness before sowing.

After this the seeds were distributed in trays measuring 596 x 396 x 60 mm (labeled 780100, produced by VEFI A/S, Drammen, Norway) with individual pots (5.5 x 5.5 x 4.8 cm) and then sowed in growth peat (Degernes Veksttorv (Degernes Torvstrøfabrikk, Degernes, Norway). Perlite was added to the growth peat to enhance the soils humectant capability. The perlite and the growth peat were mixed together with a ratio of 1:3 (perlite: peat). The seedlings were watered as needed. As fertilizer a nutrient solution containing Ammonium nitrate, Calncium Nitrate and Kristalon (YaraNorge, Skøyen, Norway) was used. These components were mixed together so the fertilizer mix reached a electrical conductivity (EC) of 1,5. The seedlings were watered with this fertilizer mix twice per week.

During the pre-growing period the seedlings were grown under 18°C and long day conditions (24 hour day length) for approximately two months (eight weeks). The light sources used during the pre-cultivation of the plants were high pressure sodium lamps (HPS; Gavita 400W HPS GAN 400 AL, Gavita, Andebu, Norway) and incandescent light bulbs (NARVA 60 W, Germany and Philips Electronics 60 W, Eindhoven, Netherlands). The incandescent light bulbs were used together with the HPS lamps to adjust the red-far red-ratio (R: FR) to 1.7, as measured with a red/far-red sensor (Skye Instruments Limited, in Llandrindod Wells, UK). This is closer to that of sunlight during the day (about 1.0) as the HPS lamps give a red-far red-ratio of 3.0. This adjustment was done since it is known that northern ecotypes of trees need a

certain amount of far-red light in the light spectrum to maintain the growth (Mølmann et al. 2006).

The irradiance of the growth chambers were set to approximately 200 µmol m^{-2} $s^{-1.}$ This was measured at the top of the plants by a Li-Cor Quantum/Radiometer/ Photometer (LI-250 Light Meter produced by LI-COR, Lincoln, Nebraska USA). The incandescent light bulbs alone gave about 8-10 µmol m^{-2} s^{-1}. In experiment 1 the main light period (HPS and incandescent lamps) was 12 h and the day was extended to 24 h with low-intensity light from incandescent lamps only. Since terminal buds then were observed in many Supalpine Fir plants, the main light period was increased to 24 h in experiment 2. In experiment 3 this was also intended, but due to failure, the first two weeks the plants got a main light period of 12 h and day-extension with incandescent lamps as in experiment 1, and thereafter the main light period was increased to 24 h.

During pre-growing for experiment 1 the air humidity was regulated to approximately 0.5 kPa water vapor pressure deficit, corresponding to about 75 % relative air humidity (RH) at 18°C. In experiment 2 the air humidity of the pre-growing period was adjusted to 0.72 kPa, corresponding to 65% RH.

2.2 Data collection and data processing

2.2.1 Experimental conditions

After eight weeks of pre-cultivation the seedlings were placed on trolleys (50 x 50 cm) distributed in different growth chambers. In experiment 1 Conviron Growth Chambers (Conviron, Winnipeg, Manitoba, Canada) was used. In experiment 2 and 3, the seedlings were grown in growth chambers manufactured by the Center for climatically regulated plant research, Norwegian University of Life sciences (Ås, Norway). In different chambers two different light treatments were provided; either a 12 hour (short day, SD) or 24 hour (long day, LD) photoperiod at 18°C. These photoperiods were obtained by light from fluorescent light tubes (Cool White, Fluorescent T12 lamps V.H.O, 215 W, Osram Sylvania, Mississauga, Ontario

Canada) in experiment 1 and high pressure sodium lamps (HPS; GAN 400 AL, Gavita) in experiment 2 and 3. In addition, incandescent light bulbs (60 W) were used to adjust the red/far-red ratio to about 1.7, as measured with a red/far-red sensor (Skye Instruments Limited). A Li-Cor Quantum/Radiometer/Photometer (LI-250 Light Meter) was used to measure the incident flux of radiant energy per unit area, the irradiance, and this was measured to be about 190 µmol m^{-2} s^{-1} in both experiment 1 and 3, and 155 µmol m^{-2} s^{-1} in experiment 2. Lower irradiance in experiment 2 was due to division of each chamber into two compartments (with and without UV-B) using a white reflecting plastic sheath, and this reduced the irradiance. Both seedlings groups were exposed to the same main daylight period of 12 hours, but for the seedlings in the LD treatment the daylight period was prolonged with 12 extra hours with low intensity light from incandescent light.

In addition to the two main light treatments, some of the seedlings were also exposed to UV-B light from Ultraviolet fluorescent tubes (60 cm Q-panel UVB- 313 EL, Q-Lab Corporation, Cleveland, USA). In the first two experiments one Ultraviolet fluorescent tube was mounted in each chamber, but two were used per chamber during experiment 3. To attenuate the shortest (i.e. UV-C) wavelengths, a 0.13 mm thick cellulose diacetate foil (DIA05.0101.0, Rachow Kunststoff-Folien GmbH, Hamburg, Germany) were wrapped around the UV-B lamps. This foil was exposed for UV-B 24 hours in advance because it ages very quickly the first 24 hours, but after 27-40 hours it stabilizes. This was done to obtain more similar UV-B exposure during the entire experiment, since less UV-B is let through as the foil ages and it was changed every second week. A spectrometer (Spectrosense 2, Skye Instruments, Llandrindod Wells, UK) was used to measure the UV-B radiation in the top of the plants in different locations within the chambers (with the cellulose diacetate foil), and was measured to be about 0.16 m^{-2} in experiment 1 and 0.10 m^{-2} in experiment 2. In the third experiment, the UV-B radiation was increased, and measured at the middle of the trolley to be approx. 0.2 Wm^{-2}. In contrast to the HPS lamps used as main light source in experiment 2 and 3, the fluorescent light tubes used in experiment 1 also gave some UV-B radiation, this was measured to be approximately 0.03 W m^{-2}.

The seedlings were divided into four different treatments in total; short days with UV-B (SD w UV-B), short days without UV-B (SD), long day with UV-B (LD w UV-B) and long day without UV-B (LD). This made it possible to study if the UV-B light modifies the response to short day, and the growth in general during long days.

In the second and third experiment, the effect of temperature was also tested. In addition to the effect of UV-B, two different temperatures were compared; 18°C against 22°C, and 18°C against 24°C. The difference between the temperatures was increased in the last experiment due to small changes in the elongation growth between the seedlings in 18°C or 22°C.

In the chambers with short day (SD) conditions the main light source and the incandescent lamps were on 12 hours a day (09.00-21.00). In the long day treatments, the main light period was also 12 hours, but the incandescent lamps were on 24 hours a day. The plants, which were treated with UV-B were exposed to UV-radiation 8 hours per day in the middle of the main light period (11.00-19.00).

In the first experiment, the air humidity was adjusted to 0.5 kPa water vapor pressure deficit, corresponding to 75 % RH at a constant temperature at 18 °C. In the later experiments were effect of temperature was also investigated, the air humidity was set to 0.72 kPa water vapor pressure deficit, corresponding to 65 % at 18 °C , 73%, at 22 °C and 76% at 24 °C.

As a biological pest control, nematodes were used which predates on garden pests such as flies (Nemasus SYS, Becker Underwood, Little Hampton, England).

2.2.1 Registrations of growth parameters

Growth and bud development were recorded at the start of each experiment, giving a starting point. The measurements then continued on a weekly basis, and the growth and bud development for each week was then calculated. In each experiment there was a different number of plants. In the first experiment there were 12-14 seedlings of

Subalpine Fir, and 19-23 seedlings of Norway Spruce. In experiment 2 there were 19 plants in each treatment for both species. In experiment 3 there were 19 seedlings of Subalpine Fir and 36 seedlings of Norway Spruce in each treatment.

2.2.3 Measuring elongation growth

The heights of the seedlings were measured in centimeters (cm) using a ruler. The length was measured from the edge of the pot to the apical meristem. These measurements were carried out once per week, the same day each time. At the end of each experimental period, the cumulative growth and average height for the trees in each treatment were calculated. At the end of experiment 1 the diameter was also measured. The length from needle tip to needle tip was measured from two angles, and the average calculated, to obtain a representative average diameter per plant.

2.2.4 Bud development

The status of terminal bud formation was recorded three times per week. To record the progress of the bud formation, the following numbers/codes were used: 0 = in growth, 1 = early bud/still green, 2 = brown, fully developed bud. The data were used at the end of the experiment to calculate the percentage of how many trees had developed buds, and the average bud stage.

2.2.5 Bud break

In experiment 3 the seedlings that had developed buds in different treatments were after 7 weeks of treatment with different UV-B, day lengths and temperatures, retransferred to long day exposure at 18°C with light and RH conditions as under the pre-growing period *(table 1)*. This made it possible to study the depth of the dormancy. This was the case for SD exposed plants, where all plants developed bud, and those seedlings that had developed buds in the long day (LD) treatment.

Tabel 1 Number of seedlings of Subalpine Fir and Norway Spruce that had developed buds in the different treatments in experiment 3, i.e. SD, SD UV-B, LD and LD UV-B, and temperatures (18℃ and 24 ℃) re-transferred to LD at 18℃ for study of bud break .

Number of seedlings				
	SD (18°C)	SD UV-B (18°C)	LD (18°C)	LD UV-B (18°C)
Subalpine Fir	19	19	9	10
Norway Spruce	36	36	0	0
	SD (24 °C)	SD UV-B (24°C)		
Subalpine Fir	19	19		
Norway Spruce	36	35		

The time it took for the different trees to exhibit bud break was registered, as well as the bud stage according to the following scale: 0 = fully developed bud, 1 = starting to break, 2 = in growth. At the end of this experiment, the average bud stage was calculated as well as the percentage of how many seedlings had broken the bud dormancy.

2.2.5 Harvesting for gene expression study

To study the gene expression of *FTL2* in the different treatments, samples were collected from each of the treatment groups; long day (LD), long day with UV-B (LD UV-B), short day (SD) and short day with UV-B (SD UV-B). Samples from the needles and the shoot apex were harvested separately, using a scalpel. Three samples were harvested from each treatment, with materials from four different seedlings in each sample. The material were immediately put in pre-freezed tubes (Biosphere plus SafeSeal Micro Tubes, SARSTEDT, Nümbrecht, Germany) and put in liquid nitrogen. The tubes were then stored at -70 °C.

2.2.6 Analysis of *FTL2* expression

Levels of transcript of the *FTL2* genes were analyzed using a real-time quantitative PCR analysis. Quantitative PCR is a technique based on the polymerase chain reaction, making it possible to amplify and quantify a targeted DNA molecule.

2.2.6.1 RNA isolation

To isolate the RNA in both the shoot apex and needles, Purelink Plant RNA reagent (Ambion, Life Technologies, Thermo Fisher Scientific, Massachusetts, USA) were used, and followed the procedure ”Small Scale RNA Isolation” with some modifications. First the samples were crushed while still being frozen, by adding lead marbles (0.5 mm) to the frozen tubes, and crushed using a Mixer Mill (MM 301, Retsch in Haan, Düsselforf, Germany) at 25 Hz for 1.5 minutes. Then 0.5 ml of Plant RNA Purification Reagent (Invitrogen, Life Technologies, Thermo Fisher Scientific) were added in each tube to the crushed frozen tissue. This was then mixed thoroughly until the sample was re-suspended, the tubes were incubated horizontally for 5 min at room temperature. Thereafter, the tubes were centrifuged for 2 min at 12000 g in room temperature, and the supernatant was then transferred to another 2.0 ml biosphere tube.

200 µl 5 M sodium chloride NaCl were added, and the tubes were tapped to mix. 600 µl chloroform $CHCl_3$ (Merck Millipore, Frankfurt, Germany) was then added and the samples were mixed thoroughly by inversion for 1 min. Then the samples were centrifuged at 4°C for 10 min at 12000 g. The tubes were then placed on ice and from here on always handled on ice.

After centrifugation 800 µl isopropanol (Isapropanol Prima, Arcus, Vestby Norway) were added in Rnase free 1.5 ml eppendorf tubes and put on ice. After centrifugation, the top or the aqueous phase (about 800 µl) was transferred to the tube with isopropanol, then mixed and put on ice for 10 min. The samples were then again centrifuged at 4°C at 12000 g for 10 min, and a pellet formed at the bottom of the tubes. The supernatant was decanted, and added 1 ml 75 % EtOH 4°C to the pellet. The tubes were then centrifuged at 4°C for 2 min at 12000 g, and then the liquid was carefully decanted. The samples were then briefly centrifuged again to collect the residual liquid, which were removed with a pipette, always using filter tips (Biosphere®plus Filter Tips, SARSTEDT). The pellet was dried for 2 min in a laminar flow bench.

After this 40 µl Rnase-free water was added to dissolve RNA, and quickly centrifuged. Then a Dnase treatment started, using an Ambion kit (Ambion TURBO DNA-free Kit, TURBO DNase Treatment and Removal Reagents, Life Technologies). 5 µl 10x DNase buffer was added to the tubes, as well as 1.5 µl Turbo DNase enzyme. The samples were mixed, quickly centrifuged, and incubated at 37 °C for 30 min. 5 µl DNase Ambion STOP solution was added to the tubes, and then mixed for 2 min at room temperature and centrifuged at 13000 g at 4°C for 2 min. As much as possible of the supernatant was transferred to a new RNase free tube, without any of the stop solution. The samples were then divided into 2 tubes, where 20 µl was used for purification and the other tube placed on store in -70°C The samples were then quantified by using Nano-Drop (ND-1000 Spectrophotometer, NanoDrop Products, Wilmington, USA).

2.2.6.2 RNA purification

For the purification of RNA, the procedure for PureLink RNA Mini Kit (Invitrogen Total RNA Purification System, PureLink RNA Mini Kit, Life Technologies, Thermo Fisher Scientific,) was used, and followed the instructions for purifying RNA from Liquid Samples.

In RNase free eppendorf tubes 125 µl RNA were added, consisting of 20 µl of RNA from the RNA isolation, and 105 µl water from the kit (PureLink RNA Mini Kit). In the same tubes, 125 µl of Lysis Buffer was also added. This buffer was made out of 1 ml Lysis Buffer from the kit, and 10 µl 2-ME (mercaptoethanol). Finally, 125 µl of Absolute ethanol (AnalaR NORMAPUR, Radnor, USA) were added to the tubes, and the blend was mixed by pipetting up and down five times, using filter tips. The samples were transferred to RNA Spin Cartridges provided in the kit, and then centrifuged at 12000 g in room temperature for 20 sec. After this 500 µl of Wash Buffer II with ethanol was added to the Spin Cartridge, and centrifuged again at 12000 g for 20 sec in room temperature. The adding of Wash Buffer II and the centrifuge was repeated once more. The Spin Cartridges were then centrifuged at

12000 g for 1 min in room temperature, and the collection tubes and the flow-through were discarded. The cartridges were inserted into a RNA Recovery Tube supplied in the kit. The RNA was diluted by adding 40 µl DEPC- treated (RNase free) water to the spin cartridge, and incubated for 1 min at room temperature. The samples were then centrifuged at 12000 g at room temperature for 2 min, and then placed in the ultra freezer (-70°C) until analyses.

2.2.6.3 cDNA synthesis

After all the samples was verified by analysis on the Nano-drop to have levels above 70 ng μl^{-1}, the quality of the RNA was tested with a Bioanalyzer (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA, United States) giving graphs showing the sample quantification.

After this, cDNA samples were made using a cDNA synthesis kit (SuperScript VILO cDNA Synthesis Kit, Life Technologies, Thermo Fisher Scientific, Massachusetts, USA). A Master mix was mixed together by adding primers, SYBRgreen (SYBR Select Master Mix, Life Technologies, Thermo Fisher Scientific), water and 2 µl RNA; giving a total of 20 µl in each well *(table 2).* The master mix was made in a PCR cabinet (Biosan VVC/T-M-AR, Life Technologies).

Table 2. The content and different amounts (µl) of the master mix made for the cDNA synthesis. First with the content to fill one well (1x), and then the content added up for the total of 48 wells (48x), giving a total of 20 µl of master mix in each well. The template is amount of purified RNA

Master mix	1x	48x
Water	7	525
SYBR green	10	750
Primer L	0,5	37,5
Primer R	0,5	37,5
Total	18	1425
Template	2	
Total	20	

Earlier in the process RNA was isolated from DNA, and an enzyme called reverse transcriptase (rt) translates the RNA to cDNA. Half of the wells were added with +rt

(reverse transcriptase present), and the other half with –rt (without reverse transcriptase), and placed in a PCR-machine (DNA Engine Tetrad 2 Peltier Thermal Cycler by Bio-Rad Laboratories, city, country) and incubated for a pre-determined cycle *(table 3)*.

Table 3 Pre-determined incubation cycle with temperature (°C) and time (min), in a PCR-machine for samples added with master mix in the cDNA synthesis.

Temperature (°C)	Time (min)
25°C	10
42 °C	50
85 °C	5
4 °C	Forever

Samples were then chilled on ice, and diluted with Rnase free water.

Then the samples were tested in a qPCR-machine (quantitative PCR machine, 7500 Fast Real-Time PCR System, Life Technologies, Thermo Fisher Scientific) where the same +rt and –rt were tested against the master mix. This was to control that the cDNA was clean and not polluted during the process. If there was any expression of cDNA in the wells with –rt it would mean that there were traces of DNA left in the RNA, and the RNA samples were polluted, or not properly isolated, and could not be used. If the samples were clean, the procedure could continue. The samples were run through a pre-determined cycling program in the qPCR *(table 4)*.

Table 4 Pre-determined incubation cycle with temperature (°C) and time (min, sec), in a qPCR-machine for samples added with master mix in the cDNA synthesis.

Temperature (°C)	Time (min, sec)
2-step Cycling	
50 °C	2 min
95 °C	2 min
40 cycles	
95 °C	15 sec
60 °C	1 min

The same was done once more, only this time *A-TUB* (*alfa-tubulin*) was tested against *FTL-2*. *A-TUB* was used as a housekeeping gene, which is a gene required for basic cellular function and that does not vary under the experimental conditions used (Chu et al. 1993). This means that *A-TUB* should be equally expressed in all samples and serves as a control. The same cycling program as described above, was used.

CT-values was retrieved from this, giving values of how many cycles was necessary to detect the DNA. The CT stands for *the threshold cycle* and is the number of cycles needed for the fluorescent signal of the reaction to cross the threshold. An increase in the product during PCR therefore leads to an increase in fluorescence intensity (SYBR green) and is measured at each cycle, allowing the gene concentration to be quantified. A low CT-value therefor equals a high gene expression, since few cycles are needed to measure a high expression.

2.2.7 Calculating RQ-values

The RQ (relative quantification) values gives information about how much of the gene in question is expressed compared to the zero/test sample. If the RQ value is 2 when the zero/test sample is set to 1, this means the gene expression is twice as high in the sample tested.

To calculate the relative quantification values (RQ), the formula shown in table 5 was used:

Table 5 Formula used to calculate the relative quantification values (RQ), using data retrieved from the qPCR analysis. GOI= gene of interest, HKG= housekeeping gene, c= calibrator, s= sample, Ct=the threshold cycle. In this study the gene of interest was FTL2, and the housekeeping gene A-TUB.

$RQ = 2 ^ (- \Delta\Delta Ct)$
$\Delta\Delta Ct = \Delta Ct (s) - \Delta Ct (c)$
$\Delta Ct (s) = Ct (GOI\ s) – Ct (HKG\ s)$
$\Delta Ct (c) = Ct (GOI\ c) – Ct (HKG\ c)$

2.3 Statistics

To evaluate the effect of the UV-B, temperature and day length on the cumulative growth, plant diameter (only experiment 1), and days to bud burst, analysis of variance (ANOVA) in the General Linear Mode (GLM), followed by a Tukey`s multiple comparison test was used (Minitab, State College, PA, USA). In experiment 1 a two-way ANOVA was used to analyze the effect of UV-B and day length. In experiment 2 and 3 a three-way ANOVA was used to evaluate the effect of UV-B, temperature and day length.

Effects of the environmental factors on bud development and bud break (bud set and bud break stages) were tested using cumulative link models in R, which are regression models for ordinal data, with a package called ”Ordinal” (Christensen 2013). Here the effect of temperature and UV-B on photoperiodic control was tested on seedlings of Norway Spruce compared to LD (LD) without UV-B as a dummy variable. UV-B, day length (SD and LD) and bud stage were categorical variables. Temperature was set as a continuous variable in the model, meaning that the analysis provides a categorical response in bud stages to an increasing temperature.

3.0 Results

3.1 Experiment 1 The effect of UV-B radiation and daylength onSubalpine Fir and Norway Spruce

3.1.1 The effect of UV-B radiation and daylength on stem elongation

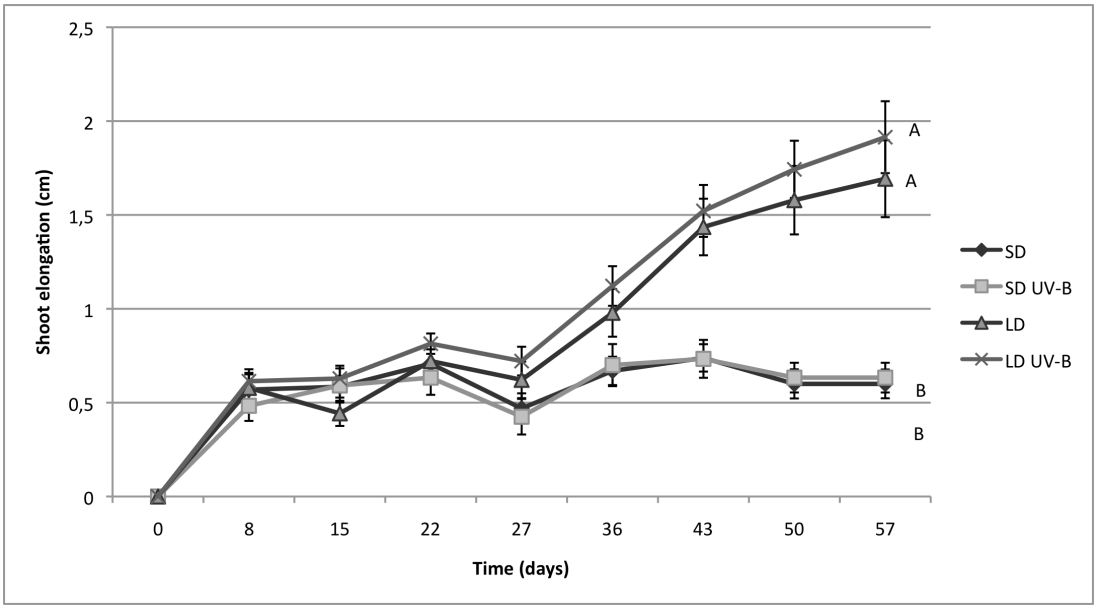

Figure 1 Average cumulative elongation growth in seedlings of Subalpine Fir exposed to different treatments in experiment 1, i.e short days (SD), SD combined with UV-B, long days (LD) and LD combined with UV-B, all at a temperature of 18 °C The results are mean ± SE of 12-14 plants per treatment. Means that do not share a letter are significantly different.

As expected, there was a significanteffect of day length on stem elongation in Subapine Fir *(p=0,0001)* with generally rapid growth cessation within the first week in SD and more elongation growth in LD. UV-B radiation did not affect the stem elongation significantly *(p>0,05)* and there was also no significant interaction between day length and UV-B *(fig. 1)*.

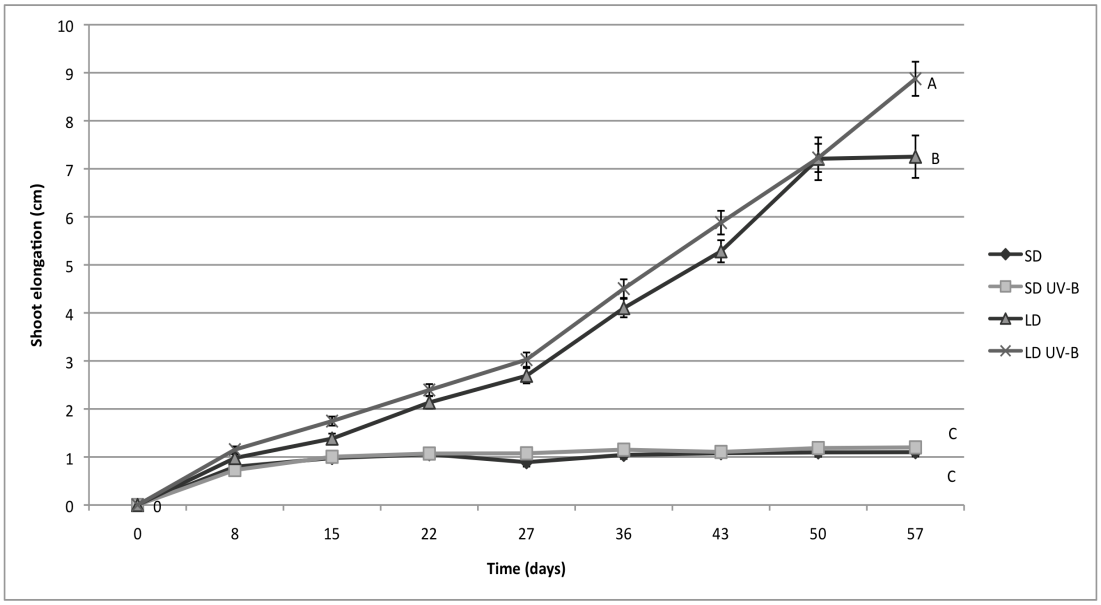

Figure 2 Average cumulative elongation growth in seedlings of Norway Spruce exposed to different treatments in experiment 1, i.e short days (SD), SD combined with UV-B, long days (LD) and LD combined with UV-B, all at a temperature of 18 °C The results are mean ± SE of 19-23 plants per treatment. Means that do not share a letter are significantly different.

The graph (*fig. 2*) shows, as expected, a clear distinction between day length on elongation growth also in Norway spruce *(p=0,000)*. The effect of UV-B treatment was significant for LD *(p=0,004)* at the end of the experiment, but this point clearly differed from the situation at the earlier time points. However there was no significant effect of UV-B radiation during SD.

3.1.2 The effect of UV-B and daylength on bud development

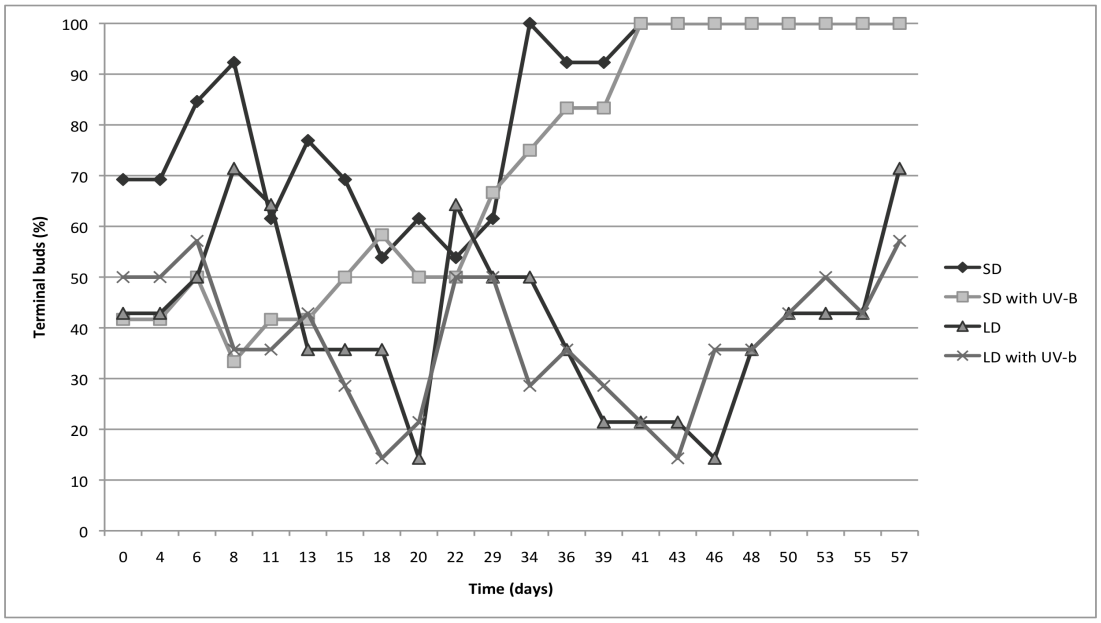

Figure 3 Percentage of seedlings of Subalpine Fir with terminal buds under different treatments in experiment 1, i.e short days (SD) and SD combined with UV-B, all at a temperature of 18 °C. The results are mean ± SE of 12-14 plants per treatment.

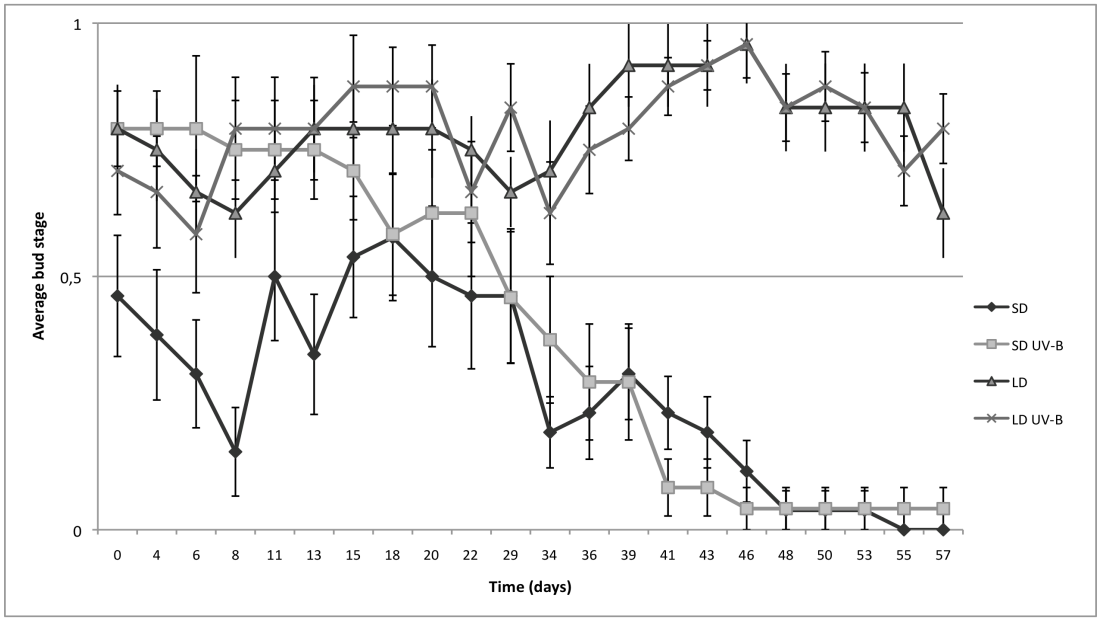

Figure 4 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) in seedlings of Subalpine Fir exposed to different treatments in experiment 1, i.e. long days (LD), LD combined with UV-B, short days (SD) and SD combined with UV-B, all at a temperature of 18°C. The results are mean ± SE of 12-14 plants per treatment.

There was a significant difference in bud development in Subalpine Fir between SD and LD. Seedlings in SD reached 100 % in buds within day 41, while 30 % of LD exposed seedlings had buds (day 41) (*fig. 3 and fig. 4*). This species formed terminal buds even under the LD conditions used, however fewer plants with buds were observed under LD than SD, i.e. 60-70% and 100 % plants with buds after 8 weeks of LD and SD, respectively. However, inspection of % bud set (fig, 3), did not reveal a clear effect of UV-B on % bud set under the different day lengths.

Table 6. Results (parameter estimates, SE and z-values) from a cumulative link model in R run to investigate the effects of UV-B (UVB) and day length (SD) on bud development (bud stages) in Subalpine Fir compared to long day (LD) without UV-B in experiment 1, at a temperature of 18 ℃. Positive estimated coefficient indicates an increased probability for bud set, while negative estimated coefficient indicates a probability for a delay in bud set.

Treatment	Coefficients	SE	z
UVB	-0,181026	0,162683	-1,113
DaySD***	2,379140	0,174339	13,647
Date***	0,026607	0,003332	7,985
UVB:DaySD*	-0,500046	0,234824	-2,129

*Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.*

However, the statistical analyzes shown in table 6 suggested a significant, positive value for SD when compared to LD without UV-B, meaning that the bud development in Subalpine Fir was enhanced during SD. There was also a significant, negative value for the interaction between UV-B and SD; meaning that bud development was delayed when given UV-B radiation during SD.

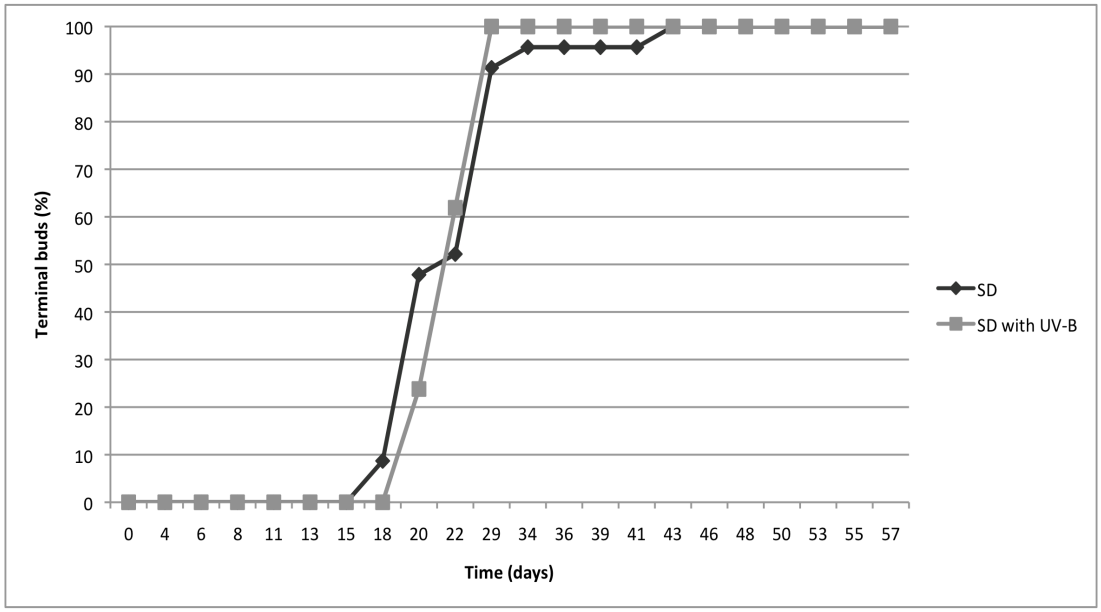

Figure 5 Percentage of seedlings of Norway Spruce exposed to different treatments in experiment 1, i.e short days (SD) and SD combined with UV-B, all at a temperature of 18 °C. Observations during long days (LD) and LD combined with UV-B) were not included due to no bud development. The results are mean ± SE of 19-23 plants per treatment.

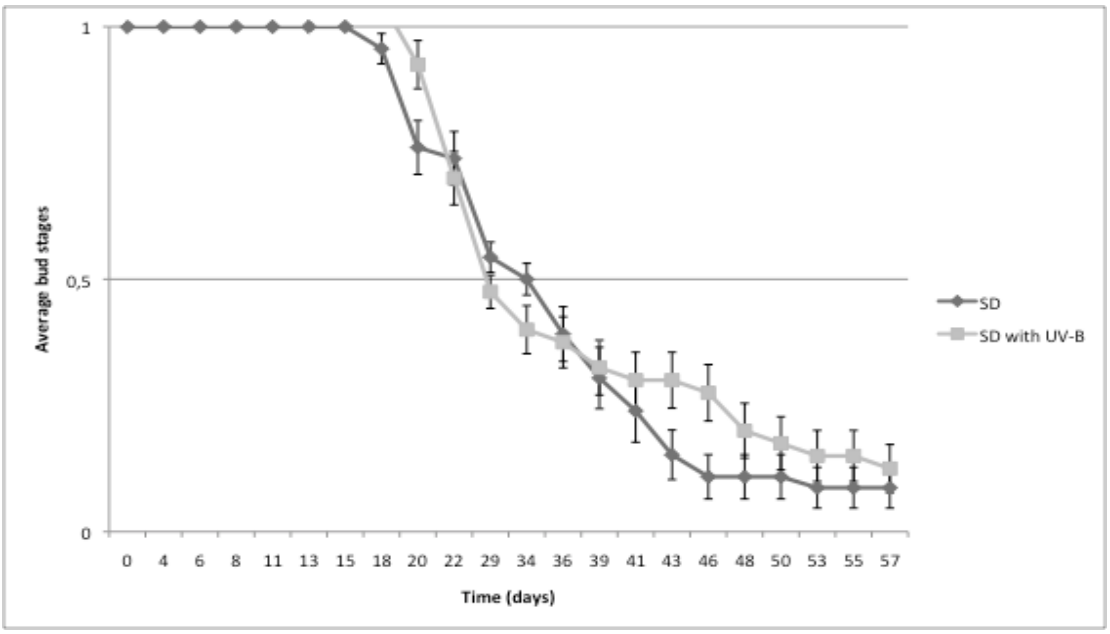

Figure 6 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) in seedlings of Norway Spruce exposed to different treatments in experiment 1, i.e short days (SD) and SD combined with UV-B all at a temperature at 18°C. Observations during long days (LD) and LD combined with UV-B were not included due to no bud development. The results are mean ± SE of 19-23 plants per treatment.

In Norway Spruce there appeared to be a slight delay in bud development when given UV-B radiation during SD unlike during LD (*fig. 5 and fig. 6*).

Table 7. Results (parameter estimates, SE and z-values) from a cumulative link model in R run to investigate the effects of UV-B (UVB) and day length (SD) on bud development (bud stages) in Norway Spruce compared to long day (LD) without UV-B in experiment 1, at a temperature of 18℃. Positive estimated coefficient indicates an increased probability for bud set, while negative estimated coefficient indicates a probability for a delay in bud set.

Treatment	**Coefficients**	**SE**	**z**
UVB*	-0,35345	0,17545	-2,015
Date***	0,22244	0,01109	20,062

*Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.*

The statistical analysis shown in table 7 indicated a significant delay in terminal bud development in response to UV-B radiation during SD.

3.1.3 The effect of UV-B radiation and daylength on diameter of the seedlings

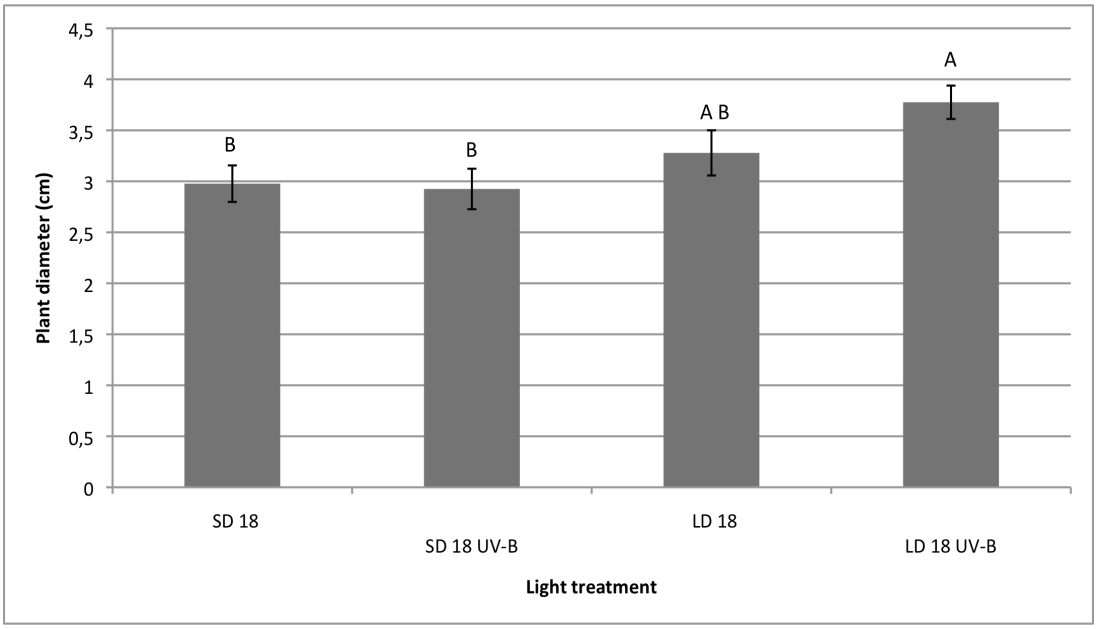

Figure 7 Average diameter (cm) measured at the end of the experiment (day 57) for seedlings of Subalpine Fir exposed to different treatments in experiment 1, i.e short days (SD) and SD combined with UV-B, long days (LD) and LD combined with UV-B all at a temperature at 18 °C. The results are mean ± SE of 12-14 plants per treatment. The treatments that do not share a letter are significantly different.

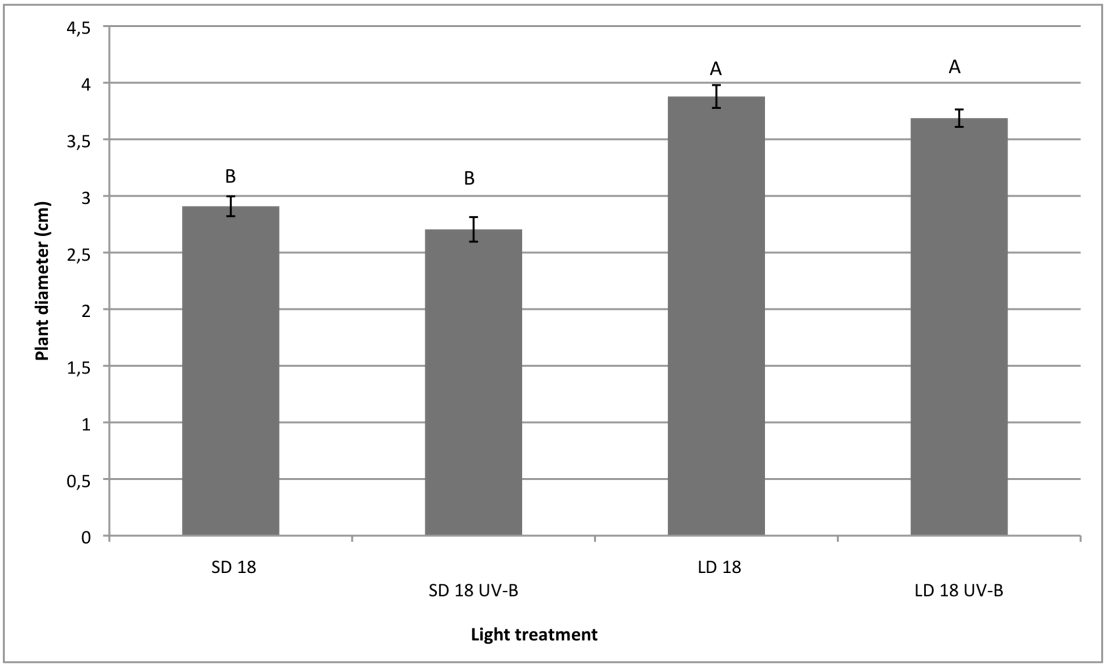

Figure 8 Average diameter (cm) measured at the end of the experiment for seedlings of Norway Spruce exposed to different treatments in experiment 1, i.e short days (SD) and SD combined with UV-B, long days (LD) and LD combined with UV-B all at a temperature at 18 °C. The results are mean ± SE of 19-23 plants per treatment. The treatments that do not share a letter are significantly different.

The average plant diameter of Subalpine Fir (*fig. 7*; *p = 0.050)* and Norway Spruce (*fig. 8; p= 0.000*) was significantly larger in LD compared to SD.

There was no significant effect of UV-B on plant diameter, neither under SD or LD ($p \leq 0.05$) in either of the plant species.

3.1.4 The effect of UV-B radiation and daylength on the accumulation of the *FTL2* transcript

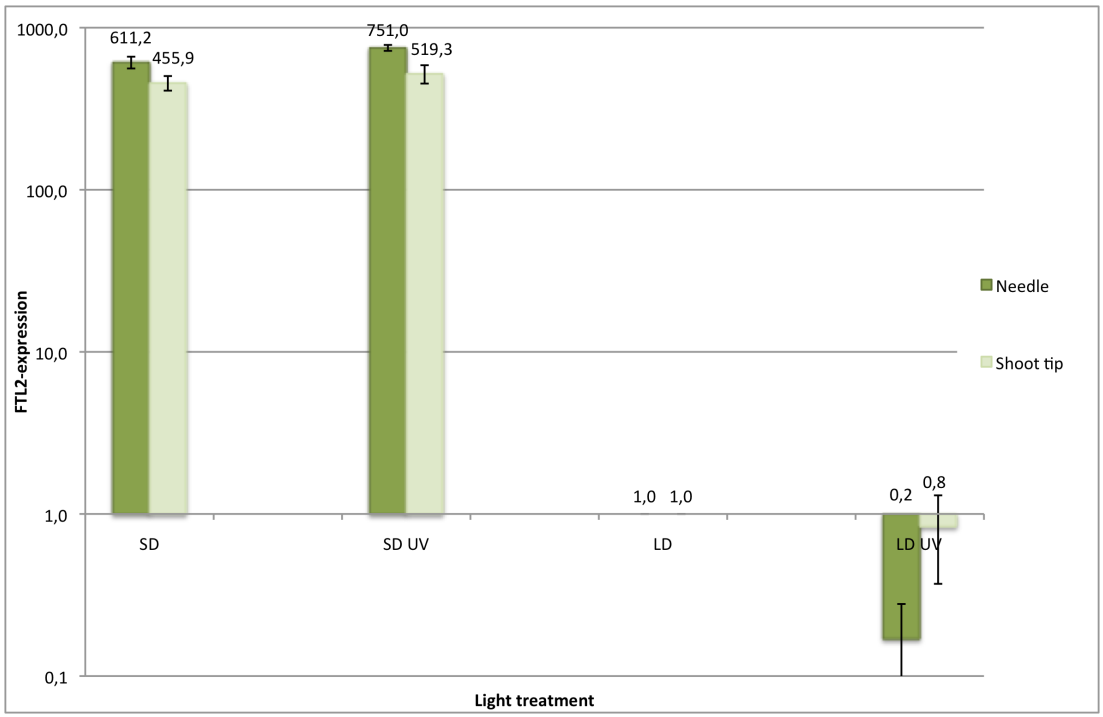

Figure 9 The effect of UV-B radiation and daylength on relative transcript levels of *FTL2* in shoot tips and young needles of seedlings of Norway Spruce exposed to short days (SD), SD combined with UV-B, long days (LD), LD combined with UV-B for 8 weeks, all at 18°C exposure). The values were normalized to α-tubulin and are shown relative to the LD treatment without UV-B. The values are mean ±SE of three samples consisting of four seedlings in each.

Transcript levels of *FTL2* in seedlings of Norway Spruce were up regulated during SDs both in needles (*p=0,026*) and the shoot apex *(p=0.001) (fig. 9)*. The *FTL2*-expression was approx. 600 x higher in SD compared to LD in the shoot apex and 400 x higher in needles. There was no significant effect of the UV-B radiation in the needles or in the shoot apex *(p>0,05)*. However, in LD with UV-B the transcription of *FTL2* showed a slight trend of being down regulated, in the needles with 5 x lower levels than in LD without UV-B. In shoot tip there was no such trend *(fig. 9)*.

3.2 Experiment 2; The effect of temperature, day length and UV-B radiation on stem elongation growth, bud development in seedling of Subalpine Fir and Norway Spruce

3.2.1 The effect of temperature, day lenght and UV-B on stem elongation growth

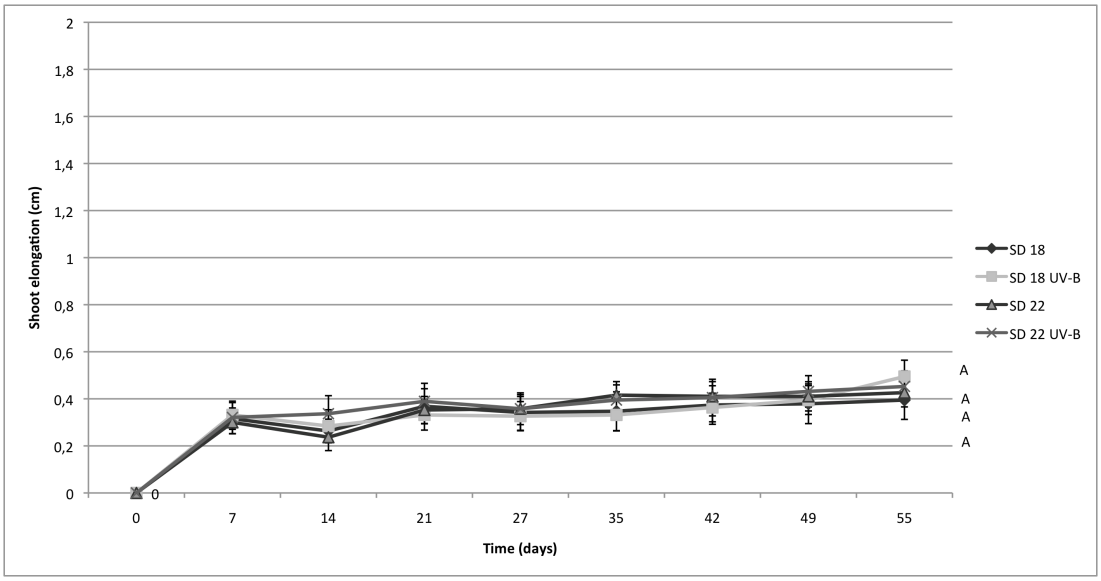

Figure 10 Average cumulative stem elongation growth in seedlings of Subalpine Fir exposed to different treatments in experiment 2, i.e short days (SD) and SD combined with UV-B, both at two different temperatures 18°C and 22°C). The results are mean ± SE of 19 plants per treatment. Means that do not share a letter are significantly different.

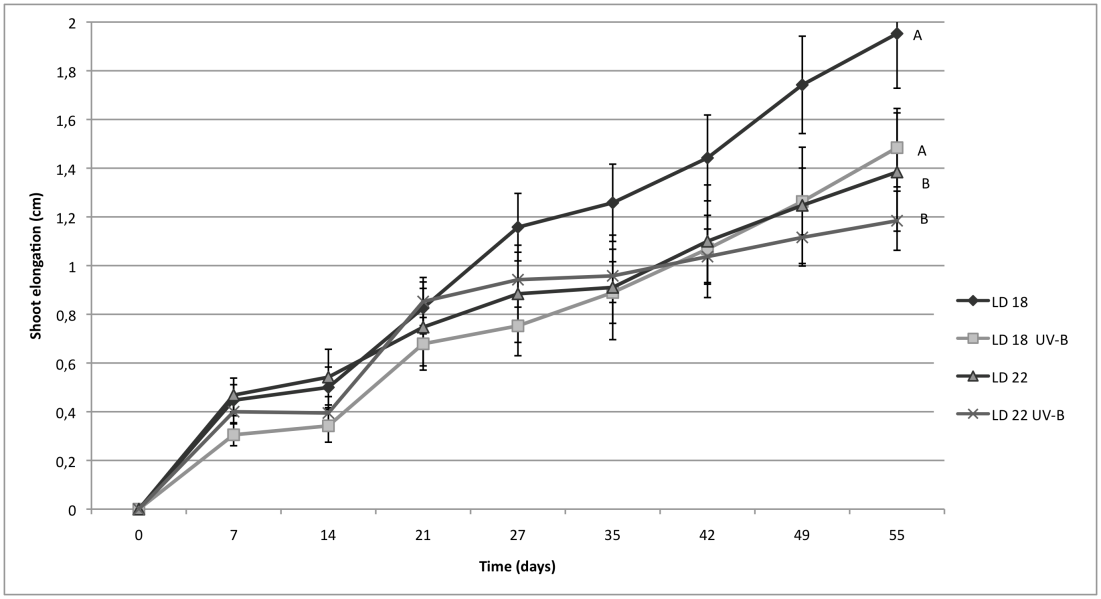

Figure 11 Average cumulative stem elongation growth (cm) for seedlings of Subalpine Fir exposed to different treatments in experiment 2, i.e long days (LD) and LD combined with UV-B at two different temperatures (18°C and 22°C) The results are mean ± SE of 19 plants per treatment. Means that do not share a letter are significantly different.

In Subalpine Fir there was no significant effect of UV-B when provided under SD in experiment 2 *(fig. 10)*.

Under LD, the plants, as expected, grew significantly more than under SD *(p=0,0001)* (*fig. 10 and fig. 11*). Although not statistically significant, the Subalpine Fir grown under 18°C showed a trend of more growth (appox. 0.5 cm longer) when exposed to LD only, compared to LD combined with UV-B *(Fig 11)*. Also at 22°C it appeared to be a similar trend of a slightly more growth without UV-B; seedlings in LD without UV-B were approx. 0.2 cm longer compared to LD with UV-B.

The different temperatures (18° C and 22° C) were significantly different in LD (p=0,041), with seedlings being longer in 18° C than in 22°C *(fig. 10),* although the growth in seedlings in 18° C with UV-B seemed to be somewhat inhibited until day 49.

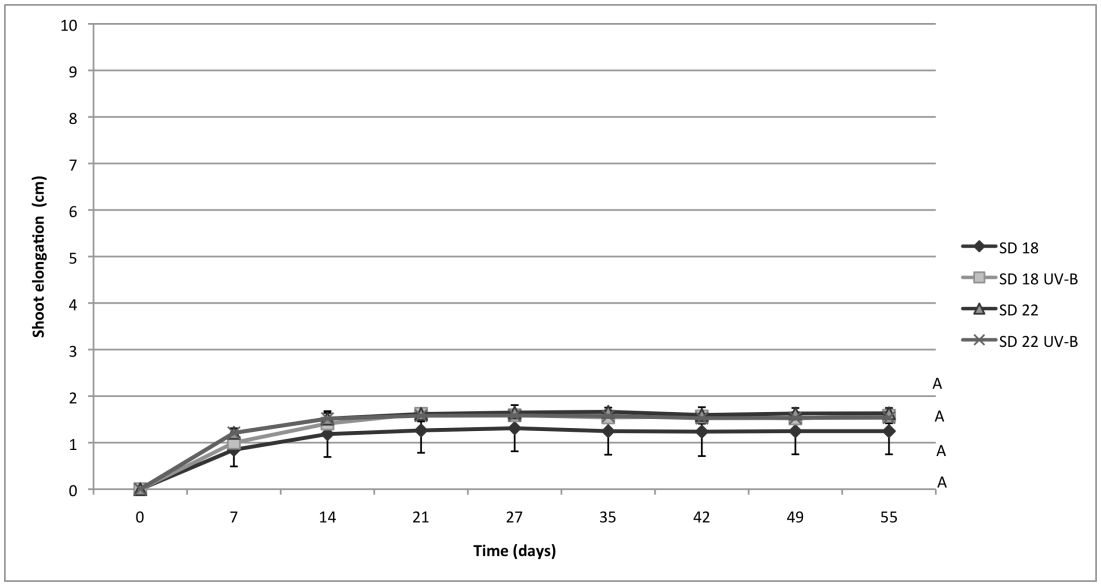

Figure 12 Average cumulative stem elongation growth in seedlings of Norway Spruce exposed to different treatments in experiment 2, i.e short days (SD) and SD combined with UV-B, both at two different temperatures 18 °C and 22 °C. The results are mean ± SE of 19 plants per treatment. Means that do not share a letter are significantly different.

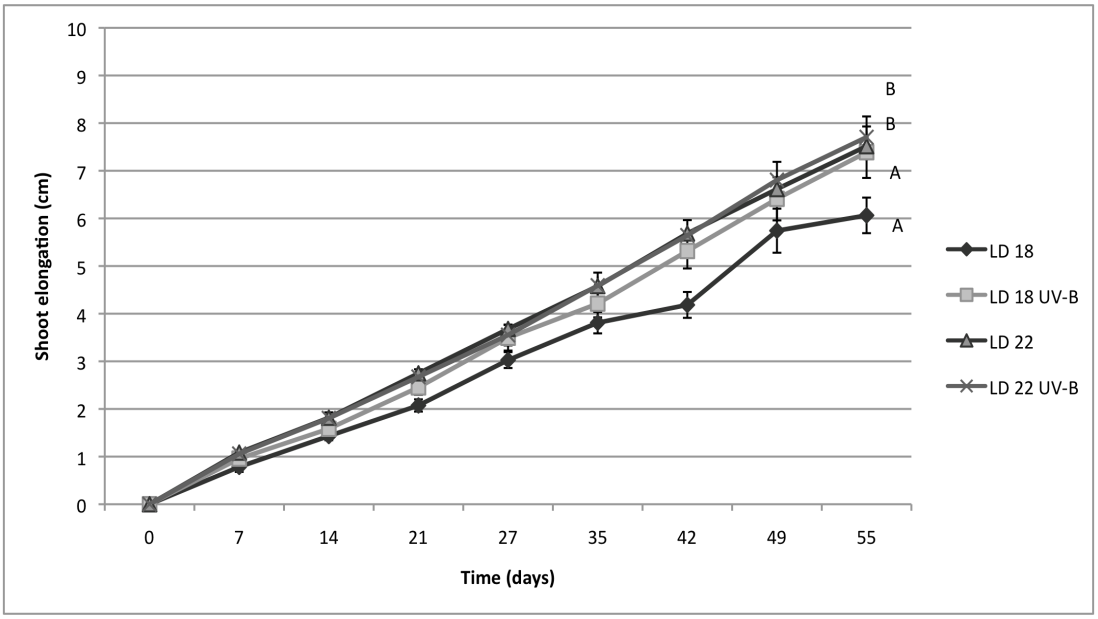

Figure 13 Average cummulative stem elongation growth in seedlings of Norway Soruce exposed to different treatments in experiment 2, i.e long days (LD) and LD combined with UV-B, both at two different temperatures 18 °C and 22 °C. The results are mean ± SE of 19 plants per treatment.

For Norway spruce seedlings there was no significant effect of UV-B or temperature (18 versus 22°C) on shoot elongation in SD (*fig.12)*. Growth ceased after about one week in all cases.

Under LD the Norway spruce seedlings showed sustained elongation growth, as expected *(fig. 13)*. There was no significant effect of the UV-B –treatment under LD, although there was a trend at 18°C that LD without UV-B har a reduced growth *(p= 0,055)* compared to the treatment with UV-B. Temperature had a slight, but significant effect *(p=0,016)*.

3.2.2 The effect of temperature on responses to UV-B radiation and day length on bud development in Subalpine Fir and Norway Spruce Bud

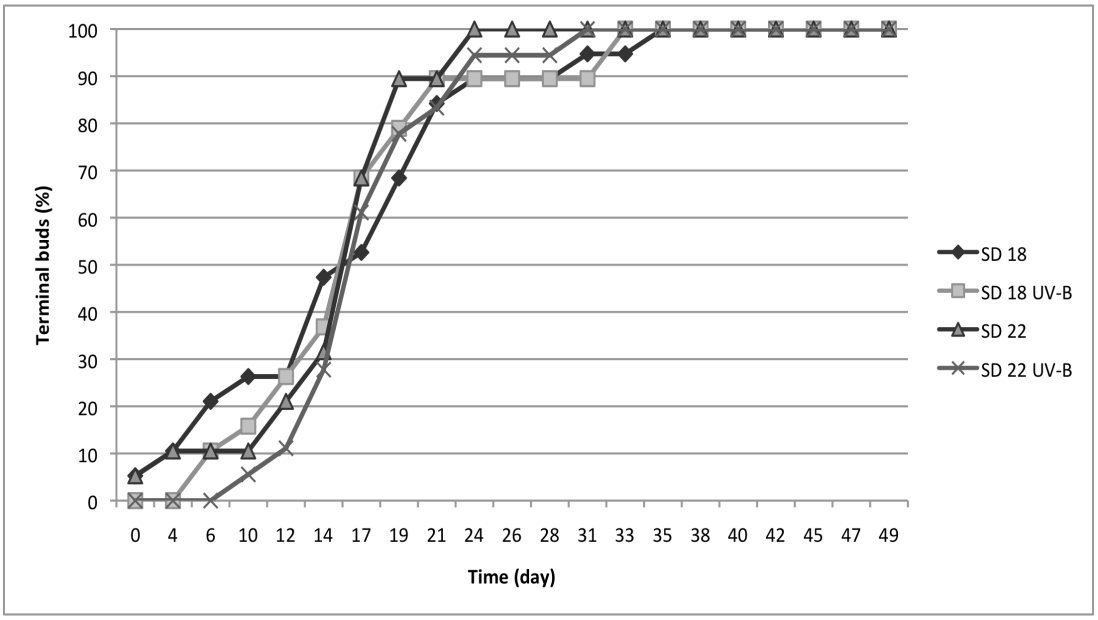

Figure 14 Percentage (%) seedlings of Subalpine Fir forming terminal buds in response to different treatments in experiment 2, i.e short days (SD) and SD combined with UV-B, both at 18°C or 22°C. The results are mean ± SE of 19 plants per treatment.

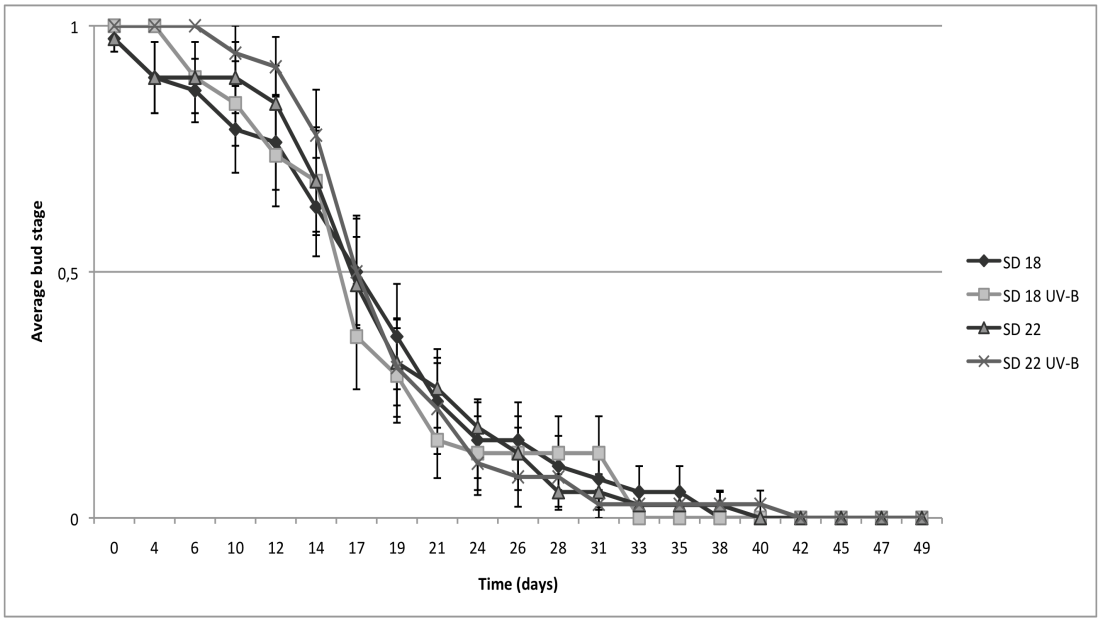

Figure 15 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) for seedlings of Subalpine Fir exposed to different treatments in experiment 2, i.e short days (SD) and SD combined with UV-B, both at 18°C or 22°C. The results are mean ± SE of 19 plants per treatment.

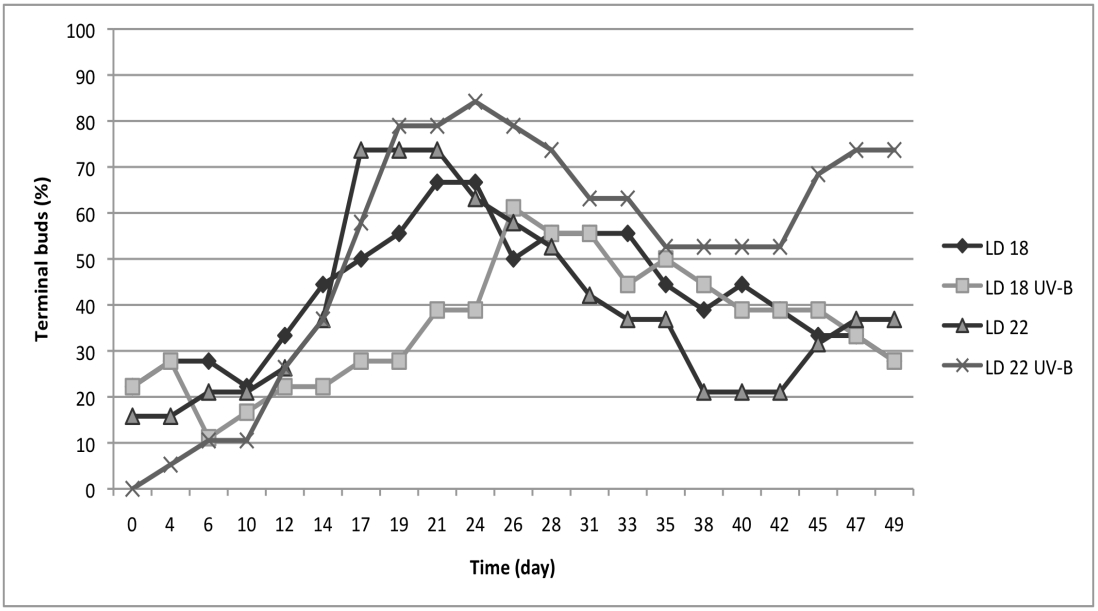

Figure 16 Percentage (%) seedlings of Subalpine Fir forming terminal buds in response to different treatments in experiment 2, i.e long days (LD) and LD combined with UV-B, both at 18°C or 22°C. The results are mean ± SE of 19 plants per treatment.

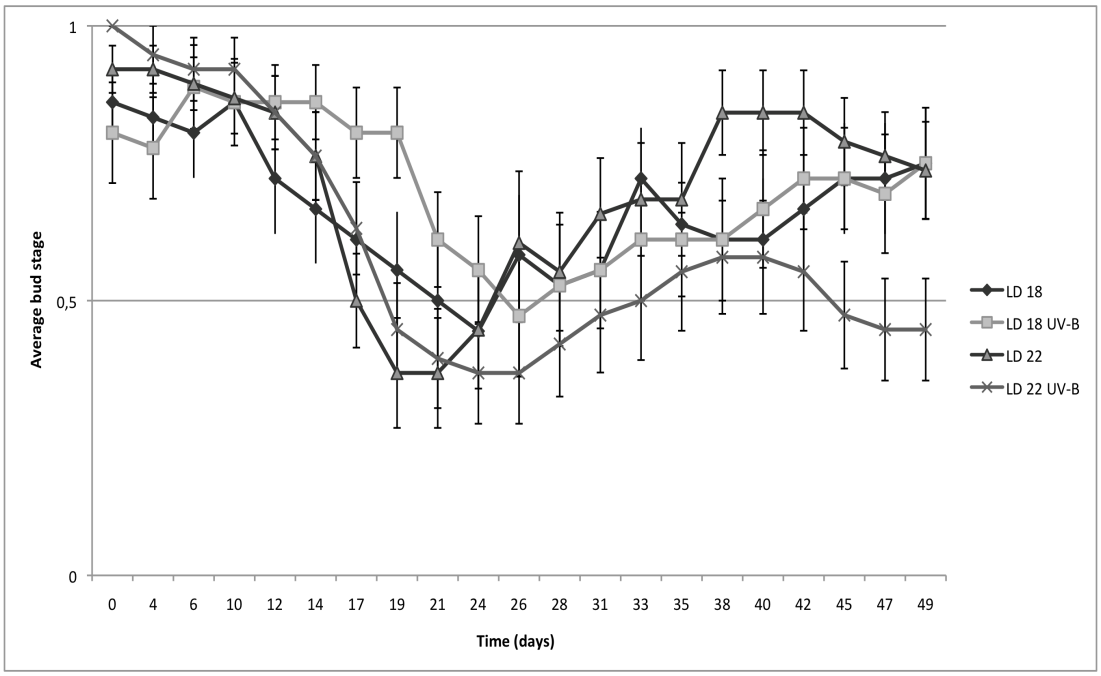

Figure 17 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) for seedlings of Subalpine Fir exposed to different treatments in experiment 2, i.e long days (LD) and LD combined with UV-B, both at 18°C or 22°C. The results are mean ± SE of 19 plants per treatment.

For Subalpine Fir there seemed to be no substantial effect of UV-B treatment or temperature on % plants with bud set under SD, except a possible trend of delay in bud set with UV-B present approximately the first two weeks after the transfer to the SD and UV-B treatments *(fig. 14)*. The effect of UV-B on the bud set under LD was unclear, although at 22°C there was a higher proportion of plants with buds after 20-25 days of UV-B treatment (± 80 % in bud) compared to at 22°C without UV-B (± 65 % in bud). Also, 75 % of the plants in UV-B had developed buds after 7 weeks, and nearly 40 % without UV-B under LD 22 °C. Under 18°C there seemed to be a delay in bud set under UV-B during the 2nd and rd week of treatment, but thereafter the proportion of plants with buds appeared similar irrespective of exposure to UV-B *(fig.16)*.

In this species, a substantial proportion of the plants formed buds even in LD *(fig. 15)*. However, several of these buds showed bud break within the experimental period of 7 weeks.

Table 8. Results (parameter estimates, SE and z-values) from a cumulative link model in R run to investigate the effects of UV-B (UVB) and day length (SD) on bud development (bud stages) in Subalpine Fir compared to long day (LD) without UV-B in experiment 2, under temperatures (Temp) of 18℃ and 22 ℃. Positive estimated coefficient indicates an increased probability for bud set, while negative estimated coefficient indicates a probability for a delay in bud set.

Treatment	Coefficients	SE	z
UVB***	-4,083573	1,115369	-3,661
Temp	-0,062711	0,039395	-1,592
DaySD	0,971191	1,114900	0,871
Date***	0,076054	0,003094	24,578
UVB:Temp***	0,208508	0,055156	3,780
UVB:DaySD**	4,611045	1,585497	2,908
Temp:DaySD	0,044872	0,055371	0,810
UVB:Temp:DaySD**	-0,0234965	0,078589	-2,990

*Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.*

The statistical analysis of the stages of bud development in Subalpine Fir, showed that UV-B together with higher temperature (22°C versus 18°C) delayed the bud

development during SD, compared to the control, LD (*fig. 15, Table 8*). The statistical analysis also showed that UV-B given at higher temperature alone enhanced the bud set in seedlings, while UV-B alone delayed *(fig. 15)*.

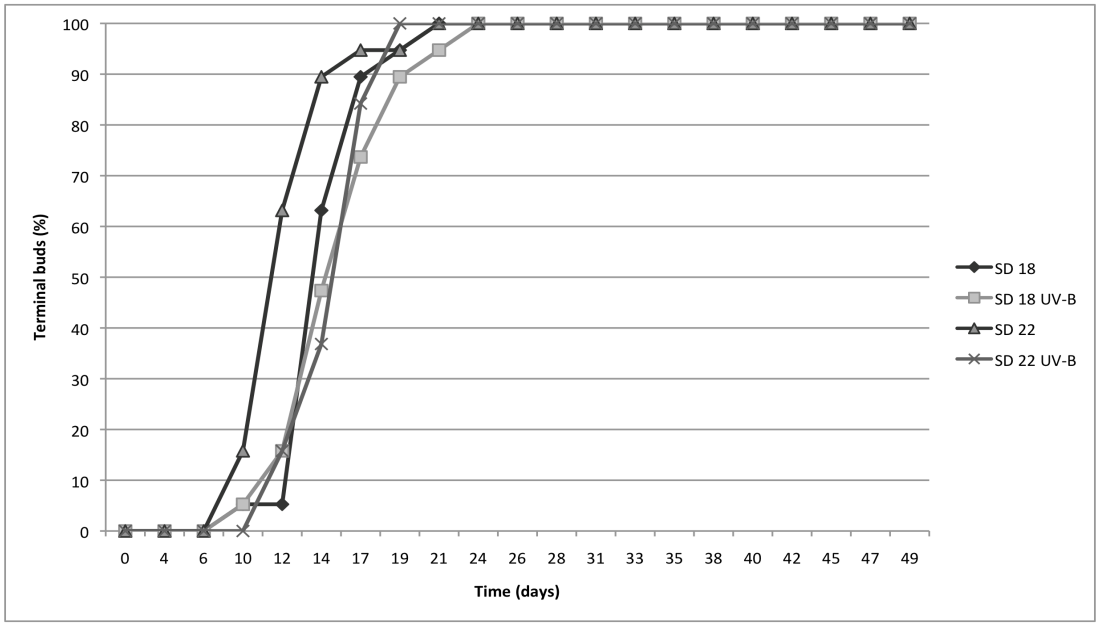

Figure 18 Percentage (%) seedlings of Norway Spruce forming terminal buds in response to different treatments in experiment 2, i.e short days (SD) and SD combined with UV-B, both at 18°C or 22°C. The results are mean ± SE of 19 plants per treatment.

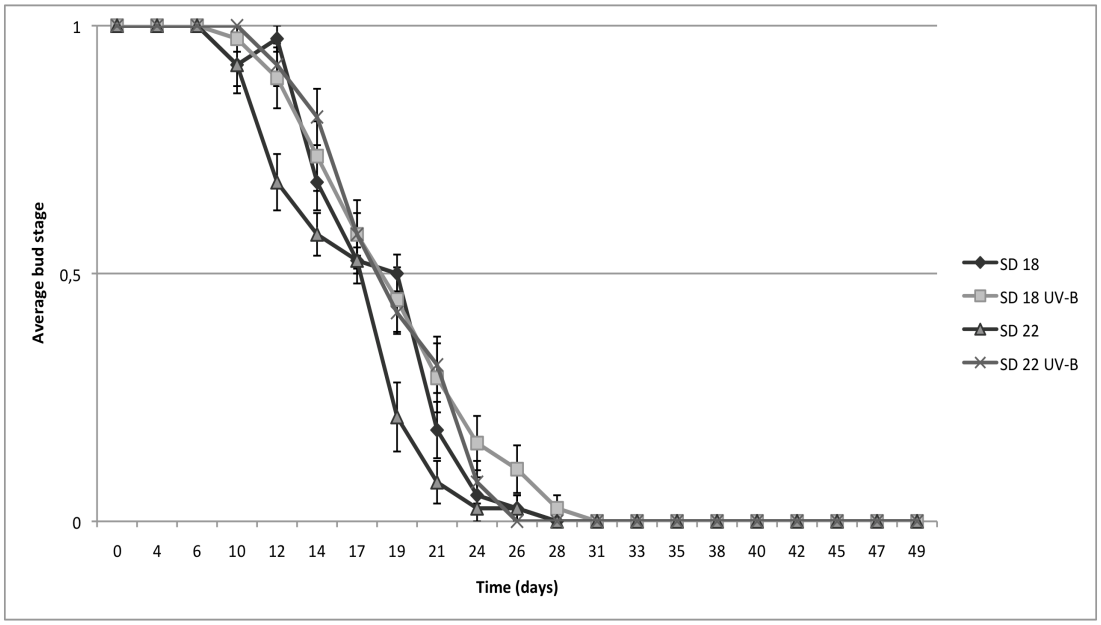

Figure 19 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) for seedlings of Norway Spruce exposed to different treatments in experiment 2i.e short days (SD) and SD combined with UV-B, both at 18°C or 22°C. The results are mean ± SE of 19 plants per treatment.

In Norway Spruce there was no bud development during any of the LD treatments. In SD, buds were developed within 25 days for all the seedlings. There appeared to be a delay to some degree in bud development when given UV-B radiation in 22°C during SD. The first buds were then observed after about 12 days, compared to after about 9 days without UV-B. In SD 18°C, the seedlings exposed to UV-B showed a slight delay compared to the treatment with no UV-B, i.e. 16-20 days after start. Seedlings seemed to develop buds earlier in SD 22°C compared to SD 18°C. SD 18°C and 22°C with UV-B were fairly similar, but after 14-20 days there might be a delay in 18°C *(fig. 18)*.

No apparent differences were detected in average bud stage for Norway Spruce in the different treatments during SD *(fig. 19)*. It was not possible to conduct an analysis of bud stages because of too few variations in the data set, making it unable to analyze any differences between the treatments.

3.3 Experiement 3 The effect of temperature (18 and 24°C) on responses to UV-B radiation and day length in Subapine Fir and Norway Spruce

3.3.1 The effect of temperature on responses to UV-B and day length on stem elongation growth

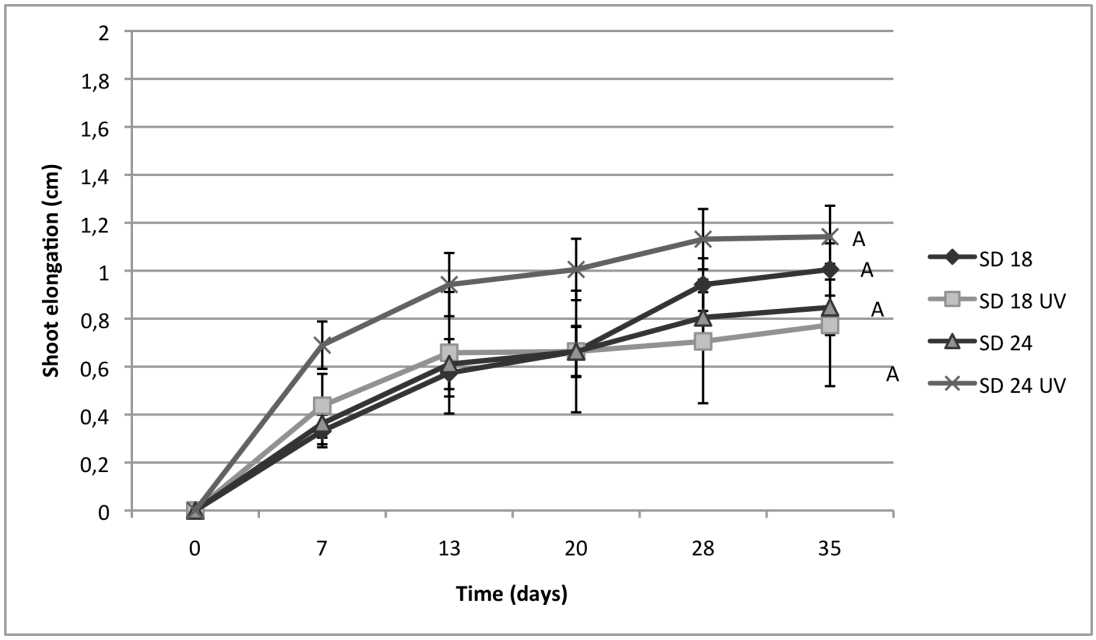

Figure 20 Average cumulative stem elongation growth in seedlings of Subalpine Fir exposed to different treatments in experiment 3, i.e short days (SD) and SD combined with UV-B, both at two different temperatures 18 °C and 24 °C.The results are mean ± SE of 19 plants per treatment. Means that do not share a letter are significantly different.

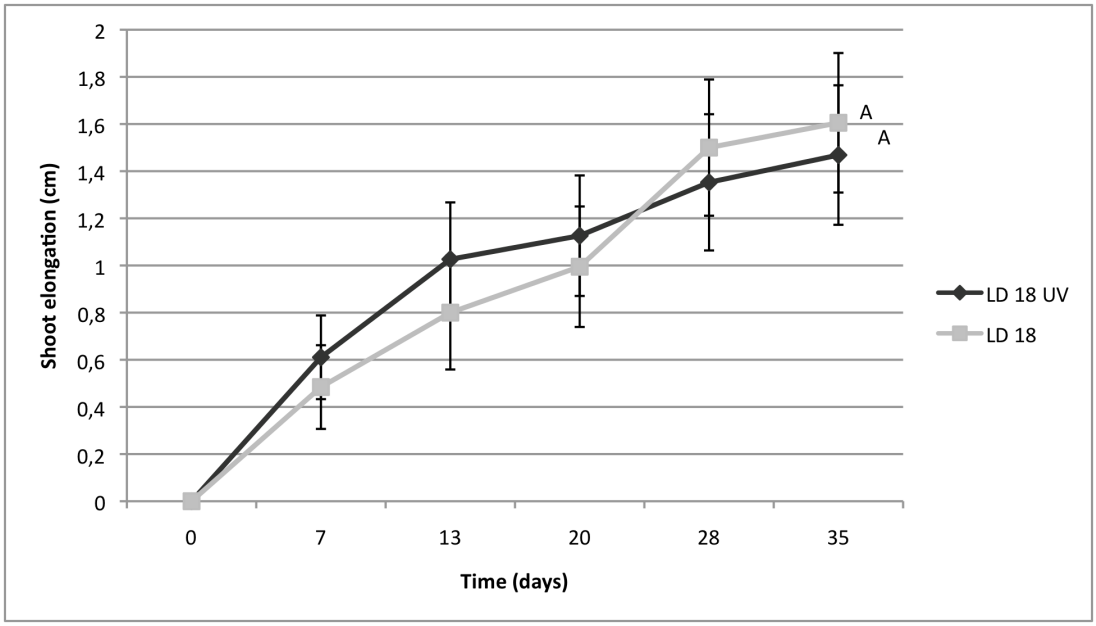

Figure 21 Average cumulative stem elongation growth in seedlings of Subalpine Fir exposed to different treatments in experiment 3, i.e long days (LD) and LD combined with UV-B, both at two different temperatures 18 °C and 24°C. The results are mean ± SE of 19 plants per treatment. Means that do not share a letter are significantly different.

As expected, the seedlings of Subalpine Fir grew significantly more *(p= 0.004)* under LD than SD (*fig. 20 and fig. 21*). There was no significant difference between the different temperatures (18°C and 24°C) or for the UV-B treatments *(p>0,05),* although there seemed to be a trend of more growth under 24 °C + UV-B as compared to 24°C without UV-B.

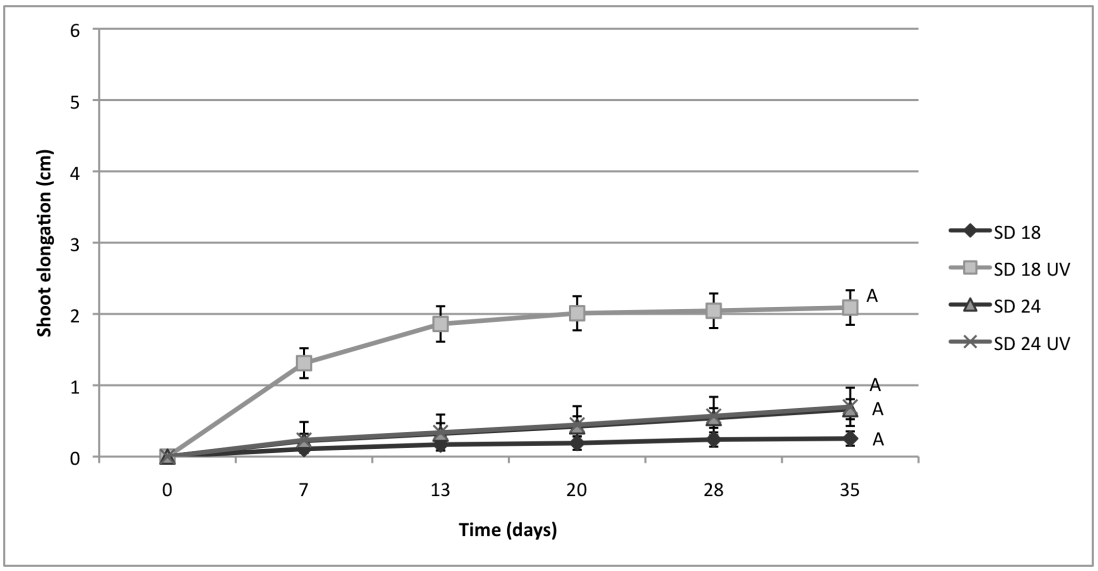

Figure 22 Average cumulative stem elongation growth in seedlings of Norway Spruce exposed to different treatments in experiment 3, i.e short days (SD) and SD combined with UV-B, both at two different temperatures 18 °C and 24 °C.The results are mean ± SE of 36 plants per treatment. Means that do not share a letter are significantly different.

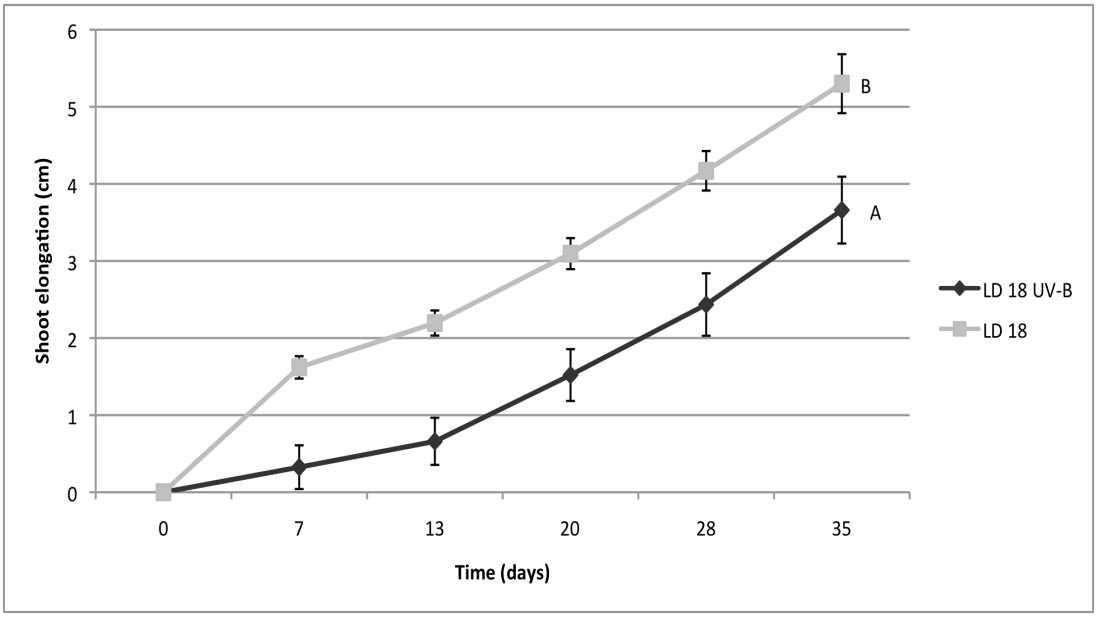

Figure 23 Average cumulative stem elongation growth in seedlings of Norway Spruce exposed to different treatments in experiment 3, i.e long days (LD) and LD combined with UV-B, both at two different temperatures 18 °C and 24 °C.The results are mean ± SE of 36 plants per treatment. Means that do not share a letter is significantly different.

As expected, elongation growth in the seedlings of Norway Spruce differed significantly ((*p= 0,0001*), and they showed growth cessation after 1-2 weeks under SD and sustained growth under LD *(fig. 22 fig. 23).* There was a significant effect between the interaction between day length and UV-B (*p=0,001*). The seedlings exposed to UV-B during LD 18°C showed significantly less growth compared to those without UV-B radiation *(fig. 22 and fig. 23).*

On the contrary, the seedlings in SD 18°C exposed to UV-B showed markedly more elongation than those without UV-B (*fig. 22)*. However, there was no significant general effect of the temperatures (18 °C and 24 °C) or from an interaction with UV-B and temperature *(p<0,05*). Furthermore, a Tukey test showed no significant differences at all in Norway Spruce during SD. The variations between SD treatments (18 °C and 24 °C) *(fig. 22)* are difficult to explain, but could be caused by undetected errors in the growth chambers.

3.3.2 The effects of temperature, day length and ultraviolet UV-B on winter bud development

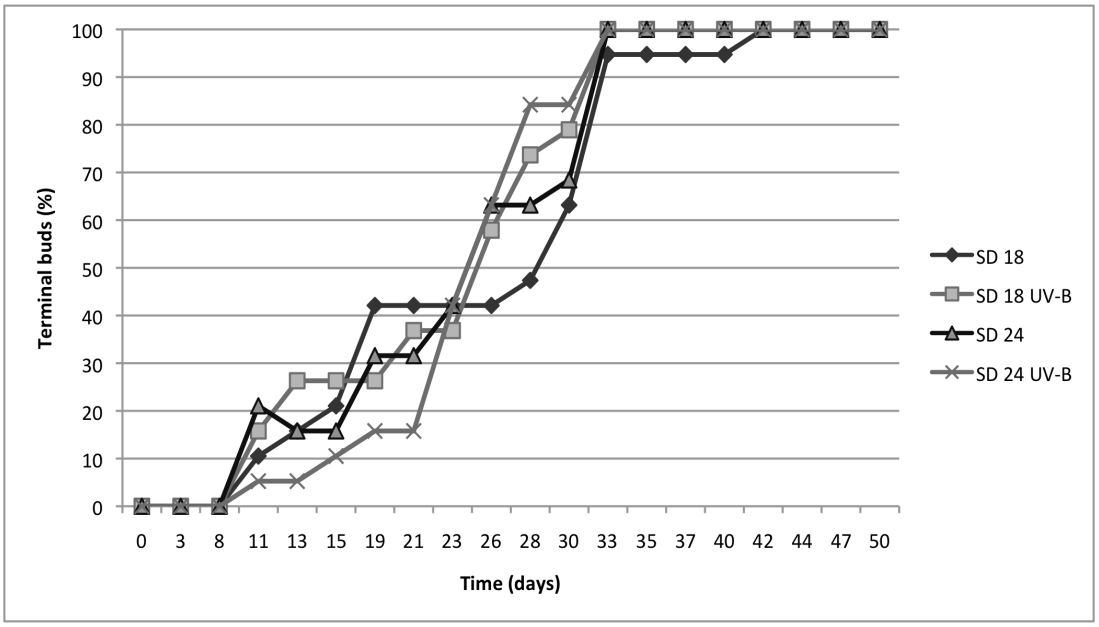

Figure 24 Percentage (%)seedlings of Subalpine Fir forming terminal buds g. in response to different treatments in experiment 3, i.e short days (SD) and SD combined with UV-B, both at 18°C or 24°C. The results are mean ± SE of 36 plants per treatment.

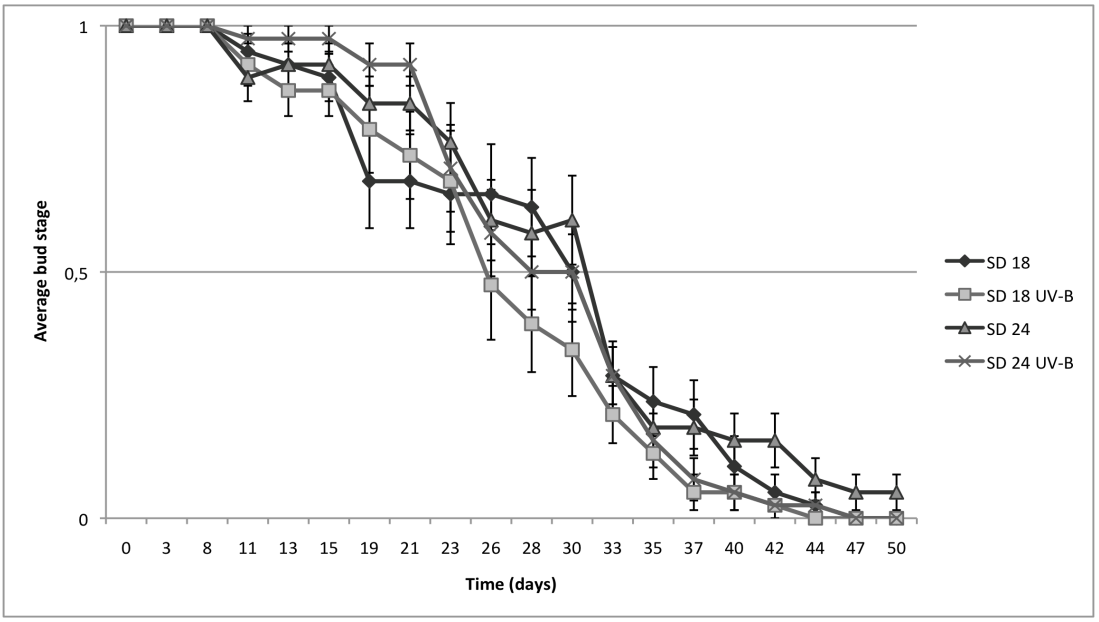

Figure 25 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) for seedlings of Subalpine Fir exposed to different treatments in experiment 3, i.e short days (SD) and SD combined with UV-B, both at 18°C or 24°C. The results are mean ± SE of 36 plants per treatment.

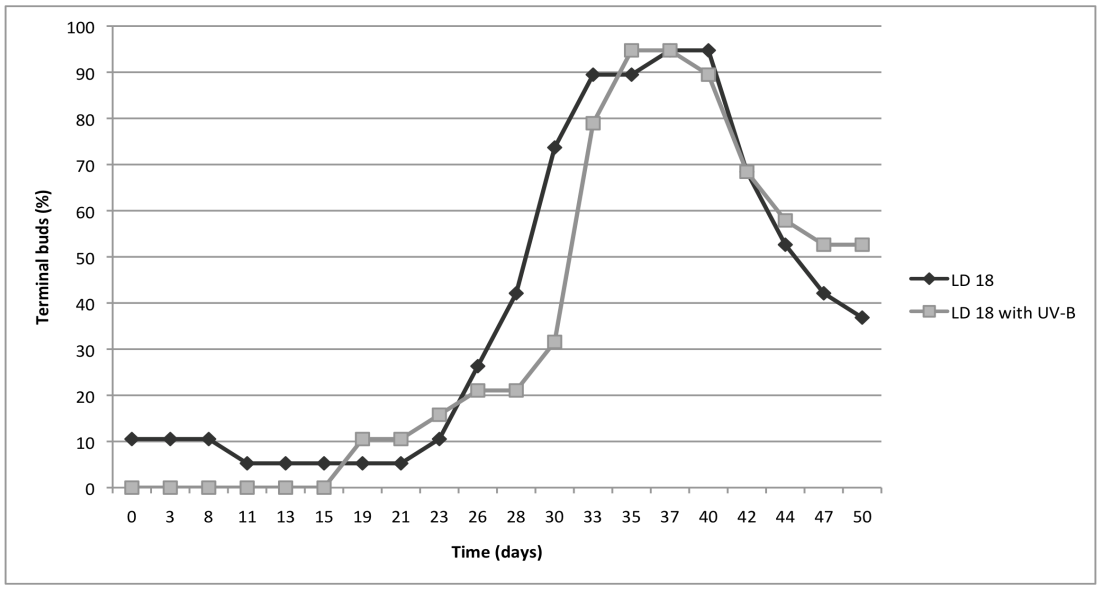

Figure 26 Percentage (%) seedlings of Subalpine Fir forming terminal buds g. in response to different treatments in experiment 3, i.e long days (LD) and LD combined with UV-B, both at 18°C or 24°C. The results are mean ± SE of 36 plants per treatment.

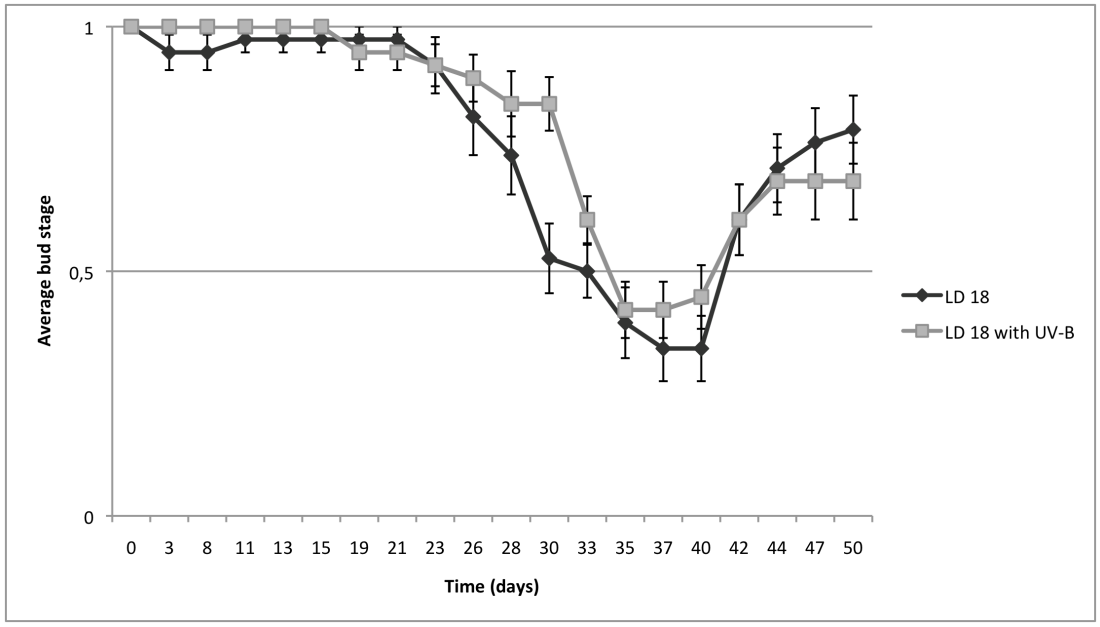

Figure 27 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) for seedlings of Subalpine Fir exposed to different treatments in experiment 3, i.e LD and LD UV-B with temperatures 18°C. (LD= long day, LD UV-B= long day and UV-B exposure) The results are mean ± SE of 19 plants per treatment.

Seedlings of Subalpine Fir developed buds faster in SD compared to seedlings in LD (*fig. 24 and fig. 26).* Also, a while after starting to develop buds seedlings in LD exposure showed bud break. Whereas after about 35 days about 95 % of the plants had terminal buds, this was reduced to about 40-50 % of the plants at the end of the experiment at day 50.

In SD the bud development showed no overall, consistent pattern distinguishing the treatments with UV from those without UV-B. However, seedlings in 18°C with no UV-B however seemed to be slightly delayed compared to the other treatments from day 24-40. In LD 18°C with UV-B there seemed to be a slight delay in bud development compared to no UV-B *(fig. 26)*

Seedlings in SD developed buds more consistently compared to in LD, and did not show bud burst during the experimental period, like was the case for buds formed in LD. Many of the seedlings in SD had fully developed buds within 36 days of treatment *(fig. 25 and fig. 27).*

Table 9. Results (parameter estimates, SE and z-values) from a cumulative link model in R run to investigate the effects of UV-B (UVB) and increasing temperatures (Temp) and day length (SD) on bud development (bud stages) in Subalpine Fir compared to long day (LD) without UV-B in experiment 3, with a temperature (Temp) at 18℃ and 24 ℃. Positive estimated coefficient indicates an increased probability for bud set, while negative estimated coefficient indicates a probability for a delay in bud set. Registrations from seedlings in LD were removed from the analysis due to little/no variation in the data set, making the progamme unable to run an analysis.

Treatment	Coefficient	SE	z
UVB*	0,311950	0,134742	2,315
Temp**	-0,065184	0,022511	-2,896
Date***	0,215879	0,008511	25,365

*Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.*

The statistical analysis of bud stages showed that the UV-B exposure in SD affected bud development in Subalpine Fir significantly, and increased the probability of bud development *(fig. 27, table 9).* There was also a significant effect of temperature in SD with decreased probability of bud development with increasing temperature *(table 9).* However, there was no significant interaction between UV-B radiation and temperature.

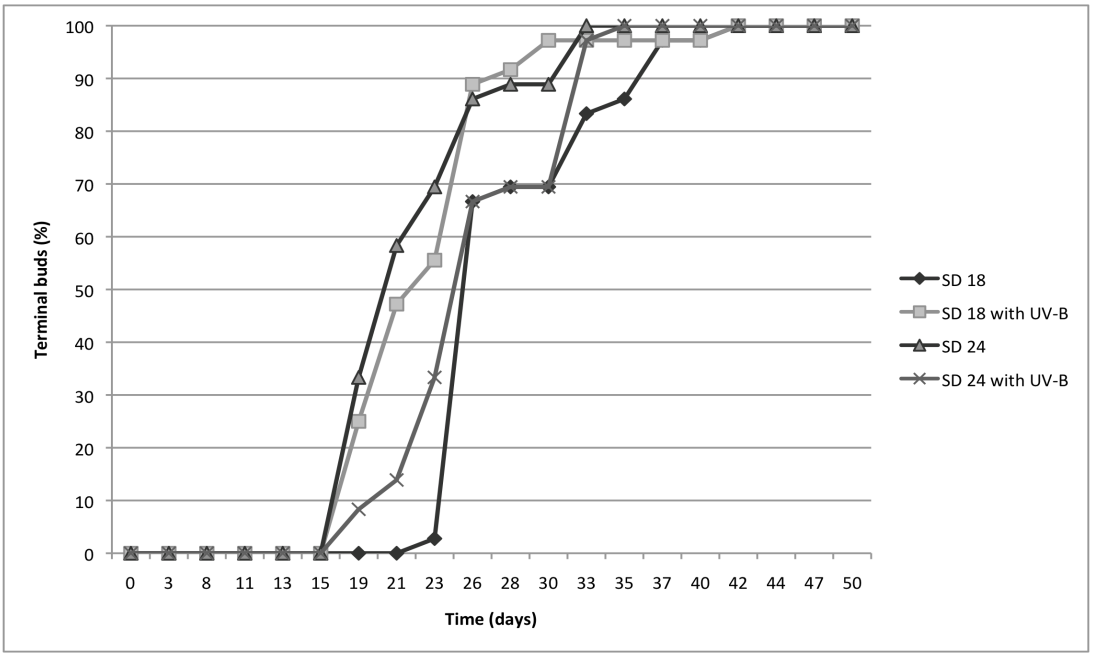

Figure 28 Percentage (%) of how many seedlings of Norway Spruce had developed buds at each timepoint for measuring. The seedlings were exposed to different treatments in experiment 3, i.e short days (SD) and SD combined with UV-B. The results are mean ± SE of 36 plants per treatment. LD observations is not presented due to no seedlings developed buds in these treatments.

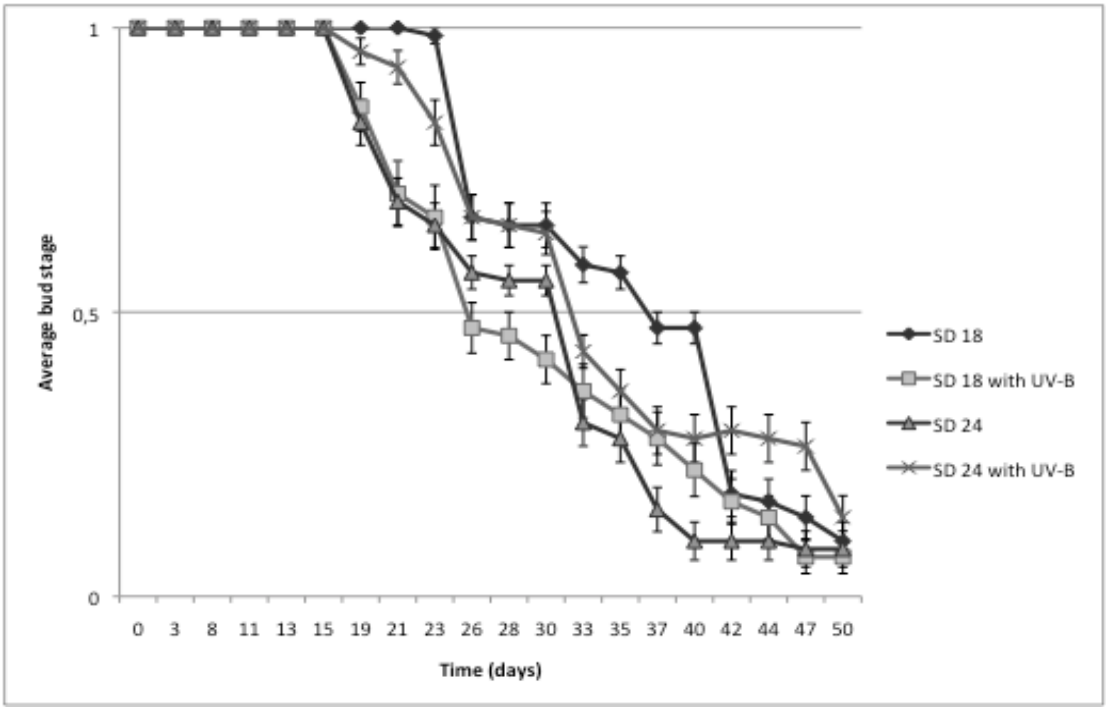

Figure 29 Average bud stages (0=fully developed bud, 0,5 =green bud, 1= in growth) for seedlings of Norway Spruce exposed to different treatments in experiment 3, i.e short days (SD) and SD combined with UV-B. The results are mean ± SE of 36 plants per treatment. Observations from LD is removed due to no bud development.

No seedlings of Norway Spruce developed buds in the LD treatments, but in SD all the seedlings developed buds consistently within the 7 weeks of the experiment. In SD during 18°C the seedlings exposed for UV-B seemed to develop buds earlier (approx. day 16), compared to without UV-B (approx. day 22) *(fig. 28)*. In 24°C however, the UV-B treatment seemed to delay the bud set *(fig. 29)*.

Seedlings in SD 18°C developed buds later than in SD 24°C, i.e after 23 and 16 days of treatment, respectively *(fig. 29)*.

Table 10. Results (parameter estimates, SE and z-values) from a cumulative link model in R run to investigate the effects of UV-B (UVB) and increasing temperatures (Temp) on day length (SD) on bud development (bud stages) in Norway Spruce compared to long day (LD) without UV-B in experiment 3, with temperatures (Temp) at 18℃ and 24 ℃ over a period of 8 weeks. Positive estimated coefficient indicates an inreased probability for bud set, while negative estimated coefficient indicates a probability for a delay in bud set. Registrations from seedlings in LD were removed from the analysis due to litte/no variation in the data set, making the progamme unable to run an analysis.

Treatment	Coefficient	SE	z
UVB***	10,742371	0,765422	14,04
Temp***	0,293513	0,024755	11,86
Date***	0,278891	0,008244	33,83
UVB:Temp***	-0,506538	0,035999	-14,07

*Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.*

The statistical analysis of bud development (bud stages) showed that higher temperatures increased the probability for buds forming in Norway Spruce *(fig. 29, table 10*). UV-B given under higher temperatures however, delayed the bud maturation, and is a significant correlation effect.

3.3.3 The effect of temperature, day length and UV-B radiation during bud set on subsequent bud burst

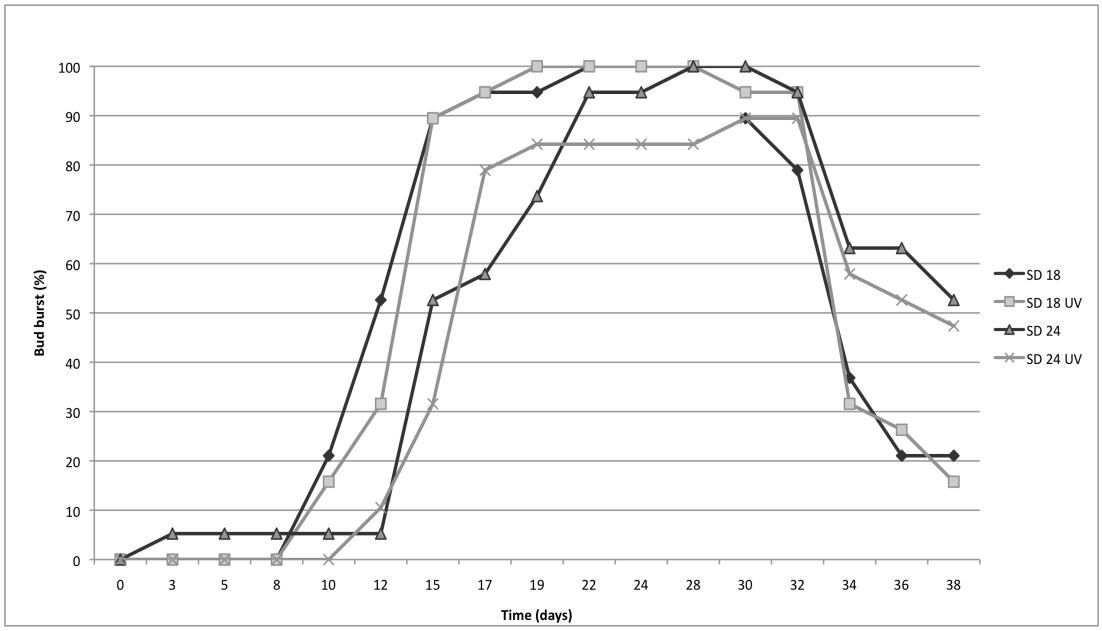

Figure 30 Percentage (%) of bud break in seedlings of Subalpine Fir after re-transfer to long days at 18°C following exposure to different treatments in experiment 3, i.e short day (SD) and SD UV-B at different temperatures (18°C and 24°C). The results are mean ± SE of 19 plants per treatment.

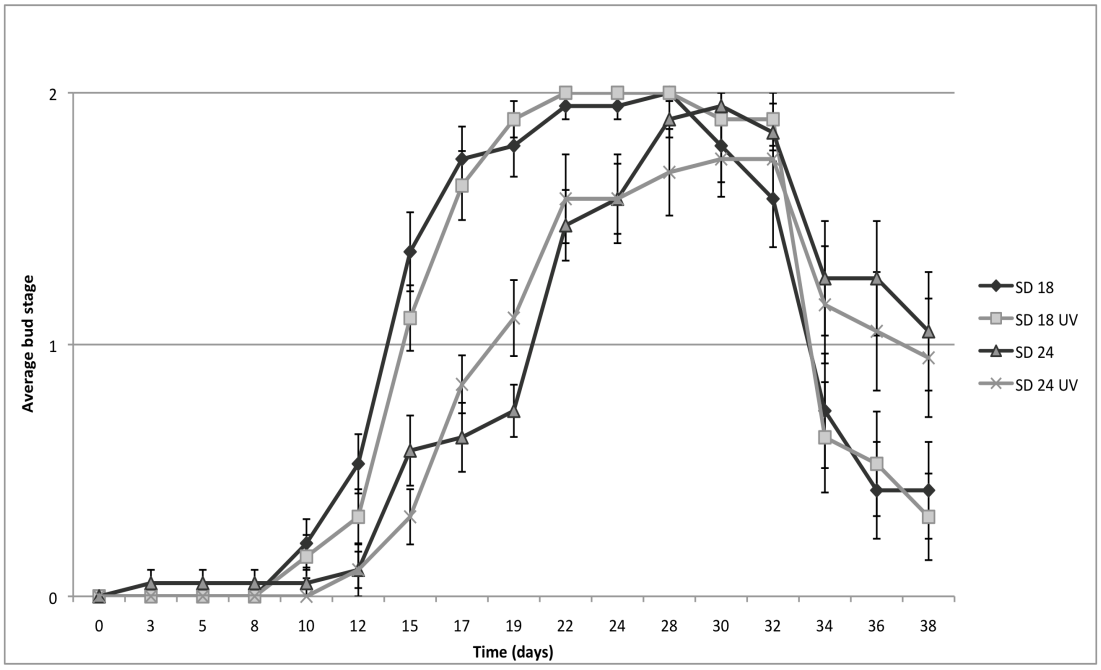

Figure 31 Stages of bud burst (0=fully developed bud, 0,5 =green bud, 1= in growth) and regrowth in Subalpine Fir seedlings after retransfer to long days following exposure to different treatments in experiment 3, i.e short day (SD) and SD UV-B with different temperatures (18°C and 24°C).The results are mean ± SE of 19 plants per treatment.

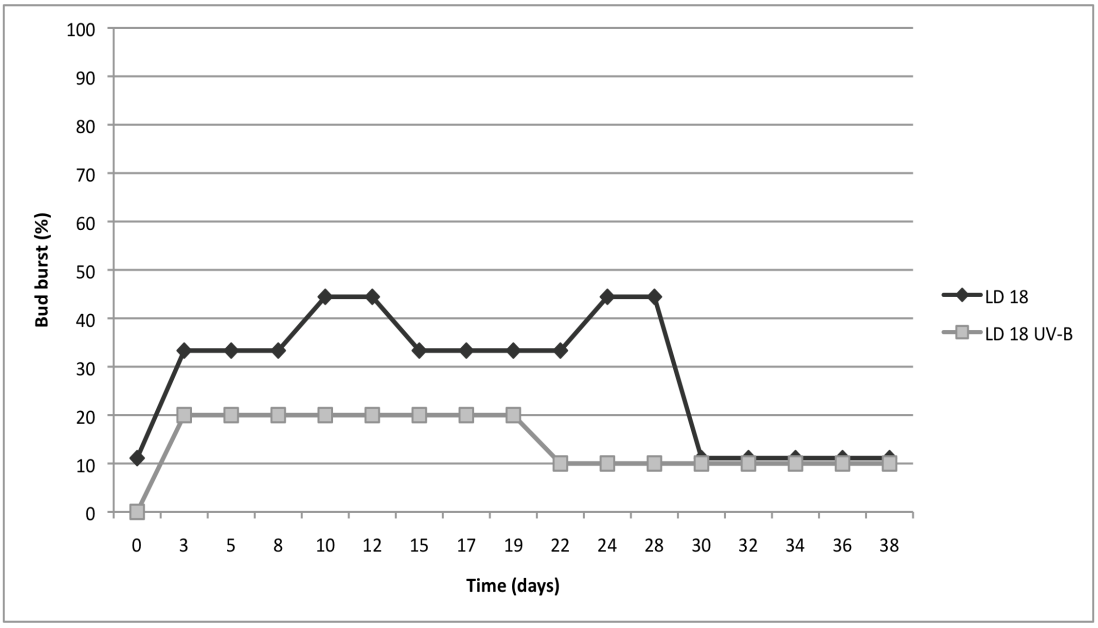

Figure 32 Percentage (%) of bud break in seedlings of Subalpine Fir after re-transfer to long days at 18°C following exposure to different treatments in experiment 3, i.e long day (LD) and SD UV-B at different temperatures (18°C and 24°C). The results are mean ± SE of 19 plants per treatment.

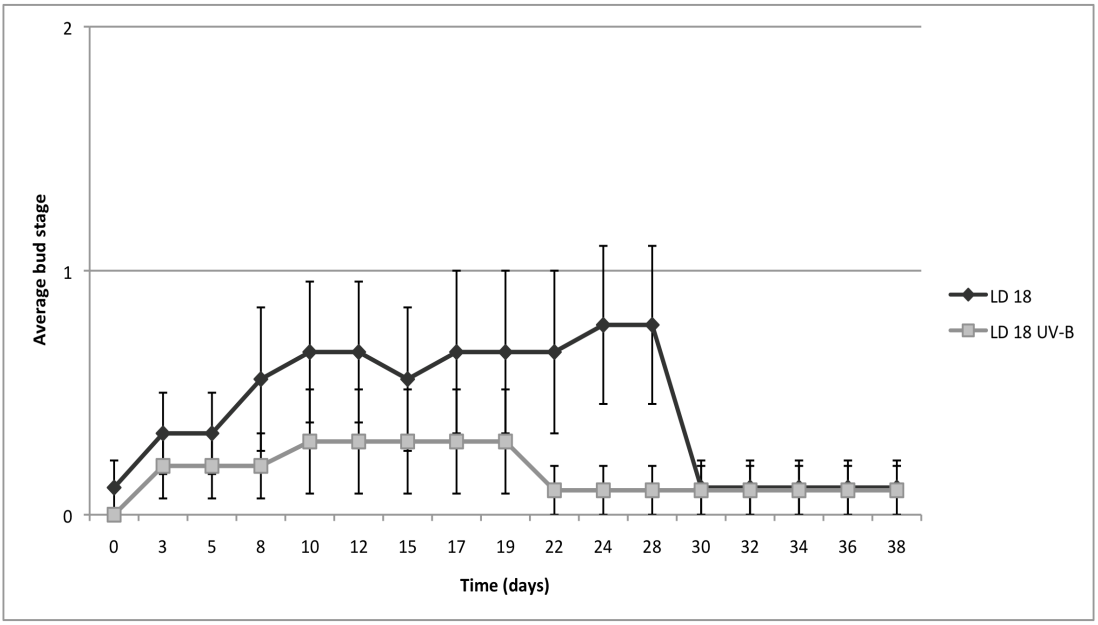

Figure 33 Average bud stages (0=fully developed bud, 1 =green bud, 2= in growth) and the amount of time for seedlings to break bud, for seedlings of Subalpine Fir exposed to different treatments in experiment 3, i.e long day (LD) and LD UV-B with temperature 18°C. The results are mean ± SE of 9-10 plants per treatment.

In seedlings of Subalpine Fir there seemed to be no consistent after-effect of UV-B in SD on bud break after re-locating to LD 18°C *(fig. 30 and fig. 32)*. In the plants exposed to SD at 18°C under bud set, the seedlings started breaking buds earlier, around day 8, and reached 100 % bud break after 22 days of LD-treatment, compared to SD 24°C, where bud burst started after 12 days. In 24°C, only seedlings without UV-B treatment reached 100 % bud break (after 28-30 days), where seedlings with UV-B seemed to be delayed; after 28-30 days 80-90 % had resumed growth *(fig. 30 and fig. 32)*. In plants forming buds under LD with UV-B the bud break was delayed compared to LD without UV-B; 20 % in UV-B and 35-45 % without UV-B resumed growth before re-forming bud *(fig. 32)*.

Only a few seedlings showing bud set under LD resumed growth, but a while after breaking some of these developed buds again *(fig. 33)*.

Table 11. Results (parameter estimates, SE and z-values) from a cumulative link model in R run to investigate the effects of UV-B (UVB), increasing temperatures (Temp) (18℃ and 24 ℃) and day lenght (SD) at subsequent bud break in Subalpine Fir compared to long day (LD) without UV-B in experiment 3. Positive estimated coefficient indicates an inreased probability for bud set, while negative estimated coefficient indicates a probability for a delay in bud set. A Drop1-test was performed to get a lower A´C, and UV-B and UV-B:Temperature interaction were removed from the analysis since these were not significant. Registrations of seedlings from LD treatments were not included in the analysis due to little/no variation in the data set.

Treatment	Coefficient	SE	z
Temp**	-0.065057	0,020122	-3,233
Date***	0,110755	0,006196	17,876

*Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.*

The statistical analysis of bud break stages *(Table 11)* showed that seedlings of Subalpine Fir in SD exposed to higher temperature had a higher probability for a delay in bud break at elevated temperatures, i.e. 24°C against 18°C *(fig. 31)*.

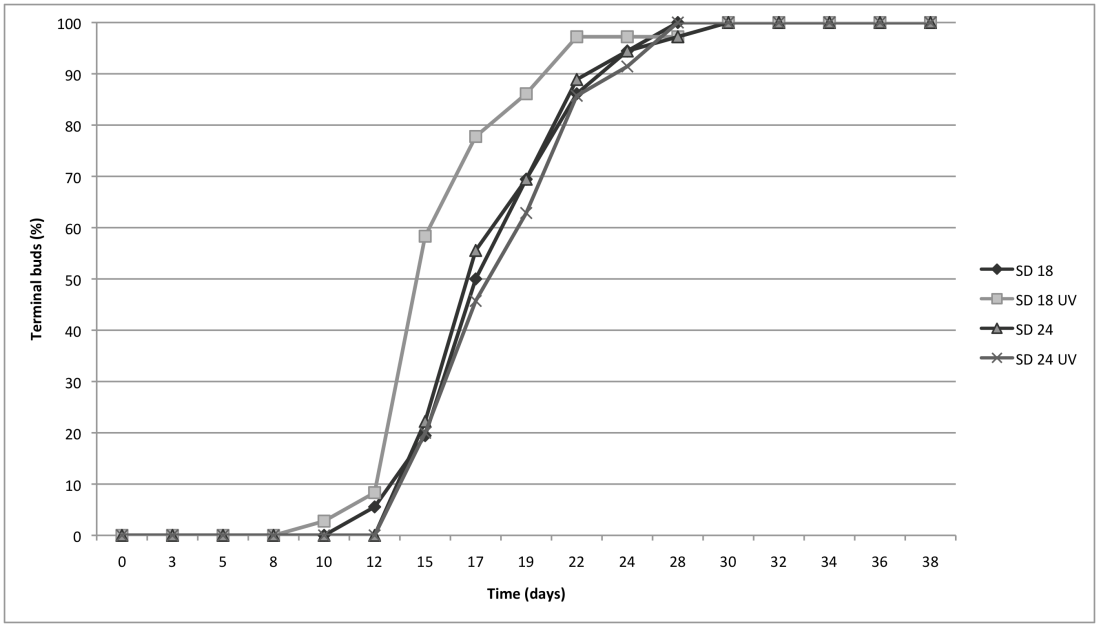

Figure 34 Percentage (%) of how many seedlings of Norway Spruce were breaking bud and returning to growth at each timepoint for measuring. The seedlings were exposed to different treatments in experiment 4, i.e short day (SD) and SD UV-B with different temperatures (18°C and 24°C). The results are mean ± SE of 36 plants per treatment.

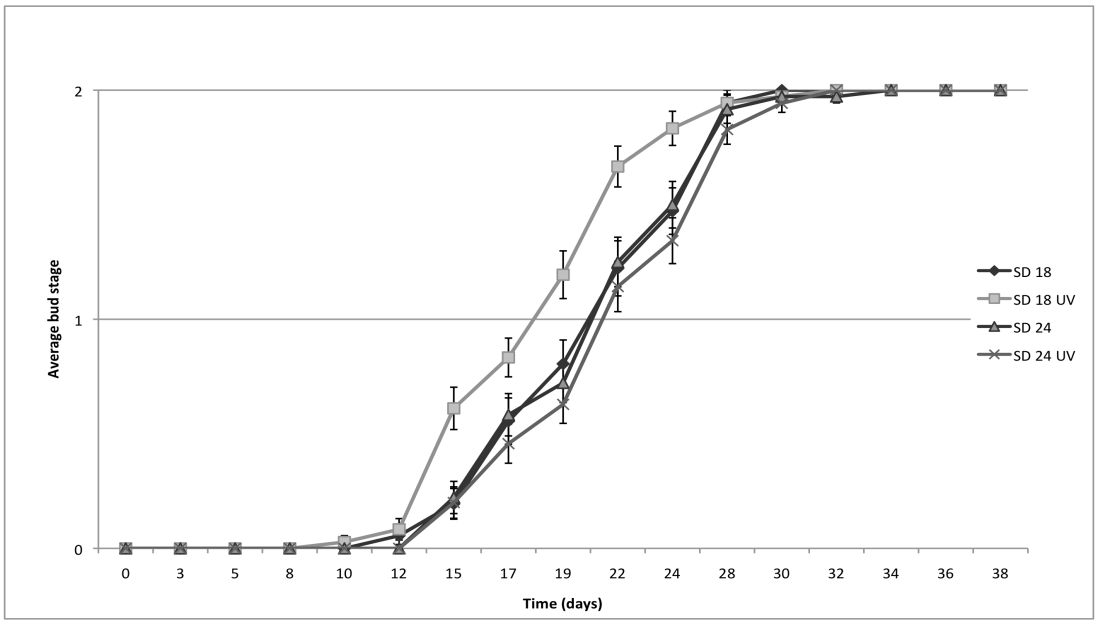

Figure 35 Stages of bud burst (0=fully developed bud, 0,5 =green bud, 1= in growth) and regrowth in Norway Spruce seedlings after retransfer to long days following exposure to different treatments in experiment 3, i.e short day (SD) and SD UV-B with different temperatures (18°C and 24°C).The results are mean ± SE of 19 plants per treatment.

100 % of all the seedlings of Norway Spruce which had formed buds in SD resumed growth within 32 days after being re-transferred to LD at 18°C. The bud break showed a similar pattern for all treatments, but in SD 18 with UV-B there seemed to be break buds earlier *(fig. 34)*. Seedlings exposed for SD 24°C with UV-B was slightly delayed in showing the first occurrence of bud break *(fig. 35)*.

Table 12. Results (parameter estimates, SE and z-values) from a cumulative link model in R run to investigate the effects of UV-B (UVB), inceasing temperatures (Temp) (18℃ and 24 ℃) and day lenght (SD) for bud break in Norway Spruce compared to long day (LD) without UV-B in experiment 3. Positive estimated coefficient indicates an inreased probability for bud break, while negative estimated coefficient indicates a probability for a delay in bud break. LD is not included in this analysis since none of the seedlings exposed for LD treatments developed buds, hence no registrations on bud break were possible.

Treatment	Coefficient	SE	z
UVB***	6,464865	1,058539	6,107
Temp	-0,008081	0,034402	-0,235
Date***	0,552227	0,021528	25,652
UVB:Temp***	-0,506538	0,035999	-5,768

*Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.*

The statistical analysis of bud break stages *(fig. 35, table 12)* showed that seedlings of Norway Spruce exposed for UV-B in higher temperatures (24°C against 18°C) had an increased probability for a delay in bud break, i.e. seedlings of Norway Spruce in these treatments may have had a deeper dormancy state.

4.0 Discussion

In order to investigate the effects of UV-B and temperature on the photoperiodic control of growth and bud set, seedlings of Norway Spruce and Subalpine Fir were exposed for UV-B during two different temperatures (18°C, 22°C) in both SD and LD. The effect of UV-B in 24°C was only tested in SD due to limited access to growth chambers. The UV-B radiation was measured to be approximately 0.16 W m^{-2} in experiment 1, and 0.10 W m^{-2} in experiment 2. The UV light was set slightly lower in experiment 2 to correspond to a lower PAR irradiance (155 µmol m^{-2} s^{-1}; due to division of chamber by plastic sheath) compared to experiment 1 (irradiance 190 µmol m^{-2} s^{-1}). In the third experiment the UV-B was increased to approx. 0.2 W m^{-2} (PAR light 190 µmol m^{-2} s^{-1}). In the two first experiments the ratio between the irradiance and UV-B was similar to what could be found in full sunlight in the summer (≈ 2000 µmol m^{-2} s^{-1}/ 1 W m^{-2}) (Solhaug, K.A, pers. com.). In the third, the UV-B was somewhat higher. Growth parameters were tested, as well as development of terminal winter buds. The average diameter was investigated in experiment 1, and the effects on bud break were tested at the end of experiment 3. In Norway Spruce the transcript levels of *FTL2,* which encodes a protein thought to act in growth cessation and bud set, were investigated in seedlings from experiment 1.

4.1 The effect of UV-B and temperature on photoperiodic control of growth and bud set in Subalpine Fir

4.1.1 The effects of UV-B

Although LD conditions are well known to sustain growth in a range of woody plant species of the boreal and temperate zone (e.g. (Nitsch 1957; Olsen 2010; Olsen & Lee 2011) in this study the Subalpine Fir formed terminal buds also in response to LD. However, seedlings exposed to LD showed overall more elongation growth compared to seedlings in SD *(fig.1).* The bud set also occurred later than in SD, and the plants thus had a longer growing period *(fig. 3 and fig. 4)*. This is in agreement with

previous studies; SD induces growth cessation and development of terminal buds (Garner 1923; Lee et al. 2014; Mølmann et al. 2006).

When considering all three experiments, there was no consistent effect of daily UV-B for 8 weeks in 18°C on elongation growth and plant diameter *(fig.7)* in Subalpine Fir, in either SD or LD. However, it appeared that the UV-B delayed the bud development during SD *(fig. 3 and fig. 4)*, and since the formation of terminal winter buds ceases the growth of the apical meristem (Gyllenstrand et al. 2007; Lang 1987; Rohde & Bhalerao 2007) this indicates a longer growing period. In previous studies, UV-B is known to decrease the stem elongation in herbaceous plants (Robson et al. 2014) and a recent study done in aspen showed that UV-B accelerated the development of terminal buds (Stømme et al. 2014). This indicates an opposite effect of UV-B in Subalpine Fir. A possible explanation for this difference could be that many seedlings of the Subalpine Fir already had or were about to develop terminal buds at the start of the experiment compared to seedlings in SD with UV-B. Furthermore, in this species many seedlings had already developed buds and had an unstable bud pattern even in LD throughout the experiment; showing alternation between bud set, bud burst, bud set and so forth. In experiment 1, the main light period in the pre-growing conditions was 12 h (about 200 µmol m^{-2} s^{-1}; HPS lamps and incandescent lamps) and the day was extended to 24 h with light from low-intensity light from incandescent lamps. This might be too low irradiance for Subalpine Fir, which could explain the early bud set. In the other two experiments the main light period during pre-growing was 24 h at about 200 µmol m^{-2} s^{-1}, but bud set then still occurred to a certain degree or was probably about to be initiated in several seedlings when the experimental treatments started, maybe still due too low irradiance. After all, 200 µmol m^{-2} s^{-1} is far lower than full light at a clear, sunny summer day. Thus, possibly too low irradiance was a strong growth inhibiting signal, which may have overshadowed an eventual effect of UV-B.

Because of the early bud formation in Subalpine Fir, it was not possible to know if the effects observed were induced in the experimental treatments, or if the plant already had started the process of bud development in the pre-growing conditions, and when.

This was an ongoing problem for all the experiments with Subalpine Fir, although the amount of seedlings with buds at experiment start was reduced in experiment two and three when the length of the main light period was increased to 24 h compared to the 12 h in experiment 1.

4.1.2 The effect of temperature on the UV-B responses

In SD no consistent effects of UV-B or temperature were observed in the elongation growth (22°C and 24 °C vs. 18°C) for Subalpine Fir *(fig. 10 and fig. 20)*. The growth was not altered by the UV-B in LD either, but temperature had an effect; seedlings were slightly, significantly shorter in higher temperatures (22°C vs. 18°C; experiment 2, *fig. 11*) in LD. Earlier experiments have shown that compared to lower temperatures, higher temperatures in short days induce more rapid bud development and growth cessation (Kalcits et al. 2009; Nitsch 1957; Olsen et al. 2014). It could be mentioned that in these previous studies the highest temperature was 21°C. However, another study done in *Prunus* species showed an insensitivity to photoperiod when exposed for higher temperatures, where 21°C was the highest, indicating that interaction between photoperiod and temperature can differ between species (Heide 2008).

In SD, the bud development appeared slightly delayed by UV-B exposure in higher temperatures, i.e. there seemed to be a longer growing period in 22°C (experiment 2, *fig. 14 and fig. 15*) and 24°C (experiment 3, *fig. 24 and fig. 25*) with UV-B compared to without UV-B. Higher temperature (24°C versus 18°C; experiment 3) in SD also delayed the bud formation in the beginning of the experiment *(fig. 25)*. In the third experiment, the levels of UV-B were increased relative to the PAR light, as compared to in experiment 2, and the possible delay in bud set in response to UV-light appeared slightly larger *(fig. 23, fig. 25, fig. 26 and fig. 27).* An experiment done with aspen (*Populus temula*) where the plants were exposed to extra UV-light in the field, showed that increased temperature delayed the bud set in aspen, while enhanced UV-B accelerated the development of buds (Stømme et al. 2014). The unstable bud

development with bud break and new bud development within the experiment period under LD, made an effect of UV-B and temperature in LD difficult to analyze. This considered, there was a higher percentage of seedlings with buds in 22°C with UV-B during LD compared to the LD treatment without UV-B, and compared to LD 18°C with or without UV-B (experiment 2) *(fig. 16)*. This might also explain why seedlings in LD were shorter in higher temperatures (22°C vs. 18°C; experiment 2, *fig. 11*).

In bud burst the most profound effect was a delay in bud burst after elevated temperatures during SD *(fig. 30 and fig. 31)*. Seedlings previously grown in SD 24°C, broke the buds later than those in SD 18°C. The seedlings earlier exposed to LD did not return to full growth; after some time these seedlings started developing new terminal buds *(fig. 32 and fig. 33)*. This correlates with earlier studies of bud burst in Norway Spruce, where higher temperature during bud development delayed the bud burst (Olsen et al. 2014; Søgaard et al. 2007). This might also suggest that even though bud set occurred even in LD, the development of bud dormancy proceeded more slowly in LD than SD. Also, as suggested in (Olsen et al. 2014), it might be that bud development has to pass a certain stage before buds are able to exhibit bud burst.

4.2 The effect of UV-B and temperature on photoperiodic control of growth and bud set in Norway Spruce

Unlike Subalpine Fir, seedlings of Norway Spruce had a stable bud development in both SD and no bud development in LD in all three experiments. Thus Norway Spruce grew more in LD compared to SD, as expected.

4.2.1 The effects of UV-B

In Norway Spruce, the effect of UV-B was unclear. In some cases it seemed to induce more elongation growth for seedlings in LD exposure (*fig 13*, experiment 2), whereas less growth was observed with UV-B present than without in experiment 1 and 3 *(fig. 2 and fig. 23)*.

No consistent significant effect of UV-B was detected in SD, except a delay in bud set under SD with UV-B in experiment 1 and 3 *(fig. 6 and fig. 29),* which might indicate a longer growing period for seedlings exposed to UV-B. This was the same delaying reaction to UV-B as discovered in Subalpine Fir, which was opposite of what the earlier study of *Populus* have shown; UV-B accelerates bud development and decreases stem elongation (Strømme et al. 2014). In Norway Spruce no seedlings developed buds under the pre-growing before start of the experimental treatments. Norway Spruce is a evergreen species adapted to tolerate high solar energy in low temperatures during in the spring, autumn and winter. This gives a requirement to safely displace excess energy when the tree lowers its photosynthetic activity through the winter when entering dormancy; via a xanthophyll cycle (Adams-Demmig & Adams 1996). This might also explain why *Populus* (Stømme et al. 2014) were more effected by UV-B than spruce, as well as Subalpine Fir. *Populus* is not evergreen, and stops photosynthesis in the autumn when shedding the leaves. A recent study investigating the metabolite profiles of Norway Spruce during SD compared to LD, found that trees exposed to SD had a higher amount of a wide range of antioxidants including specific flavonoids known to act in protection towards UV (Olsen et al. 2014). In other words, conifers like Norway Spruce is a robust tree highly adapted to tolerate environmental stress.

The *FTL2* was up regulated during SD as expected *(fig. 9).* This is consistent with earlier studies (Asante et al. 2011; Gyllenstrand et al. 2007; Karlgren et al. 2013) where up regulation of *FTL2* during SD induced growth cessation and bud development was observed. There was no effect of UV-B on the expression of *FTL2.*

4.2.2 The effect of temperature on the UV-B responses

During SD there was no consistent effect of UV-B or temperature (22 °C or 24 °C vs. 18 °C) on the elongation growth of Norway Spruce *(fig. 12 and fig. 22).* However, in experiment 3 *(fig. 22)* the seedlings in SD 18°C exposed to UV-B showed markedly more elongation than those without UV-B . It is a small tendency in experiment 2 that SD 18 with UV-B is also slightly longer than without, but this is not markedly nor

significant *(fig. 12)*. These variations in experiment 3 could have been caused by undetected errors in the growth chamber, or other errors, but these are differences that are difficult to explain. In LD, the seedlings exhibited a longer growth in higher temperatures (22 °C vs. 18 °C) *(fig. 11)*. Like shown in *Prunus*, as mentioned above in the discussion of responses in Subalpine Fir, responsiveness to photoperiod might be affected by temperatures (Heide 2008).

Temperature and UV-B appeared to affect bud development during SD. In the second experiment higher temperature (22°C) with UV-B exposure had a delayed bud development *(fig. 16 and fig. 17)*. This was similar to what was found for 24°C in the third experiment; the development of terminal buds was delayed with increased UV-B *(fig. 24 and fig. 25)*. In experiment 2 there was no clear effect of UV-B on bud set at 18°C, whereas in experiment 3 bud set appeared enhanced with UV-B present. Thus, UV-B appeared to affect bud set differently at 18°C versus higher temperatures, unlike in Subalpine Fir. Modulations on temperature have a significant impact on photoperiodic responses (Heide 2008). Therefor it could be a possibility that the sensitivity to UV-B in bud development also is affected by temperature in Norway Spruce.

A three-way interaction was found in Norway Spruce between higher temperatures, UV-B and SD exposure, which delayed bud break after being relocated to LD exposure in 18°C, i.e. seedlings of Norway Spruce in these treatments may have had a deeper dormancy state *(table 12)*. In 18°C there was an opposite effect; UV-B induced earlier bud break *(fig. 34 and fig. 35)*. Previous studies of Norway spruce have shown that higher temperatures gives a deeper dormancy state and later bud break (Olsen et al. 2014; Søgaard et al. 2007). This was not evident for Norway spruce in the present study, although the Subalpine Fir exhibited such a pattern. These previous studies of Norway Spruce tested only temperatures up to 21°C, and it cannot be excluded that still higher temperature affects Norway Spruce differently.

4.3 Concluding remarks

4.3.1 Subalpine Fir

Subalpine Fir showed no consistent effect on elongation growth of UV-B or higher temperatures in SD (22°C and 24°C vs. 18°C). In LD Subalpine fir was significantly shorter in higher temperatures (18°C vs. 22°C), although this does not correlate with the rest of this study.

Subalpine Fir was very unstable in the bud development, and developed buds even in LD, making conclusions with respect to effect of UV-B and temperature difficult. However, UV-B exposure in higher temperatures during SD delayed the bud set in this species.

Seedlings exposed to higher temperatures during bud set had a delayed bud burst, i. e a deeper dormancy. The effect of UV-B under bud set on bud burst was not significant. Seedlings earlier in LD treatment had a deeper dormancy state than those in SD, which could suggest that the development of bud dormancy proceeded more slowly in LD than in SD, and had to pass a certain stage before buds were able to exhibit bud burst.

4.3.2 Norway Spruce

In Norway Spruce the effect of UV-B under LD was not consistent, i.e. depending on experiment, growth was slightly enhanced or reduced, which could not be easily explained. In experiment 2, UV-B enhanced the growth under LD, while in experiment 1 and 3 the UV-B reduced the elongation.

Under SD, there was also no significant effect of UV-B on elongation growth. Furthermore, UV-B had no effect on plant diameter was observed (investigated only under 18°C). Under LD, plants were longer in 22°C than 18°C (experiment 2), whereas under SD no significant effect of temperature on elongation growth was

observed. UV-B and higher temperatures during SD delayed the bud development. Also, UV-B in higher temperatures given in SD delayed the bud burst, i.e. seedlings exposed for this treatment developed a deeper dormancy. The *FTL2* gene was up regulated during SD in Norway Spruce but there was no significant effect of UV-B on its expression.

4.3 Future perspectives

The signaling pathways for effects of UV-B in trees is not yet clarified, but a possible explanation for the UV-B effects in both Norway Spruce and Subalpine Fir could be a change in the hormone levels of gibberelin (GA). A reduction in levels of GA is known to stop apical growth and induce bud development (Olsen et al. 1997). Also, levels of abscisic acid (ABA) was shown to increase under SD conditions resulting in bud set in Norway spruce and a range of tree species (Lee et al. 2014). Therefore a study to further investigates the signaling pathways in conifers, and the different hormone levels when exposed to UV-B could reveal more about the metabolic responses.

The fact that both Norway Spruce and Subalpine Fir are robust, evergreen species with a high tolerance for high irradiance in combination with low temperature in the spring and autumn, could explain the small effect of UV-B in this study. The chambers used in this study had non-reflecting walls, where UV-B just came from above. The levels of UV-B were also relatively low, fairly similar to in natural light. For further experiments higher levels of UV-B could be recommended to test. UV has been shown to increase robustness of several plants species with respect to protection towards oxidative stress, herbivores and pathogens (Robson et al. 2014). Thus, further experiments aiming at exploiting this when growing seedlings of conifers in nurseries might be of interest. To explore this, studies of protective mechanisms such as contents of phenylpropanoids and antioxidants in response to exposure of conifer seedlings to different levels of UV-B should be conducted.

Temperature could also affect the metabolism in Norway Spruce, and it could be possible that *FTL2*-expression is affected by temperature. Examining the effect of temperature on the up regulation of *FTL2* could therefore be an important future study.

The SD of 12 h used in this study is a strong, growth-inhibiting signal, which may have overshadowed the effects of UV-B in this study. Therefore a study where UV-B is examined with a day length closer to the critical day length could also be of interest to evaluate the effect of UV-B on the bud set.

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Appendix

Table 13 Experiment 1, light condition in growth chambers (18℃, 75 % RH)

Treatments	UV-B (W m^{-2})	Growth light (µmol m^{-2} s^{-1})	R/FR ratio
SD 18	0,030	190	1,70
SD 18 UV-B	0,154	199	1,69
LD 18	0,030	195	1,70
LD 18 UV-B	0,173	190	1,75

Table 14 Experiment 2, light condition in growth chambers (18℃, 65 % RH)

Treatments	UV-B (W m^{-2})	Growth light (µmol m^{-2} s^{-1})	R/FR ratio
SD 18	0,03	156	1,65
SD 18 UV-B	0,103	154	1,68
LD 18	0,03	159	1,66
LD 18 UV-B	0,102	154	1,61

Table 15 Experiment 2, light conditions in growth chambers (18℃, 75 % RH)

Treatments	UV-B (W m^{-2})	Growth light (µmol m^{-2} s^{-1})	R/FR ratio
SD 22	0,030	158	1,71
SD 22 UV-B	0,092	153	1,72
LD 22	0,030	157	1,79
LD 22 UV-B	0,108	156	1,64

Table 16 Experiment 3, light conditions in growth chambers (18℃, 65 % RH)

Treatments	UV-B (Wm^{-2})	Growth light ($\mu mol\ m^{-2}\ s^{-1}$)	R/FR ratio
SD 18	0,03	181,7	1,70
SD 18 UV-B	0,22	190,5	1,72
LD 18	0,03	193,9	1,72
LD 18 UV-B	0,23	188,5	1,65

Table 17 Experiment 3, light conditions in growth chambers (24℃, 75 % RH)

Treatments	UV-B (Wm^{-2})	Growth light ($\mu mol\ m^{-2}\ s^{-1}$)	R/FR ratio
SD 24	0,03	181,7	1,72
SD 24 UV-B	0,20	193,5	1,71

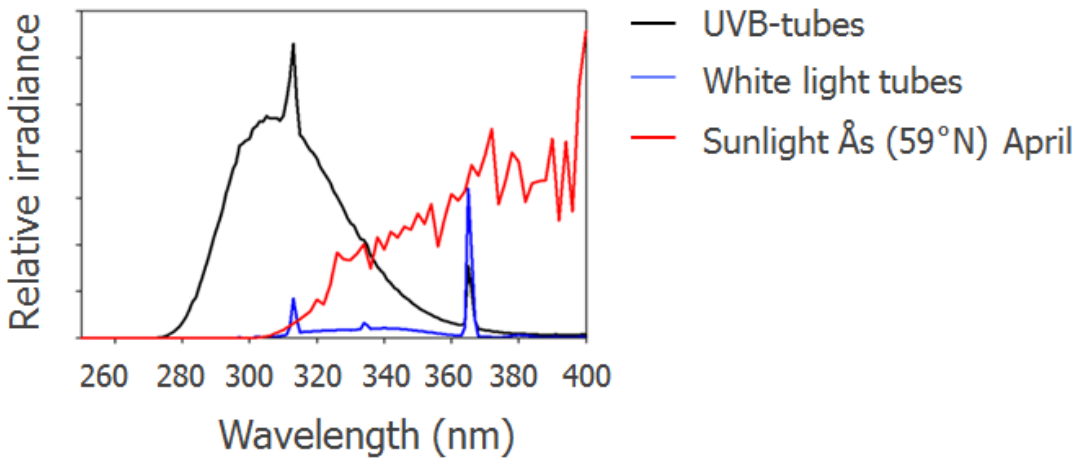
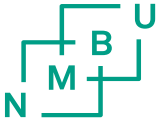


Figure 41 Sprectrum for the UV-lamps (Q-panel) used in this study, compared with flourescence light tubes (as used in experiment 1) and natural sunlight measured in Ås (Torre et al. 2012).



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