Norwegian University
of Life Sciences

Master's Thesis 2020 60 ECTS
Faculty of Biosciences

Effect of temperature-photoperiod interaction on growth and winter bud development in Norway spruce (*Picea Abies)*

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Norges miljø- og biovitenskapelige universitet

Master thesis

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Ås, 2020

Abstract

Woody species growing in temperate and boreal regions, like Norway spruce (*Picea abies)* have to enter dormancy to survive winter and freezing temperatures, while simultaneously maximizing their growing period. Dormancy is a temporary inability to resume growth, even though the plant experiences favourable growth conditions. Dormancy is usually initiated by growth cessation and bud set. Many species, including Norway spruce, use the daylength, also called photoperiod, as a signal to induce seasonal life events. Such plants respond to photoperiods shorter than a certain daylength, called the critical daylength with growth cessation, bud set and further dormancy development in the autumn. In such plants photoperiods longer than a certain daylength, called the critical daylength, sustain growth. In some species, a long photoperiod is also required for bud break and re-growth. The length of the critical daylength varies both between species, but especially between provenances. Provenances are local populations that have adapted to local climatic conditions and daylength. Provenances from higher latitudes usually have a longer critical daylength for growth than those from lower latitudes. Temperature is also an important environmental factor affecting both dormancy and regrowth. With temperature both increasing in the past and predicted to increase further in the future, it is highly relevant to study the effect of temperature on the phenology in plants. Several studies have been conducted on plants responses to temperature and short days (SD) and contradictory results have been found: Studies on several species conducted in growth chambers have found that bud set occurs earlier when the plants are exposed to warmer compared to colder temperatures, while a number of field studies have found opposite results, with colder temperatures resulting in faster bud formation. In growth chamber studies, the plants have commonly been placed directly to SD shorter than the critical daylength for growth under constant temperature or alternating day and night temperature involving rapid changes. Such daylength and temperature regimes may possibly stress the plants since daylength and temperature changes are gradual in nature.

The aim of this MSc thesis has been to study the effect of temperature on seedlings from the Halden (59°N) and Rana (66°N) provenances (both from Norway) of Norway spruce exposed to different bud set-inducing SD conditions. Specifically, it was tested whether the growth cessation and bud set response to temperature differed in plants exposed to gradually decreasing daylengths (24 h to 12 h) and plants exposed to constant SD conditions of 12 h photoperiod. The temperature regimes were either a) constant temperature of 12, 18 or 24°C

under SD of 12 h and LD of 24 h photoperiod for comparison, or b) 12 or 18°C under gradually decreasing daylength, or c) gradually changing, alternating day and night temperatures of 18/12°C or 24/18°C day/night temperature in combination with gradually decreasing daylengths. In addition, the effect of the different temperatures on various other growth parameters was studied. Afterwards, all plants were re-transferred to LD and 18°C to study the after-effect of the temperature and daylength treatments on bud break and re-growth.

The results showed that both the plants given decreasing daylengths (2 h per week down to 12 h photoperiod) and plants exposed to SD of a 12 h constant photoperiod, ceased growth and showed earlier bud set when grown at warmer temperature. The plants given decreasing daylengths had a delayed bud set response, compared to the constant 12 h SD, and plants from the northern provenance (Rana) showed faster bud set than the plants from the more southern provenance (Halden). In addition, more growth was generally observed (for most growth parameters) when the plants were kept at warmer temperatures, and plants from Halden generally grew more than those from Rana. Under constant daylengths, the plants that were exposed to 24°C did not differ from those at 18°C as much as plants at 12°C differed from those at 18°C, both with respect to growth and bud set. Furthermore, in plants exposed to alternating, gradually changing day and night temperature and decreasing daylength, bud set was more rapid under 24/18°C day/night than 18/12°C, with the Rana-plants showing earlier bud set than the Halden-plants. In both daylength treatments (combined with constant temperatures), bud break after subsequent transfer to LD and 18°C was the fastest in the plants that had been exposed to 12°C, indicating less deep dormancy in these plants compared to those from the higher temperature regimes. However, re-growth in plants from 12°C was only faster in the plants that were exposed to the decreasing daylengths.

In conclusion, the response to the different tested temperature regimes was similar with earlier bud set at the highest temperature both under gradually decreasing daylengths and constant SD of 12 h photoperiod. Thus, the specific daylength regimes tested did not affect the overall bud set response to temperature.

Sammendrag

Trearter som vokser i tempererte og boreale strøk slik som gran (*Picea abies)* må gå i vinterhvile for å overleve vinteren og minusgrader, mens de samtidig skal maksimere sin vekstperiode. Vinterhvile er en midlertidig manglende evne til å gjenoppta vekst, selv om planten opplever gunstige vekstvilkår. Vinterhvile er vanligvis innledet av vekstavslutning og knoppsetting. Mange arter, inkludert gran, bruker daglengden, også kalt fotoperiode, som et signal for å indusere sesongavhengige livshendelser. Disse plantene responderer på fotoperioder kortere enn en viss daglengde, kalt kritisk daglengde, med vekstavslutning, knoppsetting og videre hvileutvikling på høsten. Er fotoperioden lengre enn en viss daglengde, opprettholdes veksten. I noen arter er en lang fotoperiode også nødvendig for knoppbrytning og for å gjenoppta vekst. Lengden av den kritiske daglengden varier mellom arter, men særlig mellom provenienser. Provenienser er lokale populasjoner som har tilpasset seg til lokale klimatiske forhold og daglengder. Provenienser fra høyere breddegrader har vanligvis en lengre kritisk daglengde for vekst enn de fra lavere breddegrader. Temperatur er også en viktig miljøfaktor som påvirker vinterhvile og gjenvekst. Siden temperaturer både har økt i fortiden og forutsees å øke mer i fremtiden, er det høyst relevant å studere effekten av temperatur på fenologien i planter. Flere studier har blitt utført på planters respons på temperaturer og korte dager (KD) og her har motstridende resultater blitt funnet: Studier av flere arter utført i vekstkamre har funnet at knoppsetting skjer tidligere hvis planten utsettes for varmere, sammenlignet med kaldere temperaturer, mens noen feltstudier har funnet motsatte resultater, det vil si at kaldere temperaturer resulterer i raskere knoppdannelse. I vekstkammerstudier har planter vanligvis blitt plassert direkte til KD kortere enn den kritiske daglengden for vekst, under konstante temperaturer eller vekslende dag- og natt-temperaturer med raske endringer. Slike daglengde- og temperaturregimer stresser muligens plantene siden daglengden og temperaturen endrer seg gradvis i naturen.

Målet med denne masteroppgaven har vært å studere effekten av temperatur på frøplanter av granprovenienser fra Halden (59°N) og Rana (66°N) (begge fra Norge) eksponert for forskjellige knopp-induserende KD-forhold. Nærmere bestemt ble det testet om vekstavslutnings- og knoppsettingsrespons på temperatur varier mellom planter eksponert for gradvis minkende daglengder (24 t til 12 t) og planter eksponert for konstante KD-forhold med 12 t fotoperiode. Temperaturregimet var enten a) konstante temperaturer ved 12, 18 eller 24°C under KD med 12 t fotoperiode og LD med 24 t fotoperiode til sammenligning, eller b) 12 eller 18°C under gradvis minkende daglengder, eller c) gradvis endrende, vekslende dag-

og natt-temperaturer ved 18/12°C eller 24/18°C dag/natt-temperaturer i kombinasjon med gradvis minkende daglengder. I tillegg ble effekten av forskjellige temperaturer på diverse andre vekstparametere studert. Etterpå ble alle plantene tilbakeført til LD og 18°C for å studere etter-effekten av temperatur- og daglengdebehandling på knoppbrytning og gjenvekst.

Resultatene viste at både plantene som ble gitt minkende daglengder (2 t i uka ned til 12 t fotoperiode) og plantene som ble eksponert for KD med 12 t fotoperiode, avsluttet vekst og dannet knopper tidligere når de fikk varmere temperaturer. Plantene som ble gitt minkende daglengder hadde en forsinket knoppsettingsrespons, sammenlignet med de som fikk konstant 12 t KD. Planter fra den nordlige proveniensen (Rana) viste raskere knoppsetting enn plantene fra den mer sørlige proveniensen (Halden). I tillegg ble det observert mer vekst for de fleste vekstparameterne når plantene fikk varmere temperaturer, og plantene fra Halden vokste mer enn de fra Rana. Under konstante daglengder var forskjellene i vekst og knoppsetting mellom plantene som ble eksponert for 24°C og 18°C mindre enn mellom plantene fra 12°C og 18°C. I plantene som ble eksponert for forskjellige, gradvis skiftende dag- og natt-temperaturer og minkende daglengde, var knoppsettingen raskere under 24/18°C dag/natt-temperatur enn 18/12°C, og Rana-plantene dannet knopper tidligere enn plantene fra Halden. I begge daglengdebehandlinger (kombinert med konstante temperaturer) var knoppbrytning etter tilbakeføring til LD og 18°C raskest i plantene som ble eksponert for 12°C, noe som indikerer en vinterhvile som er mindre dyp sammenlignet med planter fra høyere temperaturregimer. Gjenvekst i plantene fra 12°C derimot, var bare raskere i plantene fra minkende daglengder.

Samlet sett viste plantene lignende respons på de forskjellige temperaturregimene med tidligere knoppsetting ved den høyeste temperaturen både under gradvis minkende daglengde og konstant KD med 12 t fotoperiode. De spesifikke daglengderegimene som ble testet, påvirket derved ikke den generelle knoppsettingsresponsen på temperatur.

Acknowledgements

First, I would like to thank my main supervisor Jorunn E. Olsen for the dedicated guidance, both throughout the experimental phase and through the writing of the master thesis. Then I would like to thank my co-supervisor YeonKyeong Lee with the help and guidance in microscopy of the buds. I would like to thank Marit Siira for helping with registrations, and for watering and taking care of the plants during the experimental treatments and also thanks to SKP for taking care of all the technical set up during the experiments. Thanks to Marcos Viejo for letting me use his data. And thanks to Christian Strømme for helping with the statistical analyses in R. And lastly a special thanks to Daniel Flaten Sunde for technical support in Word.

Abbreviations

SD	Short day*
LD	Long day*
R	Red light
FR	Far red light
PHY	Phytochrome gen
B	Blue light
GA	Gibberellin
ABA	Abscisic acid
m.a.s.l.	Metres above sea level
RH	Relative air humidity
VPD	Water vapour pressure deficit
EC	Electrical conductivity
PBS	Sodium phosphate buffer
ANOVA	Analysis of variance

Clmm	Cumulative link mixed model
AICc	Akaike information criterion corrected

* The length of long and short day described in the introduction varies and depends on the study that is being discussed, while long and short day in this study generally refers to 24 h and 12 h respectively.

Keywords

Bud break, bud set, growth, growth cessation, Norway spruce, photoperiod, provenances, temperature,

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Introduction

Preface

The daylength varies both within a year and across the globe and with increasing distance from the equator, the cycles of daylengths are more extreme. These daylight cycles contribute to the seasonal temperature variations. In temperate and boreal regions, the winters are too cold for plants to be able to grow. Not being able to grow all year round, makes it important to utilize the growing period effectively, while simultaneously developing frost tolerance in time before the winter and freezing temperatures. The temperature fluctuates within seasons and between years and is therefore not a reliable indication of the time of the year. Daylength on the other hand, cycles annually and is a more accurate indicator of the time of the year. Therefore many lifecycle events in plants that depend on a certain season respond to daylength signals, but other environmental factors like temperature and light quality are also important signals (Olsen, 2010; Taiz et al., 2015).

Photoperiodism

The specific daylength experienced by a plant, is referred to as photoperiod, while the ability to detect the photoperiod and respond to it is called photoperiodism (Garner & Allard, 1923; Taiz et al., 2015). Garner and Allard were pioneers in studying how plants use photoperiod to control flowering (Garner & Allard, 1922; Taiz et al., 2015). Since then photoperiodism has been extensively studied, most often with respect to flowering, but plants also use photoperiods to control other life-cycle events that depend on a specific season, like dormancy in the autumn or regrowth in the spring (Clapham et al., 1998; Nitsch, 1957; Olsen, 2010; Taiz et al., 2015). The response to photoperiods largely depends on a critical daylength. With respect to flowering, plants are usually divided into short-day and long-day plants, depending on whether the induction of flowering depends on a photoperiod that is shorter (short-day plant) or longer (long day plant), then a critical daylength (Jackson, 2009; Taiz et al., 2015). In a wide range of woody plants from the temperate and boreal zone, growth is controlled in a similar way; short days (SD) induces growth cessation, and long days (LD) promotes growth. The main focus from here on out will be photoperiodism in growth control. Plants can also be divided into dark-dominant and light-dominant plants, depending on whether the night length (dark-dominant) or the length of the day and light quality (light-dominant) is the most important factor. In woody plant species, often populations from higher latitudes tend to be more light-dominant, while plants from lower latitudes tend to be more

dark-dominant (Clapham et al., 1998; Olsen, 2010). Light quality will be further addressed below.

Plants keep track of time with the circadian clock, which results in an internal daily cycle that is being set both by environmental factors but is also able to keep the rhythm by endogenous factors, at least for a while after transfer to constant conditions. The circadian clock is among others a part of the mechanisms behind photoperiodism (Eriksson & Millar, 2003; Taiz et al., 2015). Phytochromes are important photoreceptors, known to be involved in photoperiodism. In flowering control cryptochromes are also known to be important, in woody species this is yet to be confirmed (Eriksson & Millar, 2003; Olsen, 2010). Phytochromes mostly respond to wavelengths in the red (R)/far red (FR) spectrum. There are several types of phytochrome genes, e.g. the angiosperm model plant *Arabidopsis thaliana* has *PHYA-PHYE*, but not all angiosperm species have all 5, e.g. *Populus* species appear to have 3 types; a *PHYA* gene, and 2 variants of *PHYB*. The gymnosperm woody species Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) have *PHYN, PHYO* and *PHYP*, which are homologs to *PHYA* and *PHYC* and *PHYB, PHYD* and *PHYE* respectively (Clapham et al., 1999; Olsen et al., 1997; Opseth et al., 2016; Taiz et al., 2015). Olsen et al. (1997) showed that overexpression of *PHYA* in the woody hybrid aspen (*Populus tremula x tremuloides*), changed the critical daylength, and the plant continued to grow under SD. *PHYA* therefore seems to be important photoperiodic control in growth.

Besides photoperiod, light quality is also important in growth control. As mentioned above, light quality is relatively more important at higher latitudes, compared to lower latitudes (Mølmann et al., 2006; Olsen, 2010). In a study of Norway spruce FR was more effective in maintaining growth than R but a 1:1 ratio of R:FR was the most effective in this respect. Blue light (B) did not stop growth cessation, but delayed bud formation (Mølmann et al., 2006).

Phenology

Phenology is the study of the life cycle events a plant goes through in a year or a lifetime and the seasonal environmental factors that induce these changes (Körner, 2012; Njoku, 2014; Rathcke & Lacey, 1985). These changes can be dormancy release, bud burst and leafing in the spring, flowering and fruiting, or growth cessation, bud formation and dormancy induction and development as well as leaf senescence and abscission during autumn. The phenology of

a plant is important for the optimal utilization of resources, e.g. maximizing growth, while avoiding the risk of freezing in the winter (Chuine, 2010; Delpierre et al., 2016).

Dormancy

Woody plants grow above ground for many years, even up to thousands of years for some species, and in temperate and boreal regions they therefore depend on surviving the winter. Winters are often both too cold and too dark for growth and freezing damage is a potential risk. Plants in such areas therefore cease growth, form buds, enter dormancy and get frost tolerant (Olsen, 2010; Rohde et al., 2000; Welling & Palva, 2006).

Lang et al. (1987) defined dormancy as "a temporary suspension of visible growth of any plant structure containing a meristem". Furthermore three different types of dormancy were recognized depending on the cause of growth suspension: Ecodormancy, where unfavourable environmental conditions cause lack of growth. Paradormancy and endodormancy are growth suspension caused by endogenous factors; in paradormancy the signal originates in another organ, while in endodormancy growth suspension is caused by signals within the affected organ. Before Lang et al. (1987) defined these three types of dormancy, a range of different terms were used (Lang et al. (1987) recognized 54 different terms used in literature) to describe the three types of dormancy, leading to confusion. Examples of terms used among many others are: Imposed dormancy and quiescence (ecodormancy), summer-dormancy and correlative inhibition (paradormancy) and winter-dormancy and rest (endodormancy). Junttila (1988) commented that the Lang et al. (1987) definition was to wide and included growth suspension caused by unfavourable conditions, which was not considered as dormancy earlier. To make the definition easier to understand Rohde and Bhalerao (2007) proposed a new definition of dormancy that is now widely approved: "The inability to initiate growth from meristems (and other organs and cells with the capacity to resume growth) under favourable conditions", presupposing that there is an ability for re-growth. By this definition, ecodormancy is not considered a type of dormancy. Further on, the terms from Rohde and Bhalerao (2007)will be used.

Apical growth cessation is often induced by the short photoperiods of the autumn and is usually the first event to happen, and important to enable dormancy (Nitsch, 1957; Olsen, 2010; Rohde & Bhalerao, 2007; Wareing, 1956). Short photoperiods mostly induce growth cessation in species and developmental stages that exhibit a free growth pattern, i.e. plants

that form leaf initials simultaneously with internode elongation (Olsen, 2010). Species and developmental stages that exhibit a fixed growth pattern i.e. plants where formation of leaf initials occurs separate from internode elongation, will usually stop growing while there still is a long photoperiod. Although this varies between species, a free growth pattern is typically a juvenile trait, and older plants more often have a fixed growth pattern (Olsen et al., 2004). Also, the formation of buds is often induced by short photoperiods (Olsen, 2010; Wareing, 1956). It usually takes a few weeks from induction, until the first bud can be seen, depending on the species and its latitudinal origin (Olsen, 2010). Buds contain leaf primordia, that are protected by bud scales (Delpierre et al., 2016; Lee et al., 2017). Although a short photoperiod is the most important signal, temperature also affects dormancy (Junttila et al., 2003; Kalcsits et al., 2009; Strømme et al., 2017; Tanino et al., 2010). Effects of temperature is further described below. Together with growth cessation and bud formation, plants start to build up freezing tolerance, which is vital for surviving winter. Cold acclimation is also induced by short photoperiods, this is also the case for plants with a fixed growth pattern. After induction by a short photoperiod, both the exposure to cold temperatures and prolonged short photoperiods increases the frost tolerance substantially (Howe et al., 2003; Olsen, 2010; Welling & Palva, 2006).

In the beginning of growth cessation and bud formation, the plants normally have not entered dormancy yet (Junttila et al., 2003; Rohde & Bhalerao, 2007). As time progresses, the plant will gradually enter a dormant state. It takes about 1-3 weeks to induce growth cessation, this varies both between species and within species, while it takes another 2-3 weeks to induce dormancy.

The phytohormones that are involved in dormancy regulation are still not fully identified. However, down-regulation of gibberellin (GA) has been shown to be involved in growth cessation but does not play a further role in dormancy development (Mølmann et al., 2005). Ethylene is likely to be involved in bud development, so is abscisic acid (ABA) (Olsen, 2010). It seems that ABA is involved in the differentiation of bud scales and leaf primordia as well as cold acclimation (Basler & Körner, 2014; Olsen, 2010).

In the spring, as the length of the photoperiod and temperature increases, plants exit dormancy and break buds and resume growth (Basler & Körner, 2014; Olsen, 2010; Welling & Palva, 2006). In many plants species dormancy release requires chilling, which is a certain time period, below a certain temperature (Basler & Körner, 2014; Junttila et al., 2003; Rohde &

Bhalerao, 2007). Chilling does not promote growth; it only restores the ability to grow (Rohde & Bhalerao, 2007). The temperature required for chilling is not fully understood, but it is believed that temperatures just above freezing, are most effective (Basler & Körner, 2014). Spring phenology with bud burst and start of growth is complex and very species-dependent (Basler & Körner, 2014; Roberts et al., 2015). Temperature, photoperiod and chilling are all factors that affect growth start and bud burst, but to varying degrees in different species (Basler & Körner, 2014; Heide, 2003). Both autumn temperature and spring temperature can affect the spring phenology. Autumn temperature can affect the depth of dormancy, and thereby the requirement for chilling. In some species, exposure to long photoperiods for some time, replaces the requirement for chilling (Heide, 2003). Increased temperature in spring accelerates bud burst in many species (Basler & Körner, 2014; Heide, 2003).

Provenances

As mentioned above, seasonal temperature and light fluctuations depend on latitude. Many species have a distribution range across many latitudes and will therefore face different climatic conditions along a latitudinal gradient. Populations therefore adapt to local conditions, and these populations are called ecotypes or provenances (Heide, 1974; Körner, 2012; Vaartaja, 1959). While temperatures generally decrease at higher latitudes, the photoperiod has a more extreme cycle with longer days in summer and shorter days in winter, compared to lower latitudes. The critical daylength that plants respond to is therefore among the local adaptations (Vaartaja, 1959). Usually the critical daylength increases with increasing latitude, both because the plants need to enter dormancy earlier and because the days usually are longer when the plants start the induction towards dormancy (Heide, 1974; Olsen et al., 1997; Vaartaja, 1959).The altitude the plants grow at can also alter the critical daylength, since temperature decreases with altitude. As mentioned earlier, plants from higher latitudes are also increasingly dependent on the light quality rather than just the critical daylength (Olsen, 2010).

Norway spruce – *Picea abies*

Norway spruce (*Picea abies* (L.) Karst.), is a coniferous tree species, which is one of the main species in temperate and boreal forests of Europe (Jansson et al., 2013). The species has its natural distribution in large parts of Scandinavia and eastern Europe, in mountains of central and south eastern Europe, where it can grow at altitudes higher than 2300 m.a.s.l., and has

also been planted outside its natural range, like in Denmark, northern Germany, Scotland, France, Iceland and parts of North America. Norway spruce is an important tree in forestry in Europe. Its wood is being used for several different purposes, such as paper production and timber construction, and it is commonly used as Christmas tree (Aarnes, 2014; Anderberg, 2004; Jansson et al., 2013). Norway spruce is a climax species and is often one of the dominating species in its ecosystem in Scandinavian forests. (Anderberg, 2004; Aune, 2019; Fremstad, 1997; Jansson et al., 2013). It is an evergreen species, and often forms dense forests. The leaves are needle-like and the needles stay on the tree for 8-10 years, although shoots with new needles are produced every year (Aarnes, 2014; Anderberg, 2004). Norway spruce displays apical dominance, giving it the characteristic cone shape. The juvenile stage is relatively long, maturity is reached at about 20 years (Gyllenstrand et al., 2007). Norway spruce can live for about 400 years and can get up to 40-50 m tall (Aarnes, 2014; Vidakovic, 2020)

Norway spruce has a growth cycle common for trees in temperate and boreal forests, where the trees enter dormancy in autumn and exit dormancy in spring (more details about dormancy described above) (Dormling et al., 1968; Gyllenstrand et al., 2007; Mølmann et al., 2006). Young seedlings of Norway spruce have a free growth pattern, while older specimens have a fixed growth pattern (Gyllenstrand et al., 2007). Norway spruce seems to have a shallow dormancy and a low chilling requirement, at least in young individuals (Dormling et al., 1968; Olsen et al., 2014). If transferred back to long photoperiods after bud formation under short days, buds will burst rapidly, within a few weeks (Olsen et al., 2014). The latitudinal range extends from about 41°N to about 72°N, and photoperiodic provenances have evolved (Jansson et al., 2013). The critical daylength for provenances at around 55°N is about 16 h while provenances at 63°N have a critical daylength of about 18 h (Thomas & Vince-Prue, 1996)

Climate change and how temperature affects the growth cycle

Since 1880 the average global surface temperature has increased with 0.85°C and is predicted to increase even further in the future, and this is most likely due to anthropogenic greenhouse gas emissions (Pachauri et al., 2014). It is predicted that the temperature will continue to increase in the future, though it is uncertain how much, as it largely depends on future greenhouse gas emissions.

As already mentioned briefly above, temperature is also involved in the control of the growth cycle (Olsen, 2010; Olsen & Lee, 2012; Tanino et al., 2010). Therefore, an increase in temperature will potentially affect the phenology of plants. Several studies have been conducted to figure out how temperature affects growth control in various woody species. Some woody species from the *Rosacea* family like apple (*Malus pumila*) and pear (*Pyrus communis*) do not respond to short photoperiods, and it seems that cold temperatures (<12°C) induces dormancy (Heide & Prestrud, 2005). Studies on other species have shown contrasting results. Growth chamber studies conducted on several woody species, including Norway spruce, white spruce (*Picea glauca*), silver birch (*Betula pendula*), downy birch (*Betula pubescens*) and hybrid poplar (*Populus* x spp.) have shown faster bud formation or entered dormancy faster under higher compared to colder temperature treatments (Hamilton et al., 2016; Junttila et al., 2003; Kalcsits et al., 2009; Olsen et al., 2014; Tanino et al., 2010). On the other hand, field research have shown that higher temperature delays bud formation in Eurasian aspen (*Populus tremula*) and hybrid poplar (Rohde et al., 2011; Strømme et al., 2015; Strømme et al., 2017; Strømme et al., 2018). It could be noted that studies of *Populus* species in the field and growth chambers have shown contradictory responses with respect to effect of temperature under short days on the timing of bud set. Zohner and Renner (2019) studied how increased temperature affected 8 woody species, grown in a greenhouse. They increased the temperature in parts of the year, or all year around relative to the ambient temperature, with control plants that received the ambient temperature, and found that increase in temperature during summer and autumn delayed bud set compared to the control, whereas an increase in temperature all year around or during the winter and spring accelerated bud set compared to the control.

The dynamics of temperature in dormancy release and bud break has been studied in greater detail. High temperatures during short day treatment in growth chambers has been shown to deepen dormancy, increase chilling requirement and delay bud burst during long day treatment, in a range of species like Norway spruce, downy birch, silver birch, black alder (*Alnus glutinosa*) and Norway Maple (*Acer platanoides*), indicating that increased temperature during autumn will delay bud flushing during spring (Dormling et al., 1968; Heide, 2003; Olsen et al., 2014; Søgaard et al., 2008; Tanino et al., 2010; Westergaard & Eriksen, 1997). Increased temperatures during spring have shown to cause earlier bud break in different species including sycamore (*Acer pseudoplatnaus*), European beech (*Fagus sylvatica*), Norway spruce, sessile oak (*Quercus petraea*) and Eurasian aspen (Basler &

Körner, 2014; Strømme et al., 2015; Strømme et al., 2018; Strømme et al., 2019). Roberts et al. (2015) analysed the Marsham phenology time series, which was a time course of the first leafing dates in 13 tree species, which was recorded in the period 1753-1947 in Norfolk, England. They found that the winter and spring temperature affected the different species to various degree, and that some species were more affected than others by temperature and showed larger differences between colder and warmer temperature. Also, some species started leafing earlier than others in response to warmer temperatures.

In growth chamber studies, plants are commonly raised under long day treatment and then transferred directly to a short day treatment (Hamilton et al., 2016; Junttila et al., 2003; Kalcsits et al., 2009; Olsen et al., 2014). It is therefore possible that the sudden change in day length may cause a stress response in the plants, and that plants kept at warmer temperatures respond faster to this, than plants kept at colder temperatures. It is also common to grow the plants under a constant temperature or under different day and night temperatures, keeping each of them constant.

Aims of the study

This study aimed to:

- Evaluate the effect of temperature on growth and bud set under short days of 12 h in seedlings of a southern and northern provenance of Norway spruce, (from Halden at 59°N latitude and Rana at 66°N latitude, respectively) and the effect of temperature on growth under long days of 24 h daylength.
- Investigate if growth and bud development in seedlings of these southern and northern Norway spruce provenances respond differently to temperature treatment if they are given a gradual shortening of the daylength, compared to plants transferred to a 12 h day length directly from 24 h daylength.
- Evaluate if alternating day and night temperatures affect the growth and development of these southern and northern Norway spruce provenances differently than constant temperature during gradual decrease in daylength shortening.

Materials and methods

Study species and seed provenances

The study species was Norway spruce (*Picea abies* (L.) H. Karst.). Seeds from two different provenances were used (figure 1). One provenance was from the seed collection area CØ/1, Halden, Østfold, Norway, which is located at 59 °N (seed lot 980863, Skogfrøverket, Hamar, Norway). The other provenance was from P/1, Rana in Nordland, Norway, located at 66°24'N (seed lot 4145, Skogfrøverket, Hamar, Norway). The letters in CØ/1 and P/1 represent location and the numbers indicate 0-149 metres above sea level (m.a.s.l) (figure 1).

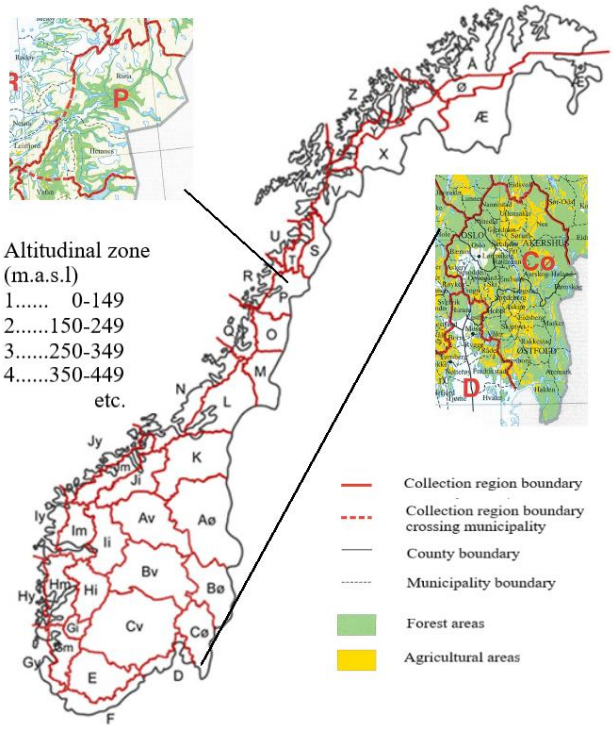

Figure 1 *Map showing the collection areas for forest tree seeds in Norway, including the origin of the 2 provenances Rana and Halden of Norway spruce used (P/1 and CØ/1), used for studying the effect of temperature and daylength treatments in seedlings. The altitudinal zones indicate height above sea level categories, m.a.s.l. = metres above sea level. (Modified from maps by Skogfrøverket and Statens kartverk; http://www.skogfroverket.no/artikkel.cfm?Id_art=10&kanal=3)*

Sowing and pre-growing before the experimental treatments

Seeds were sown either in S-soil (Hasselfors, Örebro, Sweden) (experiment 1a and 1b, Table 1) or a growth peat (Degernes torvstrøfabrikk, Degernes, Norway) and perlite mixture (1.5-6 mm, Agro, Ankara, Turkey), with a ratio of 1:3 perlite to peat (experiment 2 and 3, Table 1). The seeds were sown in pots with a dimension of 5.5 x 4.5 x 4 cm (upper diameter, height, lower diameter). The pots were placed in trays with 12 pots in each. Two seeds were

sown in each pot, to ensure germination of at least one plant per pot, since the germination rate was estimated to be about 60%.

After sowing, the trays were placed in a growth chamber (manufactured by the Norwegian University of Life Sciences), where the seeds had 6 (experiment 2 and 3) and 8 (experiment 1a and 1b) weeks to germinate and grow. In the growth chamber, the temperature was 18 °C, and the relative air humidity (RH) was 76%, which gives a water vapour pressure deficit (VPD) of 0.5 kPa. The plants were given 24 h of light treatment per day called long day (LD). Of these, 12 h were with 180-200 µmol m^{-2} s^{-1} irradiance, using metal-halide lamps (Master HPI-T Plus 400 W/64 E40 1SL, Phillips, Amsterdam, Netherlands) and light from incandescent lamps (mixture of Osram, Munich, Germany and Narva, Brown & Watson International Pty Ltd, Knoxfield, Australia); called full light. During the remaining 12 h light of the diurnal cycle, only incandescent lamps were used, with an irradiance of 8-10 µmol m^{-2} s^{-1}, called day extension light. At full light the ratio between the red (R) and far red (FR) light was adjusted to 1.7 with the incandescent light.

The plants were watered as needed. The plants were fertilized after one week of growth and were thereafter fertilized twice a week. The nutrient solution contained calcium nitrate, ammonium nitrate and Kristalon (Yara, Oslo, Norway), with an electrical conductivity (EC) of 1.5. The plants were watered with nematodes, in the case of insects. Flies were trapped with fly paper.

Treatment for winter bud formation

After 6-8 weeks (as described above) of pre-growing, treatments for bud formation started. In pots with two or more seedlings, excess seedlings were removed using a pair of scissors, keeping the most centred, the straightest and most evenly sized seedlings, leaving one seedling per pot. Thus, as similar plants as possible were used in each of the experiment. The treatments and the number of plants per treatment and provenance are summarized in Table 1. The experiments described in Table 1 where conducted in the order indicated by the table.

Table 1. *Overview over treatments in experiments studying the effect of temperature and daylength treatments on growth and development in seedlings of Norway spruce.*

Experiment	**Light treatment**		**Provenance**		**Temperature treatment**	**Relative humidity[1] VPD[2] = 0.5 kPa**	**Length of bud set treatment**	**Length of bud break treatment**	**Number[3] of plants per treatment/ provenance**
1a	12 h photo-period (SD)	24 h photo-period (LD)	Halden		12°C	64%	50 days	28 days	20 plants
					18°C	76%			
					24°C	83%			
1b			Rana		12°C	64%		25 days	
					18°C	76%			
					24°C	83%			
2	22 h -12 h photoperiod, decreased with 2 h a week		Halden	Rana	12°C	64%	63 days	35 days	36 plants
					18°C	76%			
3					18°C day/ 12°C night	76% day/ 64% night	56 days	No bud break treatment	
					24°C day/ 18°C night	83% day/ 76% night			

1: The relative humidity (RH) was set to the values in the table, but during alternating day and night RH, it was not possible to achieve the set RH, (described in more detail in the text below).
2: VPD = water vapour pressure deficit
3: The number of plants listed is the initial number of plants used, the number decreased in some cases due to various reasons, and in experiment 2, there were also two destructive studies, where additional plants were used as described further below.

There were more plants, than those used for measurements of growth and developmental parameters (as mentioned in Table 1). The extra plants where evenly distributed between the growth chambers and used for destructive measurements at the end of the winter bud treatment. The number of plants used in destructive measurements is mentioned under biomass, and microscopy of buds.

During the experimental treatments, the VPD conditions were the same as during the pre-growing conditions. RH was adjusted to keep VPD at 0.5 kPa (Table 1). The irradiance conditions were also the same as during the pre-growing conditions, but the photoperiod varied (table 2), the length of the full light period and the day extension light period for the different experiments were as described in the following:

In experiment 1a and 1b (Table 1), half of the plants were transferred to a 12 h photoperiod, called short day (SD), for induction of bud set. These plants got full light as described above, followed by 12 h of darkness. For comparison, the other half of the plants were kept at a 24 h photoperiod, LD and got the same 12 h full light followed by 12 h low-intensity day extension light, i.e. the same light treatment as during the pre-growing treatment. The plants were distributed among three temperature treatments; 12°C, 18°C or 24°C and the temperatures were kept constant. The RH was adjusted to keep a VPD of 0.5 kPa (Table 1). In total experiment 1a and 1b had 6 different combinations of treatments each with 2 different light treatments and 3 different temperature treatments.

Experiment 2 and 3 (Table 1) started with 22 h photoperiod, and 2 h of darkness. In experiment 2 the daylength was shortened by 2 h per week for 5 weeks, until SD was reached, i.e. a 12 h photoperiod and 12 h of darkness, followed by keeping the plants at 12 h photoperiod for 4 weeks, i.e. the rest of the bud set treatment. The shortening of the photoperiod was conducted during the day extension light period, i.e. the plants got 12 h full light + day extension light and the dark period was in the middle of the day extension light period. In experiment 2 there were two temperature treatments; 12°C or 18°C and the temperature was kept constant like in experiment 1a and 1b, and the VPD was also here kept at 0.5 kPa. The light treatment was supposed be the same in experiment 3 as in experiment 2, but due to technical failure in daylength shortening the plants got 22 h light for 3 weeks instead of 1 week. This increased the period of shortening the daylength from 5 to 7 weeks, followed by 1 week with SD, i.e. 12 h light. Also, in the chamber with 18°C/ 12°C (temperature treatments described below) the plants had a total of 4 days without incandescent light, because a fuse went out twice during the period of 22 h light. Due to a lack

of access to growth chambers, the experiment could unfortunately not be repeated. Because of this, results from experiment 3 were emphasized less than the other results, and the experiment was cut short. Experiment 3 also had two temperature treatments, here the treatments were alternated between two temperatures in each treatment; 24°C/18°C and 18°C/12°C. The temperature was increased and decreased gradually in the course of 3 h. Including the time it took to change the temperatures, the 2 temperatures where kept for 12 h each, with the highest temperature during full light, and the coldest during the day extension light and the dark period. The chambers were not able to keep the set VPD of 0.5 kPa during the colder periods since the RH increases when it gets colder. The VPD therefore decreased during the colder period. Experiment 2 and 3 had 2 different temperature treatments and both temperature treatments had 2 different provenances, giving 4 different combinations of treatments.

Treatment for bud break

After the treatment for bud set under the SD, the plants were re-transferred to LD, i.e. the condition they got during the pre-growing period, 24h light,18°C, and 76% RH. The length of the treatments varied between the experiments (Table 1).

Growth parameters recorded during treatments

During the experimental treatments, several growth parameters were recorded to evaluate the development of the plants. The recordings carried out varied between the experiments, and the frequency of measurements and when the recordings were carried out varied (Table 2). The number of plants used in each case is shown in Table 1 and the same plants were used for all the recordings, except recordings of biomass and number of leaf primordia (table 2) where excess plants where used, the number of plants used in these 2 recordings is described below.

Height and cumulative growth

Height was measured from the rim of the pot to the shoot apical meristem. Afterwards cumulative growth was calculated by subtracting the height from the first measurement (cumulative growth during bud set) in experiment 2 and 3 and subtracting the height from the measurement conducted on the day of re-transfer to LD (cumulative growth during bud break). Due to a mistake in the first measurements (different persons measured in different ways) in experiment 1b and since the measurements from experiment 1a and 1b were

supposed to be compared with each other, it was decided to present height instead of growth in experiment 1a and 1b.

Table 2 *Overview of growth parameters recorded during the different experiments, studying the effect of temperature and daylength treatments on growth and development in Norway spruce. Day 0 was the day the treatments started.*

Growth parameters	**Experiments**	**Frequency**
Height	1b	Once a week, 1st,6th and 7th week missing
	1a	Once a week
	2	
	3	
Bud formation	1a	From day 12, twice a week
	1b	From day 1, twice a week
	2	From day 0, twice a week until first bud, then 3 times a week.
	3	
Bud break	1a	From day 50 (day 0 of LD), twice a week
	1b	
	2	From day 65, (day 1 of LD), three times a week, once the last week.
Shoot diameter	2	Once a week, not the last week.
	3	Once a week.
Number of needles	2	3 times: day 1, day 25 and day 63, all during SD treatment.
	3	3 times: day 1, day 28 and day 56, all during SD treatment.
Number of lateral buds	2	Once, last day of SD (Day 63 and day 56, for experiment 2 and 3 respectively)
	3	
Stem diameter	2	
	3	
Biomass	2	
Number of leaf primordia	2	

Bud set

During the SD conditions in experiment 1a and 1b and during the day shortening treatment in experiment 2 and 3, bud set development was recorded using a magnifier, and divided in to 3 categories: 0 = no visible bud and shoot elongation (Figure 2 A), 1 = white bud or light green bud (Figure 2 B and C), 2 = bud has turned brown (Figure 2 D and E).

Bud break

After the re-transfer to LD and 18°C (conditions as during pre-growing) bud break was registered, the registration was also done using a magnifier. The following 3 categories where used: 2 = intact bud (Figure 3 A), 1 = hole in bud (Figure 3 B and C), all needles were still gathered and bent inwards, 0 = needles were spreading away from each other (Figure 3 D).

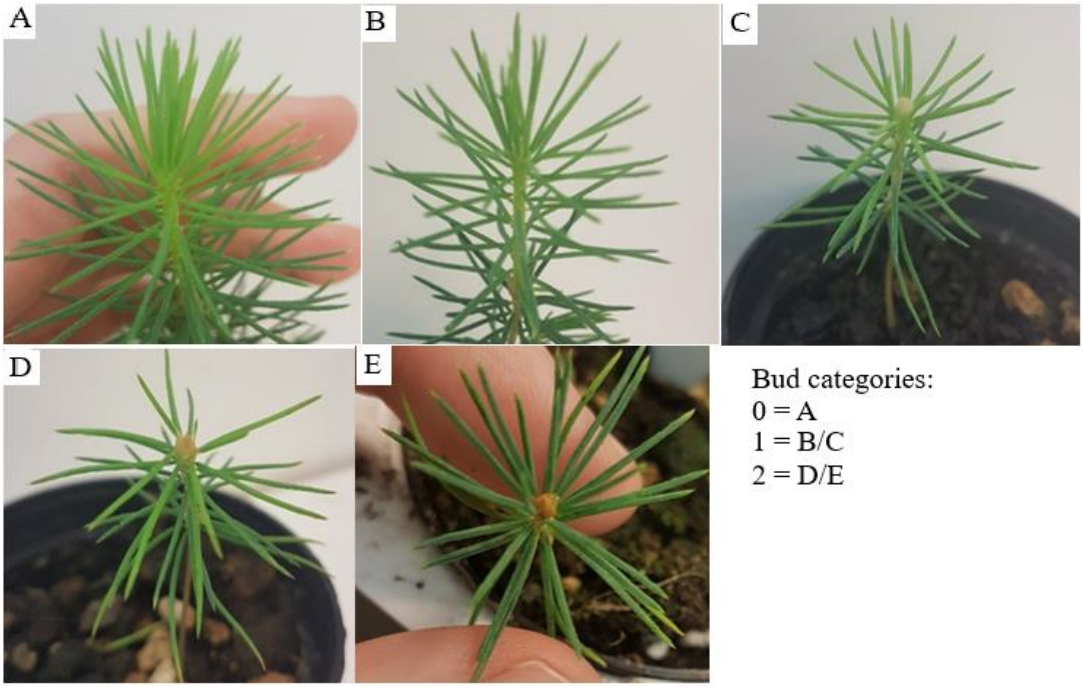

Figure 2 *Pictures of bud development during temperature and daylength treatments, as examples of bud categories. Category 0: In picture A there is no visible bud. Category 1: In picture B there is a bud starting to be visible, and in picture C there is a white bud clearly visible. Category 2: In picture D the bud is starting to get brown and in picture E the bud has fully turned brown.*

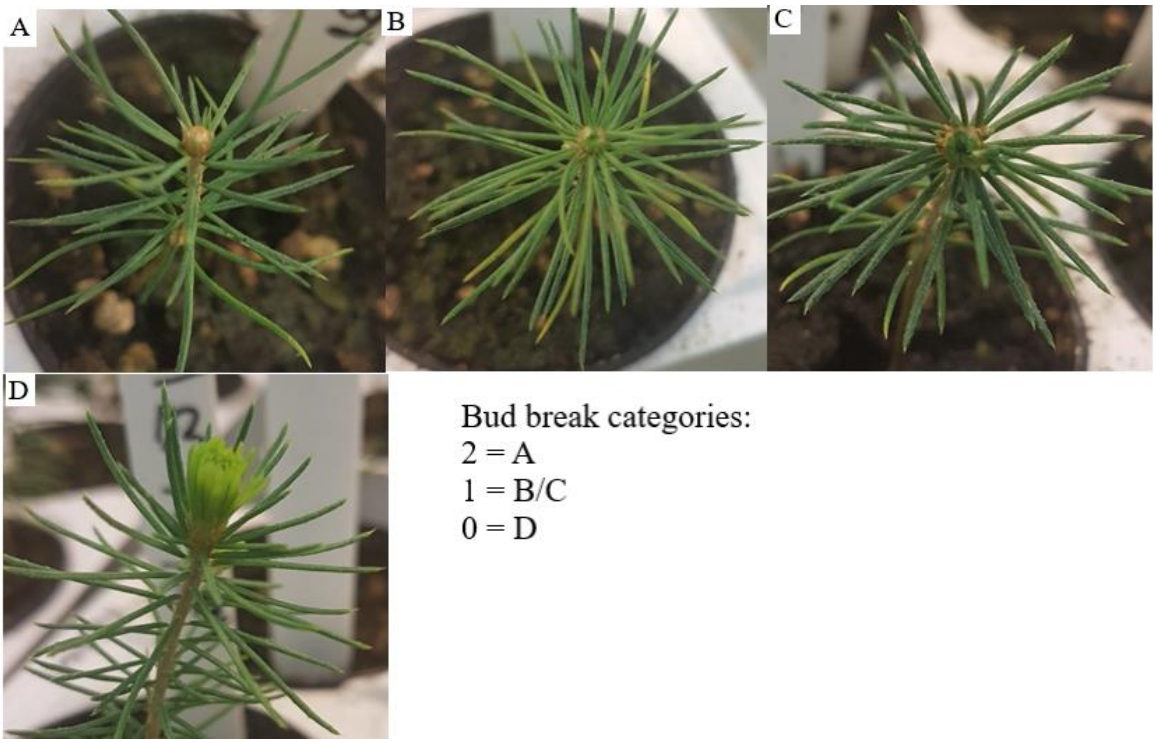

Figure 3 *Pictures of bud break development after temperature and daylength treatments, after re-transfer to long day (LD) treatment. Category 2: In picture A the bud is fully closed. Category 1: In picture B there is a hole starting to form and in picture C the needles are starting to protrude out of the bud but are still gathered. Category 0: In picture D the needles have protruded fully from the bud and are no longer gathered.*

Shoot diameter

Shoot diameter was measured from needle tip to needle tip across the top of the shoot with two, perpendicular measurements per plant. Then the average for the two measurements was calculated.

Needles

Needles longer than 5 mm were counted, but the needles that were closely gathered at the shoot tip were not counted, because this was not practically possible. Figure 2 A shows an example of needles gathered too much for counting.

Number of lateral buds

Lateral buds were counted with a distinction between closed and open buds. The total number of lateral buds was calculated afterwards.

Stem diameter

The thickness of the stem was measured right below the needles, before the stem thickened. A digital caliper (Digimatic, Pluss Mitutoyo Corporation, Kawasaki, Japan) was generally used, but during measurements in experiment 2, the digital caliper malfunctioned and a manual caliper was used for the rest of the measurements. During experiment 3 only the digital caliper was used.

Biomass

A total of 24 plants per provenance and temperature treatment were used for biomass recording. The plants were rinsed to remove soil and dried of with a paper towel. They were then separated into roots and shoots and were then dried in an oven at 70 °C for about a week, before the shoot was separated into needles and stem and weighed. The ratio between root weight and shoot weight was then calculated. Because this was a destructive study and it was conducted at the end of bud set treatment, excess plants were used, instead of the plant used in recordings described above, since they also were used for recordings after re-transfer to LD.

Microscopy of buds with leaf primordia

From each provenance and temperature treatment, 5 buds were collected. The buds were fixed in a 4% paraformaldehyde solution with 0.025% glutaraldehyde in a sodium phosphate buffer (PBS, 1M, pH 7.0). The PBS solution with the samples were placed in vacuum for 1 h to remove air within the buds. The solution was then replaced with new PBS buffer before the samples were stored at 4°C. Then the samples were washed with PBS buffer, and gradually dehydrated by placing the buds in solutions of ethanol with increasing concentrations (30%,

50%, 70%, 90% and 100%) and water. The samples were stored at 4°C in between each concentration of ethanol for at least 1 h. Then the samples were infiltrated with LR white resin (London Resin, Basingstoke, UK), by gradually increasing the ratio of LR white to ethanol, first 1:1 overnight, then 2:1 for 4 h, and then pure LR white for 3 days, where the LR white was replaced every 12 h. The samples were stored at 4°C under infiltration. Afterwards the buds were embedded in LR white by polymerization at 60°C. Then the samples were cut longitudinally into sections with a thickness of 1 µm on an ultramicrotome (Leica, Wetzlar, Germany), with a diamond knife (Diatome, Hatfield, PA, USA). The sections were placed on positively charged microscope slides (Superfrost, Thermo Fischer Waltham, MA, USA), that were laying on a hot plate at 55°C to adhere the samples to the slide glass. The samples were then stained with Stevenel's blue (2% Potassium permanganate ($KMnO_4$), 1.3% methylene blue), then the samples were studied under a light microscope (Leica), to take micrographs and count the number of leaf primordia inside the bud. This was also a destructive study, so excess plants were used here as well.

Statistical analyses

A two-way analysis of variance (ANOVA glm) was used to analyse the data for all growth parameters in experiment 1a, 1b and 2, whereas a one-way ANOVA glm was used for experiment 3 since the daylength treatments in the two temperature regimes (in separate growth chambers) were not identical due to technical failure. The ANOVA analyses were followed by Tukey's post hoc test. For lateral buds, the total number of lateral buds was analysed. For biomass, the root weight, stem weight, needle weight, shoot (stem and needle) weight, and the root/shoot ratio was analysed. For height (experiment 1a and 1b), cumulative growth during bud set (experiment 2 and 3), shoot diameter and number of needles the last registration of the bud set treatment was analysed. For cumulative growth during the bud break treatment, the last measurement of the bud break treatment was analysed. For bud data, both bud stages during bud set and bud break on individual days were analysed. The significance level was set to $p \leq 0.05$. Minitab 19 (Minitab Inc., State College, PA, USA) was used to conduct the analyses. The data were log transformed if the requirements for normal distribution or equal variance were not met.

The overall data (entire time course) for bud development in experiment 2 were also analysed with a cumulative link mixed model (clmm), with the same significance level as the rest of the data. R, version 3.5.1 (R Development Core Team 2019) was used to analyse the data.

Two packages were used: the ordinal package (Christensen 2019), for the clmm and the MuMIn package (Bartoń 2019) to compare models using Akaike information criterion corrected (AICc).

The bud data for both provenances in experiment 1a and 1b were analysed together, and days for analysis were chosen based on days where registration were conducted on the same day, while the height and cumulative growth data in experiment 1a and 1b were analysed separately, because the last measurements were not conducted on the same day.

Since the results for experiment 3 where compromised due to technical failure, the two temperature treatments could not be compared directly since they did not experience identical daylength conditions. Therefore, the temperature treatments where analysed separately, in order to compare the responses of the provenances.

Results

Effects of constant daylength and temperature (experiment 1a and 1b)

Growth during SD vs. LD

In both the Halden (from 59°N) and Rana (from 66°N) provenances, plants kept at different daylengths (SD of 12 h and LD of 24 h) and at different temperatures (12,18 or 24°C) had significantly different height at the end of the experiment, and there was a significant interaction between daylength and temperature (all with a p-value < 0.001) (table 3, figure 4). Both the Halden and Rana-plants that were exposed to SD ceased their growth during the treatment, and subsequently had grown significantly less than the plants that got LD treatment at the same temperature that continued to grow throughout the experiment. In the plants from Halden, growth cessation occurred after about 2 (24°C and 18°C) to 3 (12°C) weeks and growth slowed down before the growth cessation (figure 4 A). In the plants from Rana, the first measurement was missing due to a mistake. Therefore, it is not possible to say whether or how much the plants grew the first week. However, growth cessation occurred after about 2 weeks, in plants kept at 12°C and 18°C, while there was no growth in the plants grown at 24°C under the SD treatment (figure 4 B).

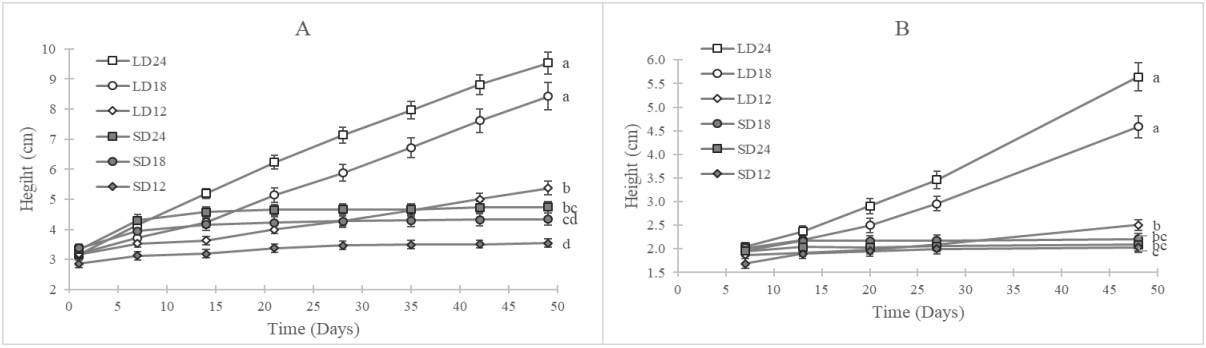

Figure 4 *Effect of constant temperature (12,18 or 24°C) under short days (SD) of 12 h photoperiod (bud set treatment) or long days (LD) of 24 h in on plant height seedlings of Norway spruce provenances from Halden (A, from 59°N) and Rana (B, from 66°N). Different lowercase letters on the right side of the graphs indicate significant differences between treatments for the last measurement for each provenance separately, based on two-way ANOVA, followed by Tuckey`s test. N=20 per treatment. Average height with ± SE displayed.*

For both provenances there was no significant difference between plants grown at 24°C and 18°C in the LD treatment, while plants kept at 12°C were significantly smaller and the height was about the same as plants from SD. In the SD treatment, the plants from the different provenances showed slightly different growth. The SD-exposed Halden-plants kept at 24°C were significantly taller than the plants grown at 12°C, while the Halden plants given 18°C were not significantly different from either of the other temperature treatments (figure 4 A). In

the SD-exposed Rana-plants, neither of the temperature treatments resulted in significantly different plant heights, but only the plants kept at 12°C and SD were significantly shorter than the plants exposed to 12°C and LD (figure 4 B).

The plants from Halden (figure 4 A) seemed to grow more than the plants from Rana (figure 4 B), in both daylength treatments and in all temperature treatments.

Table 3 *ANOVA results for the final height of seedlings of the Halden (from 59°N, at day 49) and Rana (from 66°N, at day 48) provenances of Norway spruce exposed to constant daylength and temperature treatments (12, 18 or 24°C). The plants were either given 24 h daylength, long day or 12 h daylength, short day. N=20 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Halden					
Daylength	1	2.0038	2.00376	323.12	0.000 ***
Temperature	2	0.7212	0.36061	58.15	0.000 ***
Daylength*Temperature	2	0.1360	0.06799	10.96	0.000 ***
Error	111	0.6884	0.00620		
Total	116	3.5101			
Rana					
Daylength	1	2.3913	2.39131	222.55	0.000 ***
Temperature	2	0.7373	0.36863	34.31	0.000 ***
Daylength*Temperature	2	0.5875	0.29373	27.34	0.000 ***
Error	114	1.2249	0.01074		
Total	119	4.9409			

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Bud set development

When analysing bud development under SD in Halden and Rana together, there was a significant effect of both temperature (12, 18 or 24°C) and provenance at all days analysed (day 15, 19, 22, 26, 40 and 47), and there was a significant interaction between temperature and provenance at all days except day 19 and day 26 (table 4). In both provenances, the plants kept at 24°C and 12°C had the fastest and slowest bud development, respectively. Plants from Halden (figure 5 A) had slower bud development than plants from Rana (figure 5 B).

Table 4 *ANOVA table for bud set in seedlings of the Halden (from 59°N) and Rana (from 66°N) provenances of Norway spruce treated with constant temperatures (12, 18 or 24°C) under short days of a 12 h photoperiod. Individual days were analysed. N=20 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Day 15						
Temperature	2	8.017	4.0083	29.39	0.000	***
Provenance	1	1.875	1.8750	13.75	0.000	***
Temperature*Provenance	2	1.550	0.7750	5.68	0.004	**
Error	114	15.550	0.1364			
Total	119	26.992				
Day 19						
Temperature	2	28.0167	14.0083	431.61	0.000	***
Provenance	1	0.1333	0.1333	4.11	0.045	*
Temperature*Provenance	2	0.1167	0.0583	1.80	0.170	
Error	114	3.7000	0.0325			
Total	119	31.9667				
Day 22						
Temperature	2	43.398	21.6991	480.78	0.000	***
Provenance	1	3.304	3.3043	73.21	0.000	***
Temperature*Provenance	2	4.088	2.0440	45.29	0.000	***
Error	113	5.100	0.0451			
Total	118	55.966				
Day 26						
Temperature	2	35.3915	17.6957	111.97	0.000	***
Provenance	1	5.4943	5.4943	34.77	0.000	***
Temperature*Provenance	2	0.7604	0.3802	2.41	0.095	
Error	113	17.8579	0.1580			
Total	118	59.9328				
Day 40						
Provenance	1	3.5700	3.57000	417.13	0.000	***
Temperature	2	5.9979	2.99896	350.40	0.000	***
Temperature*Provenance	2	5.9979	2.99896	350.40	0.000	***
Error	111	0.9500	0.00856			
Total	116	17.2308				
Day 47						
Temperature	2	1.5741	0.78704	17.47	0.000	***
Provenance	1	0.8095	0.80952	17.97	0.000	***
Temperature*Provenance	2	1.5741	0.78704	17.47	0.000	***
Error	111	5.0000	0.04505			
Total	116	9.1453				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

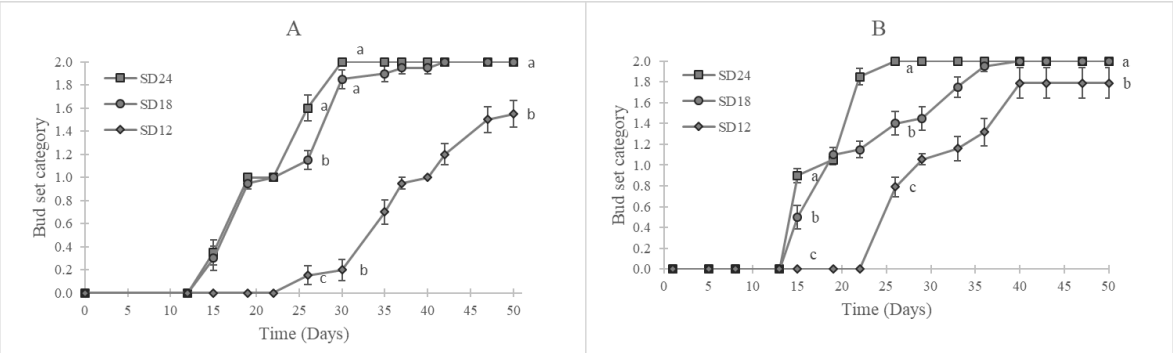

Figure 5 *Effect of constant temperature (12,18 or 24°C) under short day (SD) treatment of 12 h photoperiod on bud set in seedlings of Norway spruce provenances from Halden (A, from 59°N) and Rana (B, from 66°N). Different lowercase letters represent significant difference between treatments for the points on the left side of the letters for each provenance separately, based on one-way ANOVA followed by Tuckey`s test. When lines overlap and there are no significant differences, one letter is shown. 3 bud set categories were used: 0 = no visible bud and shoot elongation, 1 = white bud or light green bud, 2 = bud has turned brown. N=20 per treatment. Average bud set category with ± SE displayed.*

Bud break development

In both provenances (Halden and Rana) there was a significant difference in time to bud break after the re-transfer to LD and 18°C between the plants from the different temperatures (12, 18 or 24°C) during the SD treatment of 12 h photoperiod at all days analysed. There was also a significant difference between the plants from different provenances on all days except day 14 and a significant interaction between temperature and provenance on all days analysed (table 5).

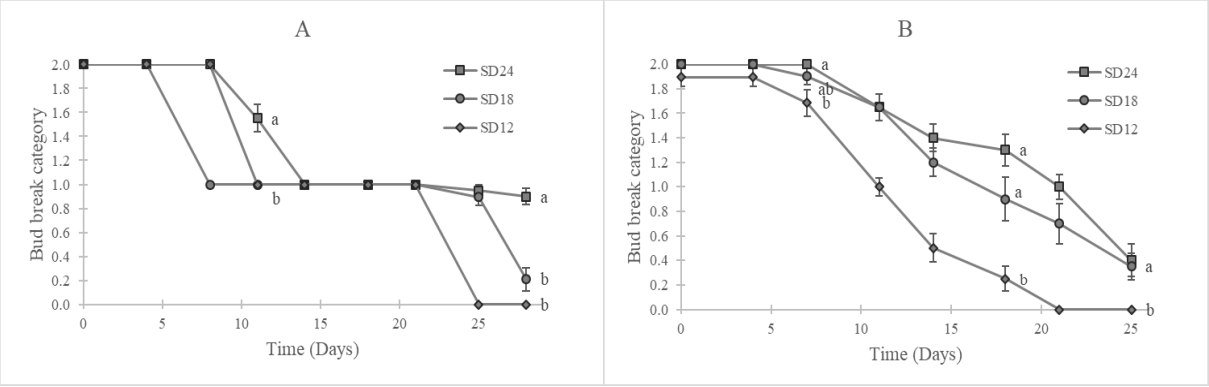

Figure 6 *After-effect of constant temperature (12, 18 or 24°C) under short day (SD) treatment of 12 h photoperiod on bud break in seedlings of Norway spruce provenances from Halden (A, from 59°N) and Rana (B, from 66°N) after re-transfer to long days of 24 h photoperiod and 18°C (bud break treatment). Different lowercase letters represent significant difference between treatments for the points on the left side of the letters for each of the provenances separately, based on the one-way ANOVA followed by Tuckey`s test. When lines overlap and there are no significant differences, one letter is shown. 3 bud break categories where used: 2 = intact bud, 1 = hole in bud, all needles were still gathered and bent inwards, 0 = needles were spreading away from each other. N=19-20 per treatment. Average bud break ± SE displayed.*

The plants from at 12°C had the fastest bud break in both provenances. The plants from 24°C were slowest in the Halden-plants, while there was no significant difference between the plants from 18°C and 24°C in the Rana-plants. The plants from Halden (figure 6 A) started to break buds earlier, while the plants from Rana had a faster development (figure 6 B), except the Rana-plants from 12°C, which were the fastest from the start.

Table 5 *ANOVA table for bud break in seedlings of the Halden (from 59°N) and Rana (from 66°N) provenances of Norway spruce after re-transfer to long days and 18°C following exposure to different constant temperatures (12, 18 and 24°C) and short days of a 12 h photoperiod. Individual days were analysed. N=19-20 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Day 11						
Temperature	2	7.216	3.6082	25.40	0.000	***
Provenance	1	1.859	1.8587	13.09	0.000	***
Temperature*Provenance	2	2.409	1.2043	8.48	0.000	***
Error	113	16.050	0.1420			
Total	118	27.496				
Day 14						
Temperature	2	4.4595	2.22974	16.80	0.000	***
Provenance	1	0.0330	0.03304	0.25	0.619	
Temperature*Provenance	2	4.4595	2.22974	16.80	0.000	***
Error	113	15.0000	0.13274			
Total	118	23.9664				
Day 18						
Temperature	2	5.6149	2.8074	14.59	0.000	***
Provenance	1	0.9996	0.9996	5.19	0.025	*
Temperature*Provenance	2	5.6149	2.8074	14.59	0.000	***
Error	113	21.7500	0.1925			
Total	118	33.9832				
Day 21						
Temperature	2	5.262	2.6310	20.94	0.000	***
Provenance	1	5.584	5.5843	44.44	0.000	***
Temperature*Provenance	2	5.262	2.6310	20.94	0.000	***
Error	113	14.200	0.1257			
Total	118	30.319				
Day 25						
Temperature	2	11.239	5.6196	45.07	0.000	***
Provenances	1	3.960	3.9601	31.76	0.000	***
Temperature*Provenance	2	1.989	0.9946	7.98	0.001	**
Error	113	14.089	0.1247			
Total	118	31.143				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Growth after re-transfer to LD and 18°C

After re-transfer to LD and 18°C from SD and LD at different temperatures (12, 18 and 24°C), plants exposed to the different daylengths and temperatures showed significantly different growth in both provenances (Halden and Rana), and there was a significant interaction between temperature and daylength treatment (p-value < 0.001) (table 6). In both provenances, LD-exposed plants had grown significantly more than plants that got SD treatment. Halden-plants exposed continuously to LD at 18°C had grown significantly more than those transferred from LD and 12°C or 24°C, which were not significantly different from each other (figure 7 A). Rana-plants from LD treatment at 24°C and at 18°C did not differ significantly after transfer to 18°C but had grown significantly more than the plants from 12°C (figure 7 B). After the SD treatment, there was no significant difference between the temperature treatments for any of the provenances. Plants from Halden (figure 7 A) seemed to have grown slightly during the last week, while this was not the case for plants from Rana (figure 7 B).

Table 6 *ANOVA results for cumulative growth at the final measurement day after re-transfer of seedlings of the Halden (from 59°N, at day 27) and Rana (from 66°N , at day 26) provenances of Norway spruce to long days (LD) of 24 h and 18°C from constant short days (SD) of 12 h photoperiod or 24 h LD under different constant temperatures (12,18 or 24°C). The data were log-transformed before analysis. N=19-20.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Halden						
Daylength	1	9.0740	9.07403	525.98	0.000	***
Temperature	2	0.6973	0.34865	20.21	0.000	***
Daylength*Temperature	2	0.7912	0.39560	22.93	0.000	***
Error	109	1.8804	0.01725			
Total	114	12.0887				
Rana						
Daylength	1	117.82	117.818	271.16	0.000	***
Temperature	2	19.93	9.963	22.93	0.000	***
Daylength*Temperature	2	19.65	9.823	22.61	0.000	***
Error	112	48.66	0.434			
Total	117	207.63				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

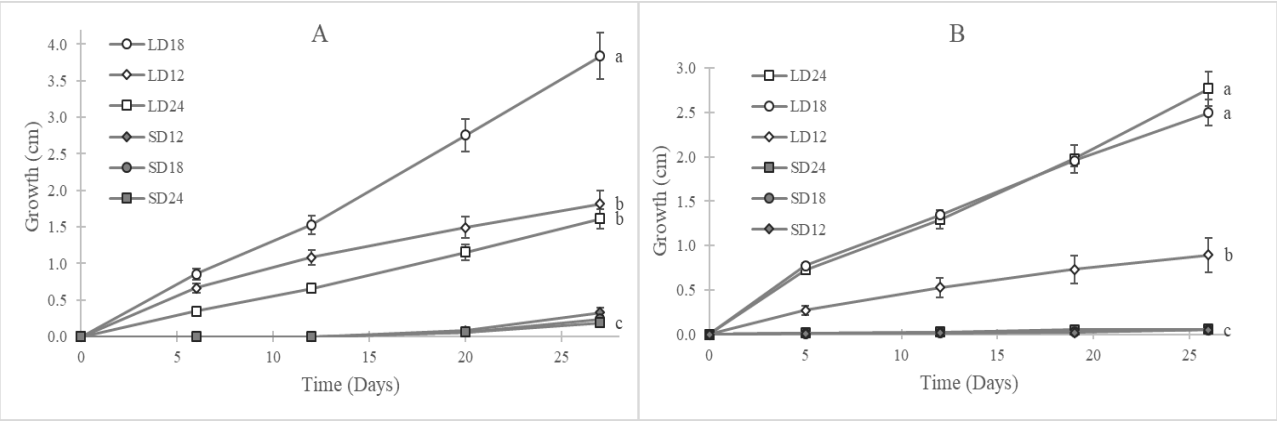

Figure 7 *After-effect of constant temperature (12,18 or 24°C) on cumulative growth after re-transfer of seedlings of Norway spruce provenances from Halden (A, from 59°N) and Rana (B, from 66°N) to long days (LD) of 24 h and 18°C from constant short days (SD) of 12 h photoperiod or 24 h LD under different constant temperatures (12, 18 or 24°C). Different lowercase letters on the right side of the graphs indicate significant differences between treatments for the last measurement for each provenance separately, based on two-way ANOVA, followed by Tuckey`s test. When lines overlap and there are no significant differences, one letter is shown. N=19-20 per treatment. Average growth ± SE displayed.*

Effects of decreasing daylength and constant temperature treatment (experiment 2)

Growth during decreasing daylength

When exposed to a gradually decreasing daylength down to a 12 h photoperiod, both plants from the Halden and the Rana provenance showed significantly different growth when exposed to different constant temperatures (12 and 18°C) (both with a p-value < 0.001), but there was no significant interaction between provenance and temperature treatment (p-value = 0.781) (table 7). The plants from the Halden provenance grew significantly taller than the plants from Rana, when comparing the temperature treatments. The Halden-plants kept at 18°C grew significantly taller than those given 12°C, while the Rana-plants at the different temperatures did not differ significantly (figure 8). However, when the data from Rana were analysed separately by a one-way ANOVA, the plants kept at 18°C were found to be significantly taller than those given 12°C.

All plants ceased their growth before the treatment was over, but at different rates. The growth rate did not seem to differ much after 1 week, but after 2 weeks the plants kept at 12°C had grown less than the plants exposed to 18°C. After 3 weeks the Rana-plants slowed down their growth compared to the Halden-plants. It seems like the plants ceased growth after about 5 weeks, except the plants from Halden, kept at 12°C, which seemed to cease growth after about 7 weeks (figure 8).

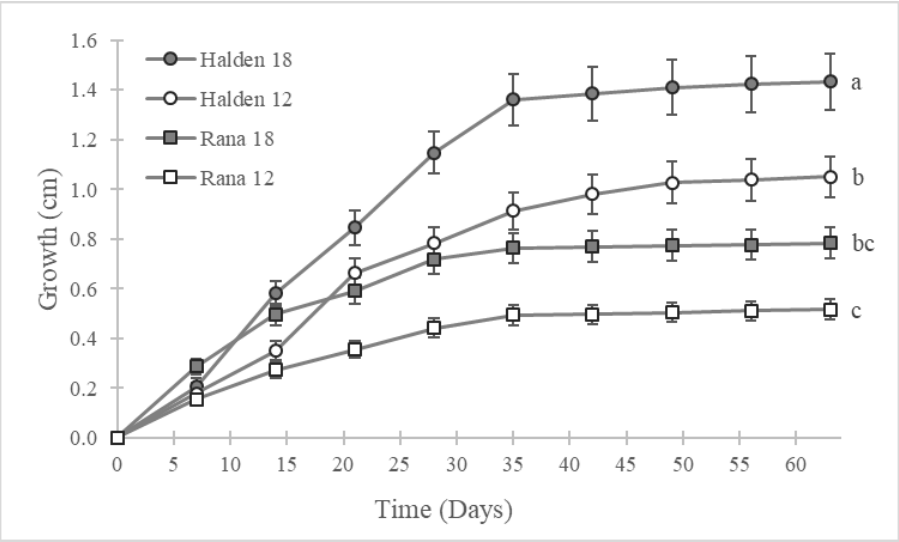

Figure 8 *Effect of constant temperature (12 or 18°C) under decreasing daylength on cumulative growth in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Daylength started with 22 h photoperiod and was decreased with 2 h a week until 12 h photoperiod was reached. Different letters on the right side of the graphs indicate significant differences between treatments for the last measurement based on two-way ANOVA followed by Tuckey`s test for both provenances together. N=36 per treatment. Average growth with ± SE displayed.*

Table 7 *ANOVA table of cumulative growth (on day 63) in seedlings of the Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N) treated with decreasing daylength and constant temperature (12 or 18°C). The seedlings were given 22 h photoperiod, decreasing with 2 h a week, until 12 h photoperiod was reached. The data were log-transformed before analysis. N=36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Provenance	1	2.5890	2.58899	49.21	0.000	***
Temperature	1	0.9441	0.94408	17.94	0.000	***
Provenance*Temperature	1	0.0041	0.00409	0.08	0.781	
Error	139	7.3129	0.05261			
Total	142	10.8261				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Bud set development

Under the gradually decreasing daylength there was a significant effect of both temperature (12 or 18°C) and provenance (Halden and Rana) on bud set, with a significant interaction between temperature and provenance, when analysing the overall data set (entire time course) with a clmm analysis. With an increasing number of days and decreasing daylength, the bud set increased (table 8). When analysing individual days with ANOVA, there was a significant difference between the plants kept at different temperatures, and between the plants from different provenances at all days analysed (day 46, 53 and 63), while there was a significant interaction between temperature and provenance only on the last day (table 9). The plants at

18°C had a faster bud formation development than the plants exposed to 12°C, and the plants from Rana had a faster bud development than the plants from Halden when kept at the same temperature (figure 9).

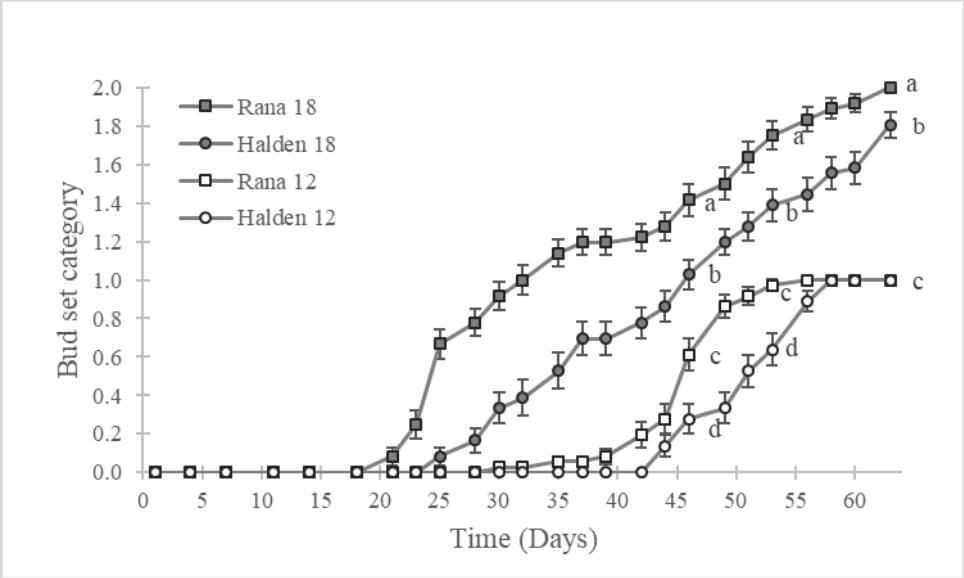

Figure 9 *Effect of constant temperature (12 or 18°C), under decreasing daylength on bud set in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Plants were given 22 h photoperiod, with 2 h decreasing a week, until 12 h photoperiod was reached. Different letters represent significant difference between treatments for the points on the left side of the letters based on two-way ANOVA followed by Tuckey`s test for both provenances together. When lines overlap and there are no significant differences one letter is shown. 3 bud set categories were used: 0 = no visible bud and shoot elongation, 1 = white bud or light green bud, 2 = bud has turned brown. N=35-36 per treatment. Average bud set category with ± SE displayed.*

Table 8 *Summary of cumulative link mixed model for bud set in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and constant temperature (12 or 18°C). The plants were given 22 h photoperiod decreased by 2 h a week until 12 h photoperiod was reached. N=35-36 per treatment.*

	Estimate Std.	Error	Z-Value	P-Value	
ProvenanceRana	1.27520	0.18190	7.011	0.000	***
fTemperature18	4.50372	0.21397	21.049	0.000	***
Daylength	-0.16862	0.05917	-2.850	0.004	**
Day	0.25772	0.01062	24.270	0.000	***
ProvenanceRana*fTemperature18	1.39521	0.22934	6.084	0.000	***

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Table 9 *ANOVA table for bud set in seedling of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N) treated with decreasing daylength and constant temperature (12 or 18°C). The seedlings were given 22 h photoperiod with 2 h decreasing a week, until 12 h photoperiod was reached. Individual days were analysed. N=35-36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Day 46						
Provenance	1	4.5188	4.5188	20.04	0.000	***
Temperature	1	21.9333	21.9333	97.27	0.000	***
Provenance*Temperature	1	0.0397	0.0397	0.18	0.675	
Error	139	31.3444	0.2255			
Total	142	57.9720				
Day 53						
Provenance	1	4.2997	4.2997	24.31	0.000	***
Temperature	1	20.8796	20.8796	118.06	0.000	***
Provenance*Temperature	1	0.0073	0.0073	0.04	0.839	
Error	139	24.5825	0.1769			
Total	142	49.9021				
Day 63						
Provenance	1	0.3379	0.3379	8.33	0.005	**
Temperature	1	29.1322	29.1322	718.12	0.000	***
Provenance*Temperature	1	0.3379	0.3379	8.33	0.005	**
Error	139	5.6389	0.0406			
Total	142	35.4545				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Shoot diameter

Under gradually decreasing daylength and constant temperature (12 or 18°C), shoot diameter from needle tip to needle tip across the shoot, did not change much during the treatments, but only increased by a few mm during the experimental period (figure 10). At the end of the SD-exposure, there was a significant difference between the provenances (Halden and Rana) (p-value < 0.001), but no significant differences between the different temperatures treatments (p-value = 0.071), and there was no significant interaction between temperature and provenance (p-value = 0.374) (table 10). Plants from Halden had a larger shoot diameter than plants from Rana (figure 10).

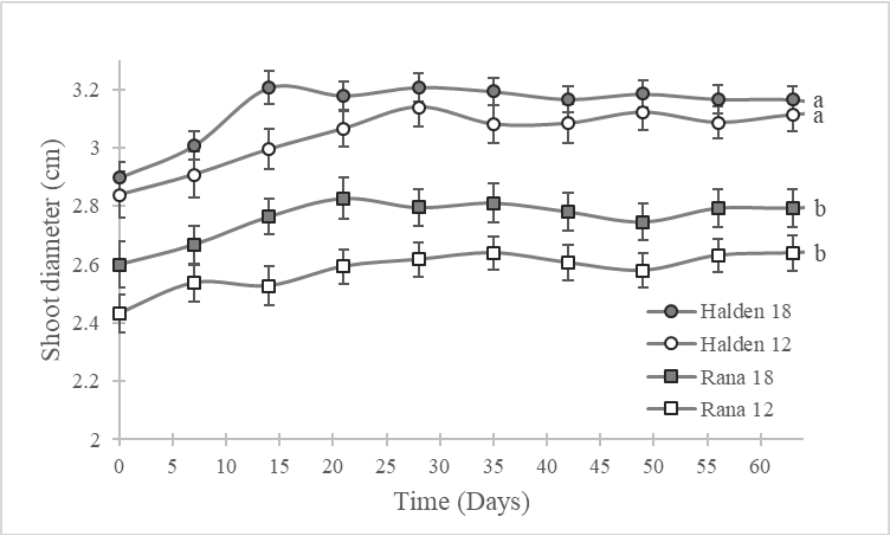

Figure 10 *Effect of constant temperature (12 or 18°C) under decreasing daylength on shoot diameter in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Plants were given 22 h photoperiod, with 2 h decreasing, until 12 h photoperiod was reached. Different letters on the right side represent significant difference between treatments on the last measurement based on two-way ANOVA followed by Tuckey`s test for both provenances together. N=36 per treatment. Average shoot diameter with ± SE displayed.*

Table 10 *ANOVA table of shoot diameter (on day 63) in Norway spruce seedlings from the provenances Halden (from 59°N) and Rana (from 66°N) treated with decreasing daylength and constant temperature (12 or 18°C). The plants were given 22 h photoperiod and decreased with 2 h a week until 12 h photoperiod (bud set treatment). The analyses were performed on the last measurement of the bud set treatment. N=36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Provenance	1	6.4389	6.43891	55.41	0.000	***
Temperature	1	0.3854	0.38543	3.32	0.071	
Provenance*Temperature	1	0.0925	0.09252	0.80	0.374	
Error	140	16.2673	0.11619			
Total	143	23.1841				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Number of needles

Under gradually decreasing daylength and constant temperature (12 or 18°C), both the plants from different provenances (Halden and Rana) and those kept at different temperatures were significantly different from each other in number of needles (p-value > 0.001), and there was a significant interaction between temperature treatment and provenance (p-value = 0.003) (table 11). At the end of the SD treatment, the Halden-plants had more needles than the Rana-plants and the plants kept at 18°C had more needles than those at 12°C. The plants from Rana, kept at 18°C did not increase the number of needles between the 2. and 3. count, while the other plants did (figure 11).

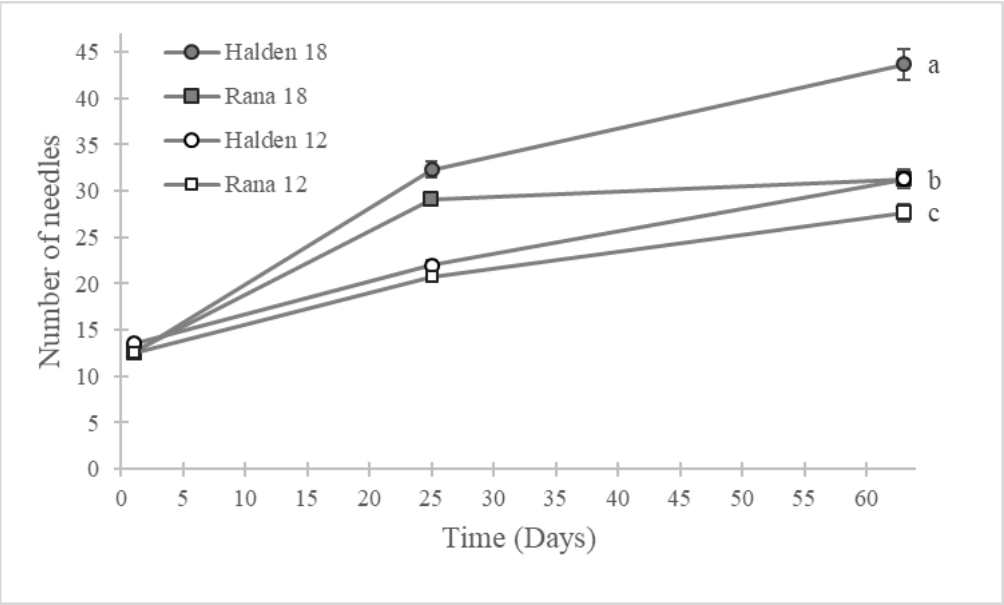

Figure 11 *Effect of constant temperature (12 or 18°C) under decreasing daylength on the number of needles in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Daylength started with 22 h photoperiod and was decreased with 2 h a week until 12 h photoperiod. Different letters on the right side of the graphs indicate significant differences between treatment for the last measurement based on two-way ANOVA followed by Tuckey`s test for both provenances together. When lines overlap and there are no significant differences one letter is shown. N=36 per treatment. Average number of needles with ± SE displayed.*

Table 11 *ANOVA tables of the last needle count (on day 63) in seedling of the Norway spruce provenances Rana (from 66°N) and Halden (from 59°N), treated with decreasing daylength and constant temperature (12 or 18°C). The seedlings were given 22 h photoperiod and decreased 2 h a week, until 12 h photoperiod was reached. The data were log- transformed before analysis. N=36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Provenance	1	0.34410	0.344100	44.91	0.000 ***
Temperature	1	0.34518	0.345177	45.05	0.000 ***
Provenance*Temperature	1	0.06815	0.068155	8.90	0.003 **
Error	140	1.07268	0.007662		
Total	143	1.83012			

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Number of lateral buds

Under gradually decreasing daylength and constant temperature (12 or 18°C), there was a significant difference in the number of visible lateral buds between the plants from different provenances (Halden and Rana) (p-value < 0.001) but not between the plants kept at different temperatures (p-value = 0.444), and there was no significant interaction between provenance and temperature (p-value = 0.444) (table 12). The Halden-plants had significantly more visible lateral buds than the Rana-plants kept at 12°C, but not more than the those at 18°C

(figure 12). Only a few plants had open lateral buds, and there was no significant difference between the treatments in this respect.

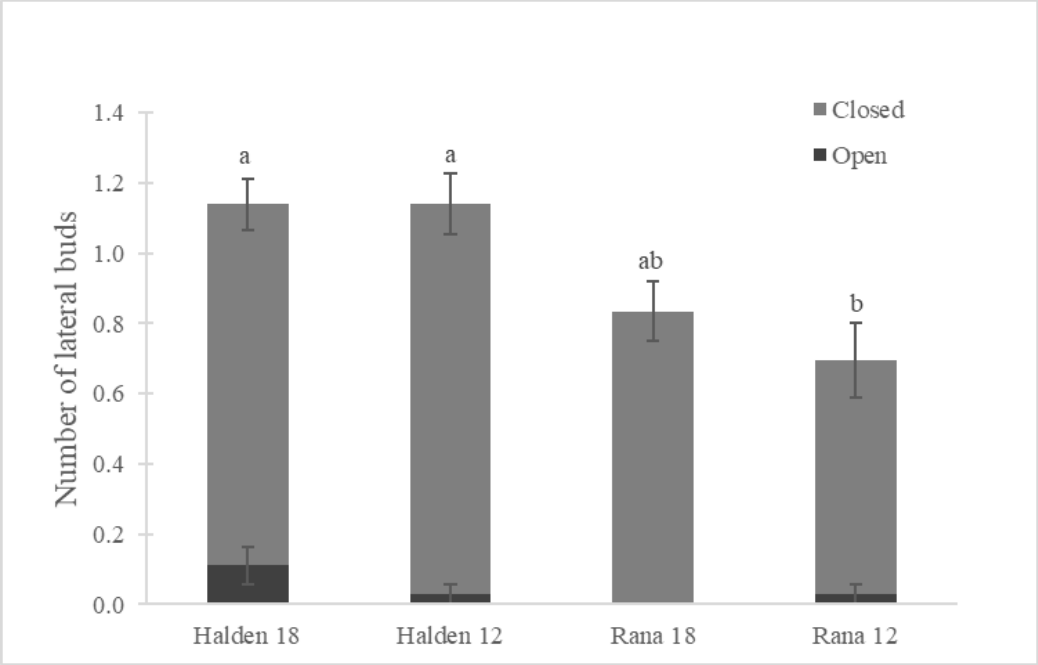

Figure 12 *Effect of constant temperature (12 or 18°C) under decreasing daylength on number of visible lateral buds in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Daylength started with 22 h photoperiod and was decreased with 2 h a week until 12 h photoperiod. Different letters above the bars indicate significant difference between treatments on the total number of lateral buds based on two-way ANOVA followed by Tuckey`s test for both provenances together. N=36 per treatment. Average number of open and closed lateral buds with ± SE displayed.*

Table 12 *ANOVA table of number of lateral buds (on day 63) in seedling of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N) treated with decreasing photoperiod and constant temperature (12 or 18°C). The plants were given 22 h photoperiod, decreasing with 2 h per week until a 12 h daylength was reached. N=36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Provenance	1	5.0625	5.0625	17.18	0.000	***
Temperature	1	0.1736	0.1736	0.59	0.444	
Provenance*Temperature	1	0.1736	0.1736	0.59	0.444	
Error	140	41.2500	0.2946			
Total	143	46.6597				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Stem diameter

Under gradually decreasing daylength and constant temperature (12 or 18°C), both the plants from the Halden and Rana provenances and the plants kept at different temperatures had significantly different stem diameter (p-value < 0.001 for both), and there was a significant

interaction between provenance and temperature (p-value = 0.001) (table 13). The plants grown at 18°C had thicker stems than those at 12°C. In the plants exposed to 18°C, the stems of the Halden-plants were thicker than the Rana-plants, while there was no significant difference between the plants from the different provenances when kept at 12°C (figure 13).

During the measurements it was also noticeable that the plants kept at 18°C had a stiffer stem and were less fragile than those grown at 12°C.

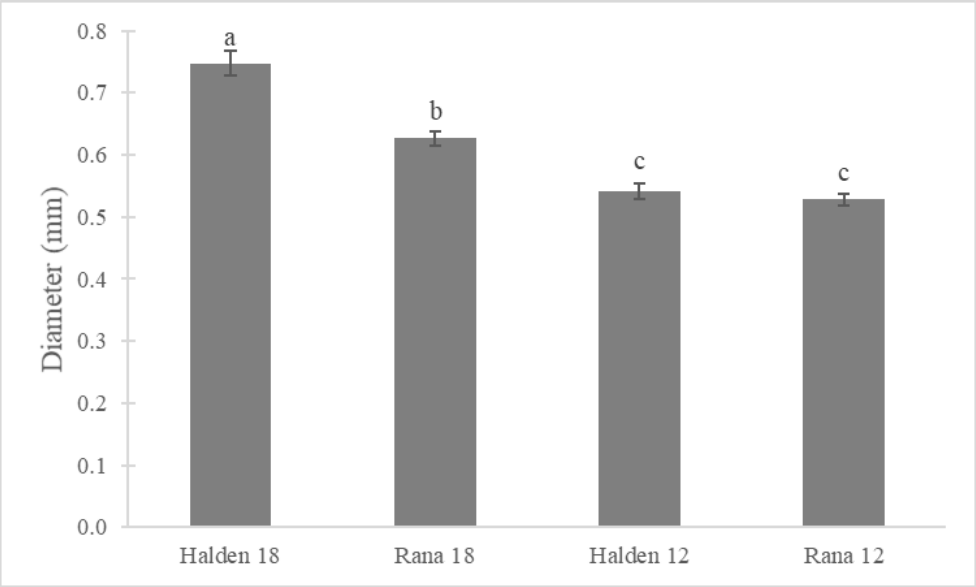

Figure 13 *Effect of constant temperature (12 or 18°C) under decreasing daylength on stem diameter in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Daylength started with 22 h photoperiod and was decreased with 2 h a week until 12 h photoperiod. Different letters above the bars indicate significant differences between the treatments based on two-way ANOVA followed by Tuckey`s test for both provenances. N=36 per treatment. Average stem diameter with ± SE displayed.*

Table 13*. ANOVA table of stem diameter (on day 63) in seedling of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N) treated with decreasing daylength and constant temperature (12 or 18°C). The plants were given 22 h daylength, decreasing with 2 h per week, until 12 h daylength was reached. The data were log-transformed before they were analysed. N=36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Provenance	1	0.06383	0.063832	19.21	0.000	***
Temperature	1	0.40699	0.406986	122.47	0.000	***
Provenance*Temperature	1	0.03603	0.036035	10.84	0.001	**
Error	140	0.46526	0.003323			
Total	143	0.97211				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Biomass

For both the root, stem, shoot (stem and needle together) and total weight, there was a significant difference between the plants from the different provenances (Halden and Rana) as well as between the plants kept at the different constant temperatures (12 or 18°C), when given gradually decreasing daylength. However, for the needle weight, there was only a significant difference between the plants from the different provenances. There was no significant interaction between the provenance and temperature for any of the plant organs, or total weight (table 14).

For the root weight, the plants kept at 18°C were significantly heavier than those at 12°C, and the Halden-plants were significantly heavier than the Rana-plants, when kept at the same temperature. For the needle weight, the plants from Halden were significantly heavier than the plants from Rana. For the stem weight, the Rana-plants, exposed to 12°C were significantly lighter than the rest of the plants. For the total weight, the plants from Halden, kept at 18°C and the plants from Rana, given 12°C, were the heaviest and the lightest respectively (figure 14 and 15).

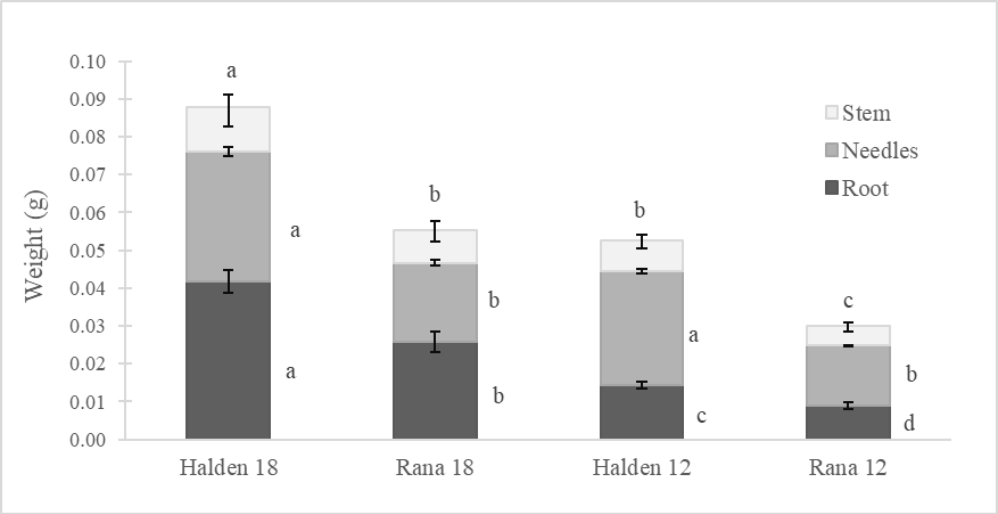

Figure 14 *Effect of constant temperature (12 or 18°C) and decreasing daylength on dry weight in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Daylength started with 22 h photoperiod and was decreased with 2 h a week until 12 h photoperiod. Different letters represent a significant difference between the treatments. The letters on the side represent significant differences of the needles (top) and roots (bottom), and the letters above the bars represent significant difference of the total weight based on two-way ANOVA followed by Tuckey`s test for both provenances together. N=23-24 per treatment. Average stem, needle and root weight with ± SE displayed.*

The ratio between the root and shoot weight was significantly different between the plants grown at the different temperatures, with plants at 18°C having a higher root/shoot ratio than those at 12°C (table 14 and figure 14 and 15).

Also notable, the plants exposed to 18°C had darker needles than the plants at 12°C (figure 15).

Table 14 *ANOVA table of dry weight (on day 64) in seedlings of Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and constant temperature (12 or 18°C). The plants were given 22 h photoperiod, with 2 h decrease a week, until 12 h photoperiod was reached. The data were log-transformed before they were analysed. N=23-24 per treatment.*

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	
Root weight						
Provenance	1	1.4776	1.47759	32.87	0.000	***
Temperature	1	5.0853	5.08534	113.12	0.000	***
Provenance*Temperature	1	0.0001	0.00009	0.00	0.963	
Error	92	4.1359	0.04496			
Total	95	10.6989				
Stem weight						
Provenance	1	0.45581	0.45581	10.31	0.002	**
Temperature	1	0.68634	0.68634	15.52	0.000	***
Provenance*Temperature	1	0.01613	0.01613	0.36	0.547	
Error	92	4.06914	0.04423			
Total	95	5.22743				
Needle weight						
Provenance	1	1.53148	1.53148	48.92	0.000	***
Temperature	1	0.08484	0.08484	2.71	0.103	
Provenance*Temperature	1	0.00919	0.00919	0.29	0.589	
Error	91	2.84902	0.03131			
Total	94	4.48065				
Shoot weight (stem and needle combined)						
Provenance	1	1.23926	1.23926	44.79	0.000	***
Temperature	1	0.19017	0.19017	6.87	0.010	*
Provenance*Temperature	1	0.01237	0.01237	0.45	0.505	
Error	91	2.51766	0.02767			
Total	94	3.96853				
Total weight						
Provenance	1	1.29020	1.29020	44.79	0.000	***
Temperature	1	1.18783	1.18783	41.24	0.000	***
Provenance*Temperature	1	0.00254	0.00254	0.09	0.767	
Error	91	2.62107	0.02880			
Total	94	5.12850				
Root/shoot ratio						
Provenance	1	0.00895	0.00895	0.30	0.586	
Temperature	1	6.11735	6.11735	204.04	0.000	***
Provenance*Temperature	1	0.05812	0.05812	1.94	0.167	
Error	91	2.72826	0.02998			
Total	94	8.90687				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Figure 15 *Sample picture of seedlings of the Norway spruce provenances Rana (from 66°N) and Halden (from 59°N), treated with decreasing daylength and constant temperature (12 or 18°C). The plants were given 22 h daylength, decreasing with 2 h a week, until 12 h was reached. From the left Rana and Halden 18°C and Rana and Halden 12°C. The scale marks 10 cm.*

Number of leaf primordia

Under gradually decreasing daylength and constant temperature (12 or 18°C), the number of leaf primordia within the terminal buds were significantly different in the plants kept at the different temperatures, while there was no significant difference between the plants from the different provenances (Halden and Rana) (table 15). There were significantly more leaf primordia in the plants grown at 18°C, than in those at 12°C (figure 17), and the buds were also visibly larger (figure 16).

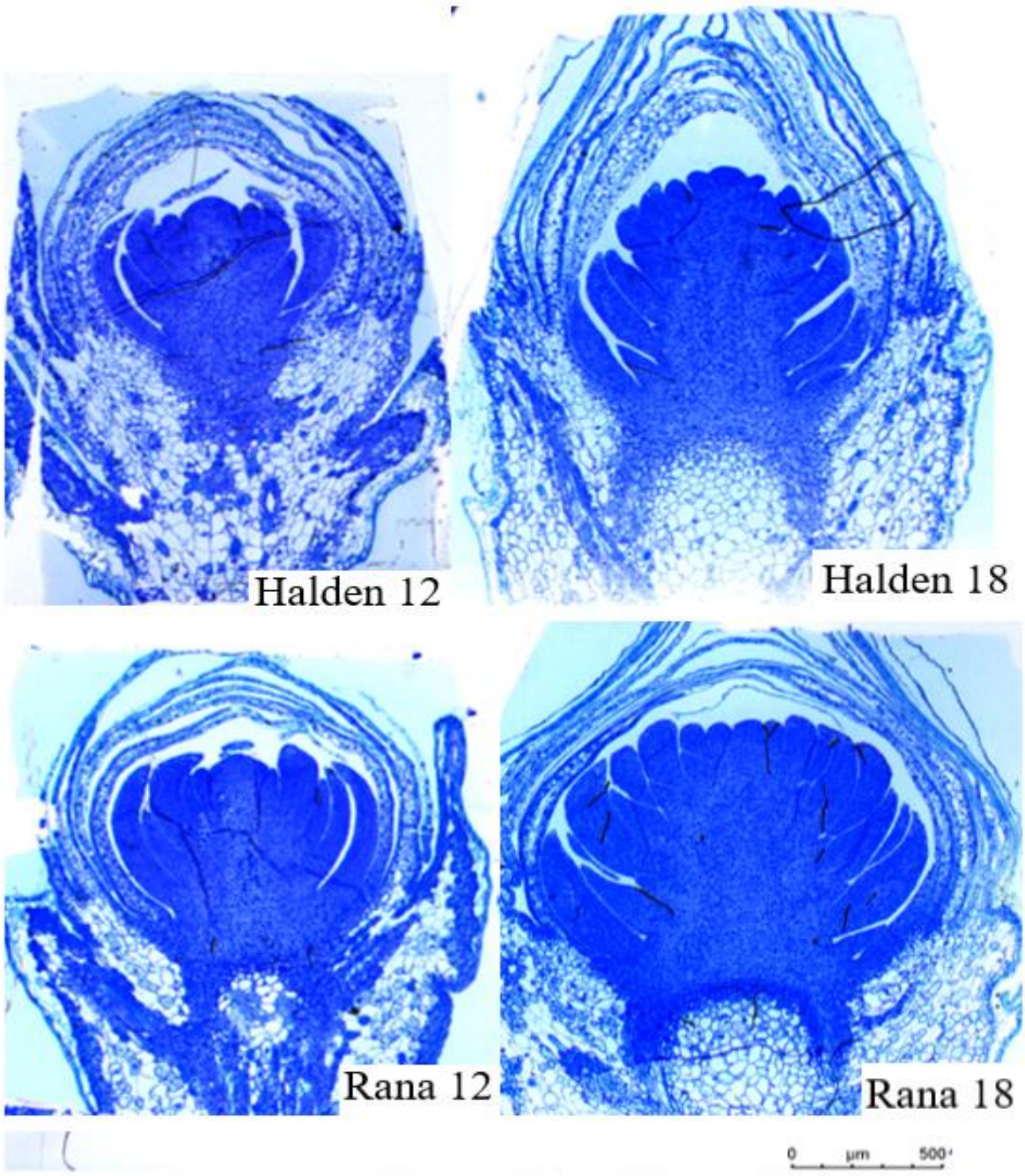

Figure 16 *Micrographs of cross sections of terminal buds from seedlings of Norway spruce from the Halden (from 59°N) and Rana (from 66°N) provenances treated with decreasing daylength and different constant temperature (12 or 18°C). The plants were given 22 h daylength, decreasing with 2 h a week, until 12 h daylength was reached. The scale marks 500 µm.*

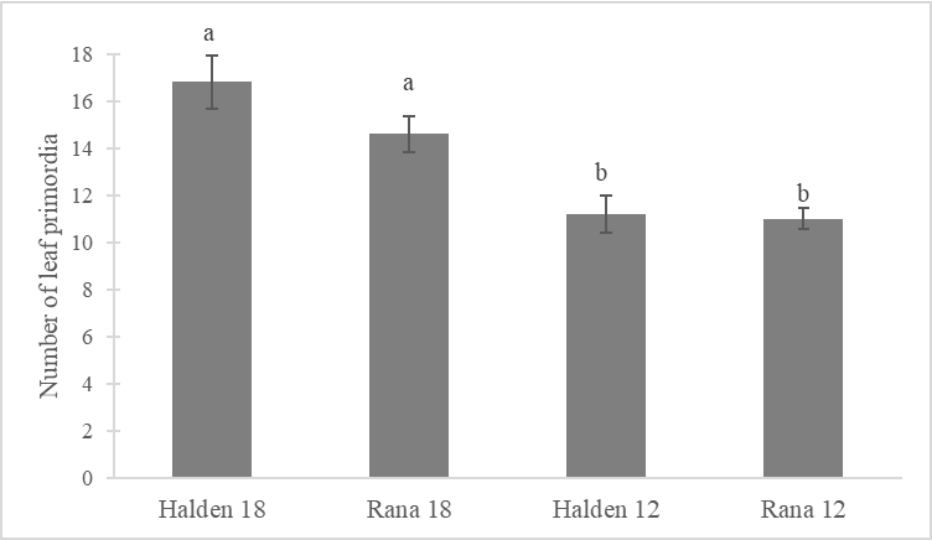

Figure 17 *Effect of constant temperature (12 or 18°C) and decreasing daylength on number of leaf primordia in terminal buds in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Daylength started with 22 h photoperiod and was decreased with 2 h a week until 12 h photoperiod. Different letters over the bars indicate significant difference between treatments based on two-way ANOVA followed by Tuckey`s test for both provenances together. N=5 per treatment. Average number of leaf primordia with SE displayed.*

Table 15 *ANOVA table of number of leaf primordia in terminal buds (on day 63) in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and constant temperature (12 or 18°C). The plants were given 22 h daylength, with decreasing 2 h a week, until 12 h daylength was reached. The data was log-transformed before analysis. N=5 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Provenance	1	0.005167	0.005167	1.54	0.233	
Temperature	1	0.111252	0.111252	33.11	0.000	***
Provenance*Temperature	1	0.003696	0.003696	1.10	0.310	
Error	16	0.053766	0.003360			
Total	19	0.173881				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Bud break development

There was a significant difference in time to bud break between the plants from the two different provenances (Halden and Rana) after the re-transfer to LD and 18°C following the different temperatures (12 or 18°C) under a gradually decreasing daylength, but no significant difference between the plants kept at different temperatures. However, there was an interactive effect of temperature and day number when the results for the entire time course were analysed with clmm. There was also a significant interaction between provenance and day (table 16). When analysing individual days with ANOVA there was a significant difference between plants from the different temperatures in all days analysed but only a

significant difference between the provenances on day 16, and there was no significant interaction between temperature and provenances for any of the days analysed (table 17). The plants from 12°C under SD had a faster bud break than the those at 18°C. Of the plants exposed to 12°C under SD, the plants from Rana started bud break faster, but the plants from Halden had a faster development, and caught up with Rana. In the plants from 18°C, there were no significant differences between the plants from different provenances (figure 18).

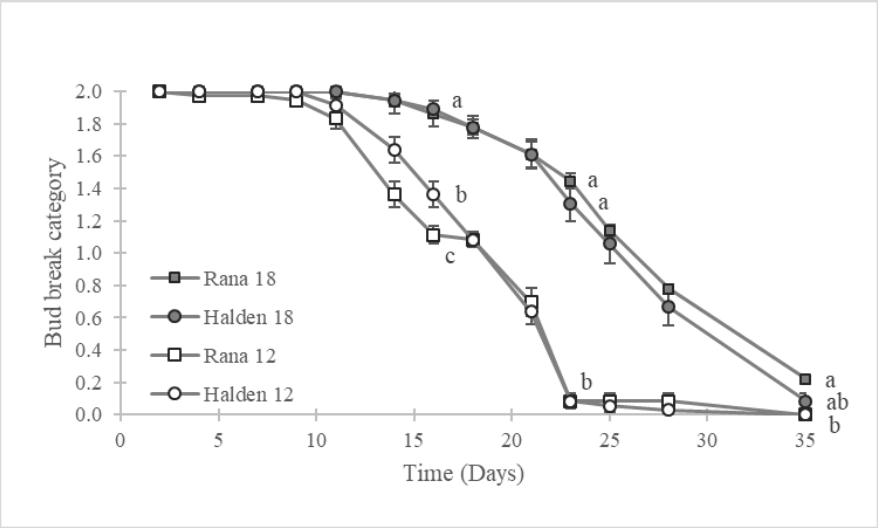

Figure 18 *After-effect of constant temperature (12°C or 18°C) under decreasing daylength on bud break in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Plants were given 22 h photoperiod, with 2 h decrease a week, until 12 h photoperiod (bud set treatment), followed by re-transfer to 18°C and 24 h photoperiod (bud break treatment). Different letters represent significant difference between treatments for the points on the left side of the letters based on two-way ANOVA followed by Tuckey`s test for both provenances together. When lines overlap and there are no significant differences one letter is shown. 3 bud break categories where used: 2 = intact bud, 1 = hole in bud, all needles were still gathered and bent inwards, 0 = needles were spreading away from each other. N=36 per treatment. Average bud break category with ± SE displayed.*

Table 16 *Summary of cumulative link mixed model for bud break in Norway spruce seedlings from the provenances Halden (from 59°N) and Rana, treated with decreasing daylength and constant temperature (12 or 18°C). The plants were given 22 h daylength, with 2 h decrease a week until 12 h daylength was reached (bud set treatment). Afterwards all plants were transferred to 24 h photoperiod and 18°C (bud break treatment). N=36 per treatment.*

	Estimate Std.	Error	Z-Value	P-Value	
Day	0.62414	0.03234	19.300	0.000	***
fTemperature18	-0.89541	0.60630	-1.477	0.14	
ProvenanceRana	2.22248	0.55581	3.999	0.000	***
Day*fTemperature18	-0.14501	0.02924	-4.959	0.000	***
Day*ProvenanceRana	-0.10542	0.02631	-4.007	0.000	***

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Table 17 *ANOVA table of bud break in Norway spruce seedlings from the provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and constant temperature (12 or 18°C). The seedlings were given 22 h daylength with 2 h decrease a week, until 12 h daylength was reached. Afterwards the plants were given 24 h daylength and 18°C (bud break treatment). Individual days were picked for analyses. N=36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Day 16						
Provenance	1	0.6944	0.6944	4.93	0.028	*
Temperature	1	14.6944	14.6944	104.31	0.000	***
Provenance*Temperature	1	0.4444	0.4444	3.15	0.078	
Error	140	19.7222	0.1409			
Total	143	35.5556				
Day 23						
Provenance	1	0.1736	0.1736	0.76	0.385	
Temperature	1	60.0625	60.0625	262.55	0.000	***
Provenance*Temperature	1	0.1736	0.1736	0.76	0.385	
Error	140	32.0278	0.2288			
Total	143	92.4375				
Day 35						
Provenance	1	0.1736	0.17361	2.71	0.102	
Temperature	1	0.8403	0.84028	13.11	0.000	***
Provenance*Temperature	1	0.1736	0.17361	2.71	0.102	
Error	140	8.9722	0.06409			
Total	143	10.1597				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Growth after re-transfer to LD

After re-transfer to LD following the bud set treatment under different temperatures (12 and 18°C) and gradually decreasing daylength, there was a significant difference in growth between the plants from the different temperatures (p-value < 0.001), but there was no significant difference between the plants from the different provenances (Halden and Rana) (p-value = 0.105) and no significant interaction between provenance and temperature (p-value = 0.279) (table 18). The plants from 12°C grew significantly more than those at 18°C (figure 19).

Both plants from 12°C and 18°C seemed to start growing in the last week of treatment (figure 19).

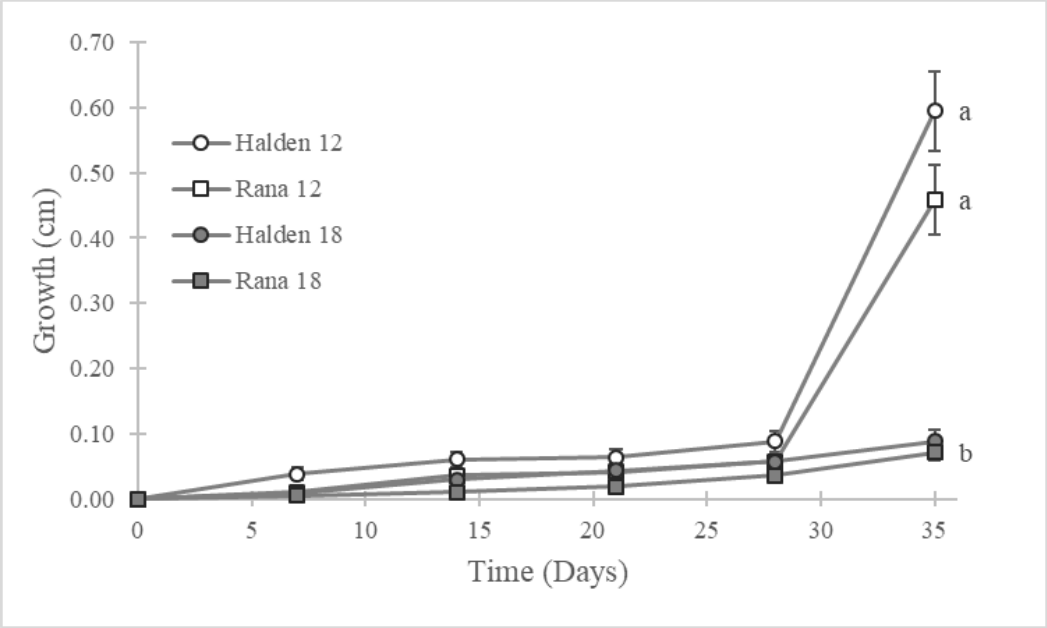

Figure 19 *After-effect of constant temperature (12 or 18°C) under decreasing daylength on cumulative growth under bud break treatment in seedlings of Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N). Daylength started with 22 h photoperiod and was decreased 2 h a week until 12 h photoperiod (bud set treatment), followed by re-transfer to 24 h photoperiod and 18°C (bud break treatment). Different letters on the right side of the graphs indicate significant differences between treatments for the last measurement based on two-way ANOVA followed by Tuckey`s test for both provenances together. When lines overlap and there are no significant differences one letter is shown. N=35-36 per treatment. Average growth with ± SE displayed.*

Table 18 *ANOVA table of cumulative growth (on day 35) during bud break treatment in seedlings of the Norway spruce provenances from Halden (from 59°N) and Rana (from 66°N) treated with decreasing daylength and constant temperature (12 or 18°C). The seedlings were given 22 h photoperiod, decreasing with 2 h a week, until 12 h photoperiod was reached. Afterwards the plants were transferred to 24 h daylength and 18°C (bud break treatment). The data were log-transformed before analysis. N=35-36.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Provenance	1	0.03464	0.03464	2.67	0.105	
Temperature	1	1.86026	1.86026	143.13	0.000	***
Provenance*Temperature	1	0.01532	0.01532	1.18	0.279	
Error	139	1.80663	0.01300			
Total	142	3.71857				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Effects of decreasing daylength and alternating temperature (experiment 3)

As stated above, the daylength treatment should have been gradually decreased as in experiment 2 but was compromised due to technical failures in different ways in two growth chambers with different day and night temperature treatments (24/18°C and 18/12°C with gradual changes over 3 h). The temperature treatments were therefore analysed separately with respect to the response of the two provenances (Halden and Rana).

Growth

Both when kept at 24/18°C day/night temperature (p-value < 0.001) and 18/12°C (p-value = 0.004) under decreasing daylength, the plants from Halden grew significantly more than the plants from Rana (figure 20 and table 19).

While all the other plants eventually ceased growth, the plants from Halden that were kept at 24/18°C, showed increased growth during the last couple of weeks (figure 20).

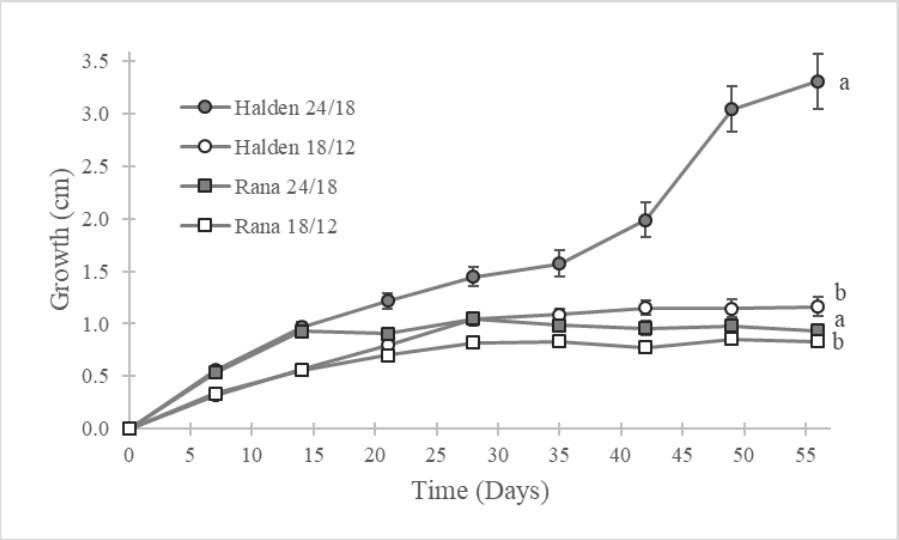

Figure 20 *Effect of alternating day and night temperature (24/18°C or 18/12°C with gradual changes over 3 h) under decreasing daylength on cumulative growth in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N). The plants were given 22 h daylength for 3 weeks, then decreasing 2 h a week, until 12 h daylength was reached. Different letters on the right side of the graph indicate significant difference between provenances at the last measurement for each temperature treatment separately (due to technical failure in daylength treatment in 18/12°C), based on one-way ANOVA followed by Tuckey`s. N=35-36 per treatment. Average height with ± SE displayed.*

Table 19 *ANOVA table of cumulative growth (on day 56) in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and alternating temperature (24°C/18°C or 18°C/12°C with gradual changes over 3 h). The plants were given 22 h daylength for 3 weeks, then decreasing 2 h a week, until 12 h daylength was reached. The data were log-transformed before analysis. Due to technical failure in daylength treatment in 18/12°C, the temperature treatments were analysed separately. N=35-36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
18°C/12°C					
Provenance	1	0.2863	0.28630	8.91	0.004 **
Error	69	2.2183	0.03215		
Total	70	2.5046			
24°C/18°C					
Provenance	1	5.281	5.28076	149.80	0.000 ***
Error	70	2.468	0.03525		
Total	71	7.748			

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Bud set development

When exposed to gradually decreasing daylengths, there was a significant difference in bud set between plants from the different provenances (Halden and Rana), in both day/night temperature treatments (18/12°C and 24/18°C) on all days analysed (table 20).

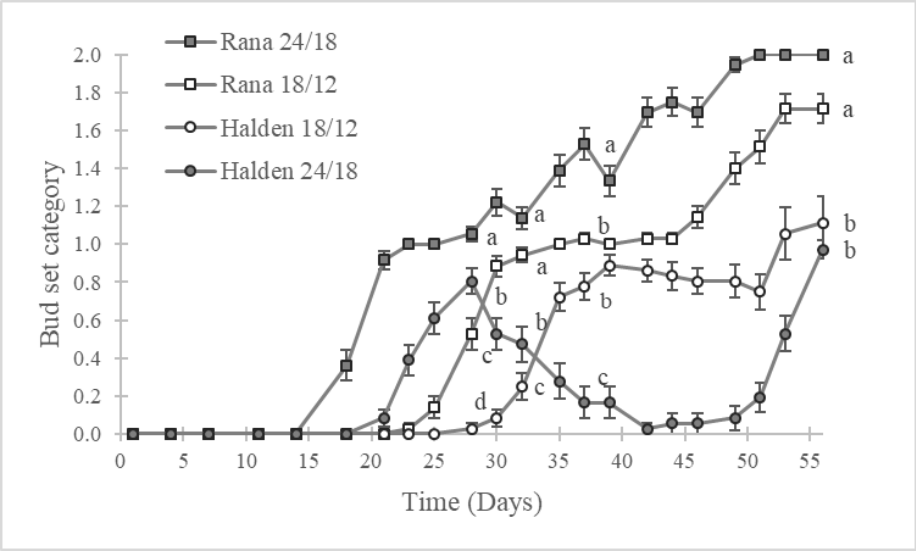

Figure 21 *Effect of alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h) under decreasing daylength on bud set in seedling of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N). The plants were given 22 h daylength for 3 weeks, then decreasing 2 h a week, until 12 h daylength was reached. Different letters represent significant difference between provenances for the points on the left side of the letters for each temperature treatment separately (due to technical failure in daylength treatment in 18/12°C), based on one-way ANOVA followed by Tuckey`s. 3 bud set categories were used: 0 = no visible bud and shoot elongation, 1 = white bud or light green bud, 2 = bud has turned brown. N = 35-36 per treatment. Average bud set category with ± SE displayed.*

The plants from Rana had a faster bud development than the plants from Halden in both temperature treatments. Most of the plants from Halden that were kept at 24/18°C, started to develop buds, before almost all buds broke again (figure 21).

Table 20 *ANOVA table of bud development (on day 56) in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h). The plants were given 22 h daylength for 3 weeks, then decreased with 2 h a week, until 12 h daylength was reached. Individual days were analysed. Due to technical failure in daylength treatment in 18/12°C, the temperature treatments were analysed separately. N=35-36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Day 28 (18°C/12°C)						
Provenance	1	4.708	4.7083	33.64	0.000	***
Error	69	9.658	0.1400			
Total	70	14.366				
Day 28 (24°C/18°C)						
Provenance	1	1.125	1.1250	10.46	0.002	**
Error	70	7.528	0.1075			
Total	71	8.653				
Day 32 (18°C/12°C)						
Provenance	1	8.519	8.5192	68.07	0.000	***
Error	69	8.636	0.1252			
Total	70	17.155				
Day 32 (24°C/18°C)						
Provenance	1	8.000	8.0000	36.65	0.000	***
Error	70	15.278	0.2183			
Total	71	23.278				
Day 37(18°C/12°C)						
Provenance	1	1.116	1.1162	10.71	0.002	**
Error	69	7.194	0.1043			
Total	70	8.310				
Day 37(24°C/18°C)						
Provenance	1	33.35	33.3472	129.88	0.000	***
Error	70	17.97	0.2567			
Total	71	51.32				
Day 56 (18°C/12°C)						
Provenance	1	6.457	6.4565	13.62	0.000	***
Error	69	32.698	0.4739			
Total	70	39.155				
Day 56 (24°C/18°C)						
Provenance	1	19.014	19.0139	447.80	0.000	***
Error	70	2.972	0.0425			
Total	71	21.986				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Shoot diameter

The shoot diameter from needle tip to needle tip across the shoot did not increase more than a few mm during the treatments with gradually decreasing daylength and alternating day and night temperature (18/12°C or 24/18°C). In both temperature treatments, the plants from Halden had a significantly larger shoot diameter than the plants from Rana (p-value < 0.001) (figure 22 and table 21).

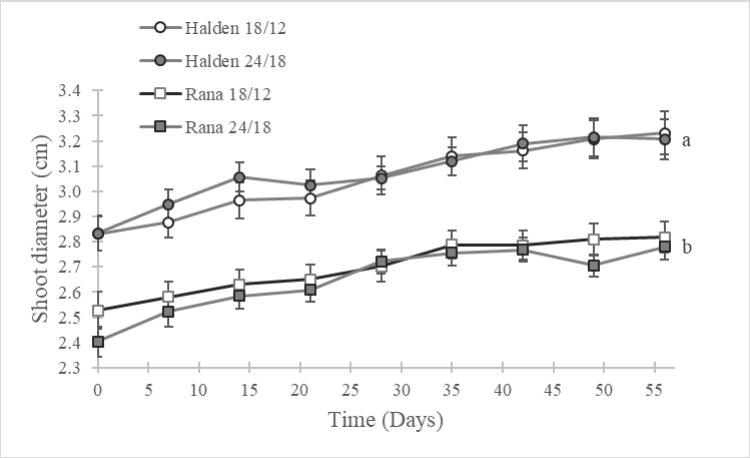

Figure 22 *Effect of alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h) under decreasing daylength on shoot diameter in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N). The plants were given 22 h daylength for 3 weeks, then decreasing 2 h a week until 12 h daylength was reached. Different letters on the right side of the graph indicate significant difference between provenances at the last measurement for each of the temperature treatments separately (due to technical failure in daylength treatment in 18/12°C), based on one-way ANOVA, followed by Tuckey`s test. When lines overlap and there are no significant differences, one letter is shown. N=31-36 per treatment. Average shoot diameter with ± SE displayed.*

Table 21 *ANOVA table of shoot diameter (on day 56) in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h). The plants were given 22 h daylength for 3 weeks, then decreased with 2 h a week, until 12 h was reached. The data were log-transformed before analysis. Due to technical failure in daylength treatment in 18/12°C, the temperature treatments were analysed separately. N=31-36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
18°C/12°C						
Provenance		0.05841	0.058413	16.47	0.000	***
Error	69	0.24467	0.003546			
Total	70	0.30309				
24°C/18°C						
Provenance	1	0.09592	0.095917	35.30	0.000	***
Error	70	0.19020	0.002717			
Total	71	0.28612				

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Number of needles

The number of needles increased during gradually decreasing daylength and alternating day and night temperature (18/12°C or 24/18°C) (figure 23). There was a significant difference between the plants from the different provenances (Halden and Rana) in both temperature treatments (p-value > 0.001) (table 22). The Halden-plants had more needles than the Rana-plants (figure 23). In both temperature treatments, the Rana-plants did not increase the number of needles from the 2. to the 3. count, while the plants from Halden did. The Halden-plants kept at 24°C/18°C increased the number of needles noticeably more than all the other plants on the last count (figure 23).

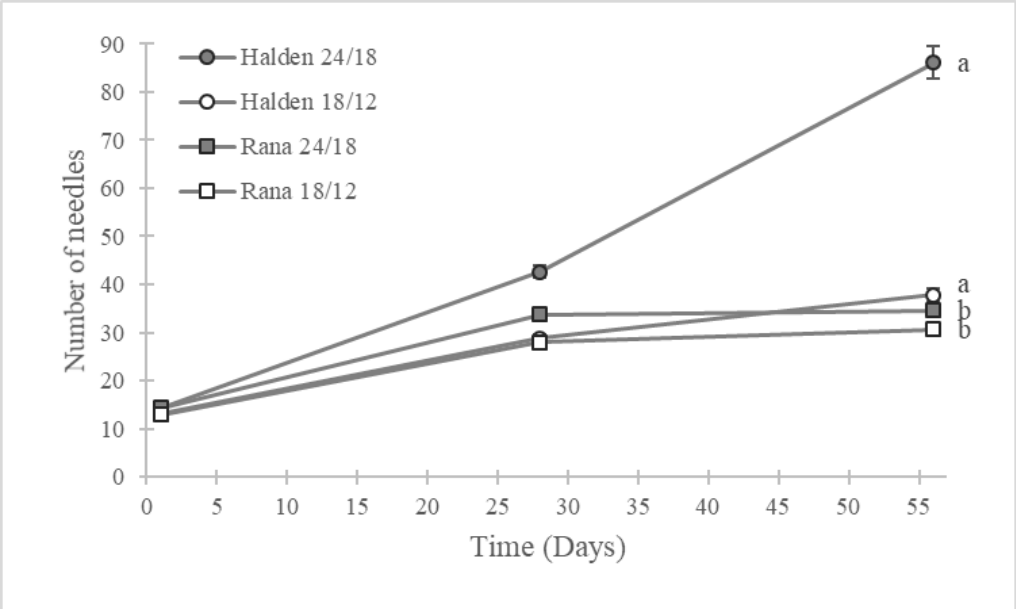

Figure 23 *Effect of alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h) under decreasing daylength on number of needles in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N). The plants were given 22 h daylength for 3 weeks, then decreasing 2 h a week, until 12 h daylength was reached. Different letters on the right side of the graph indicate significant difference between provenances at the last measurement for each temperature treatment separately (due to technical failure in daylength treatment in 18/12°C), based on one-way ANOVA followed by Tuckey`s test. N=31-36 per treatment. Average number of needles with ± SE displayed.*

Table 22 *ANOVA table of the number of needles (on day 56) in seedling of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h). The plants were given 22 h for 3 weeks, then 2 h decreasing a week, until 12 h daylength was reached. The data were log-transformed before analysis. Due to technical failure in daylength treatment in 18/12°C, the temperature treatments were analysed separately. N=31-36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
18°C/12°C					
Provenance	1	0.1399	0.139932	18.00	0.000 ***
Error	63	0.4898	0.007775		
Total	64	0.6298			
24°C/18°C					
Provenance	1	2.7404	2.74041	277.64	0.000 ***
Error	70	0.6909	0.00987		
Total	71	3.4313			

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Number of lateral buds

Under gradually decreasing daylength and alternating day and night temperature (18/12°C or 24/18°C), there was a significant difference in number of visible lateral buds between the plants from the different provenances in the plants kept at 24/18°C (p-value < 0.001), but not in the plants at 18/12°C (p-value = 0.129) (table 23 and figure 24).

Table 23 *ANOVA table of lateral buds (on day 56) in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h). The plants were given 22 h daylength for 3 weeks, then decreased with 2 h a week, until 12 h daylength was reached. The data were log-transformed before analysis. Due to technical failure in daylength treatment in 18/12°C, the temperature treatments were analysed separately. N=31-36 per treatment.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
18°C/12°C					
Provenance	1	0.1279	0.12791	2.36	0.129
Error	69	3.7447	0.05427		
Total	70	3.8726			
24°C/18°C					
Provenance	1	0.4778	0.47785	14.15	0.000 ***
Error	70	2.3646	0.03378		
Total	71	2.8425			

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

In the Halden-plants exposed to 24/18°C almost half of the lateral buds were open, while almost none of the other plants had open lateral buds.

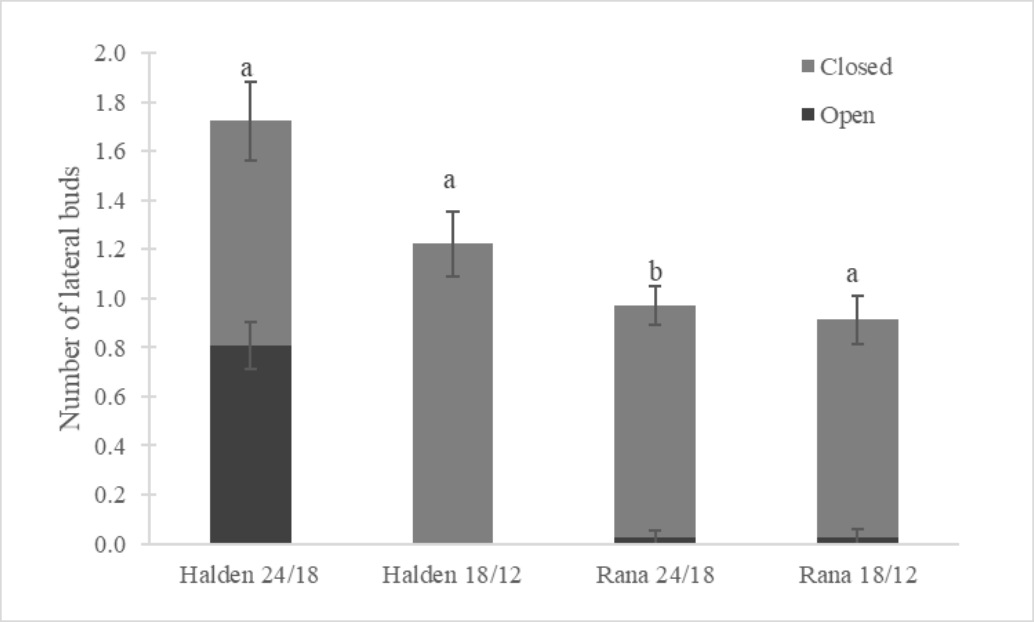

Figure 24 *Effect of alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h) under decreasing daylength on number of lateral buds in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N). The plants were given 22 h daylength for 3 weeks, then decreasing 2 h a week, until 12 h daylength was reached. Different letters over the bars indicate significant difference between provenances for each of the temperature treatments separately (due to technical failure in daylength treatment in 18/12°C), based on one-way ANOVA followed by Tuckey`s test. N=31-36 per treatment. Average number of open and closed lateral buds with ± SE displayed.*

Stem diameter

Under decreasing daylength treatment the stems of the plants from Halden kept at 18/12°C, were thicker than stems of the Rana-plants (p-value = 0.043), while there was no significant difference between the plants from the different provenances (p-value = 0.062), exposed to 24°C/18°C (table 24 and figure 25).

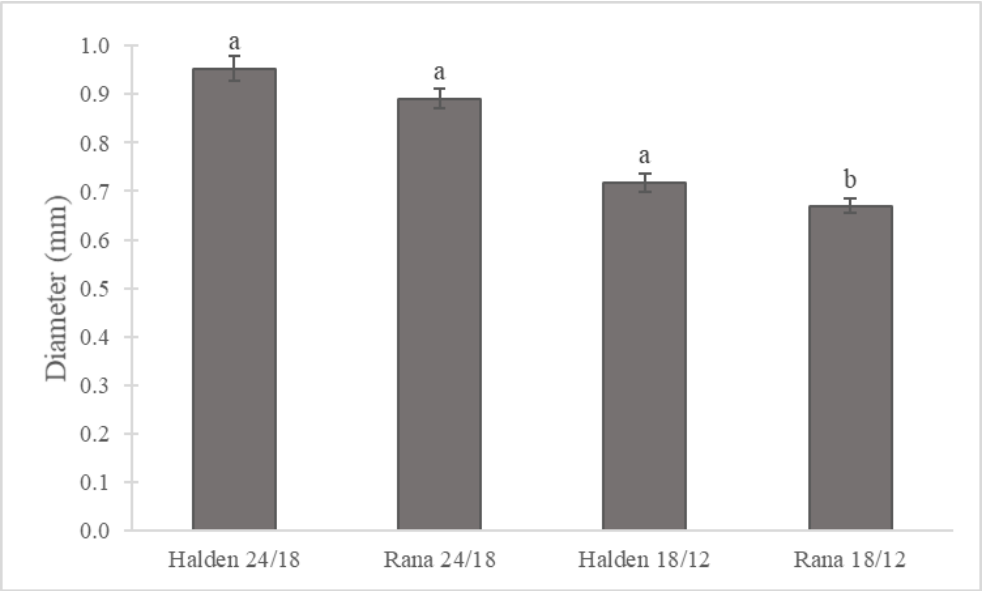

Figure 25 *Effect of alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h) under decreasing daylength on number of needles in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N). The plants were given 22 h daylength for 3 weeks, then decreasing 2 h a week, until 12 h daylength was reached. Different letters above the bars indicate significant difference between provenances for each of the temperature treatments separately (due to technical failure in daylength treatment in 18/12°C), based on one-way ANOVA followed by Tuckey`s test. N=31-36 per treatment. Average stem diameter with ± SE displayed.*

Table 24 *ANOVA table of stem diameter (on day 56) in seedlings of the Norway spruce provenances Halden (from 59°N) and Rana (from 66°N), treated with decreasing daylength and alternating temperature (24/18°C or 18/12°C with gradual changes over 3 h). Then plants were given 22 h daylength for 3 weeks, then decreased by 2 h a week, until 12 h daylength was reached. The data was log-transformed before analysis. Due to technical failure in daylength treatment in 18/12°C, the temperature treatments were analysed separately. N= 31-36.*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
18°C/12°C					
Provenance	1	0.01452	0.014522	4.27	0.043 *
Error	64	0.21786	0.003404		
Total	65	0.23238			
24°C/18°C					
Provenance	1	0.01346	0.013460	3.58	0.062
Error	70	0.26291	0.003756		
Total	71	0.27637			

Significance codes: *** < 0.001, ** < 0.01, * ≤ 0.05

Discussion

Previous studies of woody species including *Populus* species and Norway spruce conducted in growth chambers have shown that plants kept at warmer temperature ceased growth and set buds earlier. This is in contrast to results from studies of *Populus* species conducted in the field were plants grown at warmer temperature showed delayed growth cessation and bud set as compared to plants under lower temperature (Kalcsits et al., 2009; Olsen et al., 2014; Rohde et al., 2011; Strømme et al., 2015; Strømme et al., 2017; Strømme et al., 2018; Tanino et al., 2010). Although no such field studies have been conducted on young Norway spruce seedlings as of our knowledge, it may be assumed that they will behave similar to *Populus* in the field, since the growth chamber studies showed a similar response. In previous growth chamber studies plants have generally been place directly to SD treatments shorter than the critical daylength (Hamilton et al., 2016; Junttila et al., 2003; Kalcsits et al., 2009; Olsen et al., 2014), and the sudden change in daylength could possibly cause a stress response accelerating bud set.

So, in this study, gradually decreasing daylength treatments were used to investigate if this would affect the growth cessation and bud development response as well as subsequent bud break and re-growth in Norway spruce seedlings differently compared to constant daylength treatments. Olsen et al. (2014) studied the effect of alternating day and night temperatures treatment in Norway spruce but did not observe an effect of alternating temperatures in itself but found that day temperature altered the time to bud set. However, there was a correlation with the daily mean temperature with warmer temperatures resulting in faster bud set. In the Olsen et al. (2014) study, direct transfer to SD was used to induce bud set, and the day and night temperature was changed abruptly.

Furthermore, in this study a combination of gradually decreasing daylength and gradually alternating day and night temperatures with gradual change over 3 h were used to evaluate if this would affect the mentioned growth-dormancy cycling parameters. Unfortunately, the results were compromised with respect to the gradually changing daylength treatment due to technical failure, but at least the response of the two provenances studied could be compared.

A number of previous studies conducted on Norway spruce have used temperatures up to 21°C (Olsen et al., 2014; Søgaard et al., 2008) and it has been suggested that the optimum growth for Norway spruce is about 20°C (Dormling et al., 1968). To also evaluate the effect of a higher temperature, in this study the effect of a constant temperature of 24°C on growth

and bud set as well as subsequent bud break was compared with 18°C and 12°C (giving an interval of 6°C between in each temperature).

Growth and bud set

When comparing the growth and bud set between the different daylength treatments, similar results were found with respect to the effect of temperature. Plants given warmer temperature generally grew more, but also showed earlier bud set than those at colder temperatures. This could also be seen in the plants that were given alternating day and night temperatures, were the plants kept at 24°C/18°C started developing buds faster than the plants kept at 18°C/12°C, even though there was a malfunction in the day extension light in the plants kept at 18°C/12°C (these unintendedly got 4 SD of 12 h photoperiod). This is consistent with previous findings in other studies which have found similar patterns with warmer temperature resulting in earlier growth cessation and bud set in several woody species, both coniferous and deciduous species when grown in growth chambers (Hamilton et al., 2016; Heide, 2003; Heide, 1974; Kalcsits et al., 2009; Olsen et al., 2014; Tanino et al., 2010). Increased growth in plants at warmer temperature may be explained by the photosynthetic rate, which increases with warmer temperatures and thereby increases growth up to an optimum temperature, which in Norway spruce was reported to be about 20°C (Dormling et al., 1968; Hamilton et al., 2016; Stinziano & Way, 2014).

In this study, there was a tendency of earlier growth cessation at warmer temperatures, but not fully. In the plants that got constant SD of 12 h photoperiod, the plants from Rana seemed to be about a week faster in their growth cessation than the plants from Halden, except in the plants kept at 18°C, which ceased growth about at the same rate (figure 4 A). The plants kept at 24°C ceased growth the fastest in both provenances, although the plants kept at 18°C ceased their growth at about the same time as those at 24°C in the plants from Halden. In the plants given decreasing daylengths, and constant temperature, the plants from Rana seemed to slow down their growth more before they ceased their growth than the plants from Halden, although they ceased growth at about the same time, after about 5 weeks (14 h daylength). An exception to this was observed for the plants from Halden kept at 12°C, which ceased growth after about 7 weeks (12 h daylength for 2 weeks). As mentioned previously, the critical daylength is longer the higher the latitude is, and the plants from Rana therefore have a longer critical daylength than the plants from Halden (Dormling et al., 1968; Gyllenstrand et al., 2007; Thomas & Vince-Prue, 1996; Vaartaja, 1959). In the plants receiving a gradually

decreasing daylength it can therefore be expected that the plants from Rana cease growth earlier, while for the plants receiving a constant daylength it is possible that the plants from Rana have a stronger response to SD than the plants from Halden, therefore ceasing growth faster.

Growth cessation in plants that received alternating day and night temperature, was faster than expected, based on comparison with the plants given constant temperatures and gradually decreasing daylengths. Although the plants due to technical failure received 3 weeks of 22 h daylength instead of the planned 2 h decrease in daylength per week, the plants from Rana kept at 24°C/18°C ceased their growth after about 2 weeks , which was 3 weeks faster than the plants from Rana, kept at constant 18°C and 12°C and decreasing daylength, which ceased growth at about 14 h photoperiod (8 h shorter photoperiod). Possibly the 22 h photoperiod was short enough for the induction of growth cessation and combined with a higher temperature at 24/18°C growth cessation happened faster than the plants at 18°C and 12°C. A critical daylength of 22 h is longer than reported for Norway spruce provenances from between 63°N and 70°N (Thomas & Vince-Prue, 1996). Although the plants from Halden kept at 24/18°C never ceased growth completely and instead had a growth spurt at the end, most of the plants started setting buds earlier than would be expected, between day 21 and day 28, at which point the plants received between 22 h and 20 h daylength. The early growth cessation in the Rana-plants and the early bud set may possibly have been due to a stress factor like drought (possibly the plants needed more water than expected, due to a higher temperature than in earlier treatments and therefore may have been watered insufficiently at some occasions). The buds in the Halden plants probably formed when they still were exposed to longer photoperiods than their critical daylength, which probably resulted in induction of bud break, almost all the Halden-plants kept at 24/18°C reflushed while receiving decreasing daylength. It seemed that these plants when induced to break buds, where not able to respond to the shortening days, resulting in new bud set not starting before the last week of treatment. Reflushing also occurred in a few of the Halden-plants kept at 18°C and decreasing daylengths. Several earlier studies have reported reflushing occurring in Norway spruce even under short day conditions (Dormling et al., 1968; Mølmann et al., 2006). In previous studies, Norway spruce seedlings has been shown to have a shallow dormancy, at least when grown indoors under controlled conditions (Olsen et al., 2014; Søgaard et al., 2008). The situation in this study is consistent with this in that plants showed bud break upon re-transfer to LD and 18°C without any chilling treatment. The plants kept at 18°C/12°C ceased growth after about 4 (Rana) to 5 (Halden) weeks, at which point they received 20 h to 18 h daylength. This was 1

week faster for the plants from Rana, and for the plants from Halden the same amount of time as the plants kept at constant temperatures (excluding the plants from Halden kept at 12°C), but with 6 h to 4 h longer daylengths. Since the plants kept at 18°C/12°C unintendedly received 12 h daylength for 4 days during the 22 h treatment, due to malfunction of the day extension light, it is possible that this was sufficient to induce growth cessation.

Plants from Halden generally grew more than the plants from Rana (figure 4 A and B and 8 and 20), while plants from Rana showed earlier bud set (figure 5 A and B and figure 9). Less growth in northern provenances compared to southern provenances has also been observed earlier in Norway spruce (Dormling et al., 1968). Mølmann et al. (2006) found a similar bud set pattern, where the northern provenance set buds first, while Olsen et al. (2014) did not find such a pattern of bud set.

Growth cessation and bud set took longer in the treatments with decreasing daylength compared to the plants that were transferred directly to SD. The critical daylength for northern provenances at 70°N (Rana is located at 66°N) is about 20 h (Thomas & Vince-Prue, 1996). It was therefore to be expected that plants that received decreasing daylengths had a slower growth cessation and bud set, since they received daylengths above the critical daylength when the treatments started.

As expected, the plants that received LD treatment, never ceased growth or set buds during the treatment. In other studies of Norway spruce and several other species, growth cessation and bud set have occurred during LD treatment, when northern provenances simultaneously got cold temperature treatments, but these plants received such treatments for a longer period than the plants in this study and also colder temperatures. (Dormling et al., 1968; Hamilton et al., 2016; Heide, 1974; Tanino et al., 2010). For both provenances, the plants kept at 24°C and LD did not grow significantly taller, than those at 18°C and LD (figure 4 A and B). This corresponds with the optimal growing temperature, which, as mentioned above, is reported to be about 20°C for central European and northern provenances of Norway spruce (Dormling et al., 1968). Also, although the plants kept at 24°C under SD of the 12 h photoperiod developed buds the fastest, the difference between the plants kept at 24°C and at 18°C under the 12 h SD is not as big as the difference between the plants kept at 12°C and 18°C (figure 5 A and B). This was more prominent in the plants from Halden (figure 5 A) than the plants from Rana (figure 5 B). The reasons for this is not clear although it has been shown that in northern provenances, cold temperatures in itself are able to induce bud set (Tanino et al., 2010). It could be that both the temperature and SD induced bud set in plants from Rana grown at

12°C, and although these plants did not develop buds as fast as the plants exposed to warmer temperatures, the difference between the temperature treatments was not as large as in the Halden-plants. However, if a temperature of 12°C contributes to bud set in the Rana provenance, it may have been expected that the difference between the Halden and Rana-plants when grown at 12°C under a gradually decreasing daylength would be more pronounced, and possibly that Rana-plants at 12°C would set buds first. However, as described above, this was not the case. Although the optimum temperature for growth is probably about 20°C for both provenances (Dormling et al., 1968), it may be that the plants from Rana are better adapted to grow and develop at colder temperatures. However, no such clear trend was observed for any of the observed growth parameters in decreasing daylength treatments.

Shoot diameter and number of lateral buds

Shoot diameter and lateral buds was only registered in the plants that got decreasing daylength. Measuring the shoot diameter is a way to measure the elongation growth of the needles. For both growth parameters there was no difference between the plants kept at the different temperatures, only a difference between the Halden and the Rana-plants. The plants from Halden had both more visible lateral buds (figure 12), and a wider shoot diameter (figure 10). It therefore seems like temperature does not have an effect when it comes to shoot diameter and number of visible lateral buds. However, it is possible that since the shoot diameter did not increase much in any of the plants, that more time would have been needed for a difference to become significant as there seems to be a tendency of a larger shoot diameter in the plants kept at 18°C. As mentioned above, plants from northern provenances tend to grow less than plants from southern provenances (Dormling et al., 1968), and it seems this was the case for these two parameters too. In the plants that were given alternating day and night temperatures, the plants from Halden also had a wider shoot diameter (figure 22) than the plants from Rana. However, the plants from Halden only had significantly more lateral buds than the plants from Rana when kept at 24°C/18°C, there was no significant difference in those at 18°C/12°C.

Number of needles, stem diameter and number of leaf primordia

These growth parameters were also only registered in plants that got decreasing daylength treatments. In all these growth parameters, temperature had an effect, and the plants grown at

18°C had the highest number of needles (figure 11), thickest stems (figure 13) and highest number of leaf primordia (figure 17) and thicker buds (figure 16). As mentioned above, higher temperatures increase the photosynthetic rate up to 20°C, which is considered the optimal temperature for Norway spruce (Dormling et al., 1968; Stinziano & Way, 2014), and it seems that these growth parameters are affected by temperature too. Provenance also played a role, the plants from Halden had more needles than the plants from Rana, and in the plants kept at 18°C, the plants from Halden had thicker stems than the plants from Rana. There was no significant difference in plants from Halden and Rana in the number of leaf primordia within the terminal buds, although there seemed to be a tendency that Halden had more leaf primordia than the plants from Rana at least in the plants that received 18°C. As mentioned above, less growth in northern compared to southern provenances has been shown previously (Dormling et al., 1968), and this seems to be the case for number of needles and stem thickness too. In the plants that were given alternating day and night temperatures, the plants from Halden had more needles. In the plants kept at 18°C/12°C, the plants from Halden had a thicker stem, while there was no difference between the plants from Halden and Rana when kept at 24°C/18°C.

Biomass

The biomass was only registered in plants that received gradually decreasing daylength. The biomass followed the same pattern as seen in several other growth parameters; plants kept at 18°C were heavier than at 12°C and the Halden-plants were heavier than Rana-plants. The exception was the needle weight were the only difference was between provenances; the plants from Halden had a higher needle weight than the plants from Rana. Most notable though, was the ratio between root and shoot weight, which was most likely influenced by the temperature treatment. The plants kept at 18°C had a higher root to shoot ratio than those at 12°C (figure 14), meaning that the plants exposed to 18°C had stored more photosynthates in the root system compared to plants given 12°C. Hamilton et al. (2016) studied growth cessation in roots in white spruce, and found that growth cessation only seemed to occur in plants that were kept at colder temperatures (8°C day /4°C night). Growth cessation in the roots of plants kept at 12°C may be an explanation for the small roots compared to the plants kept at 18°C or it could be due to decreased photosynthetic rate as seen in growth.

Notable, while number of needles showed a significant effect of temperature, needle weight did not. There are a few factors that could explain this: First, it should be noted that different

plants were used for the biomass studies and for the needle count. So, it could possibly be that there was a difference between the two samples used. On the other hand, the plants from Rana kept at 18°C, had the same number of needles as the plants from Halden at 12°C, and the plants from Halden had a significant larger shoot diameter, compared to the plants from Rana, meaning they had a longer needles, and therefore most likely also heavier needles.

Bud break and re-growth

For plants from both daylength treatments (12 h SD and gradually decreasing daylength), the plants kept at 12°C were the fastest to break their buds after transfer to LD and 18°C (figure 6 A and B and figure 18). This supports several findings in earlier studies that has found that woody species that entered dormancy at colder temperature had a shallower dormancy and broke the buds earlier (Dormling et al., 1968; Heide, 2003; Olsen et al., 2014; Søgaard et al., 2008). In the plants from constant daylength of 12 h SD, the plants from 24°C were slower to break their buds, but only in the plants from Halden, and only at two registration days. Like for growth and bud set, there were small differences between the plants from 24°C and 18°C compared to the plants from 12°C, further supporting the findings of Dormling et al. (1968), that Norway spruce has an optimum growing temperature at 20°C.

Previous studies have shown that Norway spruce seedlings from more northern provenances start breaking their buds earlier than plants from more southern provenances (Dormling et al., 1968; Olsen et al., 2014; Søgaard et al., 2008). The plants from this study did not all follow the same pattern. In the plants from constant daylength of 12 h SD and 24°C or 18°C, the plants from Halden started breaking buds earlier, while the plants from Rana broke the buds faster. However, in the plants from this daylength treatment and 12°C, the plants from Rana broke the buds both earlier and faster than the Halden-plants. During decreasing daylength, in the plants kept at 12°C, those from Rana started breaking their buds earlier, but the Halden-plants had a faster development, so they finished the bud break at about the same time. There was no significant difference between the plants from Rana and Halden, from 18°C. The difference in response in this study, compared to earlier studies may possibly be due to difference in registration of bud break. Olsen et al. (2014) and Søgaard et al. (2008) registered only % bud break and no bud break categories.

In the plants from the constant daylength treatment during bud set, re-growth was measured the last time at day 26 (Rana) and day 27 (Halden) during bud break (figure 7 A and B), while the plants that were given decreasing daylength during bud set, were measured for the last

time at day 35 during bud break (figure 19). The letter thus had more than a week longer with bud break treatment. In the plants from constant SD, the plants from Halden had grown 2-3 mm during bud break treatment (figure 7 A), while the plants from Rana had less than 1 mm growth (figure 7 B), with only a few plants starting to grow, and there was no difference between the temperature treatments. The plants from decreasing daylengths had grown less than 1 mm growth until the second to last measurement during the bud break treatment (on day 28), and on the last measurement (on day 35) the plants kept at 18°C still had not grown more than 1 mm, while the plants from 12°C had grown about 5-6 mm (figure 19), indicating that the plants from 12°C, resume growth faster than the plants from 18°C. Although most studies have focused on bud break after re-transfer of plants to LD treatment after SD, some studies have also shown that colder temperatures during SD result in faster regrowth in at least Norway spruce and Norway maple (Dormling et al., 1968; Westergaard & Eriksen, 1997). In this study only the plants from decreasing daylengths showed a difference in temperature treatment, but only during the last week, so possibly the plants from constant daylength would have shown a difference between the temperature treatments, if they were given an extra week of treatment.

As expected, the plants that were given different temperatures under LD from the start of the experiment, continued to grow after transfer to 18°C. The plants from Rana and Halden showed a different pattern in growth. The plants from Rana showed a similar pattern as before the transfer to 18°C, i.e. the plants from 18°C and 24°C grew significantly more than the plants from 12°C, while in the Halden-plants from 18°C (not subjected to temperature change) grew significantly more than the plants from 12°C and 24°C. It may seem that the Halden-plants from 12°C and 24°C had a stress response to the temperature change, and that this retarded their growth, while those at 18°C, which were kept at the same conditions throughout the experiment, continued growing as before. The reason for the difference between the provenances in growth after re-transfer to LD at 18°C remains elusive although it may be speculated that plants from the northernmost provenance are adapted to more changing temperature conditions.

Conclusions

The effect of temperature under decreasing daylength resembled the effect of temperature under a constant SD of 12 h photoperiod, with the plants given the warmest temperatures

ceasing growth and setting buds first, although with a few weeks delay compared to the plants exposed to constant SD of 12 h.

The plants grown at warmer temperatures also showed increased growth in most of the growth parameters registered, except for shoot diameter, lateral buds and needle weight, indicating that higher temperature increases the photosynthetic rate.

The plants grown at 24°C grew more and ceased growth and set bud earlier than those at 18°C during constant daylength, but the difference was not as pronounced as the difference between the plants grown at 12°C and 18°C, indicating that the Norway spruce seedlings are reaching a temperature limit for growth.

The plants from Halden generally grew more in most of the growth parameters tested, while the plants from Rana ceased growth and set buds earlier, showing that there are clear differences between provenances, with the Halden-plants showing a larger growth potential, while the Rana plants have a faster response to both gradually decreasing daylength and constant SD of 12 h photoperiod.

After re-transfer to LD and 18°C the plants exposed to 12°C under the SD treatment, broke the buds the fastest in both daylength treatments. Only in the plants that were exposed to gradually decreasing daylengths the plants at 12°C showed the most re-growth. There was no clear difference in re-growth between the temperature treatments for the plants from constant daylengths, but this was possibly because these plants received a week less of growth than in the experiment with gradually decreasing daylength. Both in gradually decreasing daylength and at constant SD of 12 h photoperiod there were also no clear differences in bud break and re-growth between the two provenances.

The results from plants grown at alternating day and night temperature were somewhat compromised due to technical failure of the growth chambers, and the results from this experiment could therefore not be compared directly with the results from the experiment with constant temperature under the gradually decreasing daylength. However, also this experiment showed more rapid growth cessation and bud set under the highest temperature and supports the general finding from the other experiments of this thesis that bud set is induced more rapidly under higher compared to lower temperature.

It could be noted that previous studies showing different response to temperature under induction of bud set in growth chambers and in the field, have been performed with *Populus* species and not Norway spruce. To shed light on the effect of temperature under natural short-

day conditions on Norway spruce provenances, further research should be conducted by e.g. employing heating lamps or a natural altitudinal gradient in temperature, like was done in *Populus* (Strømme et al., 2017; Strømme et al., 2018).

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