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Preface and acknowledgements

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Abstract

This study on Norwegian populations of beech was investigating different environmental factors influencing the winter dormancy stage and bud burst time, as the first known phenological study on these populations. Bud burst date was found to happen earlier than what has been considered normal during the last decades. Our findings question the assumption of beech being a late-flushing tree species with low variation in bud burst dates between years. A shift towards earlier bud burst time has been predicted for beech in northern areas due to increasing spring temperatures, and is likely to alter the fitness and competitive status of beech. In a scenario of increasing temperatures, the shift towards earlier bud burst time is assumed to stabilize within some time, as the necessary chilling phase during autumn is likely to be disturbed. The dormancy removal experiment found bud burst to be limited until a certain day length during spring, but we were unable to conclude whether this observation was due to phenological control or other factors influencing the experiment. The frost tolerance experiment showed a peak in frost tolerance by mid-winter (in January), estimating bud damage to occur below -15°C at this time.

Sammendrag

Denne studien har tatt for seg ulike påvirkningsfaktorer på vinterhvile og tidspunkt for knoppsprett hos bøk, som første kjente fenologiske forsøk på de norske populasjonene. Knoppsprett skjedde opptil to uker tidligere i 2014 enn hva som har vært ansett som normalt de siste tiår, noe som står i kontrast til tidligere antakelser om bøk som et stabilt, sentskytende treslag. Et skift mot tidligere gjennomsnittlig tidspunkt for knoppsprett for bøk i nordlige områder har blitt ansett som sannsynlig i tidligere studier, primært som et resultat av økte vårtemperaturer. Dette vil sannsynligvis påvirke bøketrærnes vekstforhold og konkurransestatus. I et scenario med ytterligere økende temperaturer vil skiftet mot tidligere vekstsesong antas å stabilisere seg, ettersom hvilestadiets kjøleperiode på høsten vil forstyrres i større grad. Resultater fra hvilebrytingseksperimentet indikerer sterk hemming av knoppsprett før en nødvendig daglengde er tilstede, men det er usikkert om observasjonen skyldes en fenologisk kontrollmekanisme eller utenforliggende faktorer ved selve eksperimentet. Frostherdighetsforsøket indikerte høyest herdighet midt på vinteren (i januar), der det ble estimert at knoppskade av betydelig grad skjedde ved eksponering for -15°C eller kaldere.

Introduction

Distribution range of beech

European beech (*Fagus sylvatica*) is one of the dominating deciduous tree species in temperate regions of western and central Europe (Bolte et al. 2007). In northern Europe, populations of natural origin (i.e. not naturalized provenances of foreign origin or spread from beech plantations), is found throughout Denmark and southern Sweden. In Norway, isolated populations are found in two separate areas in the south: on the east coast as several dispersed populations in the Vestfold area, and on the west coast as one isolated population at Seim, north of Bergen. Genetic- and pollen analysis suggest that the Norwegian populations in both areas have origin from Danish beech populations (Myking et al. 2011). They are believed to have been introduced in the period 500-1000 AD, most likely along with human migrations rather than being a natural extension of the south Scandinavian range. However, due to the presence of these populations over a long time span, they are normally characterized as indigenous populations today, in contrast to beech trees spread from planted beech trees of foreign origin.

According to Bolte et al. (2007), recent paleoecological and forest management studies show that beech has not yet recolonized its natural distribution range after the last ice age, and it is spreading northwards through unmanaged forests in southern Sweden. However, beech is not an invasive tree species in old growth- or in cultivated forests, and the recolonization of new areas by the species is limited (Bolte et al. 2007). The limitations for spreading northwards are not fully understood. Human management activities, like burning and clearing of forest areas, or cultivation of more competitive tree species, is shown to have influenced the presence of beech forests in Scandinavia during the period after the last ice age, therefore the potential distribution range in this area is not known (Bradshaw & Lindbladh 2005).

In addition to limitations caused by anthropogenic activity, climatic factors determine the potential distribution range of beech. Modellation studies have predicted the future distribution of beech in northern Europe to expand with increasing temperatures (e.g. Hickler et al 2012). In north-eastern Europe, several studies have been conducted trying to determine the natural limiting factors for beech dispersal, collected in the review study by Bolte et al. (2007). In general, both drought conditions in summer and long, cold winters are believed to be limiting factors for the distribution range. The tree species is thought to prefer a maritime, temperate climate with mild winters and moist summers. Five minimum environmental requirements for the presence of beech are summarized: (1) precipitation rates of 500 mm per year, or ~250 mm between May and September, (2) a July mean temperature of less than 19°C, (3) fewer than 141 days of a daily minimum temperature below 0°C, (4) a January mean temperature above -3°C, and (5) more than 217 days of daily mean temperature of 7°C or more. These requirements are defined as the long-time prevalent climatic conditions in areas within the beech populations' potential distribution range in north-eastern Europe. In addition, stressful climatic events, like severe heat, drought or frost (below -35°C) are listed as essential limiting factors for the distribution range of beech.

Norway's populations of beech are found in coastal areas, where climatic conditions usually fulfill those defined as the minimum requirements above. Our study will be looking at adaptations to winter conditions in beech trees from populations along the northern distribution range, both on the south-eastern and south-western coast of Norway. We investigate some of the environmental factors determining the potential northern limit for the geographical distribution of the species. Important traits related to this are the characteristics of the species' winter dormancy, i.e. the timing of the physiological processes related to the growing season, and the adaptations that make the

species able to tolerate cold temperatures and frost during winter. Our study is limited to these processes in vegetative buds of beech trees, as these are the main plant tissues undergoing dormancy adaptations, and hence are most important in determining the ability to endure winter conditions.

Winter dormancy of buds

Perennial plants in temperate areas are facing a trade-off between a long growing season and the avoidance of plant tissue damage during frost events (Kramer et al. 2010; Caffarra & Donnelly 2011). A longest possible growing season is important in inter- and intraspecific competition. At the same time, dormancy release and bud burst too early in the spring increase the risk of frost damage, and can be fatal for bud and plant survival. The time around bud burst, when new leaves are developing, is the most frost sensitive period of the plant's growth cycle (Vitasse et al. 2014). Deciduous plant species have evolved traits that influence the timing of bud burst in relation to the species' specific frost tolerance, and are from this characterized as early- or late-flushing species. The former group is generally opportunist/pioneer tree species that are able to start vigorous growth early in spring if temperatures are high, the latter group usually long-lived, late-successional species with a more conservative regulation of bud burst (Caffarra & Donnelly 2011). Beech is considered a late-flushing species in most of its distribution range, and has been shown to show low variation in bud burst dates between years compared to other broad-leaved tree species (Vitasse & Basler 2013). The explanation for this is a complex interplay of environmental factors, influencing and controlling the timing of the dormant state of the buds.

In general, winter dormancy in buds consists of physiological adaptations that prevent the plant from being in active growth or development during the winter period, and remain so until environmental conditions are favorable in spring (Welling & Palva 2006). The dormancy adaptations influence e.g the timing of growth cessation and bud set during late summer, tolerance to frost during the winter period, and the timing of bud burst during spring.

Dormancy induction and frost tolerance development

The dormant stage consists of different phases, which are related to the physiological processes throughout the period. First, dormancy is induced by the same signal triggering the events of growth cessation and bud set during late summer (Rinne et al. 2001). These signals are e.g. shorter day length and changes in the solar spectral composition, which are sensed by photoreceptors in the plant (Olsen 2010). In addition, a complex set of other factors influence the dormancy induction, e.g. the concentration of different hormonal substances (e.g. abscisic acid, ethylene), the amount of sugars accumulated in the buds, drought exposure etc. (Welling & Palva 2006; Olsen 2010).

This first phase of dormancy is termed endodormancy, and is characterized by an inability of the newly formed buds to obtain bud burst, even after exposure to flushing conditions (long day length and high temperatures), (Welling & Palva 2006). The ability to reach bud burst will not be acquired by the buds until they have experienced a substantial period of cold temperatures, after which they enter the ecodormancy phase. This necessity of exposure to cold temperatures is named a chilling requirement (Lang et al. 1987). The duration of chilling needed for releasing the endodormancy phase varies between tree species, and also between populations. A fully chilled state is generally not acquired until exposure to low, but not freezing, temperatures for long, undisturbed periods

during endodormancy (Welling & Palva 2006; Tanino et al. 2010). If the period is not sufficiently long for the plant's chilling requirement, the buds exit the endodormancy phase in a partially chilled state.

Winter hardening of the buds is induced at the same time as dormancy induction, and has many common environmental influencing factors (Welling & Palva 2006). The newly formed buds get prepared to both endure a cold winter climate, and later be able to develop new leaves and make the plant able to resume growth as soon as possible the following spring. Hence, it is important for the buds to avoid damage caused by ice formation in the plant tissue, and withstand the stresses related to this, e.g. dehydration. The development of frost tolerance consists of physiological adaptations that make the buds able to either avoid or survive exposure to freezing temperatures (Welling & Palva 2006; Gusta & Wisniewski 2012). These mechanisms vary between different plant parts and species, and can consist of e.g. osmotic regulation, production of substance that prevents ice formation, changes of cell structural composition, etc.

Winter hardening is mainly thought to be induced by the combination of short day length and low temperatures, occurring through autumn (Welling & Palva 2006). The process is similar to the chilling phase in many respects, and is happening at the same time. Tolerance to freezing temperatures is gradually acquired during the hardening process, and can induce resilience to very low temperatures if the hardening process is undisturbed by high temperatures during the period (Welling & Palva 2006; Olsen 2015). As the buds enter the ecodormancy phase, frost tolerance is maintained at the acquired level, and the buds can withstand large temperature fluctuations for a long period after. As the dormant state is gradually released through spring, frost tolerance also decreases, reaching a minimum as the buds are bursting and new leaves are developing (Vitasse et al. 2014).

Dormancy duration

After a sufficient duration of chilling, dormancy enters the ecodormant phase. From this stage, bud burst can be achieved after the buds have been exposed to a certain thermal sum (threshold level of warm temperatures), named a thermal (or forcing) requirement (Welling & Palva 2006; Vitasse & Basler 2013). The duration of the ecodormancy stage is assumed to be influenced by the amount of chilling the buds acquired through their endodormant phase. A longer period of chilling is thought to lead to a reduced thermal requirement, i.e. a lower heat sum necessary before the dormant state is released and bud break can happen (Vitasse & Basler 2013). However, if buds are only partially chilled during autumn, the thermal requirement will be higher, which might delay the release process later in spring (Vitasse & Basler 2013; Fu et al. 2014; Olsen et al. 2015). This has been termed as increased thermal time.

In areas with high autumn temperatures, the chilling requirement can be hard to fulfill in warm years. This has been observed as an increasingly occurring phenomenon of prolonged dormancy in plant species with high chilling requirements, especially in southern areas (e.g. southern Europe and the Middle East). This is considered a problem especially in the commercial cultivation of some plant species, e.g. in fruit trees or varieties of grapevines (*Vitis vinifera)*, because prolonged dormancy can lead to shifts (or rather, lack of) in the timing of bud burst and further development (e.g. flowering) in relation to increasing spring temperatures (Ophir et al. 2009; Zheng et al. 2015). With increasing autumn temperatures, full chilling is assumed to occur less often also in beech, which has a chilling requirement that is very high compared to other deciduous species (Vitasse & Basler 2013). Research from southern Europe indicates that chilling in beech is only partially obtained most years, except at high altitudes (Vitasse et al. 2009). For northern areas, it is unknown whether current autumn temperatures are leading to a high or low degree of chilling.

The phenomenon of high autumn temperatures delaying bud development in spring might seem a paradox. However, it underlines the sophisticated mechanisms that make many plants able to detect seasonal variations within a year, and also seasonal variations between years. This makes it possible for frost sensitive plants to adjust the timing of their growing season, and prevent bud burst from happening until a substantial part of the cold season has been endured. This is an adaptation especially important in temperate and boreal biomes, where the climatic variations between seasons can be large, and where there is a risk of frost events over a long period, until late in the spring.

Dormancy release and time of bud burst

Low temperatures through winter and early spring maintain the ecodormancy state (Welling & Palva 2006). It is released as daytime temperatures rise and the necessary thermal sum. For fully chilled buds, the temperatures during the winter and early spring will determine the timing of dormancy release. Higher temperatures lead to faster fulfillment of the thermal requirement, and will result in earlier dormancy release and bud burst. If the buds are not fully chilled, the thermal requirement is higher, which may prolong the duration of the dormancy period, even if winter temperatures are high. Partially chilled buds are believed to be inhibited in dormancy release until they detect a certain day length (photoperiod), whereas fully chilled buds are not believed to be controlled by the same mechanism (Vitasse & Basler 2013). As a result, fully chilled buds can reach bud burst earlier if temperatures are high during spring.

However, partially chilled buds have been thought to be able to partly compensate this delaying effect of the photoperiod. The mechanism behind this is unknown, but it is believed that these buds are experiencing a reduction in the thermal requirement (alternatively, an increased rate of thermal accumulation), resulting in significantly shortened thermal time, after the necessary day length has been detected (Vitasse & Basler). The combined effect of the photoperiod can be characterized as a feedback loop, at the same time delaying and enhancing dormancy release, where the total effect on the timing of the release is neither much delayed nor advanced. This is characterized as a bud burst conservative mechanism, and is thought to be the reason for why trees have been seen to usually experience bud burst time at similar time of the year, even if they are believed to have a different degree of chilling during the dormancy.

However, with increasing spring temperatures, this similar time of dormancy removal regardless of chilling state can be altered. Fully chilled buds, mainly controlled by the level of their thermal requirement, will be able to reach bud burst early in warm springs, and later in cold. For partially chilled buds, however, the bud burst time is not likely to be changed much due to increasing spring temperatures, due to their conservative mechanism. Indeed, for beech in central and southern Europe, very low variation in bud burst dates have been observed for beech over the last three decades, despite increased spring temperatures (Vitasse et al. 2009; Vitasse & Basler 2013). Conversely, for trees at high altitudes, where the chilling degree is believed to be to be higher, a trend of earlier bud burst time has been observed.

In northern areas (e.g. Scandinavia), the effects of increased spring temperatures are assumed to be similar to those for high altitudes, since the chilling of the buds during autumn most likely are high most years (Vitasse & Basler 2013). Hence, larger variation in bud burst dates between years can be expected in these areas, depending on the variation in winter- and spring temperatures between the years.

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Bud burst time for the Norwegian populations

Indeed, a general trend of earlier bud burst time for deciduous tree species has been observed over the last 40 years in northern areas, including beech (Nordli et al. 2008). Phenological garden observations from western- and mid-Norway show that beech varieties on average reach bud burst one to four days earlier by the end of the period, with average bud burst dates during the second or third week of May.

For beech populations in southern Norway, no similar trend analysis of bud burst dates are known. No recent recordings of previous bud burst dates for Vestfold were known to us prior to the study. However, some estimations of what was considered normal bud burst time over the last decades have been presented to us. For a managed beech stand approximately 5 km from the Tjølling population (Bøkeskogen in Larvik municipality), local sources stated that bud burst usually was observed during the second week of May, occasionally also during the week before or after (Nyhus 1999; Nyhus, pers. comm.). Bud burst before May was not reported by the mid-1990s. This was stated to be the general picture for beech populations both south and north in Vestfold up until the last decade, with slight observed differences within populations, most likely due to environmental differences at a local scale (e.g. aspect), (Bakka, pers. comm). However, for the last decade, we lack sufficient records to say whether bud burst dates have been observed earlier.

In Ås, Heide (1993) registered bud burst dates of beech of central European origin in the 1990 and 1991 seasons, and reported it to occur between May 5 and 8, following both a winter with mild and normal average temperatures (the period January to March 1990 was reported to be 6.5°C above the 30-year normal). Heide stated that this stability between years was common, also between years with high and low average temperatures.

Future bud burst time

Due to these observations, there seems to be an indication of a shift towards earlier bud burst time in northern areas of the beech distribution, although it has been considered to be a late-flushing species during the last decades. In a scenario of climate warming, beech in southern areas are to expect an even stronger conservative effect on bud burst time, as sufficient chilling becomes less common. This will most likely maintain the stable bud burst dates between years.

Compared to more photoperiod-insensitive species that are more likely to experience shifts towards earlier bud burst time, beech might face an altered competitive status during the growing season. It might also face advantages by maintaining late bud burst time in a more unstable climate, e.g. in reduced risk of frost damage during spring (Gu et al. 2008). Beech in northern areas might expect a different situation, with a likely shift towards earlier bud burst time, and a possible change in the ranking of early- vs. late-flushing tree species (Vitasse & Basler 2013). However, if autumn temperatures also are rising, the same effect as in southern Europe (a lower chilling degree of the buds becoming common), bud burst time is likely to stabilize at the new level also in northern areas.

As temperature increase is happening at a different rate than what genetic adaptation in trees can compensate, beech is likely to face an altered competitive status with phenological shifts due to increasing temperatures. This will be seen towards opportunist species that are getting an earlier start for their growing season, e.g. in the competition for nutrients and light before canopy closure, and towards other photoperiod-controlled species, that might adapt less to the increased temperatures than beech (Körner & Basler 2010).

Aim of the present study

Knowledge about dormancy characteristics and influencing factors is important for understanding and predicting effects on the future ecological status for beech at its northern distribution range. Potential shifts in the dormancy timing and bud burst time will have implications for various factors influencing the fitness of both beech and other tree species. This includes altered competition status, ability to adapt to a longer growing season, changes in frost risk, etc, in addition to shifts relative to other temperature dependent processes, e.g. soil mineralization and availability of nutrients (Gu et al. 2008; Vitasse & Basler 2013). Hence, the total effect on tree growth and fitness might be altered both positively and negatively (Körner & Basler 2010). Changes in growing season time and duration may also have important implications on a larger scale, on ecosystem functions like surface albedo, carbon balance, evapotranspiration etc. (Fu et al. 2014).

The aim of this study was to investigate the relationship between dormancy and environmental factors both under natural and controlled conditions, for a set of Norwegian populations of beech. These are the northernmost beech populations in Europe, which to our knowledge never has been subject to any physiological or phenological studies.

Through field observations and controlled experiments, we investigate questions related to the bud burst time that is observed for these populations today compared to previous decades. Are the Norwegian beech populations following other deciduous tree species towards an earlier start of the growing season, or are they subject to a conservative control of the dormancy release process, as are observed in central Europe? What can be expected in a scenario of increasing temperatures? With a joint interpretation of our results we will try to assess the likely implications for the growing season of the Norwegian beech populations.

Materials and methods

Study area and species

Two parallel studies of dormancy release in beech (*Fagus sylvatica*) buds were conducted with field observations and growth chamber experiments, both using material from the same populations (Table 1). Buds from the same trees were also tested under controlled conditions for frost tolerance characteristics throughout the dormancy period of 2014.

Figure 1. Location of the beech populations (Bergen, Vestfold and Ås) used in the dormancy release- and frost tolerance experiments (Geonorge 2015). For detailed locations of the Vestfold populations (Falkensten, Holmestrand, Melsom and Tjølling), see Figure 2.

Table 1. Locations for the beech populations used in the field study (all Vestfold populations) and the dormancy release- and frost tolerance experiments (all populations). The given population name abbreviations are as given in all further tables and figures.

Population	Region	Coordinates
Falkensten (F)	Vestfold	59°25'25.3"N, 10°26'11.4"E
Holmestrand (H)	Vestfold	59°31'43.2"N, 10°11'56.7"E
Melsom (M)	Vestfold	59°13'15.0"N, 10°21'10.4"E
Tjølling (T)	Vestfold	59°3'45.1"N, 10°6'28.3"E
Bergen (B)	Hordaland	60°38'17.0"N, 5°12'50.9"E
Ås (Å)	Akershus	59°41'35.3"N, 10°44'53.6"E

Table 2. List of replicate plots used in the field study. The plots were either in unmixed beech stands (Beech) or in mixed stands of beech and spruce (Mixed). Altitude (meters above sea level) and aspect (slope direction, if any) are given. All plots are in the Vestfold region.

Population	Replicate plot	Plot type	Altitude (m.a.s.l.)	Aspect
Falkensten (F)	Plot F1	Beech	30	SW
	Plot F2	Beech	20	W
	Plot F3	Beech	60	None
Holmestrand (H)	Plot H1	Beech	170	None
	Plot H2	Beech	200	SW
	Plot H3	Beech	207	W
Melsom (M)	Plot M1	Beech	51	W
	Plot M2	Beech	48	SW
	Plot M3	Beech	35	SE
	Plot M4	Mixed	8	SW
Tjølling (T)	Plot T1	Mixed	59	S
	Plot T2	Mixed	73	None
	Plot T3	Mixed	74	None
	Plot T4	Beech	66	SW
	Plot T5	Beech	93	SW

Field observations

For the field study, we selected beech saplings of 1 - 2 m in height from 15 replicate plots, representing different beech populations on four locations in Vestfold, southern Norway: Hillestad, Holmestrand municipality (WGS84 59°31'N, 200-207 m altitude), Falkensten, Horten municipality (59°25'N, 20-60 m altitude), Melsom, Stokke municipality (59°13'N, 8-51 m altitude) and Tjølling, Larvik municipality (59°03'N, 60-90 m altitude). The locations are situated approx. 20 km apart. Plots were situated in semi-open stands of either unmixed beech forest (beech trees dominating in and around the plot), or in mixed stands of beech and spruce (*Picea abies*). All plots were 3*3 m (9 m^2), facing south or southwest, and had a density of beech saplings more than 1/m^2 (Table 2).

Dormancy release- and frost tolerance experiments

The material used in the dormancy release- and frost tolerance experiments consisted of twigs collected from adult trees from the same locations in Vestfold as in the field study (Figure 2). In addition, beech trees from two other regions were used: one site with a more continental climate near Årungen (59°41'N) in Ås municipality, Akershus and one site near Norway's western coast, in Vollom nature reserve (60°38'N), Lindås municipality, Hordaland (named "Bergen" in this text, Figure 1). The twigs were collected with a pole pruner from branches facing south, at heights up to 7 m. Buds on the outer 10 cm of the twigs were used for the frost tolerance experiments, whereas all buds studied in the growth chamber experiments were from the inner section of the twigs.

Figure 2. Location of the Vestfold beech populations (Falkensten, Holmestrand, Melsom and Tjølling) used in the field study, dormancy release- and frost tolerance experiments (Geonorge 2015).

Data collection and processing

Field observations

We studied the saplings in Vestfold throughout the growing season of 2014, from early April to mid-September. The approximate date of bud burst was recorded for each replicate plot, defined as the time when buds showed green foliage on more than half the saplings. Sapling length measurements were conducted monthly to investigate environmental differences between the plots, and as indication of dormancy initiation timing. Local temperature measurements were conducted in each plot type on the Vestfold locations, using Tinytag Plus 2 temperature loggers (Intab, Stenkullen, Sweden). The loggers were placed at ground level at the center the plots, and recorded the temperature with 10 minute intervals in the period from April 3 to Sept 19. The temperature data was analyzed with the manufacturer's software Easy View version 5.7.0.1.

In addition, canopy density for each plot was estimated, taking 180° hemispherical (fisheye) canopy photographs, using a Pentax K-5II D-SLR camera with Sigma 4.5mm F2.8 EX DC HSM Circular Fisheye lens (projection distortion provided by the camera lens manufacturer). The photographs were taken from the center of each plot at the time of assumed maximum canopy foliage during the growing season (20 August). Fisheye photos were also taken at the time of minimum canopy foliage (25 April), for comparison of light conditions in the plots at the time around bud burst. The photos were analyzed using the software Gap Light Analyzer version 2.0 (Frazer et al. 1999), relating each plot to

its geographical position and tracking the specific solar trajectory for that plot. The software estimated the total amount of solar radiation (direct and diffuse) transmitted to each plot, calibrated for the percentage canopy cover.

Dormancy release experiment

Twigs were sampled from 3 adult replicate trees on all 6 locations around the 15th of each month in January, February, March, October, November and December 2014. Samples were stored dark under cold conditions (4°C) until preparation. Twig samples with length 10-15 cm, containing 2-3 dormant vegetative buds, were set in aluminum wrapped glasses with distilled water 1-3 days after sampling. For the October sampling some twigs still had live green foliage, which was removed before the start of the experiment.

Replicate twigs (3 samples from each tree, in total 9 samples from each location) were subjected to either short (SD) or long (LD) photoperiod, and a testing temperature of either 9, 15 or 21°C. The combination of LD and 21°C (LD 21°C) was considered sufficient as flushing conditions for beech buds. The growth chambers provided no natural daylight, all having 400 W high pressure sodium (HPS) bulbs. In addition, transparent Osram 60 W incandescent bulbs were used for day length extension in all LD chambers. The SD program consisted of 8 h day length with irradiance 200 µmol photons m^{-2} s^{-1}, followed by 16 h darkness. The LD program consisted of 24 h continuous light, first 8 h with irradiance 200 µmol photons m^{-2} s^{-1}, followed by 16 h with 5 µmol photons m^{-2} s^{-1} using incandescent light only. The difference in total irradiance between the SD and the LD treatments were considered negligible.

The 3 different temperatures were tested for each of the sampling months, except for the samplings in January and February, where only 21°C were tested. The temperatures were maintained throughout the testing period, with brief variations (of 8 h or less) of ± 8°C on two occasions (for the March samples in the 9°C growth chambers and the November samples in the 21°C growth chambers). Relative humidity (RH) was 65% in all experiments.

We recorded visually the number of days from the onset of treatment until bud burst occurred on at least one of the buds per twig. A categorical scale of 5 distinguishable bud stages were used (as described by Murray et al. 1989), where 0 = dormant, 1 = swelling started, 2 = fully swelled, 3 = green foliage visible, 4 = leaf fully developed, and bud burst was defined as reaching stage 3 or higher. The experiments were run for approx. 35 days, then terminated due to drying out or deterioration of the samples (e.g. algae growth preventing water uptake in the twigs). Hence there was an overlap in time of approximately five to ten days for samples of subsequent testing months in the growth chambers. Samples were removed from the chambers after they reached bud development stage 4, to avoid leaf maturation hormonal influences on other samples as much as possible. Calculations of bud burst percentages and day of bud burst were done, based average data for the samples that reached bud burst only.

Our experiment setup was made similar to a previous study conducted at Ås during the winter seasons of 1989/90 and 1990/91 (Heide 1993). This is the only phenological study on beech growing in Norway known to us. However, the trees used were of central European origin, and might not be representative for the beech populations found in Vestfold and Bergen. Our replicate trees at Ås were from the same populations as the one used in Heide's (1993) study, making it possible to detect any major differences in this provenance.

Frost tolerance experiment

From the 3 replicate trees on each location, we also collected 16 twig samples in each of the sampling months, and tested them for frost tolerance during the dormancy period. The twig samples, each containing two to three vegetative buds, were prepared in bundles and covered with moist quartz sand in open aluminum boxes. The samples were given freezing treatment of temperatures 5 (control), -5, -10, -15, -20, -25, -35 and -45 °C in temperature test chambers (Weiss Umwelttechnik simulationsanlagen, customary made). All treatments followed a basic, stepwise cooling/decooling program, differing only in target temperatures and amount of cooling/decooling:

1) 8 h at -0.5°C

2) Temperature lowered by 3°C/h until -17°C (or higher target temperature)

3) Temperature lowered by 10°C/h until -45°C (or higher target temperature)

4) Target testing temperature for 4 h

5) Temperature increased by 2°C/h until 5°C

6) Temperature maintained at 5°C for 2-3 h.

After finished temperature treatment, the twigs were placed in moist, transparent boxes and placed in natural daylight near windows in room temperature for 5 days. This was done for bud damage to develop on a macro scale and become easily detectable. The relative amount of dead plant tissue in the buds was examined visually by a categorical scale of four distinguishable levels of frost tolerance (0 = bud completely dead, 1 = >50% of bud dead, 2 = <50% of bud dead, 3 = no visible bud damage), (Olsen et al. 1997). The lethal temperature threshold (LT_{50}, the temperature at which 50% of the plant biomass in the buds was killed) was estimated by graphical plotting of the damage distributions (Helleland 2005).

Meteorological data

Temperature data was retrieved from meteorological stations near the study areas (Eklima 2015), and are listed in Appendix 1. For some of the populations, nearby stations had not monthly registrations available and the nearest station with sufficient coverage was used. In Vestfold, one station was used only (station number 27450, Melsom), situated close to the Melsom population and geographically centered among the other populations in the region. In Bergen, the station was approximately 30 km from the population (station number 50540, Bergen – Florida), whereas in Ås it was approximately 4 km from the population (station number 17850, Ås).

Statistical analysis

Calculation of average and SE values was conducted in R version 3.1.1 (R Core Team 2014), as was also the statistical modelling, using cumulative link mixed models (clmm) in the Ordinal package (Christensen 2015). All tables and graphical figures were made in Microsoft©Excel 2010.

Statistical modelling with clmm combined numerical and categorical data, using the described categorical scales for bud development or frost tolerance as response variable (for the field observation/dormancy release experiment or the frost tolerance experiment, respectively). The digits used for the bud categories were shifted compared to the ones described (ranging from 1-5

instead of 0-4), to avoid using 0 as a digit in the statistical modelling. Populations were tested as categorical factors (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling, B: Bergen, Å: Ås), as was also plot type (Beech or Spruce mixture), photoperiod (SD or LD), and temperature (in one model, each tested temperature representing a category). Experiment month and registration day numbers were tested as numerical data, as was also temperature in the general models. Replicate plots or trees were not tested separately in the models, but were included as random factors.

The models compared all tested variables with a given reference population and a given treatment program. The Falkensten population was used as reference population in all models, as this was situated geographically centered among the Vestfold populations, avoiding using populations located at the extremities of the beech distribution range. The Falkensten population was also considered having the most homogenous sapling plots for the field observation study (unmixed beech plots only), enabling us to test for differences from mixed beech and spruce plots at other locations. The treatment program used as reference in the modelling of the dormancy release experiment was the treatment considered as flushing conditions (LD 21°C). In the frost tolerance experiment, the control testing temperature (5°C) was used as reference.

Models testing for general effects were developed for all experiments, in addition to models investigating differences between the populations. In the dormancy release experiment, models were developed separately per testing month, and results from the spring and the autumn months were analyzed as different physiological events (decreasing vs. increasing of the dormant state). For the frost tolerance experiment, models were developed separately per season (spring and autumn), also analyzed as separate physiological events (decreasing vs. increasing of the frost tolerant state).

Results

Field observations

Time of bud burst

The approximate time of bud burst was observed for saplings in the replicate plots in the Vestfold populations (Falkensten, Holmestrand, Melsom and Tjølling) in spring 2014. However, as bud burst happened earlier than we expected, registrations did not start until after visible bud development had already begun at all locations (April 25). At this time, bud burst had already occurred on at least one sapling in all plots but one, and the exact timing of bud burst start was not recorded more precisely than being within the last week before April 25.

Because of this lack of precise bud burst date registrations, comparisons of the bud burst date between the different populations were not possible. However, we observed differences in bud development between different plot types (plots in stands mixed or unmixed with spruce trees) on some locations (Figure 3). All unmixed plots had reached bud burst on all or most of their saplings by April 25, except in one of the Holmestrand plots. All mixed plots were delayed in bud development compared to the unmixed plots at the same location, except one mixed plot in Tjølling where all saplings had also reached bud burst by April 25. In another mixed plot in Tjølling, bud burst had not occurred on any of the saplings at first registration day, but occurred shortly after (Apr 28). By this date, all unmixed plots had reached bud burst, and by May 1, this was so also for all mixed plots.

Statistical testing showed some general differences between the Vestfold populations (Table 3). The model confirms the observed differences between mixed and unmixed plots ($p < 0.001$) and the accelerated bud development by later registration dates ($p < 0.001$). The Holmestrand population were shown to have later bud development than Falkensten ($p < 0.01$), whereas the Melsom and Tjølling populations did not show different bud burst time on a 0.05 level. However, note that the model is based on bud development registrations where important information on differences between the populations is missing, since the actual timing of bud burst was not registered for most replicate plots. Because of this, the results of the model should be interpreted with caution.

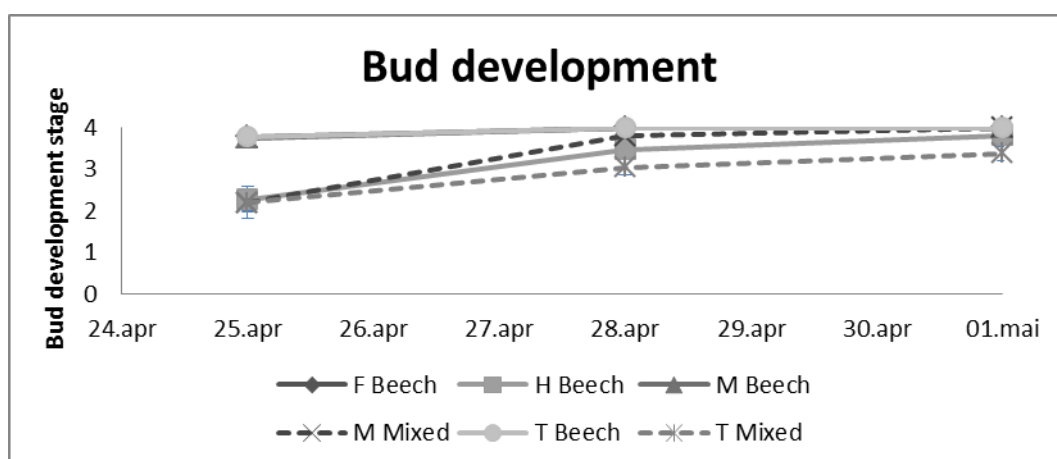**Figure 3.** Average bud development on beech saplings in replicate plots on four Vestfold locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling). The plots were either in unmixed beech stands (Beech) or in mixed stands of beech and spruce (Mixed), values shown here are averages for all plots of the same type from the same location. Bud registrations were conducted between Apr 25 and May 1, 2014.

Table 3. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of increasing registration date (Day of year) and stand characteristics (Spruce mixture) on bud development on beech saplings from four Vestfold locations (H: Holmestrand, M: Melsom, T: Tjølling. The Falkensten population is used as reference). The buds were registered three times during the period Apr 25 to May 1, 2014.

Fixed effects terms	Coefficient	SE	z
Day of year***	0.93208	0.11335	8.223
Location H**	-5.14353	1.77861	-2.892
Location M	-0.01601	1.74791	-0.009
Location T	-0.53234	1.88977	-0.282
Spruce mixture***	-5.41670	1.57534	-3.438
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 4. Total height growth (cm) for beech saplings on four Vestfold locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling) for the growing season of 2014. Plot types are classified as Beech (beech trees dominating in and around plot) or Mixed (beech and spruce mixture in and around plot). The values represent average height growth for similar plot types at each location.

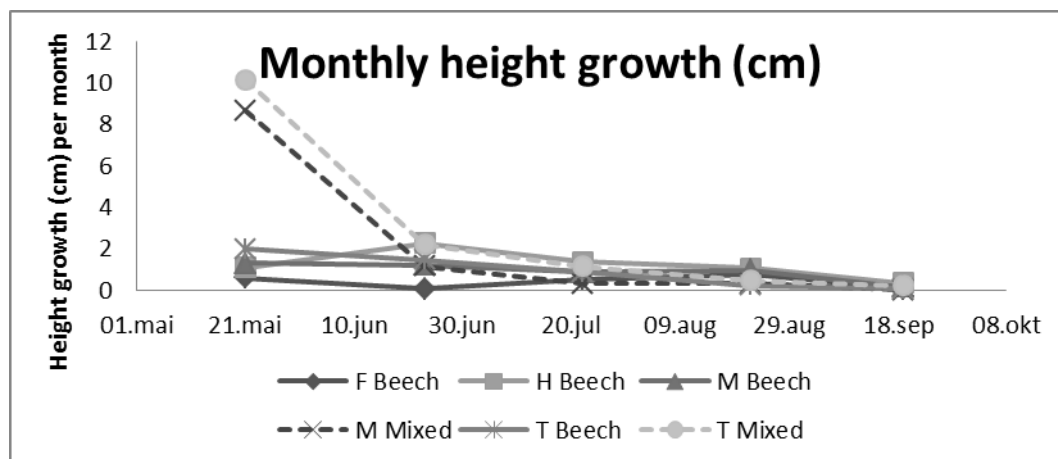| Location/ plot type	Total height growth (cm)
F Beech	2,0
H Beech	6,1
M Beech	4,5
M Mixed	10,5
T Beech	4,6
T Mixed	14,2

Figure 4. Average monthly height growth (cm) for beech saplings on four Vestfold locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling) for the growing season of 2014. Plot types are classified as Beech (beech trees dominating in and around plot) or Mixed (beech and spruce mixture in and around plot). The values represent average height growth for similar plot types at each location. Height measurements were conducted monthly throughout the vegetative season of 2014 (Apr 3 to Sept 19).

Height measurements throughout the growing season

Height growth of the saplings throughout the growing season of 2014 was low (Table 4). Of the 15 replicate plots, three plots showed an average height growth of more than 10 cm, and were all in mixed beech stands. This growth mainly happened during the first two months of the vegetative season (before mid-June), whereas growth through the rest of the vegetative season was almost none in all plots (Figure 4).

Canopy measurements for the plots

The amount of solar radiation transmitted by the canopy was estimated to differ greatly between the time of minimum and maximum canopy cover (late April and late August, respectively), and between the mixed and unmixed beech plots (Figure 5). For unmixed plots, as much as five times the amount of light was estimated to reach the plots in April than in August. Mixed plots were estimated to receive less light in April than unmixed plots: in the case of the Tjølling plots, the amount was only half the amount estimated for the unmixed plots. The mixed plot at Melsom also estimated approximately 30% lower canopy transmittance than the unmixed plots at the same location. However, in August the situation was the opposite, as mixed plots were estimated to receive up to twice the amount of light as unmixed plots.

Temperature measurements throughout the growing season

The temperature measurements for the period prior to the growing season (April 3 to May 1) and the growing season of 2014 (defined from May 2, when all buds had burst, until September 19, when no additional height growth was registered), showed further indications of microclimatic variation (Table 5). The average temperatures for the periods did not vary much between locations or plot types, however, the registered extreme (minimum and maximum) temperature values did.

In April, all plots recorded minor freezing events (down to -2.4°C), and all experienced large temperature fluctuations between day and night temperatures (Figure 6). The maximum temperatures registered in that period were as high as 32.8°C, and for some plots were the April max temperature in fact higher than for the rest of the growing season. The magnitude of the day/night temperature fluctuations varied between the plots, especially between different plot types. Within a location, the temperature difference between the mixed and the unmixed plots could be up to 20°C during daytime, whereas no difference was measured during the night. However, note that the differences between the mixed and the unmixed plots at Melsom and Tjølling are opposite, as Melsom showed largest fluctuations in the unmixed plot, whereas Tjølling showed much larger fluctuations in the mixed plot than in the unmixed. The day/night temperature fluctuations were large at the time around bud burst (between April 20 and 25) in all plots, but the magnitude varied strongly: from as much as 25°C difference in the Holmestrand plot to a difference less than 10°C in the mixed Melsom plot and the unmixed Tjølling plot.

During the growing season, the registered temperatures did not show as much variation between the locations as in April (Table 5). Within the Melsom population, almost no difference was registered between the plot types for the min and max temperatures in this period. In Tjølling, the average and min/max temperatures varied slightly more between the plot types.

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Estimation of timing of dormancy onset

As very little height growth was detectable after June (Figure 4), growth cessation was not possible to use as a measure for the timing of dormancy onset at the end of the vegetative season. However, we visually observed that winter buds had developed on most saplings by the end of August. Together with the observation that no height growth were registered between then and mid-September, we estimated that dormancy onset happened during the last half of August in 2014.
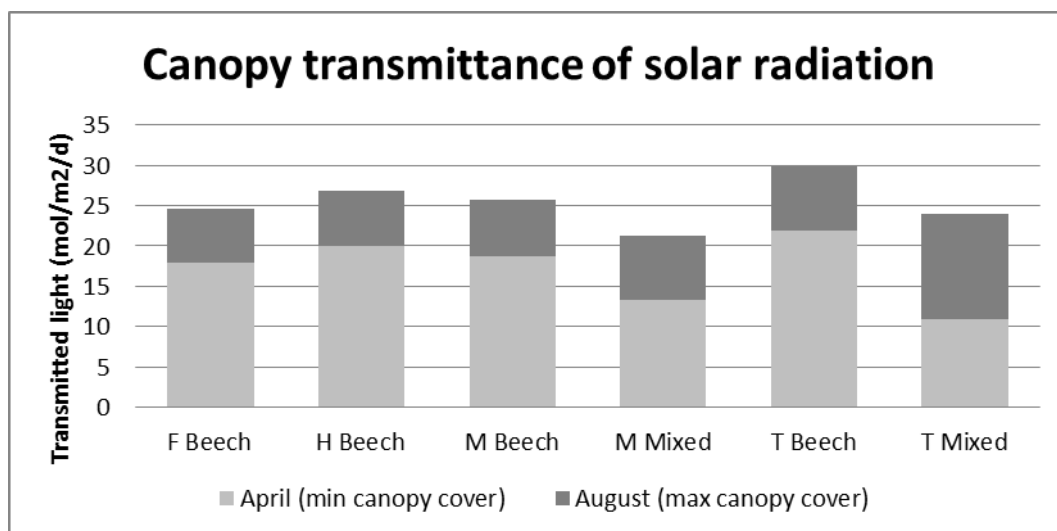

Figure 5. Estimated solar radiation transmitted by the forest canopy (mol photons/m^2/day) above replicate plots on four Vestfold locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling). Plot types are classified as Beech (beech trees dominating in and around plot) or Mixed (beech and spruce mixture in and around plot), the bars represent average values for the plot types at each location. Estimates are calculated for the time of minimum and maximum canopy cover in 2014 (Apr 25 and Aug 20, respectively).

Table 5. Average, minimum and maximum temperatures (°C) for the period prior to the growing season (Apr 3 to May 1) and the growing season (May 2 to Sept 19) of 2014. Temperatures are measured at ground level in one or more of the replicate plots at the Vestfold locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling). Plot types are classified as Beech (beech trees dominating in and around plot) or Mixed (beech and spruce mixture in and around plot).

	April			Growing season		
Location	Avg. Temp (°C)	Min Temp (°C)	Max Temp (°C)	Avg. Temp (°C)	Min Temp (°C)	Max Temp (°C)
F Beech	8.4	-2.1	26.9	14.8	3.9	30.4
H Beech	7.1	-2.4	32.8	13.4	1.4	28.4
M Beech	7.9	-1.4	29.1	14.2	3.2	25.0
M Mixed	7.0	-0.1	17.3	14.3	3.9	26.1
T Beech	7.1	-0.4	27.4	14.3	2.7	27.0
T Mixed	8.8	-2.0	32.2	13.9	2.3	35.7

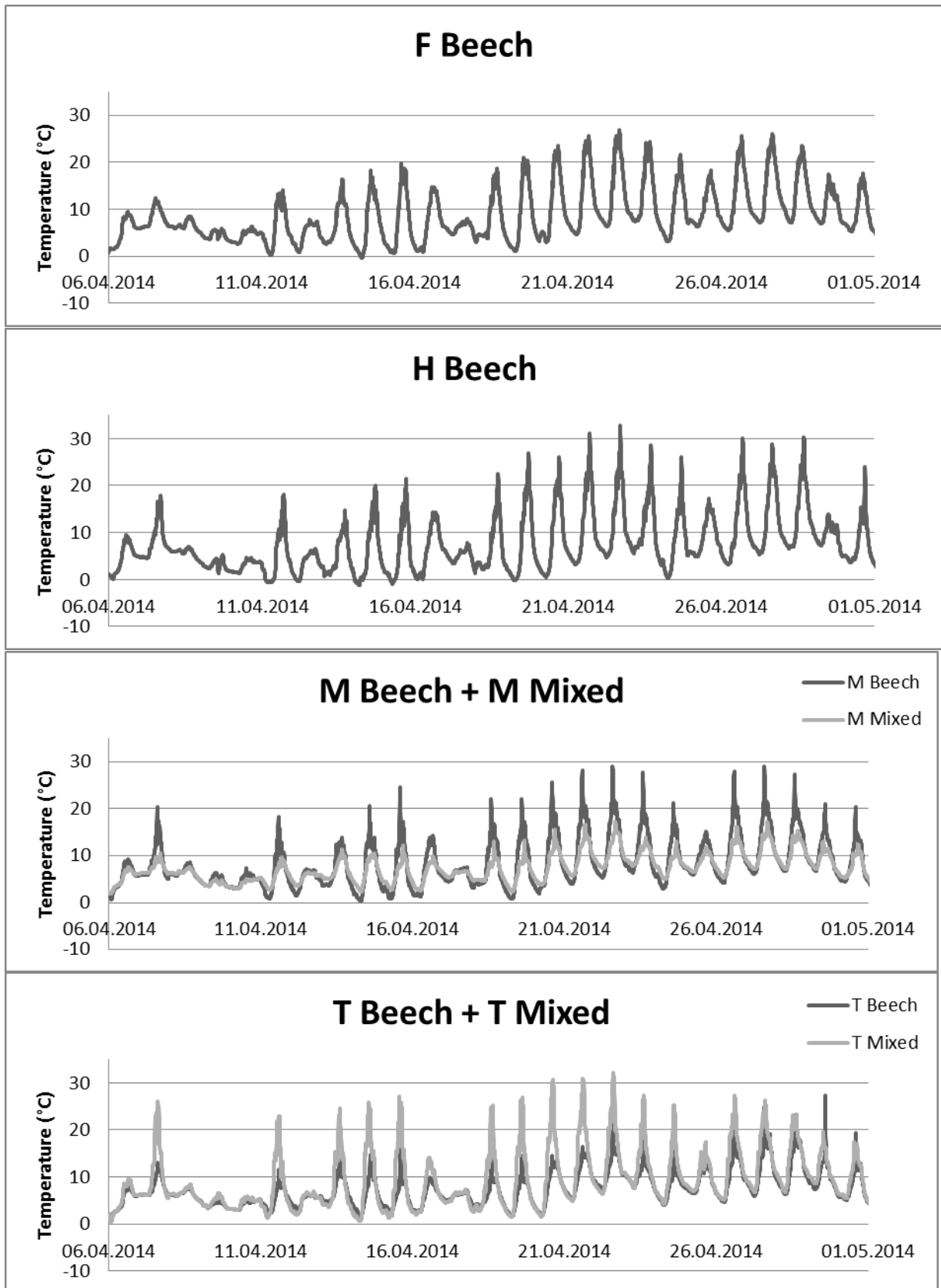**Figure 6.** Temperatures measured at ground level in selected plots at four Vestfold locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling). Measurements are done with 10 minute intervals in the period Apr 3 to May 1, 2014. Plot types are classified as Beech (beech trees dominating in and around plot, dark gray lines) or Mixed (beech and spruce mixture in and around plot, light gray lines).

Dormancy release experiment

Bud burst as affected by date of intake and photoperiod

We registered bud development and the number of days until bud burst for cuttings sampled once per month in January, February, March, October, November and December 2014 (Table 6). The percentage of samples reaching bud burst under flushing conditions (LD 21°C) varied between the testing months, and was mainly high for the spring months (January, February, March) and low for the autumn months (October, November, December). A significant delaying effect of short photoperiod (SD) was shown for all buds sampled during spring ($p < 0.001$, Tables 7-9). No significant difference between SD and LD exposure were found when tested on the buds sampled during the autumn months (Tables 10-12), but this could be due to the general low bud burst percentage for both treatments (Table 6) as much as a lack of effect.

The number of days that the samples were exposed to flushing conditions before bud burst occurred varied strongly between testing months (Table 6). The samples from the spring intake showed a decline in time until bud burst for each month, averaging from 23 d in January to 14 d in March. The decrease was even stronger for the SD 21°C samples, declining from an average of 45 d in January to no difference from the LD samples (13 d) for the March samples. However, only a very low percentage (7 and 4% of the January and March samples, respectively) reached bud burst within the testing period under SD conditions.

The samples harvested during the autumn months all needed 29 d or more under flushing conditions to reach bud burst, no difference was observed between the months (Table 6). None of the autumn SD samples reached bud burst within the testing period.

Table 6. Percentage bud burst (average for all populations) and number of days until bud burst ± SE as affected by date of intake and short and long photoperiod (SD and LD, respectively) for the buds exposed to 21°C for >30 d. n = 9 cuttings for each of the 6 populations (N = 54 in total per treatment), except for the Jan intake, where only 5 populations were tested (N = 45). For experiment months where no buds reached bud burst within the experiment period, a > sign is noted.

Date of intake	Bud burst (%)		Days to bud burst (d)	
	SD	LD	SD	LD
15 Jan	6.7 ± 4.1	84.4 ± 8.3	44.5 ± 1.6	23.1 ± 1.2
15 Feb	0.0 ± 0.0	61.1 ± 13.0	>30	19.5 ± 1.1
15 Mar	3.7 ± 2.3	79.6 ± 7.8	13.0 ± 0.6	13.5 ± 0.9
15 Oct	0.0 ± 0.0	13.0 ± 5.3	>38	29.4 ± 1.8
15 Nov	0.0 ± 0.0	3.7 ± 2.3	>35	33.5 ± 0.9
15 Dec	0.0 ± 0.0	25.9 ± 8.4	>32	30.4 ± 0.8

Table 7. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from five locations (Falkensten, Holmestrand, Melsom, Tjølling and Bergen). The buds were sampled in January 2014.

Fixed effects terms	Coefficients	SE	z
Photoperiod (SD)***	-6.33228	0.39641	-15.97
Experiment day***	0.40397	0.02548	15.86
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 8. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from six locations (Falkensten, Holmestrand, Melsom, Tjølling, Bergen and Ås). The buds were sampled in February 2014.

Fixed effects terms	Coefficients	SE	z
Photoperiod (SD)***	-6.10680	0.24234	-25.20
Experiment day***	0.25597	0.01044	24.52
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 9. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from six locations (Falkensten, Holmestrand, Melsom, Tjølling, Bergen and Ås). The buds were sampled in March 2014.

Fixed effects terms	Coefficients	SE	z
Photoperiod (SD)***	-4.895384	0.208466	-23.48
Experiment day***	0.172651	0.009519	18.14
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 10. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from six locations (Falkensten, Holmestrand, Melsom, Tjølling, Bergen and Ås). The buds were sampled in October 2014.

Fixed effects terms	Coefficients	SE	z
Photoperiod (SD)	-26.2939	484.3166	-0.054
Experiment day***	0.2440	0.0474	5.147
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 11. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from six locations (Falkensten, Holmestrand, Melsom, Tjølling, Bergen and Ås). The buds were sampled in November 2014.

Fixed effects terms	Coefficients	SE	z
Photoperiod (SD)	-25.4273	1677.7521	-0.015
Experiment day	0.5928	0.3750	1.581
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 12. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from six locations (Falkensten, Holmestrand, Melsom, Tjølling, Bergen and Ås). The buds were sampled in December 2014.

Fixed effects terms	Coefficients	SE	z
Photoperiod (SD)	-26.94835	375.14997	-0.072
Experiment day***	0.31708	0.04813	6.587
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Bud burst as affected by temperature and photoperiod

The effect of temperature on the thermal time was investigated for the cuttings sampled in March, October, November and December. Very little bud burst occurred with low temperatures and short photoperiod in all the tested months (Figure 8, for details, see Appendix 2).

The March samples showed high percentage of bud burst for the LD experiments conducted under 15 and 21°C, whereas the amount was low in the LD 9°C experiment (Table 13). Note that the bud burst percentage was not 100 for the samples subjected to flushing conditions (LD 21°C), and was in fact slightly lower than that of the samples tested at LD 15°C (80 vs. 85%, respectively). For the SD samples, only 21°C resulted in bud burst during the 33 day testing period, and less than 4% of the samples reached bud burst.

The different temperatures tested on the March samples strongly affected the number of days until bud burst (Tables 13 and 14). Higher temperature reduced the number of days until bud burst ($p < 0.001$). The SD samples did not experience shortened bud burst time under 9 and 15°C, but showed no difference from the LD samples when tested under 21°C (Table 13). A decrease in time until bud burst was also observed for the LD 9°C samples compared to the SD 9°C.

For the autumn months, none of the samples tested in 9 and 15°C reached bud burst, whereas some few of the LD 21°C samples collected in October and December did (Figure 8, for details, see Appendix 2).

Table 13. Effects of temperature (°C) and short and long photoperiod (SD and LD, respectively) on bud burst in buds harvested approx. 15 Mar 2014. Bud burst (%) shows the average percentage of samples reaching bud burst ± SE per population. Days to bud burst shows the average number of days until bud burst ± SE for the samples that reached bud burst. n = 9 cuttings for each of the 6 populations (N = 54 in total per treatment). For experiment months where no buds reached bud burst within the experiment period, a > sign is noted.

Temperature (°C)	Photoperiod	Bud burst (%)	Days to bud burst (d)
9	SD	0.0 ± 0.0	>33
	LD	25.9 ± 11.0	31.1 ± 1.1
15	SD	0.0 ± 0.0	>33
	LD	85.2 ± 6.8	19.9 ± 1.1
21	SD	3.7 ± 2.3	13.0 ± 0.6
	LD	79.6 ± 7.8	13.5 ± 0.9

Table 14. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of low temperature (9 and 15°C), shortened photoperiod (SD) and duration of experiment (Experiment day) in relation to flushing conditions (long photoperiod, 21°C) on beech bud samples from all locations. The buds were sampled in March 2014.

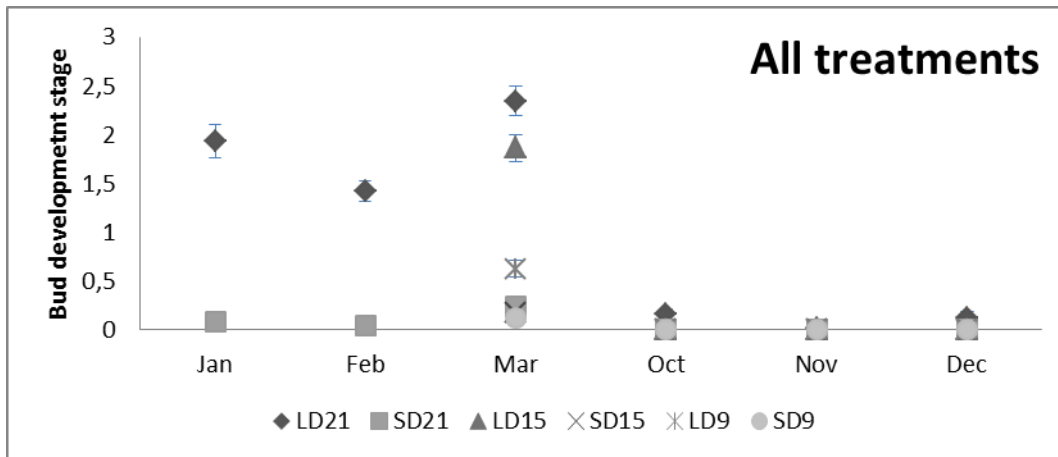| Fixed effects terms	Coefficients	SE	z
Photoperiod (SD)***	-4.920393	0.134282	-36.642
Experiment day***	0.235990	0.006632	35.581
Temp 9°C ***	-3.215509	0.122988	-26.145
Temp 15°C ***	-0.779443	0.093926	-8.299
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Figure 7. Average bud development (all populations) ± SE for all treatment programs (LD: Long photoperiod, SD: Short photoperiod, testing temperatures of 9, 15 or 21°C.) for each of the testing months (Jan, Feb, Mar, Oct, Nov, Dec) of 2014.

Variation in bud development between populations

Spring experiments

The different photoperiod and temperature treatments were tested for all Vestfold populations (Holmestrand, Falkensten, Melsom and Tjølling) and the Bergen population for all winter months of 2014 (January, February, March, October, November, December), while the Ås population lacked from the January intake. All populations mainly followed the same pattern of decreasing bud burst time with later intake date during the spring, when tested under LD 21°C (Figure 9). The samples tested under SD 21°C showed little bud burst for all populations (Figure 10).

The variation between the populations was in general low. In the January and February experiments (Tables 15 and 16) Tjølling showed earlier bud development than the other populations (p < 0.05 and < 0.01, respectively), whereas Melsom seemed to be slightly slower (not significant on a 0.05 level). In March (Table 17), Tjølling also indicated slightly earlier development, however not statistically significant (p > 0.05). There was not shown any general pattern of difference between the Vestfold populations and the Bergen or Ås populations, or any pattern within Vestfold.

Autumn experiments

As mentioned, very few of the samples harvested during the autumn months reached bud burst within the experiment period (Appendix 2), and no differences in bud development between populations were detectable with our statistical testing methods. The average bud development plots for the LD 21°C testing (Figure 11) show slight increases for the Melsom population in October and the Bergen population in December towards the end of the experiment period. However, this applied to a very low percentage of the samples from those populations, and no statistically significant differences were found.

Table 15. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from four locations (B: Bergen, H: Holmestrand, M: Melsom, T: Tjølling) in relation to the Falkensten population. The buds were sampled in January 2014.

Fixed effects terms	Coefficient	SE	z
Location B	0.84834	0.73572	1.153
Location H	0.51942	0.73275	0.709
Location M	-0.25517	0.73549	-0.347
Location T*	1.65852	0.73912	2.244
Photoperiod (SD)***	-6.33307	0.39642	-15.976
Experiment day***	0.40494	0.02549	15.887
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 16. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from five locations (B: Bergen, H: Holmestrand, M: Melsom, T: Tjølling, Å: Ås) in relation to the Falkensten population. The buds were sampled in February 2014.

Fixed effects terms	Coefficient	SE	z
Location B	0.97176	0.74931	1.297
Location H	0.79166	0.75061	1.055
Location M	-1.28749	0.76063	-1.693
Location T**	1.95446	0.75036	2.605
Location Å	0.13878	0.74932	0.185
Photoperiod (SD)***	-6.10963	0.24229	-25.216
Experiment day***	0.25603	0.01044	24.530
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 17. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of low temperature (Temp 9 and 15°C), shortened photoperiod (SD) and duration of experiment (Experiment day) on beech bud samples from four locations (B: Bergen, H: Holmestrand, M: Melsom, T: Tjølling, Å: Ås) in relation to the Falkensten population. The buds were sampled in March 2014.

| Fixed effects terms	Coefficients	SE	z
Location B	-0.528020	0.646891	-0.816
Location H	0.074308	0.646646	0.115
Location M	-0.080131	0.646105	-0.124
Location T	0.697832	0.646402	1.080
Location Å	-0.584558	0.647249	-0.903
Photoperiod (SD)***	-4.920905	0.134289	-36.644
Temp 9°C ***	-3.216661	0.122995	-26.153
Temp 15°C ***	-0.780133	0.093927	-8.306
Experiment day***	0.235988	0.006633	35.580
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			
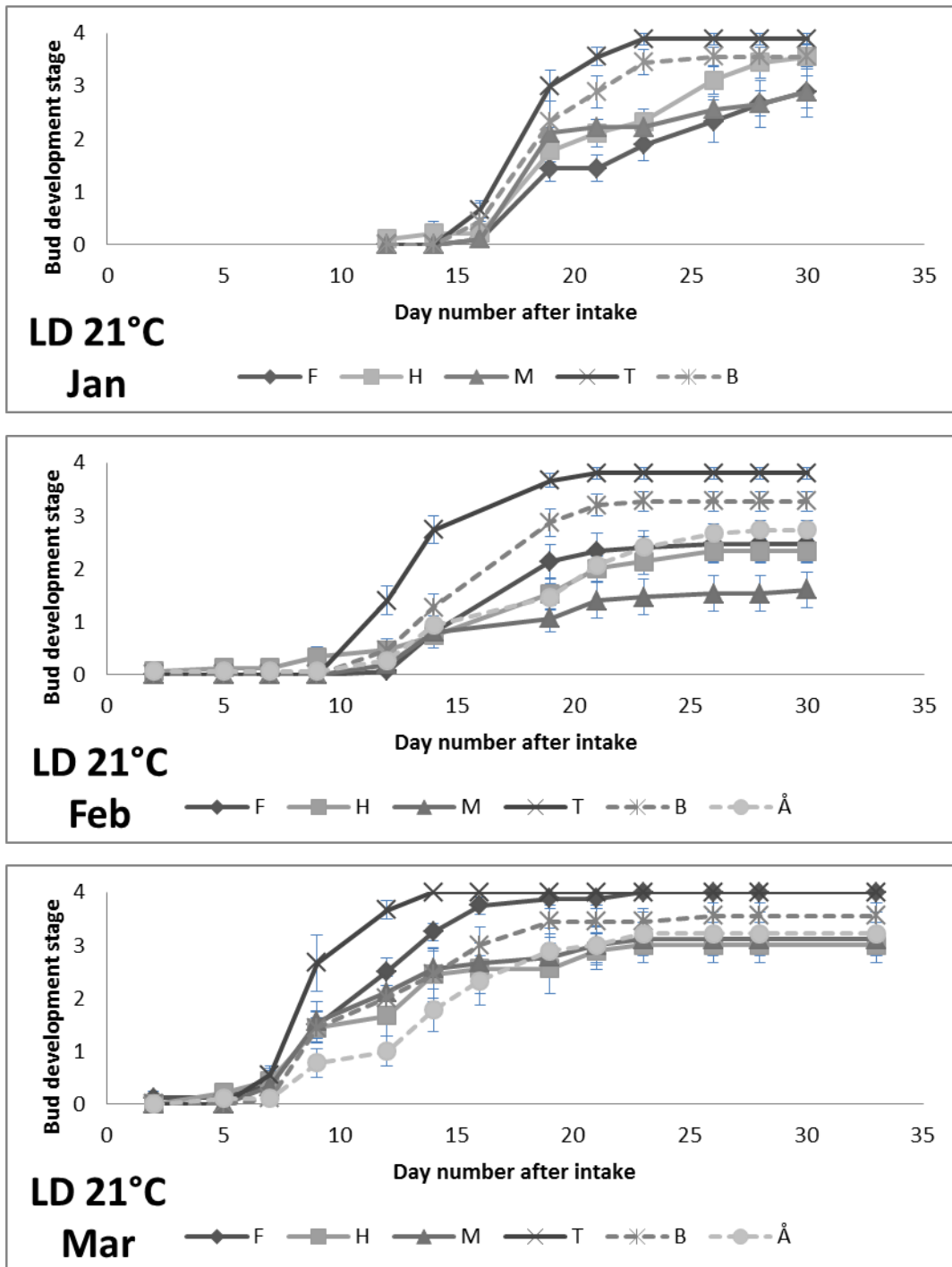
Figure 8. Average bud development ± SE per tested population under long photoperiod (LD; photoperiod 24 h) and 21°C for cuttings harvested in the spring months (Jan, Feb. Mar) of 2014. The Vestfold populations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling) are marked with whole lines, whereas the Bergen (B) and Ås (Å) populations are marked with stapled lines.
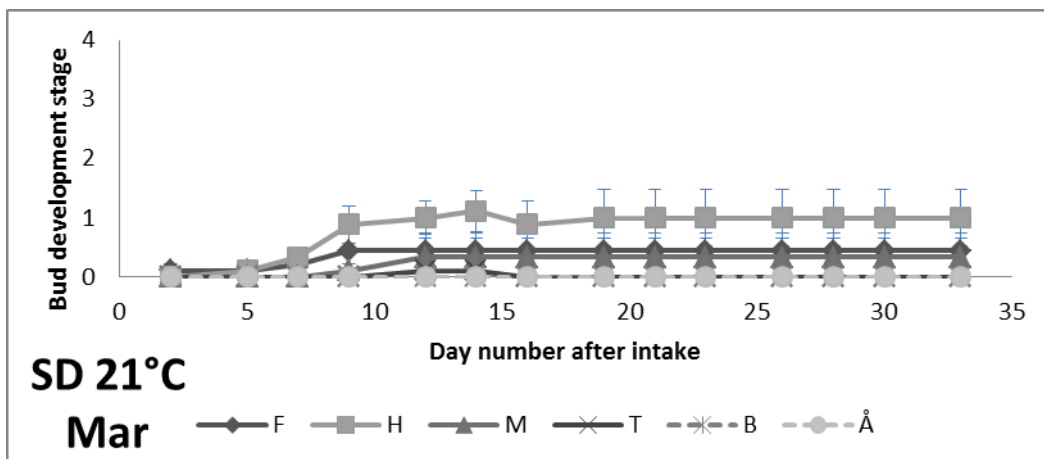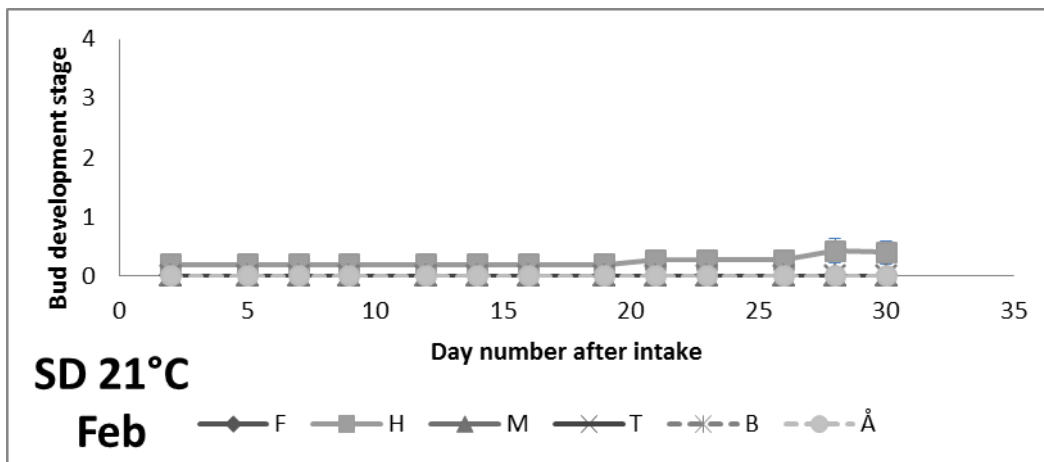
Figure 9. Average bud development ± SE per tested population under short photoperiod (SD; photoperiod 8 h) and 21°C for cuttings harvested in the spring months (Jan, Feb. Mar) of 2014. The Vestfold populations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling) are marked with whole lines, whereas the Bergen (B) and Ås (Å) populations are marked with stapled lines.
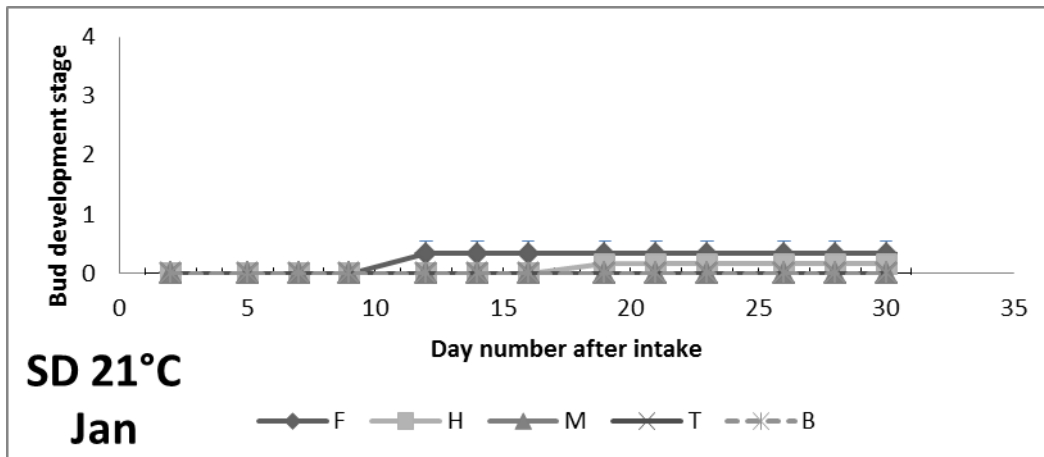
Figure 10. Average bud development ± SE per tested population under long photoperiod (LD; photoperiod 24 h) and 21°C for cuttings harvested in the autumn months (Oct, Nov, Dec) of 2014. The Vestfold populations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling) are marked with whole lines, whereas the Bergen (B) and Ås (Å) populations are marked with stapled lines.
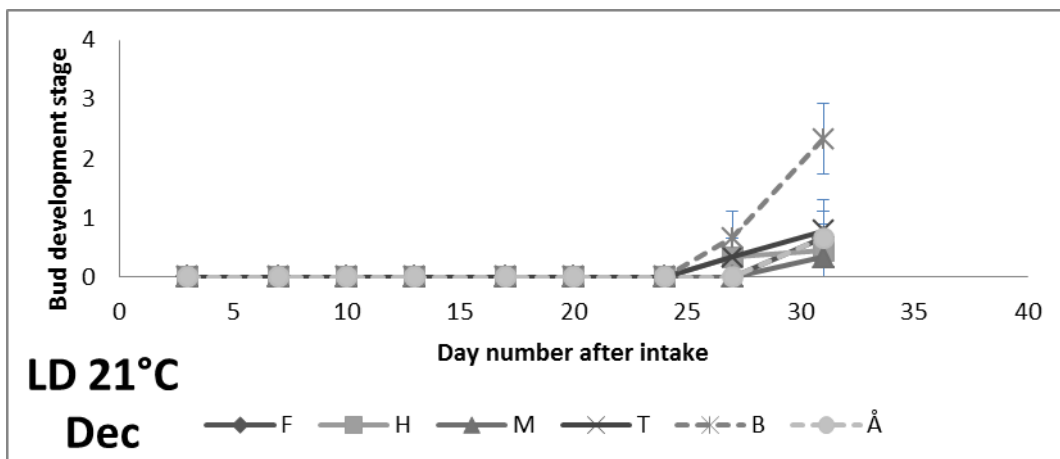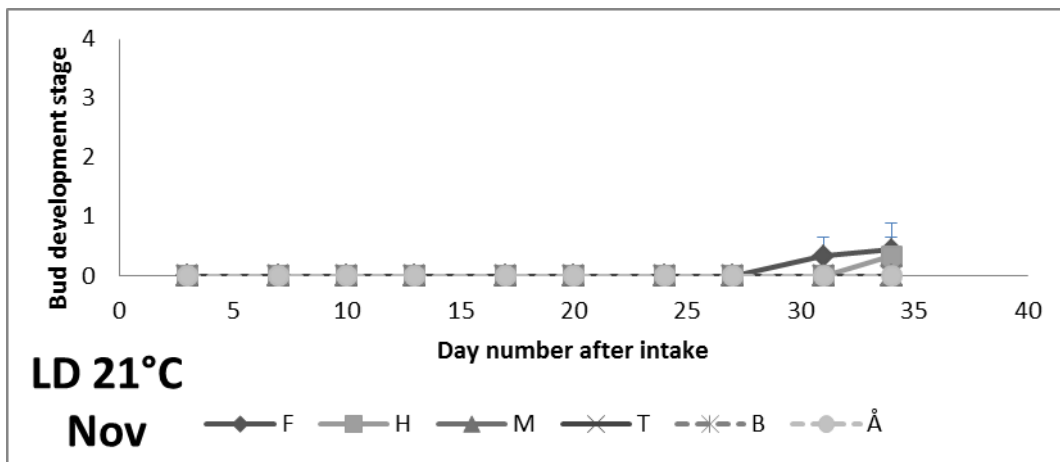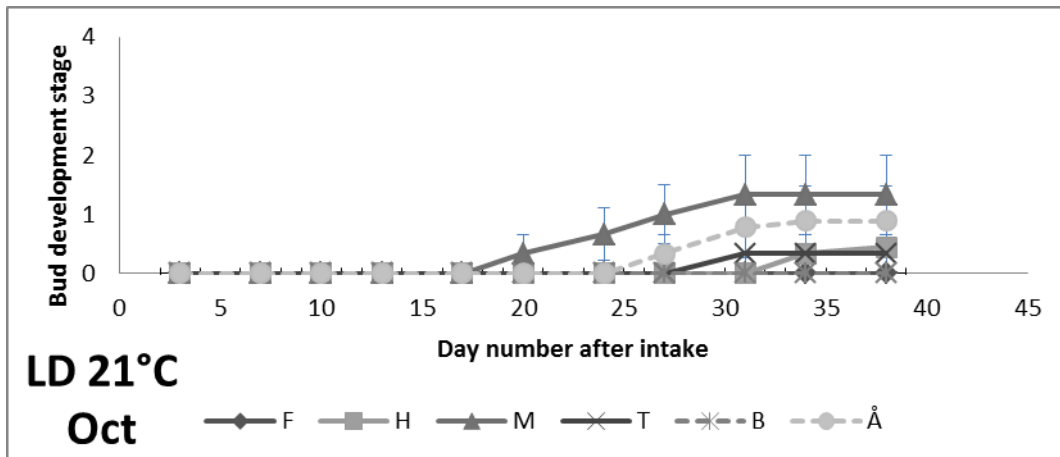
Frost tolerance experiment

Variation in frost tolerance throughout the dormancy period

Frost tolerance of the buds was found to vary between the testing months (Figure 12). The average LT_{50}-level, marked in the plot as a horizontal line, was lowest in January and February (approx. -20°C) and highest in October (approx. -10°C). In March, November and December, average LT_{50} was estimated to be approximately -15°C. Note that the testing months are all within the same calendar year (2014), and hence collected during two different winter seasons. Caution should be shown when comparing these results, as dormancy characteristics can vary between winter seasons. However, the results seem to indicate a gradual increase of frost tolerance during the autumn months (LT_{50}-levels decreasing), and a reversed process of tolerance reduction during the winter and early spring (LT_{50}-levels increasing).

In concurrence with the average results. statistical testing showed a positive relationship between increased testing temperature and frost tolerance (and conversely: lowered temperature and reduced frost tolerance) of the buds in both the spring and the autumn experiments, with significance levels of $p < 0.001$ (Tables 18 and 19). For spring, frost tolerance was reduced with later intake month ($p < 0.001$, Table 18), whereas it increased through autumn ($p < 0.001$, Table 19).

Tested separately (for the spring months only), all temperatures from -10°C and below gave significantly reduced frost tolerance ($p < 0.05$ for -10°C and <0.001 for lower temperatures, Table 20). Comparison of the estimated parameter coefficients indicate strongest decline of the frost tolerance between -10 and -15°C. Testing with -5°C did not give significantly reduced frost tolerance compared to the 5°C control on a 0.05 level.

Testing each temperature separately was not possible for the autumn data with our used statistical method, as the models were not able to calculate parameter estimates from the data. This was possibly due to too little variation in frost tolerance between the autumn testing months.

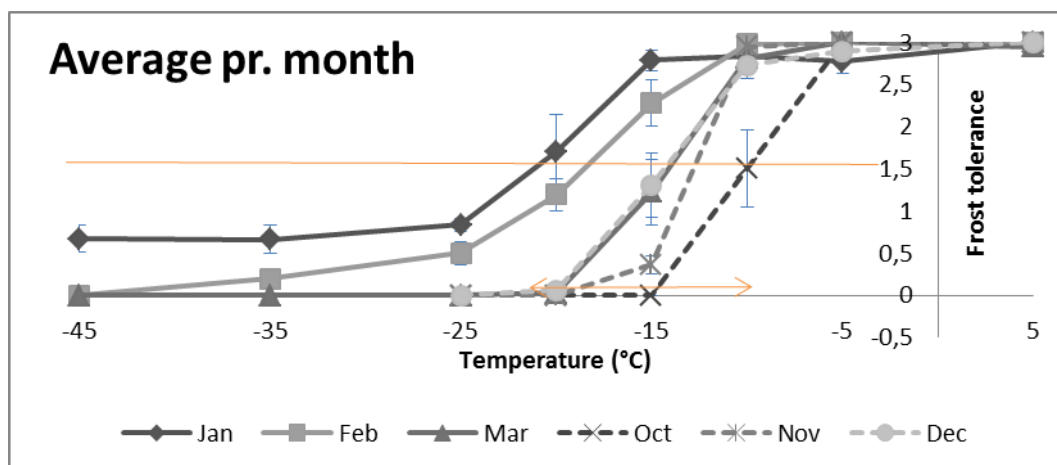**Figure 11.** Average values for frost tolerance ± SE (3 = undamaged, 0 = killed) with lowered testing temperature (°C) for all winter months of 2014 (whole lines: Jan, Feb, Mar, stapled lines: Oct, Nov, Dec). Bud damage value = 1.5 defines the Lethal Temperature 50 (LT50, the temperature level at which 50% of the bud biomass was killed), and is indicated with a horizontal line.

Table 18. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of temperature and increasing intake month on frost tolerance in buds sampled at all locations. Intake time in mid Jan, Feb and Mar, 2014.

Fixed effects terms	Coefficient	SE	z
Temperature***	0.22815	0.01273	17.92
Intake month***	-1.49399	0.13512	-11.06
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 19. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of temperature and increasing intake month on frost tolerance in buds sampled at all locations. Intake time in mid Oct, Nov and Dec, 2014.

Fixed effects terms	Coefficient	SE	z
Temperature***	0.70415	0.06074	11.594
Intake month***	1.14658	0.21152	5.421
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Table 20. Parameter estimates, SE and z-values for covariates in the cumulative link mixed model run to investigate the effects of lowered temperatures (-5, -10, -15, -20, -25, -35 and -45°C compared to control temperature 5°C) and increasing intake month on frost tolerance in buds sampled at all locations. Intake time in mid Jan, Feb and Mar, 2014.

Fixed effects terms	Coefficient	SE	z
Location B	0.4404	0.3506	1.256
Location H*	-0.7357	0.3551	-2.072
Location M*	-0.8590	0.3503	-2.452
Location T	0.3571	0.3476	1.027
Location Å	-0.3362	0.4043	-0.832
Temperature -45°C ***	-10.6843	0.8781	-12.167
Temperature -35°C ***	-10.1864	0.8620	-11.817
Temperature -25°C ***	-9.3290	0.8365	-11.153
Temperature -20°C ***	-7.7765	0.7992	-9.731
Temperature -15°C ***	-5.1074	0.7753	-6.587
Temperature -10°C *	-1.7845	0.8160	-2.187
Temperature -5°C	-1.0165	0.8678	-1.171
Intake month***	-1.8856	0.1626	-11.597
Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.			

Variation in frost tolerance between populations

The buds sampled in the populations Holmestrand and Melsom showed lower frost tolerance in the spring experiments than the Falkensten population (p < 0.05; Table 20, Figure 13), as did also the Ås population (not significant on a 0.05 level). The Tjølling and Bergen populations indicated slightly higher frost tolerance than Falkensten, although not significant on a 0.05 level. Differences between the populations for the autumn data were not detectable when testing with a cumulative link mixed model, likely due to too little variation in frost tolerance between the autumn testing months (Figure 14).

Graphical interpretation of the monthly plots (Figures 13 and 14) indicate minor differences in LT_{50}-levels between the populations, marked as LT_{50} ranges on the temperature axis in each of the plots. The range widths illustrate the variation, and are spanning over approx. 5° for all months except February and November, where the intervals are larger and smaller, respectively. However, these ranges are not tested statistically, and are mainly included as an indication of a more complex pattern of variation between the populations than what is shown in the statistical analysis covering the seasons as a whole.
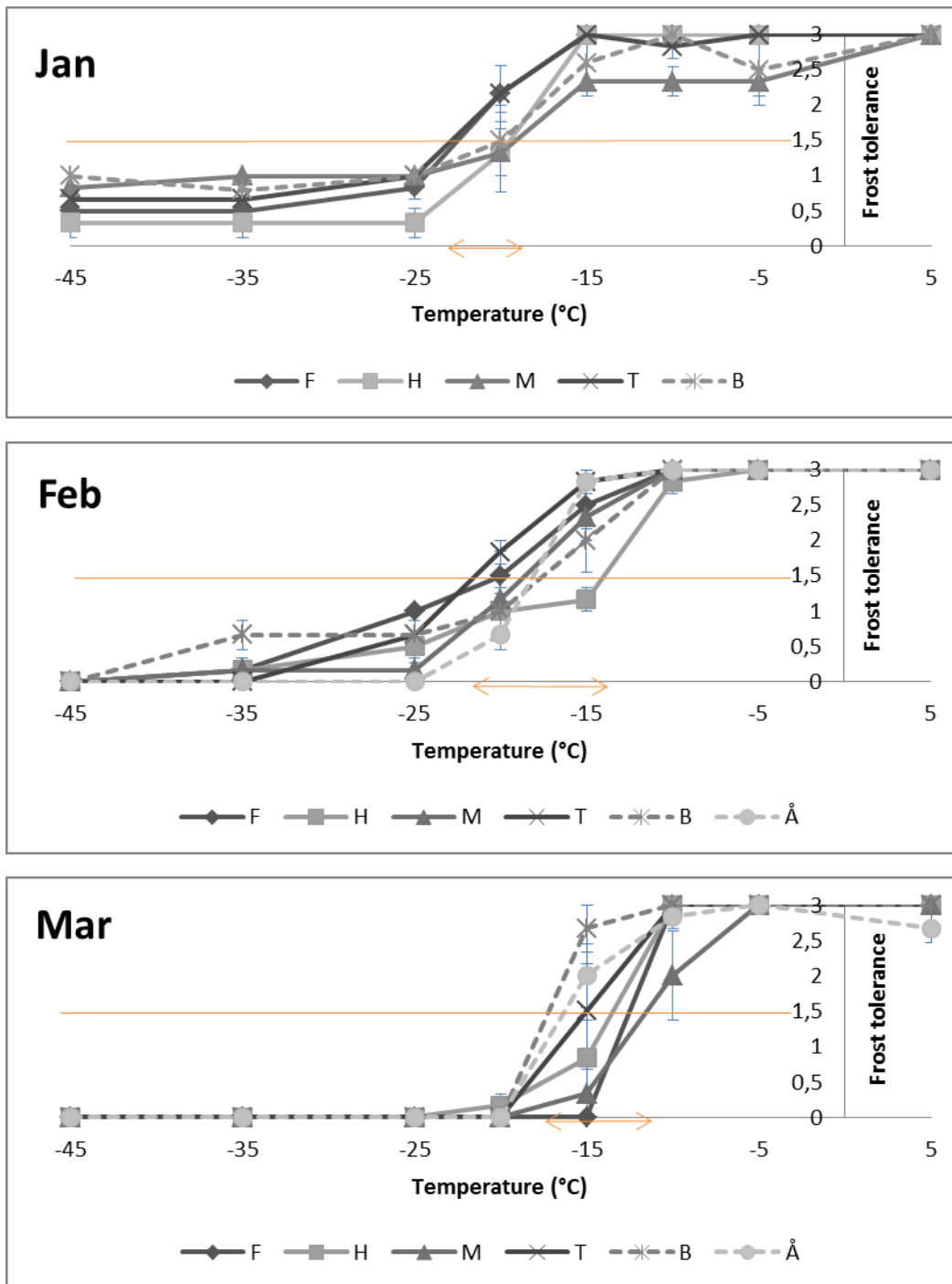
Figure 12. Average values for frost tolerance ± SE (3 = undamaged, 0 = killed) with lowered testing temperature (°C) for the spring months of 2014 (Jan, Feb, Mar). Buds are sampled on six locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling, B: Bergen , Å: Ås). Bud damage value = 1.5 defines the Lethal Temperature 50 (LT50, the temperature level at which 50% of the bud biomass was killed), and is indicated with a horizontal line. The approximate LT50 temperature intervals for all populations are marked on the temperature axis.

Figure 13. Average values for frost tolerance ± SE (3 = undamaged, 0 = killed) with lowered testing temperature (°C) for the autumn months of 2014 (Oct, Nov, Dec). Buds are sampled on six locations (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling, B: Bergen , Å: Ås). Bud damage value = 1.5 defines the Lethal Temperature 50 (LT50, the temperature level at which 50% of the bud biomass was killed), and is indicated with a horizontal line. The approximate LT50 temperature intervals for all populations are marked on the temperature axis.
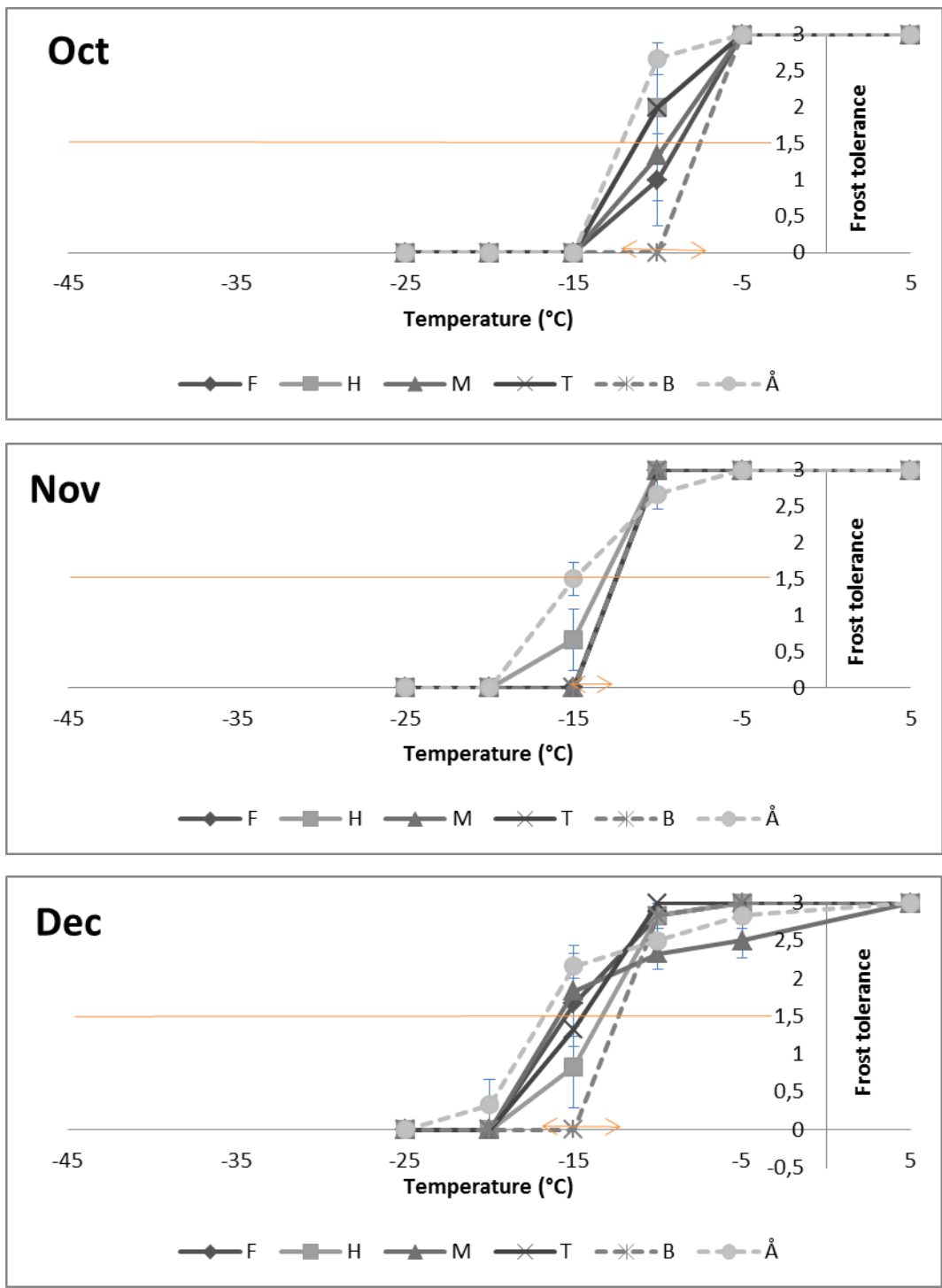
Discussion

Field observations

Time of bud burst

Bud burst was shown to have started in all Vestfold populations by Apr 25 in the growing season of 2014. The early bud burst time observed was unexpected to us, and led to imprecise bud burst date registrations which lacked information on potential population differences. Related to the various sources of previous bud burst dates, although of varying scientifically quality, it can be assumed that bud burst happened approximately two weeks earlier in 2014 than what had been considered normal during the last decades. In light of beech being explicitly stated as a late-flushing tree species 25 years ago and known to show little variation in bud burst date between years (Heide 1993), the bud burst time observed in 2014 in our study is indeed interesting. Similarly early bud burst has been recorded for the current (2015) spring in all the Vestfold populations used in our study (Strømme, pers. comm.).

Related to our investigation of photoperiod and temperature influence on bud burst time in the dormancy removal experiment, the results are also interesting. No specific estimations were done for chilling duration during the autumn season prior to neither Heide's (1993), nor our experiment, and the difference in chilling state of the buds used in the two is uncertain. There was insufficient meteorological information about the 1990 season available to compare properly. Prior to our bud burst registrations in 2014, temperature average values from nearby meteorological stations indicated mild autumn months (with temperatures above the 1961-1990 normal in the period September to December) both in Vestfold, Bergen and Ås (Appendix 1). This was also seen prior to the current spring (2015). For both seasons, spring temperatures were also found to be above the normal values for all months (January to April). These values are monthly averages only, and further details about the periods are not analyzed. Hence, they are not sufficient for conclusions about the impact on the chilling process in the buds occurring at the same time. However, they seem to indicate that the degree of chilling has been lower the recent years than what was seen some decades ago.

Bud burst variation between plots

We observed a slight delay in bud development for beech stands with spruce mixture. However, this was not consistent in all mixed plots, and also one unmixed plot at one location showed delayed bud burst. As mentioned, an experimental setup with both mixed and unmixed plots in more than two of the populations would be necessary to draw conclusions about general differences between plot types. However, our results indicate that small differences in bud burst time can be observed between some plots. As shown, microclimate and light conditions could vary greatly between different plot types (Figure 6; Figure 7), especially at the time before bud burst, and it must be expected that buds in different plots have experienced differences in temperature and light amount during the dormancy period. As mixed plot types generally received less heat and light before the vegetative season, buds here might have been subject to slight delays in the time of thermal activation and/or reduced accumulated thermal sums compared to the more exposed beech plots, which might be an explanation for the observed variation in bud burst dates. However, sufficient estimations of differences in light or temperature exposure were not done to conclude generally about the different plot types.

Plot differences in the growing season

As shown, height growth, canopy transmittance of solar radiation and local temperatures were found to vary somewhat greatly between the plots also during the growing season (Figures 3-5). Sapling height growth was low in all unmixed plots, and relatively high in mixed plots. This might be due to microclimatic differences, e.g. more received light through the growing season in the mixed plots. However, caution should be shown when studying one growing season only, as no reference of previous years' growth and growth differences are known.

It should be noted that the summer of 2014 experienced a long period of high temperatures and little precipitation (Appendix 1), resulting in drought conditions by the end of July in most of the Vestfold area. The extent of this was not studied thoroughly in the study plots, but it is likely that drought might have limited sapling growth in some or all of the plots during this period. In addition, drought conditions might have triggered early bud set and started leaf fall during this period. This suspicion advices caution when interpreting the results from the canopy cover estimations, as the time chosen as maximum canopy reference (August 20) might already be subject to started leaf fall. However, the comparative study of the plots is all based on measurements done on similar study days, and the relative differences between the plots might not have been disproportionally affected.

Dormancy release experiment

Spring experiment

Photoperiod control
Interpretation of the spring results proved somewhat difficult, as several findings were not as expected according to previous research and general assumptions about the dormancy state. Firstly, the observed amount (percentage) of bud burst was not following any clear pattern through the testing months. We found that bud burst mainly happened under exposure to LD, except for some few SD 21°C samples in January and March (Table 6). The bud burst percentages under LD were not following an increasing trend through the testing months, as the percentage in January was higher than in February, and also slightly higher than what it was in March. This was unexpected, as buds were thought to be in a deeper dormant state in January than later in the spring, and hence would require a longer period under flushing conditions to accumulate the necessary forcing temperature.

Very few of the samples tested under SD reached bud burst (Table 6; Figure 8). This indicates that there was an inhibition of dormancy release until a certain day length, even if the temperature was high (21°C). This is in line with a theory of a photoperiod control that is activating the thermal accumulation after a certain day length (Vitasse & Basler 2013). The thermal activation was most likely not acquired by the SD buds within the experiment period, with the exception of the few mentioned observations in the January and March experiments.

Unexpected results
The observed bud burst under SD in January (Table 6) was particularly unexpected, as these samples were thought to be both inhibited by SD and having acquired a very low forcing temperature level so early in the winter, and therefore would be least likely to acquire dormancy release. As no bud burst was found under SD the following experiment month (February), and the percentage in March was lower than what it was in January, this strongly indicates that the January samples were influenced by an external factor that was not present in the later months. As discussed in a later section, there

was a possibility of presence of plant hormonal maturation gases during the January experiment, which might explain the unexpected high amount of bud burst observed both in the SD and LD experiments that month. Nothing indicates that this might have had an effect during the other spring months, especially since the February experiment showed no bud burst under SD. Therefore, this might have been an influence during the January experiment only, or a different explanation is needed for the January results.

The observed bud burst under SD in March (Table 6; Table 13) was also unexpected, as the photoperiod control is assumed to prevent dormancy release until a certain day length is detected. This indicates that the required day length had already been detected by some few buds prior to the March intake, and these buds had already acquired sensitivity to forcing temperature by the experiment start. However, this was unexpected, as day length was not varying much between the populations. It seems unlikely that a required day length threshold have been passed for only a few samples at geographically separate locations (Holmestrand and Melsom). It might be possible that the populations had different photoperiod threshold levels.

The samples reaching bud burst were only representing two different replicate trees, and interpreting findings based on such few observations is dubious. Interestingly, the two samples reached bud burst in no longer time than what was observed for the LD 21°C samples in March (13 days), whereas no other SD 21°C samples reached bud burst within the 30 day testing period. This observation is difficult to explain. It might add further evidence to the suspicion of external factors influencing bud burst. However, this is hard to prove as no similar observations were found in February. It might also be that some buds responded differently due to environmental factors at the site of some of the replicate trees, e.g. differences in cold exposure leading to variation of chilling of the buds during autumn, differences in light exposure, etc., as hypothesized for the different plot types in the field study (previous section).

Chilling state of the buds

In addition to the unexpected observations, bud burst percentages were different from what was observed by Heide (1993), leading to an assumption about differences in the amount of chilling of the buds prior to his experiment and ours. Both the February and March LD experiments resulted in higher bud burst in Heide's experiment (86 and 100%, respectively) than in ours (61 and 80%, respectively, Table 6). This might partly be due to differences in experiment setup, as Heide registered bud development over a longer period after all intake dates (50 days, up to 20 days longer than in our experiment), and a higher percentage would be expected with an extended period.

Bud burst amount in beech has been shown to have little relation to chilling degree when exposed to LD, as even very short chilling exposure can lead to high bud burst percentages (Caffarra & Donnelly 2011). However, for bud burst percentage to become 100, a substantial period of chilling must be experienced by the buds (Caffarra & Donnelly registered more than 50 days with a chilling temperature of 3°C). As Heide's experiment resulted in 100% bud burst in March, and ours did not, this leads to the assumption that the buds used in his experiment had acquired a higher degree of chilling than what our buds did during the autumn of 2013. This is difficult to prove, as no estimations of chilling exposure have been done in either experiment, and meteorological data for the autumn seasons prior to Heide's intake months were not available with sufficient details for comparison. However, for our experiment, average temperature measurements from nearby meteorological stations show that all autumn months of 2013 were warmer than the 30 year normal

(based on the period 1961-1990, Appendix 1), and it is not unlikely that the chilling process might have been more disturbed for our samples than what they were before Heide's experiment.

Thermal accumulation

The results from the LD and SD 21°C experiments seem to support the assumption that a certain day length requirement must be fulfilled, after which the thermal (spring) temperature is determining the time of dormancy release (Table 6; Figure 8). This was also reflected in our testing with different temperatures in the March experiment, as exposure to high temperatures resulted in earlier bud burst than exposure to low (Table 13). The differences indicate faster fulfillment of a certain thermal requirement with higher temperatures, termed as differences in thermal time. The low variance in bud burst percentage between buds tested under LD 15 and 21°C suggest that the necessary thermal threshold was acquired under both these treatments within the experiment period, taking shorter time in 21°C. Some few (26%) of the LD 9°C samples also seemed to start acquiring the necessary thermal sum by the end of the experiment period.

Thermal time

The number of days until bud burst was found to decline through the winter (from 23 days in January to 14 days in March under LD). This clearly illustrates that the thermal accumulation process had started prior to intake in many of the samples from the later months (February and March). Heide's (1993) experiment showed higher bud burst percentage for all March temperature and photoperiod treatments. About a third of his SD samples reached bud burst, regardless of testing temperature, whereas almost none of ours did. However, his all took a very long time in doing so (49, 44 and 40 days for 9, 15 and 21°C, respectively). This was longer than the duration of our experiment period, indicating that the reason for lower bud burst percentages in our SD experiments was due to the extended study period in his experiment. An extended experiment period might also have increased bud burst percentage for some of the LD experiments, e.g. LD 9°C in March, where started bud burst was observed just before the experiment's end. However, it is unknown whether experiment duration differences can explain the low variation in bud burst percentage observed for LD 15 and 21°C, and if an extended testing period would have led to higher certainty about the chilling differences of the experiments.

Interaction photoperiod and thermal time

Heide (1993) observed shorter bud burst time for samples tested under low temperatures (LD 9°C) in March than what we did (25 days vs. 31 days, respectively, Table 13), whereas no difference was observed for the higher temperatures. This indicates that there might have been a difference in thermal time between the experiments, either in terms of a different thermal requirement, or a different thermal accumulation rate. However, why this is only observed for the low temperatures is unknown.

Except the uncertain observations previously discussed, and the shorter LD 9°C bud burst time in Heide's experiment, our results are not different from his. However, it is not possible to determine differences in thermal time in relation to chilling state based on these results when studying only one winter season. The comparison with Heide's results is also uncertain, as the relative difference in chilling state of the buds should be known to be able to hypothesize whether thermal time has been reduced due to chilling or photoperiod. It might be argued that larger differences would have been

observed in the experiments if a chilling difference was indeed the case, which cannot be proven in these experiments. As differences in the temperature conditions during the chilling phase, and later during the ecodormancy phase, are unknown, conclusions about this are uncertain. It is possible that an extended study period would have given more clear differences. However, extending the experiments can also contribute to increased uncertainties of the observations, as will be discussed later.

Experiment conclusion

Our results seem to indicate that a strong photoperiod control has been inhibiting bud burst until a certain day length in most of our experiments. However, the reduced thermal time with later intake clearly indicated that the thermal accumulation was started in some buds prior to the late intake months, which hence have been less inhibited by the photoperiod control. These buds seemed mainly controlled by the thermal temperature in determining their time of dormancy release, in line with Vitasse & Basler (2013). It is unclear whether this was due to a higher chilling degree of these buds, which as a consequence would have a lower thermal requirement than other buds, or if some buds had already received the necessary day length signal by that time. Nevertheless, this seems to indicate that our populations, isolated along the northern edge of the beech distribution, and not subject to genetic diversity or influence as in more southern populations, are responding somewhat differently to the influencing factors than what is assumed for other areas.

Comparison of experiment results with field observations of bud burst date

One clear difference between the Heide (1993) experiment and ours, was the observed bud burst dates in the field during the experiment years. Heide reported burst time in the spring seasons of 1990 and 1991 to be at May 5 and May 8, respectively, following winters with mild (1990) and normal (1991) temperatures in the period January to March (compared to the 30-year normal). Our field study, using natural populations in Vestfold, showed bud burst almost two weeks earlier (before April 25) both in 2014 and 2015. This gives a clear indication of differences in thermal time of the buds between the experiments.

Clearly, any conclusions about the mechanisms behind the different thermal times in Heide's (1993) and our experiment are uncertain, as sufficient information about the temperature conditions during the experiment years are not available. However, interestingly, the claim of beech bud burst dates showing low variance between years and being generally late seems to be under question for the northern area of its distribution, supported by the results of our study. Our early bud burst observed in the field seem to have been less limited by photoperiod control (bud burst inhibition until a certain day length) than what is observed in more southern areas (Vitasse & Basler 2013), and seems to be mainly dependent on variations in the spring temperature for determination of the bud burst time. With similar autumn temperatures as observed for the recent years, larger variations in bud burst time must be expected than what was assumed by Heide (1993).

Autumn experiment

In autumn, bud burst percentages were low for all testing months, and highest in December (Table 6). This indicates that fulfillment of the chilling requirement (necessary to release the endodormancy stage) had not been acquired until the December intake. However, a low amount (13%) of the buds harvested in October reached bud burst under LD, which is not in accordance with the expected

inability of the buds to reach bud burst during endodormancy. In November, very few samples reached bud burst, indicating that sufficient chilling was still not acquired by this intake. In December, however, 26% of the LD samples reached bud burst, indicating release of the endodormant state for some of the buds. However, no statistically significant effect was found, as the variation between the months was low (Tables 10 to 12).

Our results from the autumn LD 21°C experiments show a tendency towards increased bud development at the end of the experiment period, after more than 25 days of testing, (Figure 11), indicating that bud burst percentages would have been higher if the experiments had been run longer. No bud burst occurred under SD, which is supporting the theory of insensibility to forcing temperature and bud burst inhibition until a certain day length, regardless of chilling state.

The explanation for the bud burst observed in October is unknown, and might have to do with external factors, as hypothesized for the unexpected January results. Heide (1993) also observed a relatively high bud burst percentage for the October intake, whereas 0% for both November and December. This might indicate a lower degree of chilling of the buds used in his experiment (which was conducted within the same winter season) than we observed for out buds, which, as previously discussed, is uncertain.

Variation in bud development between populations
The statistical testing for the spring months indicated largest variation between the Tjølling and Melsom populations in January and February (Tables 15 and 16). In March, no significant differences were found (Table 17). There was not observed any general patterns of difference between the Vestfold populations and the Bergen or Ås populations, or within Vestfold.

Hence, there don't seem to be evidence for latitudinal differences, e.g. due to day length differences between the southernmost and the northernmost populations, although this was not tested for specifically. As mentioned, day length differences between the populations did not vary much, as latitudinal positions were not very different. A possible explanation for the small observed variation between locations is that there were differences in the stand characteristics where the replicate trees were found. Hence, the trees might not be true replicates in all cases, despite being of the same size and vigor, and surrounded by approximately the same stand density. This applies especially to the replicate trees in Tjølling and Ås, which were in mixed stands rather than unmixed beech stands (in Tjølling as equal mixture with spruce, in Ås as single beech trees in a stand dominated by other deciduous tree species). As shown in the sapling field study, canopy density and microclimate can vary much between different types of beech stands, which is likely to affect the adult replicate trees within the stands, in terms of both temperature and photoperiod sensing. However, it is unknown whether these environmental differences can explain the population differences observed in our experiment. The lower variation observed in the March experiment is most likely caused by a higher degree of started thermal accumulation at all locations prior to the intake.

The testing of the autumn months did not provide information on population differences, as previously explained. However, the monthly plots (Figure 11) indicate that a started increase in bud development towards the end of the experiment period was happening earlier for the Bergen population than the others. This might reflect a difference in the amount of chilling between the different parts of the country, possibly due to chilling requirement differences between the populations, which would result in release of the endodormant stage at different times.

Other factors influencing the results

Experimental design challenges

It should be noted that results may have been slightly altered due to some general challenges with this type of experiment setup. As mentioned, experiment duration was too short to give certain results for some of the treatments and testing months. A testing period of 50 days would have been ideal for a better comparison with the Heide (1993) study. However, many twig samples were showing signs of deterioration (drying out, algae growth at the excise wound etc.) by the time that we ended our experiments, and a prolonged testing period might have given results that would have been affected by this. In general, using twig cuttings over a long period for studying physiological and phenological processes are related to a high degree of uncertainty compared to studying whole plants. This is especially so compared to plants growing in the field, that are influenced by a wide range of both internal and external factors. However, in our study, plant material from the same populations were both studied *in situ* and used under controlled conditions in the experiments. Although the plant material consisted of buds on saplings in one study and cuttings from adult trees in the other, which are not necessarily similar in terms of physiological and phenological processes, this gave us a possibility to detect any major inconsistencies.

Maintaining the controlled environment at a constant level proved challenging, as the high RH in the testing chambers led to incidents of icing of the thermostat on a few occasions (in both SD and LD 9°C during the March experiment and both SD and LD 21°C during the November experiment). This led to temperature increases of up to 8°C in the affected chambers for up to 8 hours before it was detected, and may have contributed to actual higher thermal accumulation under these treatments. For the March LD 9°C samples, this might have contributed to the observed bud burst that started just before the experiment's end. For the November samples, bud burst percentage was low, and seemingly unaffected by the temperature increase.

Plant hormonal influence

A further challenge in the chambers was the overlap in time between samples from subsequent testing months. Samples were removed from the chambers after they had fully developed leaves (bud development stage 4), to avoid leaf maturation hormonal influences on other samples as much as possible. However, to which extent ongoing flushing of the previous month's samples might influence the bud development of recently harvested samples, is unknown.

However, a likely influencing factor was that the 21°C chambers were used for storage of mature apples prior to the start of the January experiment. The apples had been removed from the chambers the same day as experiment start, and although ventilation of the chambers were done before the bud samples were put in, gaseous plant maturation hormones (e.g. ethylene) produced by the apples might have been present in unknown quantities at the time of experiment start. The same might have been the case during all autumn experiment months, as storage of apples was being done simultaneously in neighboring chambers, and a risk of unknown amounts of these gases entering was present.

Ethylene has been shown as a potent plant hormone with enhanced effect on dormancy release in buds of other woody plant species, e.g. fruit trees and grapevines (Ophir et al. 2009, Zheng et al. 2015). Ethylene formation in the plants is even actively triggered in commercial cultivation of some varieties of grapevines, to promote dormancy release in southern areas where the plants' chilling requirement is often not fulfilled (Ophir et al. 2009). Ethylene is also well known to stimulate

dormancy release in seeds of some fruit tree species (Gniazdowska et al. 2007), which in many respects affects many of the same physiological processes as for dormancy release in buds. The specific effects of ethylene on these processes in beech buds are not well known, but it is possible that dormancy release can be triggered also for this species. If so, it might be a contributing factor to the high bud burst percentages observed in both the SD and the LD experiments in January. However, for the autumn experiments it seems less likely that this may have contributed to any visible effects, as autumn bud burst percentages were generally low for all LD experiments and 0 for all SD experiments.

Frost tolerance experiment

Variation in frost tolerance throughout the dormancy period

In general, frost tolerance of the buds was found to vary somewhat much between the different dormancy testing months. The spring results (January, February and March, (Table 20)) indicated that the buds tolerated moderate frost (-5°C) well, then showing increasing damage after being exposed to -10°C or below, and an especially strong decrease in tolerance between -10 and -15°C. The LT_{50}-estimations refined these findings, and indicated that tolerance was highest in January and February (LT_{50} level around -20°C), and lower towards the end of the dormancy period (around -15°C in March), (Figure 12).

The autumn experiments did not give as refined results on the frost tolerance variation between the testing months, as they were not statistically tested. As for the dormancy release experiment, we faced problems with developing statistical models for the autumn data, assumedly caused by little variation in the data for the different autumn months. This is a weakness for the interpretation of the data, as damage levels cannot be estimated as accurately as for the spring data. However, the LT_{50}-estimations (Figure 12) indicated lower frost tolerance at the beginning of the dormancy period (approximately -10°C in October), and increasing tolerance in November and December (-15°C).

These findings correspond well with what was expected, with a frost tolerance peaking during the beginning of the ecodormancy stage (January). The estimated tolerance levels indicate that beech buds tolerate mild frost fairly well until the end of the dormancy period. They also indicate that exposure to temperatures below -15°C during mid-winter might lead to some bud damage.

However, it is stressed that the damage level measure used in the analysis (the LT_{50}-estimations) only gives indications of a certain damage level (50% or more of the bud tissue killed), which are not necessarily transferable to bud survival or the tree's frost impact as a whole. It should also be noted that the damage evaluation were done visually in a macro scale only. More refined results might have been obtained using a different evaluation method (e.g. ion leakage analysis of the buds after temperature exposure), although there are uncertainties related also to these methods (Gusta et al. 2003).

Variation in frost tolerance between populations

Statistical testing (Table 20) did not indicate strong differences in frost tolerance between the tested populations. Variations were not consistent in terms of latitudinal or continental/coastal differences. Tjølling and Bergen showed highest frost tolerance in the spring experiments, whereas Holmestrand, Melsom and Ås showed lowest. However, differences were mainly not significant on a 0.05 level. The

LT$_{50}$-estimations (Figure 13) also indicated small population variations that were showing the same patterns as the statistical estimations, but no clear population differences were estimated.

As mentioned, statistical testing of the autumn results was not conducted, and potential population differences were not determined. Analysis of the LT$_{50}$-diagrams indicate generally low variation between the populations, with range widths less than 5°C for all autumn months.

Other factors influencing the results
There are several general challenges with this type of frost tolerance testing that are worth mentioning. Most importantly, results from testing under controlled environmental conditions, using a specific program of cooling/decooling, is not necessarily transferable to conditions found under natural conditions. Differences in cooling rates and duration of exposure to the target temperature, termed as varying degrees of chronic vs. acute stress, have been found to result in very different levels of damage (Gusta & Wisniewski 2012). This should be taken into account when interpreting the estimated frost tolerance levels. Type and amount of frost damage can also depend on the experimental setup, and exposure to the same temperature can give different results when tested under controlled vs. natural conditions. Also, the wound at the excise surface of the twig cuttings are exposing the interior plant tissue more to the environment (Gusta et al. 2003). This has been shown to increase the damage risk for buds on these twigs, even if the buds are not located directly above the excise wound. Cutting of the twigs from the tree might also induce physiological responses that make the buds more or less vulnerable to abiotic stress than in the uncut state. Hence, results obtained under controlled conditions are not necessarily reflecting the frost tolerance responses observed in nature.

Also, although not specifically tested for, frost tolerance might be expected to vary for buds within a tree, as they are found both exposed or more sheltered within the tree's crown or within the surrounding canopy. As all buds used in our experiments were collected from one part (approx. same height and aspect) of the replicate trees, frost tolerance levels estimated might not be transferable to the trees as a whole.

Therefore, direct interpretation of the temperature damage levels estimated in this study is not advisable. As our spring and autumn experiments were conducted over two different winter seasons, direct comparisons of temperature values between the seasons may be inaccurate. However, the experiments can provide valuable information about relative differences between testing months within the seasons, as they were conducted following the same method for all testing months. We faced some challenges with performing consistent damage evaluation between experiment months, as bud damage was sometimes hard to determine as detailed as our four level categorical scale. This may have altered the overall results slightly, and may add to the inaccuracies of the graphical LT$_{50}$ estimations. However, in most bud samples there was a clear distinction between killed and live plant tissue (category 0 and 3, respectively), and the general trends of decreasing frost tolerance during spring and increasing tolerance during autumn are most likely accurate.

Conclusion

Bud burst date was found to be earlier than what was considered normal during the last decades. This seem to question the assumption of beech being a late-flushing tree species with low variation in bud burst dates between years. A shift towards earlier bud burst dates has been predicted for the colder areas of the beech distribution range (e.g. northern areas), due to increasing spring temperatures. This seems to be the observed development during the last decades, in contrast to southern areas, where bud burst dates have been showing low variation in the same period.

The results of the field study show a complex pattern of factors influencing bud burst, leading to local differences in exposure to temperature, solar radiation etc., both within stands and single trees. This can be related to the observed variation in the experiment studies, as both chilling, heating and frost exposure of the buds seemed to have varied without consistent geographical patterns.

The dormancy removal experiment showed a complex influence of both photoperiod and temperature on the time of bud burst. The dormancy removal experiment found bud burst to be limited until a certain day length during spring, but we were unable to conclude whether this observation was due to phenological control or other factors influencing the experiment. The results were not showing consistent patterns of influencing factors between tested months or populations. This seems to indicate that our populations, isolated along the northern edge of the beech distribution, and not subject to genetic diversity or influence as in more southern populations, are responding somewhat differently to the influencing factors than what is assumed for more southern areas.

The frost tolerance experiment showed variation through the dormancy season, with a frost tolerance peaking in the middle of the winter (January). The estimated tolerance levels indicate that beech buds tolerate mild frost fairly well until the end of the dormancy period, whereas exposure to temperatures below-10°C in the early/late winter and -15°C during mid-winter might lead to a high degree of bud damage.

In a scenario of increasing spring temperatures, dormancy release in beech buds can be influenced both towards a more variable and a more conserved bud burst time between years. This will most likely depend on the temperatures during autumn, determining the chilling state of the buds. It seems likely that a shift towards earlier bud burst is already happening for beech in Norway, and that this new time will stabilize if more unstable autumn temperatures becomes common.

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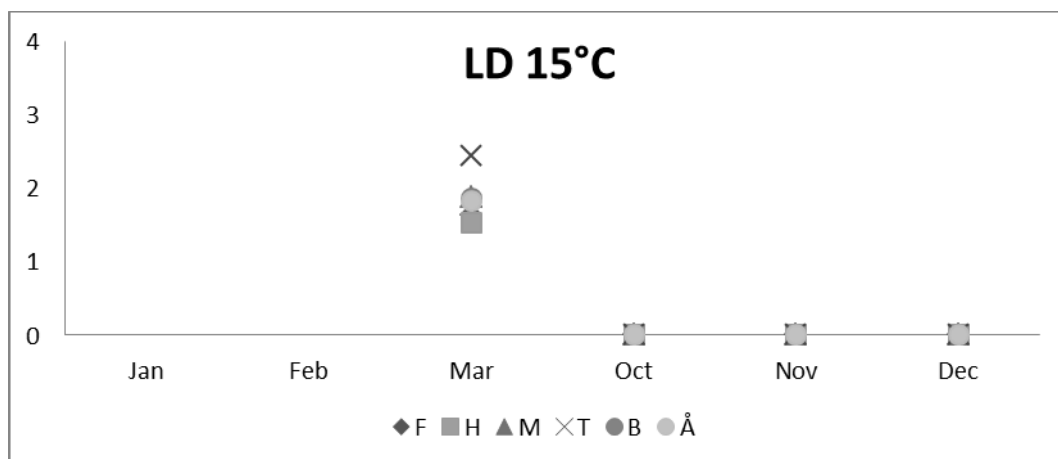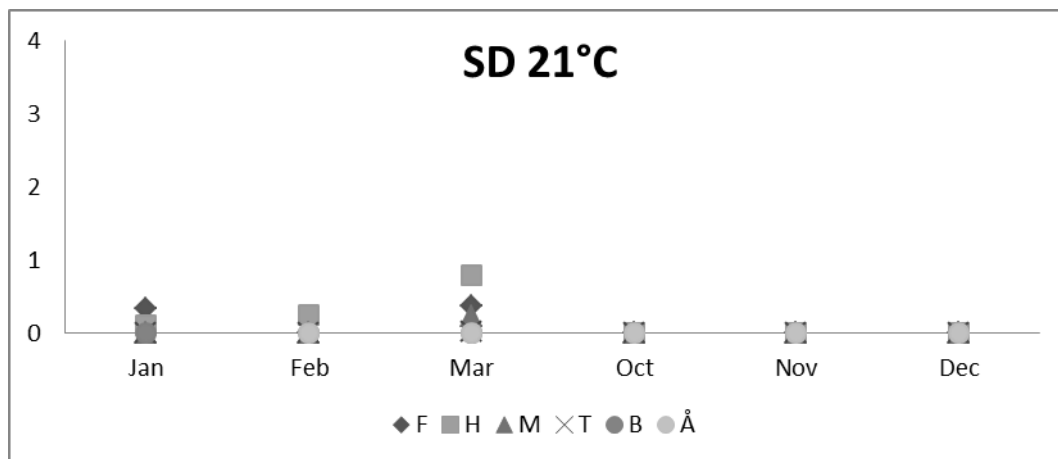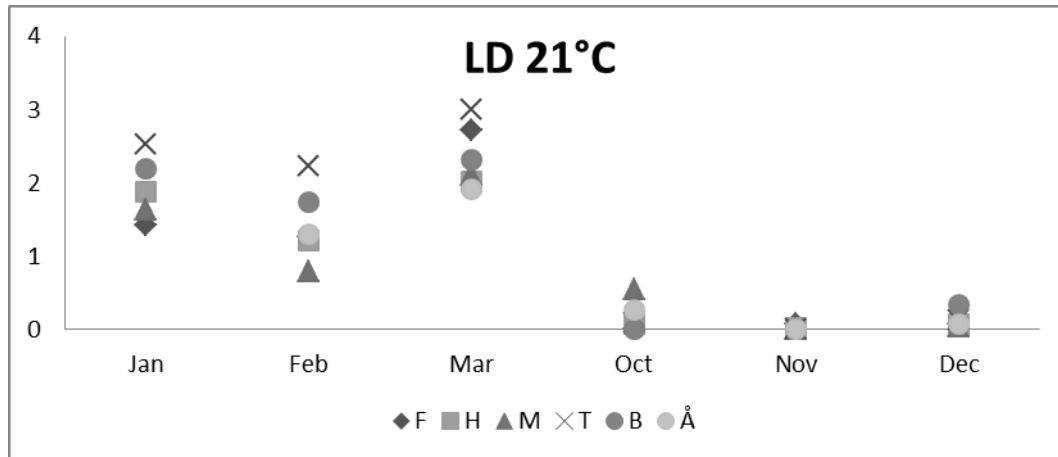
Appendix

Appendix 1
Monthly average temperatures for the areas of the beech populations (Eklima 2015). Melsom represents the Vestfold populations. Values are shown for our study year (2014) and the year before (2013), in addition to the 30-year normal (1961-1990). The periods discussed in relation to dormancy and thermal processes are marked.

Location	Year	Monthly average (°C)					
		Jan	Feb	Mar	Apr	May	Jun
Melsom	2013	-4,2	-3,3	-2,4	4,1	11,9	14,7
	2014	-1,4	2,3	4,6	7,6	11,6	15,4
	Normal 1961-1990	**-3,7**	**-3,8**	**0**	**4,4**	**10,6**	**15**
Bergen	2013	0,3	0,6	0,9	5,1	11,3	13,3
	2014	3,4	5,5	6	8,9	11,4	14,5
	Normal 1961-1990	**1,3**	**1,5**	**3,3**	**5,9**	**10,5**	**13,3**
Ås	2013	-5,2	-4,3	-3,5	3,6	12,1	14,3
	2014	-2,6	1,8	3,9	6,9	11,2	15
	Normal 1961-1990	**-4,8**	**-4,8**	**-0,7**	**4,1**	**10,3**	**14,8**
		Jul	Aug	Sep	Oct	Nov	Dec
Melsom	2013	17,9	16	11,7	7,9	2,9	3,3
	2014	20,3	15,6	12,8	9,7	4,8	-0,8
	Normal 1961-1990	**16,3**	**15,2**	**11,1**	**7**	**1,7**	**-2,1**
Bergen	2013	15,6	15,2	12,5	9,6	5,5	5,7
	2014	19	15,5	13,6	10,6	7,3	3,2
	Normal 1961-1990	**14,3**	**14,1**	**11,2**	**8,6**	**4,6**	**2,4**
Ås	2013	17,5	15,5	11,1	7	1,9	2,4
	2014	20	15,1	12,2	8,9	3,8	-2,6
	Normal 1961-1990	**16,1**	**14,9**	**10,6**	**6,2**	**0,4**	**-3,4**

Appendix 1. Monthly average and normal temperatures (°C) for the study areas (Melsom (Vestfold), Bergen and Ås). Periods of special interest to the study are marked.

Appendix 2

Average results from all dormancy removal experiments. Average bud development stage per location and testing month for the different treatments.

Appendix 2

Average results from all dormancy removal experiments. Average bud development stage per location and testing month for the different treatments.
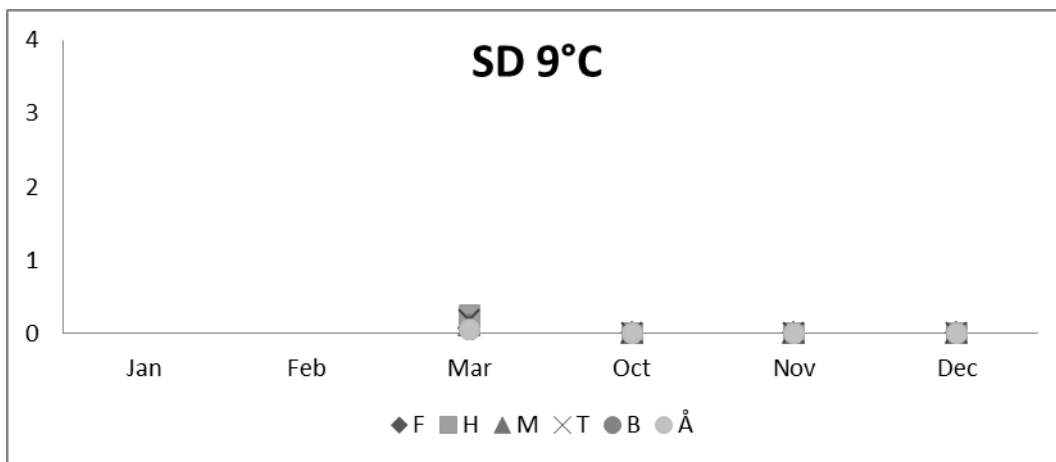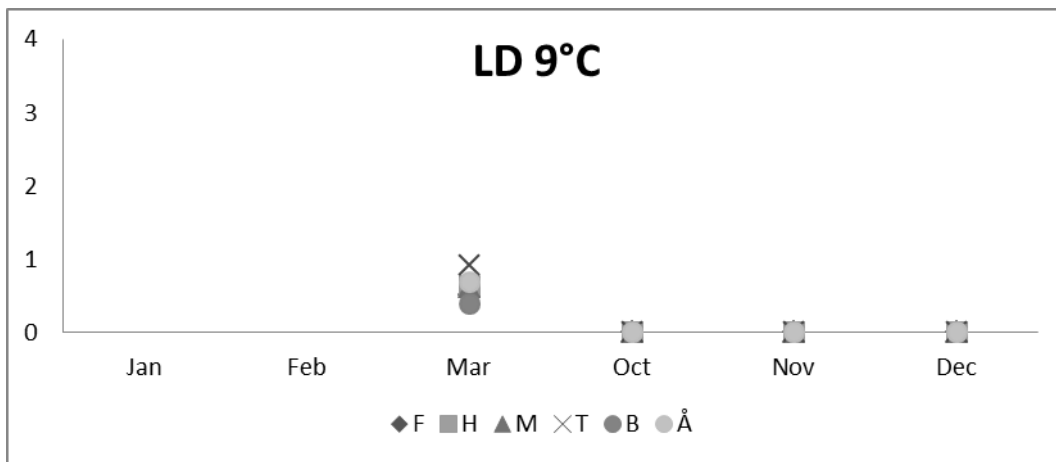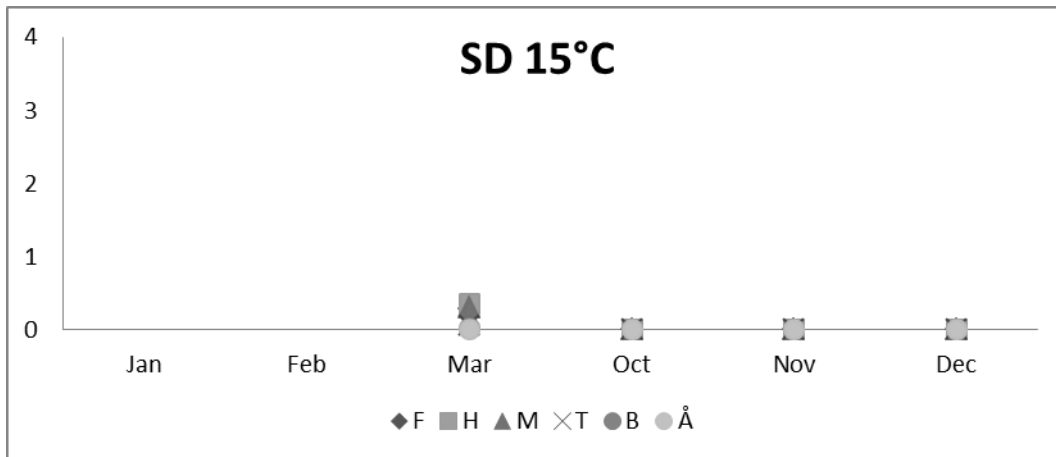
Appendix 2. Average bud development per population (F: Falkensten, H: Holmestrand, M: Melsom, T: Tjølling, B: Bergen, Å: Ås) for all treatment programs (LD: Long photoperiod, SD: Short photoperiod, testing temperatures of 9, 15 or 21°C.) for each of the testing months (Jan, Feb, Mar, Oct, Nov, Dec) of 2014.