

# Environmental control of spring phenology in mature temperate trees

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# Chapter 1

## General Introduction

David Basler

### Seasonality in plants

Extra-tropical climates are characterized by temperature-related seasonality. Long time survival and species persistence is based on the ability to cope with unfavorable and possibly fatal climatic conditions. During active phase plants are commonly less resistant to environmental stresses, such as freezing temperatures and water shortage. With higher latitude or elevation the growing season length for plants shortens, and perennial plants have to temporarily suspend growth to withstand the cold temperatures in winter. Dormancy, characterized by suspended growth and correlated with increased stress tolerance, allows the plants to survive potentially harmful seasonal environmental conditions. Hence, during the course of a year, plants undergo a series of developmental changes, which are also reflected by their appearance. The study of the timing of re-occurring, visible development stages is named phenology (from the Greek *phainō*, ‘cause to appear’, and *-logia*, ‘study of’).

### Dormancy

The rhythm of plant life in the annual cycle is the evolutive result of a risk minimization in interaction with climate (Larcher 2003). In humid

extra tropical regions, the cold winter temperature bears the risk of tissue damage in actively growing tissues. The timing of dormancy induction has to take place before the first possibly fatal temperatures occur. Likewise, dormancy should not be released when the risk for freezing damage is still high. The timing of the phenophases defining the growing season (bud burst and leaf unfolding in spring, bud set in autumn) is thus a trade-off between maximizing the growing season length and minimizing the risk of freezing damage.

To adjust their phenology to the local climatic conditions, plants have to relay to environmental signals indicating the progression of seasons. Two main environmental factors are controlling dormancy in temperate trees: temperature and daylength (photoperiod). The role of temperature is twofold: the experience of a certain amount of low temperatures make plants receptive to warmer conditions as spring is approaching ('chilling': non freezing temperatures in the range of 2-7°C), while warm temperatures are directly affecting the rates of development ('forcing') once the internal disposition is established. While the course of late winter or spring temperature is strongly variable from year to year, photoperiod is a reliable astronomic environmental signal for the progression of the season. Beside the importance for the timing of dormancy, photoperiodism is also important for reproductive events, including synchronous flowering that assures gene flow among the individuals of often scattered plant populations (Jackson 2009; Keller and Körner 2003; Thomas and Vince-Prue 1997). Photoperiod is recognized by photoreceptor systems, such as the phytochrome system, and functions as a dose-independent signal, i.e. as soon as a certain (very low) threshold of light intensity is passed, plants recognize the signal 'day-on'.

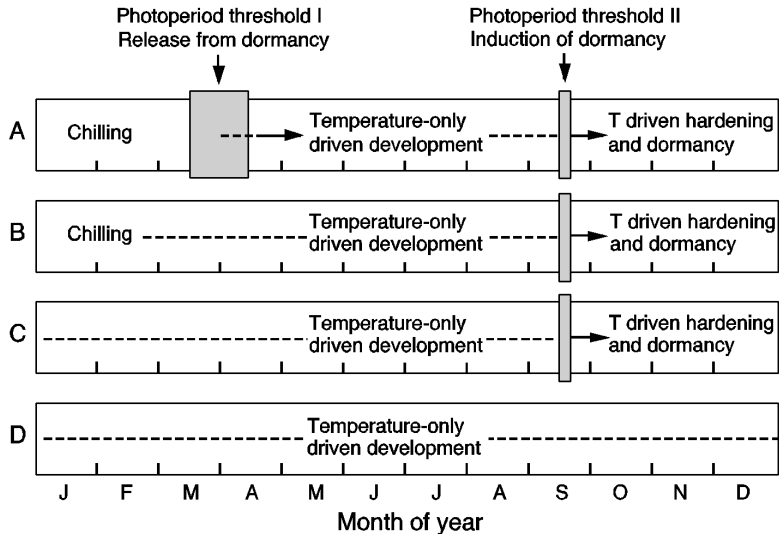
In most tree species, the shortening photoperiods in autumn (perceived in the leaves) induce dormancy, which becomes apparent in the formation of winter bud. The period of dormancy may be separated into three distinct phases (Lang et al. 1987): (1) paradormancy, where (hormonal) signals from other plant organs induce dormancy in the buds, (2) endodormancy, where physiological conditions within the bud inhibit growth and development and (3) ecodormancy, where environmental conditions (e.g. low temperature) suppress growth in the bud. In the first stage of dormancy (paradormancy) plants may readily resume growth when transferred to long-day conditions. Under the influence of low temperatures, the paradormant state develops into endodormancy. Once endodormancy

is fully established, plants will not resume growth even under favorable conditions. The autumnal leaf coloring and leaf fall are coincident phenological events, but do not reflect the status of bud dormancy, which normally precedes the leaf coloration. Temperatures may modulate the induction of dormancy, with warm temperatures either fastening or delaying the dormancy induction. Exposure to low temperatures (chilling) makes plants receptive to warmer conditions. After the chilling requirement is fulfilled, endodormancy is released and plant may enter a phase of ecodormancy, where growth is resumed as soon as the environmental conditions allow. Chilling, forcing and photoperiod are part of complex interactions, e.g., a lack of chilling may lead to increased requirement of forcing temperatures for budburst, but may also be substituted by long daylength (Heide 1993a; Heide 1993b).

## **Species-specific differences**

The transition between the different phases of dormancy is gradual and species or even ecotypes may differ in their environmental requirements for dormancy induction and release (Körner 2007; see Fig. 1.1; Perry 1971). Ecological life strategy and successional status of a species may determine the response to warm temperatures in early spring. Opportunistic species will more likely respond to temperature only, although the potential risk of freezing damage may be larger. Long-lived late successional species will more likely adopt a more conservative strategy, relying more on photoperiod to decrease the risk of freezing damage.

As is known in forestry for at least a century (Langlet 1971; Vaartaja 1959), trees of late successional species are genetically calibrated to the latitude or elevation they live in, explaining for instance the failure of low elevation genotypes planted at high elevation (e.g. Holzer 1967). Common garden experiments have evidenced this provenance differentiation.



**Fig. 1.1:** A schematic representation of the interaction of temperature and photoperiodism in photoperiod-sensitive species from cool temperate climates. Boxes illustrate the photoperiod-driven windows that permit development, the speed of which is controlled by the actual temperature. A depicts a triple control of bud burst, B a double control (no spring photoperiod effect), C an opportunistic behavior (only actual temperature matters), with A-C still adopting a photoperiod control of timely senescence or dormancy introduction in a seasonal climate. D represents a tropical ecotype with no regular threshold controls of phenology (but there may be other triggers). From Körner (2007)

## Responses to climate warming

During the last years, phenology has received increased interest in the light of global warming and many studies observed a shift in phenological phases during the last decades. Due to the recent climate warming, phenological spring events advanced globally on average by 2.3 days per decade (1971-2000; Parmesan 2006). In Europe, spring events advanced by 2.5 days per decade over the same period, corresponding to 2.5 days per °C (Menzel et al. 2006). However, phenological responses to warming is non-linear, and increased warming can even delay spring phenology, as was observed for example in steppe and meadow vegetation of the Tibetan

Plateau (Yu et al. 2010). The changing appearance of plants during the course of a year is based on environmental influences as well as on their internal disposition (genetic/hormonal). As an observational method, phenology may not distinguish between both influences. The internal disposition to react to favorable conditions are thus often overlooked and linked to the concurrent weather conditions. Whether the start of the growing season is able to track the temperatures in spring depends on the extent of the autonomous developmental control. With increasing warming, underlying photoperiodic thresholds or chilling fulfillment may become an issue and decelerate or even reverse the trend towards earlier phenology.

## **Aims of this work**

In this thesis, I aimed at

1. Screening a representative set of temperate forest trees for their photoperiod sensitivity (Chapter 2).
2. Investigate the effect of temperature and photoperiod on the rate of development prior to bud burst (Chapter 3).
3. Evaluate, whether the integration of photoperiod sensitivity into commonly used phenological models increases their accuracy and applicability (Chapter 4).

The responses of bud burst to temperature and photoperiod (Chapter 2, 3) was investigated experimentally under controlled conditions using cuttings (cut twigs from mature trees, Fig. 1.2) of 14 tree species sampled at two elevations and replicated across two regions of Switzerland. The validity of using cuttings as proxy for mature tree phenology, rather than seedlings, was tested by comparing cutting phenology and adult tree phenology in-situ (Chapter 7).

With a continued rise of temperature a further advancement of spring phenology has been projected using linear models. ‘Process-based’ phenological models are designed to simulate the response to environmental drivers and should, if the underlying assumptions are true, yield more realistic predictions of phenological onset dates. I compared and analyzed a set of over 30 existing models (and combinations thereof) using long-term observation and phenological data derived from experiments (Chapter 4).



**Fig. 1.2:** Watered cuttings used to investigate the response of spring phenology to different combinations of photoperiod and temperature under controlled conditions (see Chapter 2 and 3).

Even though a photoperiod effect on spring phenology has been documented since decades it was not widely acknowledged by the phenology community. The extensive literature search for this thesis lead to a summary on photoperiod control of tree phenology in general (Chapter 5) and for *Fagus sylvatica*, specifically (Chapter 6).

## List of publications

The chapters of this thesis have been published in peer-reviewed journals:

- Ch. 2 Basler D, Körner C (2012) Photoperiod sensitivity of bud burst in 14 temperate forest tree species.  
*Agricultural and Forest Meteorology* 165:73-81
- Ch. 3 Basler D, Körner C (2014) Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species.  
*Tree Physiology* 34:377-88
- Ch. 4 Basler D (2016) Evaluating phenological models for the prediction of leaf-out dates in six temperate tree species across central Europe.  
*Agricultural and Forest Meteorology* 217:10-21
- Ch. 5 Körner C, Basler D (2010) Phenology under global warming.  
*Science* 327:1461-1462
- Ch. 6 Vitasse Y, Basler D (2013) What role for photoperiod in the bud burst phenology of European beech.  
*European Journal of Forest Research* 132:1-8
- Ch. 7 Vitasse Y, Basler D (2014) Is the use of cuttings a good proxy to explore phenological responses of temperate forests in warming and photoperiod experiments?  
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**Part I**  
**Main studies**



## Chapter 2

# Photoperiod sensitivity of bud burst in 14 temperate forest tree species

David Basler, Christian Körner

**Abstract** The timing of spring phenology of trees reflects a trade-off between a longer growing season and a lower risk for damage by late freezing events. Temperature is driving rates of development directly, but given the high inter-annual variability in weather, it is a poor environmental cue for the progression of the season and thus, the period with low freezing risk. In contrast, photoperiod is a reliable and weather independent signal of the progression of the season. Using growth chamber experiments we assessed the photoperiod sensitivity of bud burst under artificial spring conditions in cuttings of 14 common European tree species that belong to different life-strategy types (pioneers or exotic species vs. native late-successional species; 3 conifers/11 broadleaved). Fully chilled twigs were sampled from populations along two elevational gradients in the Swiss Alps. Applying realistic contrasts in photoperiod, short photoperiods delayed bud burst in five late successional species to variable degree, whereas no distinct photoperiod sensitivity was observed in early successional species. In *Picea abies*, the photoperiod response was additionally influenced by elevation of origin, whereas in *Quercus petraea* and *Abies alba* regional differences in the photoperiod response were observed. For late successional species, photoperiod is thus an important environmental signal that will constrain responses to climatic warming because rising temperatures will drive phenology toward the species specific photoperiod threshold.

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**Key words:** Spring phenology, Daylength, Temperature, Development, Season

## Introduction

The precise timing of phenological events (bud burst, flowering, bud set) is a key factor for long-term survival, successful reproduction and species establishment (Larcher 2003). To survive the harsh winter conditions in high latitude seasonal climates, trees go through a period of dormancy and enhanced freezing resistance during winter. The timing of the induction and the release from dormancy is closely linked to three components of local climate conditions, with the amount of low temperatures experienced (chilling), photoperiod and (forcing) temperature acting as the main environmental drivers in humid extra tropical regions (Körner 2007).

The autumnal growth cessation and the induction of dormancy, including freezing resistance, is largely a photoperiodic response to the longer nights (shorter daylength) in autumn (Klebs 1903, 1914, Vaartaja 1959, Thomas and Vince-Prue 1997), although, concurrent temperatures are modulating this response (Heide 2003, Kalcsits et al. 2009). Unlike actual weather, the astronomically defined photoperiod is a most reliable indicator for the progression of the season and thus, the photoperiodic induction of dormancy ensures that trees are ready to cope with freezing temperatures well before the first freezing events occur (Körner 2007). The period of dormancy may then be separated into the three main phases (1) predormancy, (2) endodormancy, and (3) ecodormancy, based on the depth of silencing of metabolic activity (Samish, 1954). The transition from endodormancy to ecodormancy is jointly controlled by the fulfillment of chilling requirement and by photoperiod, where chilling temperatures describe a rather vaguely defined range of cool, non-freezing temperatures below 10 °C (Battay 2000), with the range of 2-5 °C being the most effective for most species (Cannell 1989). During ecodormancy (warm) temperatures accelerate bud development until bud burst marks the start of a new growing season. The transitions between the different phases of dormancy are gradual, with species or even genotypes differing in their requirements for these environmental triggers (Perry, 1971).

The timing of dormancy induction and its release always reflect a trade-off between the length of the active period ('growing season') and the risk of damage by freezing temperatures, both in early autumn and in spring (Larcher 2003, Bennie et al. 2010). The dissimilar phenological responses of different species may thus be linked to the species life-history. While opportunistic pioneer species adopt a more 'risky', often even temperatureonly driven dormancy release, late successional species generally show a more 'conservative', more complex response, with a large chilling requirement and enhanced photoperiod sensitivity (Körner 2007, Caffarra and Donnelly 2010, Körner and Basler 2010). Also nutrition influences this trade-off, with species that have high nutrient access, such as *Alnus sp.* (with N<sub>2</sub>-fixing symbionts) employing a more risky foliage life history than species operating at more restricted nutrient supply (Tateno 2003). Within species, genetic adaptation to local climate conditions, such as the differentiation into latitudinal and elevational ecotypes, is common (Morgenstern 1996, Thomas and Vince-Prue 1997).

The warming temperatures in the last decades are facilitating a longer growing season, especially an earlier onset of spring, which has been observed across many scales and taxa (Parmesan and Yohe 2003, Menzel et al. 2006). However, such a response will follow a nonlinear trend in an even warmer future, given that some species will hit their genetically fixed photoperiod or chilling constraints as warmer temperatures facilitate potential earlier leafing (Körner and Basler 2010, Morin et al. 2010). Thus, photoperiod sensitivity of bud burst may prevent some species from tracking the earlier onset of warm weather in spring as the climate gets warmer. As the timing of bud burst has a strong heritable component (Engler 1905, Burger 1926, Morgenstern 1996), a re-adaptation may take several generations (Langlet 1971, Nienstaedt 1974), which means centuries in the case of trees.

Photoperiodic responses of spring phenology were assessed in several tree species, most prominently in *Fagus sylvatica* (Wareing 1953, Falusi and Calamassi 1990, Heide 1993b, Caffarra and Donnelly 2010), but also in a few other tree species (e.g. Nienstaedt 1967, Worrall 1975, Heide 1993a, Myking and Heide 1995, Caffarra et al. 2011). However, these results are often contradictory or challenging to interpret, given the complex interactions of the three drivers, chilling photoperiod and actual temperature forcing. In addition, genotypes (provenances) of a species may also

differ in their photoperiod responses, as was observed in *Betula* (Heide 1993b, Myking and Heide 1995).

Any experimental research targeted at revealing mechanisms of tree phenology, is facing severe methodological constraints, since whole trees cannot be undertaken photoperiod manipulation in situ (e.g. shortening daylength while simulating warming). Seedlings or cuttings (cut twigs) of mature trees may be used as substitute in growth chambers, however the phenology of seedlings is known to differ from that of mature trees, whereas cuttings are per se disconnected from (potential) whole-tree signals affecting bud burst. The way experimental treatments are performed, using different fixed rather than fluctuating temperatures (Campbell and Sugano 1975, Erez and Couvillon 1987, Myking 1997, Partanen et al. 1998, Saxe et al. 2001) and constant vs. gradually lengthening photoperiods (Partanen et al. 1998), may further influence bud burst.

Given the diverse results in the literature, and aware of potential methodological limitations we made an effort to assess the basic photoperiod sensitivity of bud burst in a multi-species approach, including elevationally separated populations from geographically distinct regions. We conducted growth chamber experiments with cuttings of 14 temperate forest tree species, including species with differing leaf duration (deciduous vs. evergreen), whole tree life strategy (early- vs. late successional), bio-geographic origin (native vs. exotic), and in some species, different provenances from populations along two elevational gradients in the Swiss Alps. To our knowledge, no study has yet assessed the contemporary photoperiod sensitivity of bud burst in a wide range of temperate forest tree species after extensive chilling during winter under dynamic photoperiods. Given the above mentioned limitations, results will be conservative, that is, we may not be able to detect the full strength of in situ photoperiod control under such experimental conditions. Distinct photoperiod sensitivity is expected in late successional species.

## Methods

### *Study sites*

Three distinct sampling sites were defined along each of the two elevational gradients in the region of Chur (46°51' N/9°32' E, hereafter named 'eastern transect') and Lavey (46°12' N/7°02' E, 'western transect'), Switzerland. Temperature loggers (TidBit v2, Onset Computer Corporation, Bourne, MA, USA) were placed at the three different sites along each transect inside the forest in order to track the local air temperatures (2 m above ground, shaded) and to provide a link to long term temperature records from nearby weather stations. In order to avoid confusion between temperatures (°C) and temperature differences, we join other authors in adopting K (for Kelvin) for all differences in temperature. Both slopes are facing west and are covered by near natural forest stands (historical management could not be excluded).

### *Sampling*

Sampling of the cuttings took place on 2 and 3 March 2009: according to species distribution along the gradients, each species was sampled from two out of the three sites per gradient, a high and a low elevation site (Table 2.1). The elevational difference between the high and low sampling sites was between 400 and 500 m, which corresponds to a mean temperature difference of around 3 K. On each sampling site, dormant twigs of five individual trees per occurring species were sampled from the lower canopy (5–6 m above ground; 4 twigs per tree) using a 4 m tree pruner (Fiskars, Helsinki, Finland). The twigs were immediately labeled, watered, and transported to the Institute of Botany within 6 h where they were stored at 2 °C in the dark until the start of the experiments, once all samples had been collected (i.e. after 2 days, 4 March 2009). *Tilia cordata* and *Prunus avium* were sampled on the western transect only (no suitable trees in the eastern transect). Additionally, we sampled (as a reference) two exotic ornamental tree species, horse chestnut (*Aesculus hippocastanum*) and lilac (*Syringa vulgaris*), which are known to be photoperiod insen-

sitive and thus, are closely tracking temperature (Defila and Clot 2001, Larcher 2007; both species sampled from low elevation only). In the following we refer to species by their genus name.

**Table 2.1:** Species and number of sampled trees per species along two elevational gradients in the Swiss Alps. Each species was sampled from a high and a low elevation site per gradient, according to species distribution along the gradient. A total of 960 twigs were cut from 240 trees.

	western transect			eastern transect		
	500 m	1000 m	1450 m	700 m	1100 m	1520 m
<b>early successional native species</b>						
<i>Acer pseudoplatanus</i> L.		5	5		5	5
<i>Betula pendula</i> Roth	5	5		5	5	
<i>Corylus avellana</i> L.	5	5		5	5	
<i>Fraxinus excelsior</i> L.	5	5		5	5	
<i>Larix decidua</i> Mill.		5	5		5	5
<i>Prunus avium</i> (L.) L.	5	5				
<i>Sorbus aucuparia</i> L.	5	5		5		5
<b>late successional native species</b>						
<i>Abies alba</i> Mill.	5	5		5	5	
<i>Fagus sylvatica</i> L.	5	5		5	5	
<i>Picea abies</i> L.		5	5		5	5
<i>Quercus petraea</i> (Mattuschka) Liebl.	5	5		5	5	
<i>Tilia cordata</i> Mill.	5	5		-	-	
<b>exotic, ornamental species</b>						
<i>Aesculus hippocastanum</i> L.	5	-		5	-	
<i>Syringa vulgaris</i> L.	5	-		5	-	

Closest weather station data (1981-2010): West (Aigle, 381 m a.s.l.): 1010 mm annual precipitation, 10.1 °C mean annual T, duration of the growing season ( $T_m \geq 5$  °C) 262 d. East (Chur 556 m a.s.l.): 860 mm annual precipitation, 9.7 °C mean annual T, duration of the growing season ( $T_m \geq 5$  °C) 255 d. Data provided by MeteoSwiss (The Swiss Federal Office of Meteorology and Climatology)

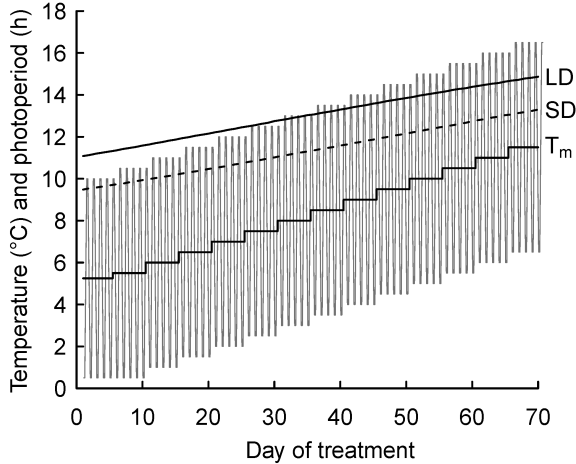


### ***Sample treatment***

Before the start of the experiment, the sampled twigs were recut to a length of around 30–40 cm. The number of buds per twig was species dependent and ranged from 2 in *Aesculus* up to around 40 buds in *Larix*. The twigs were then half dipped into a disinfectant sodium hypochlorite solution for 30 s (200 ppm active chlorine), recut a second time underwater at a steep angle using a sterile hand pruner, and finally placed into 0.5 l glass bottles filled with 0.4 l cool tap water. For *Fraxinus* and *Quercus*, the water was additionally treated with the broad-spectrum antibiotics gentamicin sulfate (40  $\mu$ g/l; Sigma–Aldrich, Germany; Larcher et al. 2010) since the xylem of these ring-porous species tends to become jammed by growing bacteria. During the experiment, the water was changed weekly and at the same time twigs were re-cut another 1–3 cm in order to assure good water supply.

### ***Growth chamber conditions***

The photoperiod sensitivity of spring phenology in a future climate was assessed with the assumption that warm temperatures will occur earlier in the season and thus, will coincide with a shorter photoperiod. Hence, we programmed fully automatic phytotron units to match such realistic dynamic climate scenarios. We defined two similar temperature treatments in combination with either long or short photoperiods (9.5 h at start of experiment resp. 11 h at start of experiment; Fig. 2.1). Temperature was set to cycle  $\sim 5$  K around the daily mean temperature, which was increased by 0.5 K every five days, simulating temperatures increase as spring progresses. The photoperiod in all treatments consisted of 8 h high intensity light from metal halide lamps (MF400LS/U, EYE Iwasaki Electric Co., Japan) providing  $506 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$  PFD (photosynthetically active photon flux density; Red:Far Red 4.2) at plant level and a low intensity extensions using incandescent lamps (Classic A 100 W, Osram AG, Munich, Germany) providing  $42 \pm 6 \mu\text{mol m}^{-2} \text{s}^{-1}$  PFD (Red:Far Red 0.8). The length of the photoperiod was extended daily using time switches, set to follow the natural (astronomical) daylength extension at the sampling latitude ( $\sim 47^\circ$  N) of around 3–4 min per day. The short photope-



**Fig. 2.1:** Experimental variation of photoperiod and temperature during the simulated late winter/spring conditions. Long day (LD; starting daylength as of 1 March) and short day (SD; starting daylength as of 1 February) treatments were continuously adjusted to simulate progression of season at a distance of ca. 90 min. Both photoperiod treatments were combined with similar stepwise-increases in temperature ( $T_m$  for daily mean) in order to simulate the natural progression of spring weather at 47° N.

riod treatment thereby corresponded to the daylengths from February to April at this latitude, whereas the long photoperiod treatment simulated daylengths of March to May. The two treatments were replicated and randomly assigned to four of these computer controlled growth cabinets (each 253 cm × 120 cm × 195 cm, Weiss Klimatechnik GmbH, Germany).

Every second day sample positions within each chamber were randomized and every 5 days the samples and photoperiod treatments were switched between the chambers, to minimize potential chamber effects. The chambers were constantly well ventilated to maintain a homogenous temperature distribution within the chamber. The set point temperature during the light period was adjusted to compensate for the heat emission of the lamps. Temperature, humidity as well as light conditions at plant level were monitored using data loggers (HOBO Temperature/RH resp. HOBO Light On/Off, Onset Computer Corporation, Bourne, MA, USA).

## ***Observations***

Given the large number of samples (in total 960 twigs of 240 trees; 4 twigs per tree; Table 2.1), observations had to be split over two day (on each day, half of the cuttings were visually inspected for bud development). The status of the uppermost buds was rated using a four stage scale, as defined by (Murray et al. 1989): (1) bud dormant, (2) bud swollen, (3) bud burst (first green leaf tip showing), (4) leaf unfolding (leaf stalk visible). For efficient observation, each bottle was bar-coded and the bud status was assigned by a bar-coded reference table using a barcode-reader.

## ***Statistical analysis***

Bud burst data was analyzed using split–split plot ANOVAs for each species. The two replicates were used as blocking factor, the photoperiod treatment was applied to whole plots (chambers), and the cuttings within each chamber were treated as elevational samples nested in their region of origin.

Temperatures at the sampling sites (elevations) before sampling were calculated using the linear regressions of the temperature data logged on-site after sampling with temperatures from nearest weather stations ( $R^2$  always  $> 0.95$ , weather station data provided by the Swiss Federal Office of Meteorology and Climatology MeteoSwiss). The degree-days at bud burst were calculated from the reconstructed daily mean temperatures at the sampling site (from 1 January until sampling) and the daily mean treatment temperatures (from sampling until bud burst), using  $0^\circ\text{C}$  as base temperature for degree-day accumulation, as recommended by Heide (1993a). Chilling days were calculated according to Murray et al. (1989) and reflect the number of days since 1 November with daily mean temperature  $\leq 5^\circ\text{C}$ . Since species differ in their individual temperature response, the absolute degree-day value calculated here should assist in explaining the influence of the climatic conditions before sampling and possible ecotypic responses.

For all data processing, statistical analysis and graphics R 2.11.1 (R Development Core Team 2010) were used. All the values mentioned are mean  $\pm$  standard deviation, unless noted otherwise.

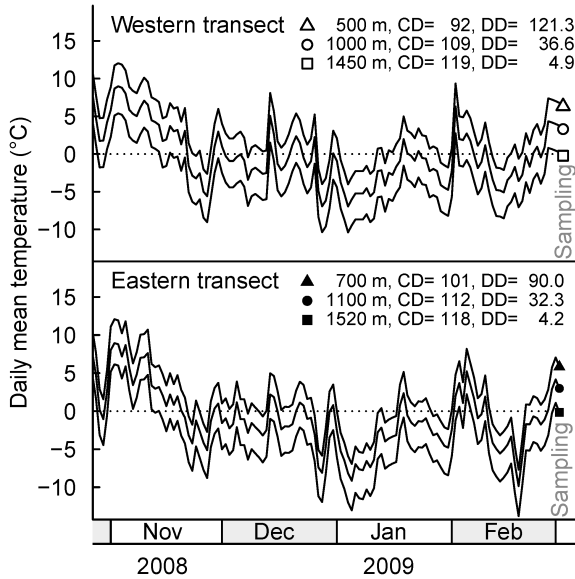
## Results

### *Climatic conditions*

The twigs were sampled as late as early March, to ensure trees were sufficiently chilled. Naturally, buds from lower elevation will have experienced less chilling and consequently also warmer temperatures during the previous winter than those from high elevation (Fig. 2.2). According to the degree-days and the number of chilling days, the winter before the experiment was slightly warmer along the eastern transect than along the western transect, however the thermal differences among the three sampling sites per transect were quite similar on both transects. During the experiment, the temperatures among the four chambers were similar, with only minute deviations between chambers (SD of daily mean temperatures always  $\leq 0.3$  K). Mean relative humidity inside the chambers was kept constant at  $71 \pm 8\%$ .

### *Sequence of bud burst among species*

We observed bud burst under our experimental conditions on all cuttings until the end of the experiment after 70 days (100% bud burst). The bud burst in all cuttings, even under the shorter photoperiods, is thus an indication of successful chilling, given that lack of chilling would have significantly reduced the fraction of bursting buds or delayed bud burst in cuttings (Heide 1993a). The species required different time to bud burst and were following approximately the natural order of early to late flushing species, as commonly found by field observations in Switzerland (Defila, 1991). The earliest species, *Prunus*, started to flush already after 17 days, followed by *Larix*, *Sorbus*, *Betula*, *Aesculus*, *Syringa*, *Corylus*, *Acer*, *Fraxinus*, *Fagus*, *Picea*, *Quercus*, *Abies* in that sequence, and finally after more than 60 days of exposure to the treatment conditions, *Tilia*. The general order of species' bud burst remained similar in both photoperiod treatments. Within-population variation in the time of bud burst was generally low.



**Fig. 2.2:** Daily mean temperatures at the sampling sites in the winter before sampling the cuttings from adult trees. CD indicate number of chilling days since 1 November with daily mean temperature  $\leq 5^{\circ}\text{C}$  and DD degree-days  $> 0^{\circ}\text{C}$  since 1 January until sampling.

## Photoperiodism

Photoperiod affected the timing of bud burst by delaying bud burst in short photoperiods in cuttings of five out of the 14 tested species, namely in the late successional species *Abies*, *Fagus*, *Picea*, *Quercus* and *Tilia* (Table 2.2), the species belonging to the late bud burst group, with the effect in *Tilia* only marginally significant ( $5.2 \pm 2.7$  days). In three of the species which show a clear photoperiod effect, we observed a significant interaction between photoperiod and either region or elevation of origin (Fig. 2 and Table 2.2): in *Abies*, and even more prominently in *Quercus*, the delay of bud burst in short photoperiods was larger in the cuttings sampled from the eastern transect than from those sampled from the western transect (*Abies*:  $10.3 \pm 3.3$  vs.  $5.3 \pm 3.8$  days; *Quercus*  $8.3 \pm 2.0$  vs.  $2.0 \pm 3.4$  days).

**Table 2.2:** Results of the split-split plot ANOVAs for the number of days to bud burst in the 14 tree species included in the experiment. The table shows  $p$ -values of  $F$ -tests, bold values are statistically significant ( $p < 0.05$ ).

Species	Photoperiod	Region	$P \times R$	Elevation	$P \times E$	$R \times E$	$P \times R \times E$
<b>early successional native species</b>							
<i>Acer</i>	0.468	<b>0.001</b>	0.621	<b>0.001</b>	0.731	<b>&lt;0.001</b>	0.961
<i>Betula</i>	0.322	0.082	0.243	<b>&lt;0.001</b>	0.381	0.459	0.315
<i>Corylus</i>	0.344	<b>0.032</b>	0.152	<b>0.009</b>	0.451	0.344	0.514
<i>Fraxinus</i>	0.182	0.893	0.256	<b>0.016</b>	0.346	0.242	0.242
<i>Larix</i>	0.758	0.146	0.184	<b>&lt;0.001</b>	0.203	<b>0.020</b>	0.203
<i>Prunus</i>	0.617	-	-	0.075	0.563	-	-
<i>Sorbus</i>	0.732	<b>0.003</b>	0.809	<b>&lt;0.001</b>	0.371	<b>0.005</b>	0.557
<b>late successional native species</b>							
<i>Abies</i>	<b>0.034</b>	0.222	<b>0.027</b>	<b>0.002</b>	0.316	0.089	0.206
<i>Fagus</i>	<b>0.032</b>	<b>0.047</b>	0.061	<b>0.007</b>	0.499	0.252	0.126
<i>Picea</i>	<b>0.045</b>	0.066	0.160	<b>0.002</b>	<b>0.006</b>	0.770	0.684
<i>Quercus</i>	<b>0.050</b>	<b>0.006</b>	<b>0.016</b>	0.127	0.352	<b>0.027</b>	0.406
<i>Tilia</i>	0.064	-	-	0.076	0.315	-	-
<b>exotic, ornamental species</b>							
<i>Aesculus</i>	0.818	0.220	0.617	-	-	-	-
<i>Syringa</i>	0.927	0.090	0.763	-	-	-	-

$P$ : photoperiod,  $R$ : region,  $E$ : elevation

Finally, in *Picea* the delay of bud burst in short photoperiods was influenced by elevation: the delay was more pronounced in samples from high ( $7.8 \pm 2.4$  days) compared to low elevation ( $3.5 \pm 2.7$  days). No photoperiod effect was observed in the cuttings early successional species (*Acer*, *Betula*, *Corylus*, *Fraxinus*, *Larix*, *Prunus* and *Sorbus*) and in the two ornamental, exotic species *Aesculus* and *Syringa*.

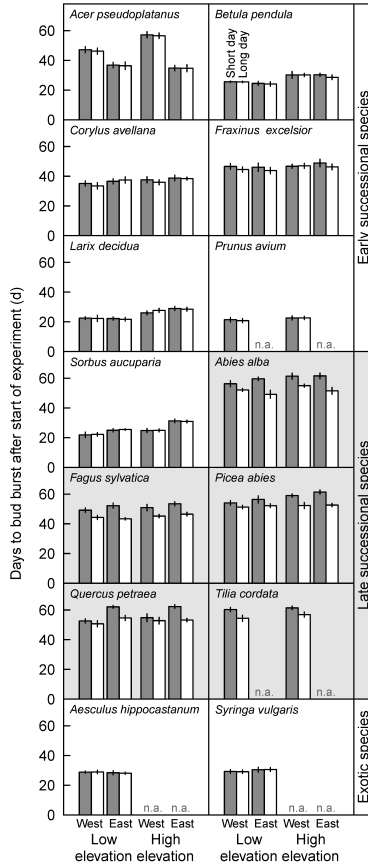
### *Ecotypic responses*

Irrespective of photoperiod, the elevation of cutting origin had an delaying effect on the time of bud burst in almost all studied species (Fig. 2 and Table 2.2): except for *Abies* sampled on the western transect, and *Acer* sampled on the eastern transect, we observed significantly earlier bud burst in the cuttings from low elevation than in those from high elevation. How-

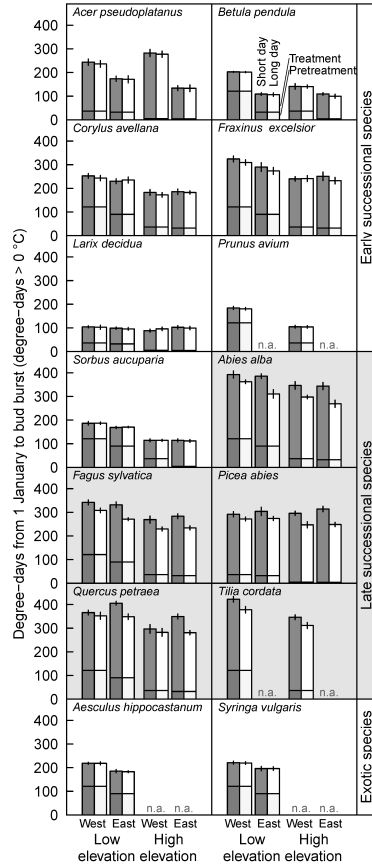
ever, this effect was quite small, mostly two to four days. In most of these species, this elevational delay of bud burst was additionally influenced by the cutting's region of origin, although the direction of the region effect was not consistent: in *Larix*, *Sorbus* and *Quercus*, the elevational delay of bud burst was larger in the cuttings from the eastern transect, whereas in *Abies* and *Acer* this difference was larger in the samples from the western transect. In four species (*Acer*, *Corylus*, *Quercus*, *Sorbus*) the regional differences found were fairly consistent: cuttings sampled at the eastern transect flushed a few days later than those originating from the western transect, although a significant region effect, in the absence of an interaction with elevation, was only present in *Corylus*.

### ***Degree-days until bud burst***

In the five photoperiod sensitive species, longer photoperiods accelerated bud burst and thus, we also found reduced degree-days at bud burst under longer photoperiods (Table 2.3 and Fig. 2.4). Additionally, the degree-days at bud burst were influenced by elevation and/or region of cutting origin in all species assessed here. As described in the preceding section, bud burst of high elevation cuttings was, with few exceptions, later than in cuttings from low elevations under our simulated spring temperatures. Consequently, the high elevation cuttings experienced more degree-days until bud burst during the experiment. However, by including the pre-sampling temperatures since 1 January at the sampling sites into the degree day calculation, we found that the high elevation cuttings of all species (except *Acer* and *Larix*) opened their buds after less degree-days than the low elevation cuttings. No clear elevational pattern was found in *Acer*, which exhibited opposite responses to elevation among regions, both, in terms of days to bud burst and degree-days at bud burst. In *Larix*, elevation of origin had no significant effect on the degree-days at bud burst, despite the highly significant effect on the time of bud burst. Similarly, the regional differences in the time of bud burst in *Corylus* were not reflected in the degree-days at bud burst of this species. Should the different populations have similar thermal requirements for bud burst, the differences in the time of bud burst observed are thus most likely associated with the pre-sampling in situ temperatures.



**Fig. 2.3:** Mean date ( $\pm$ SD) of bud burst under short and long photoperiod treatments in cuttings sampled from high and low elevation mature trees along two elevational transects (East and West; Swiss Alps) under similarly increasing temperatures.



**Fig. 2.4:** Degree days until bud burst in 14 tree species under short and long photoperiod treatments simulating future spring conditions (mean  $\pm$  SD). The degree-days experienced the sampling sites before sampling at high and low elevation sites along two elevational gradients (East and West; Swiss Alps) is indicated.



**Table 2.3:** Results of the split-split plot ANOVAs for the degree-days until bud burst (temperature sum  $>0^{\circ}\text{C}$  since 1 January) for the 14 tree species included in the experiment. The table shows  $p$ -values of  $F$ -tests, bold values are statistically significant ( $p < 0.05$ ).

Species	Photoperiod	Region	$P \times R$	Elevation	$P \times E$	$R \times E$	$P \times R \times E$
<b>early successional native species</b>							
<i>Acer</i>	0.410	<b>0.001</b>	0.526	0.880	0.732	<b>&lt;0.001</b>	0.963
<i>Betula</i>	0.404	<b>0.001</b>	0.239	<b>&lt;0.001</b>	0.344	<b>&lt;0.001</b>	0.286
<i>Corylus</i>	0.316	0.212	0.157	<b>&lt;0.001</b>	0.411	<b>0.011</b>	0.510
<i>Fraxinus</i>	0.171	<b>0.036</b>	0.250	<b>&lt;0.001</b>	0.346	<b>0.006</b>	0.244
<i>Larix</i>	0.961	0.483	0.177	0.061	0.224	<b>0.008</b>	0.196
<i>Prunus</i>	0.451	-	-	<b>0.001</b>	0.534	-	-
<i>Sorbus</i>	0.907	<b>0.018</b>	0.846	<b>&lt;0.001</b>	0.400	<b>0.003</b>	0.537
<b>late successional native species</b>							
<i>Abies</i>	<b>0.034</b>	<b>0.018</b>	<b>0.029</b>	<b>&lt;0.001</b>	0.208	0.090	0.207
<i>Fagus</i>	<b>0.033</b>	0.094	0.061	<b>&lt;0.001</b>	0.612	<b>0.002</b>	0.142
<i>Picea</i>	<b>0.043</b>	0.094	0.139	<b>0.032</b>	<b>0.005</b>	0.651	0.585
<i>Quercus</i>	<b>0.048</b>	<b>0.018</b>	<b>0.014</b>	<b>&lt;0.001</b>	0.347	0.252	0.420
<i>Tilia</i>	0.062	-	-	<b>0.003</b>	0.365	-	-
<b>exotic, ornamental species</b>							
<i>Aesculus</i>	0.588	<b>0.003</b>	0.607	-	-	-	-
<i>Syringa</i>	0.783	<b>0.011</b>	0.761	-	-	-	-

$P$ : photoperiod,  $R$ : region,  $E$ : elevation

## Discussion

This screening for interactive responses of bud burst to photoperiod, temperature and provenance, using cuttings of adult trees revealed both, genetic as well as environment induced effects. Applying as realistic as possible combinations of dynamic temperature and photoperiod conditions, the study permitted a clear ranking of species in terms of photoperiod control of spring development (as opposed to temperature-only control). In the following we will discuss the observed photoperiod and non-photoperiod related responses and their implication for phenology in a warmer climate in future.

### ***Photoperiod sensitive species***

A delayed bud burst in response to a short photoperiod was observed here in the five late successional species *A. alba*, *P. abies*, *F. sylvatica*, *Q. petraea* and *T. cordata*. In these species, photoperiod influenced bud burst despite a high degree of preceding chilling. Our results thereby confirm the photoperiod sensitivity of fully chilled buds, as was previously evidenced only in seedlings and cuttings of *Fagus* (Kramer 1936, Wareing 1953, Heide 1993b, Caffarra and Donnelly 2010) and seedlings of *Picea* (Partanen et al. 1998), while such photoperiod sensitivity has not yet been described in *A. alba* and *Q. petraea*. In *Quercus*, photoperiod sensitivity was observed previously in seedlings of the deciduous *Q. faginea*, but not in the co-occurring, evergreen *Q. ilex* subsp. *ballota* in Spain (Sanz-Perez et al. 2009). In *Tilia*, the marginally significant trend to earlier bud burst of fully chilled buds under our dynamic long photoperiods, challenges the earlier finding that photoperiod sensitivity of this species is limited to insufficiently chilled plants, as was observed in rooted cuttings under constant long photoperiod (16 h vs. 8 h; Caffarra and Donnelly 2010).

### ***Photoperiod insensitive species***

As expected, neither of the two species of exotic origin, *Aesculus* and *Syringa*, was found to be photoperiod sensitive. Also cuttings of early successional (*Acer*, *Betula*, *Corylus*, *Larix*, *Prunus*, *Sorbus*) and intermediate successional species (*Fraxinus*) revealed no measurable photoperiod response. Our finding for *Sorbus* are consistent with those by Heide (1993a) and suggest a general, photoperiod independent control of dormancy for this species, given that this species also shows a weak photoperiod influence on autumnal dormancy induction (Wareing 1956, Heide 2011). In the fully chilled cuttings of *Betula*, the absence of a photoperiod effect under our treatment conditions matches observations by Myking and Heide (1995) and Caffarra et al. (2011). These authors reported photoperiod sensitivity in *Betula* when chilling was incomplete. Our findings in *Corylus*, also belong to the *Betulaceae*, and *Prunus* however, are inconsistent with previous observations: in cuttings of *Corylus*, a 24 h photoperiod advanced bud burst by 2–3 days compared to an 8 h photoperiod, even af-

ter extensive chilling until mid March and treatment conditions of 21 °C, which may be considered as rather extreme test conditions (Heide 1993a). In *Prunus*, a 16 h photoperiod advanced bud burst in rooted cuttings from mature trees compared to seedlings, whereas no such difference was found in a 12 h photoperiod (Besford et al. 1996). We were not able to detect a photoperiod effect under our realistically small contrasts in photoperiod (ca. 90 min only), indicating that such an effect, is too weak to materialize under daylength and temperature conditions as they actually occur at our sampling latitude in spring. However, it cannot be excluded that our initial short photoperiod of 9.5 h was already beyond a potential short photoperiod threshold to break endodormancy under the temperatures employed, nor exclude that cuttings do not reveal the full response (Section 4.5).

## ***Ecotypic responses***

### **Ecotypic photoperiod responses**

Strong heritability of the timing of dormancy release and a considerable variation among provenances of a species under common growing conditions, as observed here, has been frequently evidenced in broad forest tree transplant experiments (e.g., Engler 1905, Burger 1926, but see reviews by Langlet 1971, Morgenstern 1996 and references therein). In contrast, some common garden (Vitasse et al. 2009b)/modelling studies (Chuine et al. 2000) arrived at similar temperature sensitivity of spring phenophases in seedlings of geographically separated populations from low temperate latitudes (southern France), which led the authors to conclude that local adaptation plays only a minor role for phenology under climate warming.

While common garden experiments are excellent tools to assess overall provenance responses under a common climate, it remains difficult to separate thermal responses from photoperiodic responses because both may be ecotypic. For the induction of dormancy, photoperiod ecotypes have been evidenced in many species (Klebs 1914, Vaartaja 1959, Thomas and Vince-Prue 1997, Li et al. 2003, Böhlenius et al. 2006), hence, photoperiod ecotypes may also be expected in dormancy release, although the controls of dormancy release are much more complex. However, among the photoperiod sensitive species examined here, such an ecotypic photope-

riod effect with elevation was found in *Picea* only. This species showed later bud burst under short photoperiods in the high elevation cuttings compared to those from low elevation. In the cuttings of *Abies* and *Quercus* the more pronounced photoperiod responses in the eastern provenances may relate to selective effects of the extreme ‘foehn’ wind in this area, causing exceptionally warm episodes in late winter/early spring, often followed by late freezing. In *Fagus* and *Tilia* however, we found a similar photoperiod effect across the different regions and elevations. For *Fagus*, this is in agreement with the similar responses to photoperiod in four latitudinal ecotypes (47° 59' N) observed by Heide (1993b), who tested cuttings sampled from four regions in mid March with constant photoperiods between 8 and 16 h and under a warm 21 °C temperature regime. Given that *Fagus* has been shown to have a very large chilling requirement (Murray et al. 1989), the actual weight of photoperiod and chilling for the response to follow-up warm temperatures may depend on local weather conditions.

### **Ecotypic thermal responses**

Besides photoperiod ecotypes, provenances may also exhibit heritable thermal responses, the current experiment was not primarily designed to assess. Such provenance-specific thermal responses are most prominently demonstrated by the remarkable elevation-independent (and hence temperature-pre-history independent) variation of bud burst observed here in the photoperiod insensitive species *Acer pseudoplatanus* (Fig. 2.4), a species known for its strong provenance variation in the time of bud burst (Engler 1905, Vitasse et al. 2009a). In most other species assessed here, the high elevation cuttings opened their buds later than the low elevation cuttings under similar temperature and photoperiod conditions (Fig. 2.3). However, contrary to classical common garden experiments, the cuttings used here have not experienced the whole period of dormancy under similar climatic conditions, hence our results may also reflect the contrasting natural pre-history in the field. Obviously, the low elevation trees have experienced higher temperatures before sampling than the high elevation trees (Fig. 2.2). Although the buds appeared dormant (unswollen) at sampling, buds from low elevation might still have been at a slightly advanced developmental stage, in favor of an earlier bud burst. Hence, similar re-

sponses under the same controlled conditions under otherwise substantially different natural pre-history may mask differences that might have been seen when provenances had been exposed to an identical pre-history, as irrelevant this would be from an ecological point of view.

Under our treatment conditions, high elevation cuttings open their buds later, however, at less of degree-days than low elevation cuttings (degree-days in situ since 1 January to sampling and treatment degree-days until bud burst; Fig. 2.4). Similarly, a lower thermal requirement for bud burst of high elevation provenances has also been found in common garden experiments with *Fagus* (Hjelmqvist 1940, von Wuehlisch et al. 1995, Chmura and Rozkowski 2002, Vitasse et al. 2009a) and *Picea* (Engler 1905, Burger 1926, Worrall 1983) and some other species (Acevedo-Rodríguez et al. 2006). Consequently, a lower thermal threshold for bud burst, as was also observed here, was suggested for high elevation provenances of these species (Worrall 1983, von Wuehlisch et al. 1995). In *Picea* however, seemingly inconsistent results have been found in common garden experiments: Burger (1926) observed consistent earlier bud burst of young trees (9–15 years) from high elevation grown at 380 m, 670 m and 1880 m a.s.l., with a more pronounced difference in higher elevation gardens. In a large provenance trial at low elevation (226 m a.s.l.) however, no such trend were observed in *Picea* seedlings, whereas even a reversed trend was observed in young trees (> 9 years; Holzer and Nather 1974). These differences may have been introduced by the enhanced photoperiod sensitivity of the high elevation ecotypes of this species as observed here. The reverse trend, that is earlier bud burst of low elevation provenances (and thus at less degree-days) was also found in seedlings of *P. avium* (Besford et al. 1996) and *Q. petraea* (Vitasse et al. 2009a, Alberto et al. 2011). Our observed opposite elevational pattern in the degree-days at bud burst in these species may also have been caused by species specific temperature thresholds for the progression of development, which may be higher than the 0 °C daily base temperature used here for degree day calculation. Higher thresholds would reduce the weight of the mostly cool pre-sampling temperatures in situ (Fig. 2.2) and may thus counterbalance the elevational effects on the degree-days observed here. However, also in common garden studies the presence or direction of elevational trends of bud burst under similar climatic conditions seems to be strongly species specific and may be influenced by age of the plant material used (seedling vs. mature tree) and climate (elevation) of the garden. Furthermore, eco-

typic elevational trends may be missing because of high within-population variance of bud burst or because elevation is confounded with latitude in the different studies.

### ***Ecological advantage of photoperiod sensitivity***

The obvious ecological advantage of photoperiod sensitivity is the reduced risk of freezing damage in new, but premature tissue. Thus, species specific differences are likely to be related to seasonal freezing risk in the species' natural habitat and to the species' life history. In contrast to the photoperiod sensitive late successional species, early successional species commonly reach bud burst as soon as temperatures permit, without pronounced chilling and photoperiod requirements. This opportunistic behavior will lengthen the active growing season, while enhancing the risk of freezing damage. In the photoperiod sensitive species however, photoperiod seems to be modulating the response to concurrent warm temperatures guiding bud burst into a 'safer' period. Longer photoperiods may thus either decrease the thermal requirement for bud burst, or (more likely) speed up development at a given temperature. The generally late bud burst of ring-porous species however, as observed here in photoperiod insensitive *Fraxinus* (holding an intermediate successional position), but also in the late successional *Quercus*, may be additionally associated with a slower reactivation of water supply by a new layer of xylem before bud burst, given that these species lose most of the hydraulic conductivity through embolism during winter (Sperry et al. 1994).

### ***Methodical considerations***

Photoperiod experiments with trees face two problems related to tree size and the known differences between young life stages compared to older life stages (Ununger et al. 1988, Besford et al. 1996, Partanen et al. 2001). In situ photoperiod manipulation on mature trees, without affecting thermal conditions and allowing for appropriate replication, is constrained by tree size and the dose-independency of the photoperiod signal which

would require absolute light-tight darkening of whole trees during the early/late parts of the day, should the effect of warmer temperature be tested at shorter photoperiods. Alternatively, growth chamber experiments are always limiting plant size, such that only small saplings or cuttings (cut twigs) of mature trees can be used. While saplings, in contrast to mature trees, are known to exhibit a more opportunistic behavior concerning bud burst (understory trees flush before canopy trees, utilizing light before the canopy closes; Uemura 1994, Augspurger and Bartlett 2003, Richardson and O'Keefe 2009), cuttings are, per definition, disconnected from whole-tree (hormonal) signals potentially affecting bud burst. In some tree species cuttings may respond quite autonomously and thus, can serve as an appropriate substitute for mature trees in growth chamber studies, whereas in other (unknown) cases, cuttings will not reflect whole tree responses. The direction of artifact is unpredictable, but delays in phenology may be expected in processes related to xylem pressure and tissue turgor and root plus whole crown hormonal signals. We assume that the sum of these limitations leads to a conservative picture of photoperiod signals in cuttings compared to whole tree responses. Hence many of the observed patterns may indicate direction, rather than the full signal strength that would only be seen in a whole tree approach.

## Conclusions

The observed photoperiod sensitivity in late successional tree species, demonstrates that spring phenology of most of the observed late successional tree species is not driven by temperature alone, even after experiencing substantial (natural) chilling. In a future climate with warmer springs, photoperiod will become an increasingly important factor for constraining the timing of spring phenology when warmer weather conditions are accelerating development (earlier bud burst) toward genetic photoperiod thresholds. Combined with reduced chilling in milder, low elevation winters, some late successional species are likely not to continue tracking the actual (warmer) temperatures as they currently still do. Our results evidence the considerable photoperiod influence on bud burst at otherwise weak indications for ecotypic differentiation. Our results suggest that photoperiod plays only a minor role in early successional species.

Since phenology of trees can be expected to have been selected for efficient use of the growing-season, different photoperiod and temperature sensitivities among species or genotypes are likely to affect the success of species in a warmer climate. The selection for new photoperiod genotypes will take several tree-generations ( $> 100$  years). The results obtained here in cuttings of adult trees are likely to underestimate the actual significance of photoperiod for spring phenology. Taken together, the experimental evidence for spring phenology presented here, warns at scaling trends observed in the recent past into a warmer future by accounting for temperature only. Such extrapolations need to account for temperature  $\times$  photoperiod interactions in mature, late successional trees.

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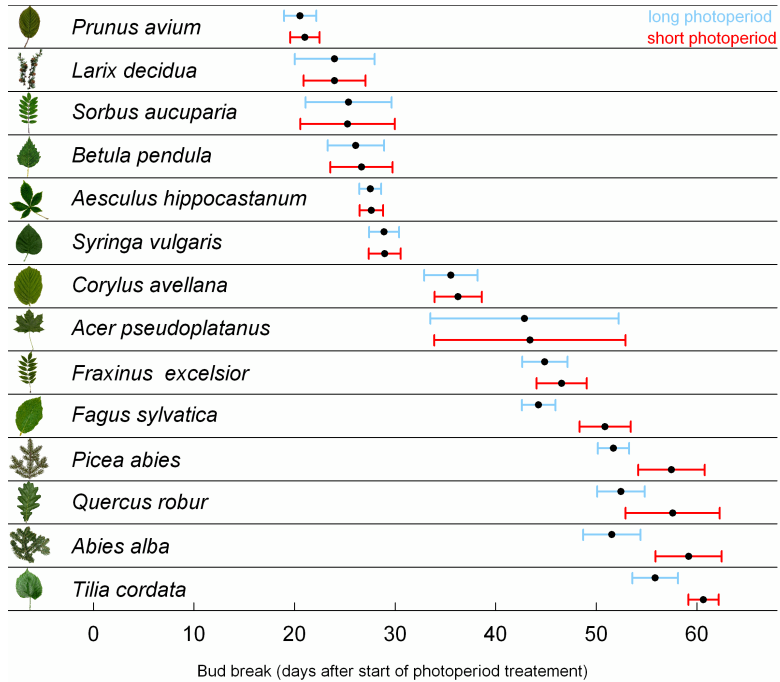
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## Supplementary material



Mean date ( $\pm$ SD,  $n=20$ ) of bud burst in cuttings under naturally extending short- and long-photoperiod treatments with similarly increasing temperatures. Cuttings were sampled from mature trees along two elevational transects in the Swiss Alps (high and low elevation) in early March.



## Chapter 3

# Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species

David Basler, Christian Körner

**Abstract** Spring phenology of temperate forest trees is optimized to maximise the length of the growing season while minimizing the risk for freezing damage. The release from winter dormancy is environmentally mediated by species-specific responses to temperature and photoperiod. We investigated the response of early spring phenology to temperature and photoperiod at different stages of dormancy release in cuttings from four temperate tree species in controlled environments. By tracking bud development, we were able to identify the onset of bud swelling and bud growth in *Acer pseudoplatanus* L., *Fagus sylvatica* L., *Quercus petraea* (Matuschka) Liebl. and *Picea abies* (L.) H. Karst. At a given early stage of dormancy release, the onset and duration of the bud swelling prior to bud burst is driven by concurrent temperature and photoperiod, while the maximum growth rate is temperature dependent only, except for *Fagus*, where long photoperiods also increased bud growth rates. Similarly, the later bud burst was controlled by temperature and photoperiod (in the photoperiod sensitive species *Fagus*, *Quercus* and *Picea*). We conclude that photoperiod is involved in the release of dormancy during the ecodormancy phase and may influence bud burst in trees that experienced sufficient chilling. This study explored and documented the early bud swelling period that precedes and defines later phenological stages such as canopy greening in conventional phenological works. It is the early bud growth resumption that needs to be understood in order to arrive at a causal interpretation

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and modelling of tree phenology at large scale. Classical spring phenology events mark visible endpoints of a cascade of processes as evidenced here.

**Key words:** Day length, Deciduous trees, Development, Phenology, Warming

## Introduction

Trees in temperate and boreal climates undergo a period of dormancy and enhanced freezing resistance to withstand the harsh climate conditions during winter. The phenological events that coincide with induction and release of dormancy (bud set and bud burst) are finely tuned to the seasonality of the tree's environment, minimizing the risk of potentially fatal freezing damage in autumn and spring, while maximising the length of the growing season. A well timed phenology is crucial for long-term survival, successful reproduction and species persistence (Larcher 2003).

Plant dormancy is characterized by suspension of growth and development (Samish 1954) that is, suppressed cell division and a strongly reduced metabolism. Three different states of dormancy are distinguished (Lang et al. 1987): (1) endodormancy, an internal, (genetically controlled) set state of inactivity, (2) ecodormancy, a state of inactivity imposed by unfavourable environmental conditions and (3) paradormancy, a state of specific bud dormancy maintained due to physiological factors outside the dormant meristems (e.g. correlative inhibition and apical dominance). The phenological changes that occur when plants perceive the environmental signals for the induction and release of dormancy are associated with physiological responses including phytohormones, phytochrome, and carbohydrates (Chao et al. 2007). The gradual transitions between the different phases of dormancy involve numerous genetic, biochemical, physiological and anatomical alterations (Faust et al. 1997, Rinne et al. 1997, Horvath 2010, Cooke et al. 2012). During the winter months, bud scales may grow minutely (Perry 1971) and cell division in the apical meristems may continue at low rates, but elongation growth is absent due to an inhibition of the sub-apical tissue (Romberger 1963).



In humid extra-tropical climates the induction and release of seasonal dormancy is triggered by environmental signals, mainly temperature and photoperiod. In most temperate and boreal trees dormancy is induced by the decreasing length of the photoperiod in autumn and cool temperatures, resulting in growth cessation and the formation of winter buds (Wareing 1956, Vaartaja 1959, Thomas and Vince-Prue 1997). The astronomically defined photoperiod serves as a reliable environmental signal for the progression of the season and may thus indicate the period with higher risk of freezing events in autumn before trees are actually exposed to such temperatures. Photoperiod and low temperature may induce dormancy through independent pathways (Welling et al. 2002) and in a few species, low temperatures alone seem to be sufficient to induce endodormancy (Heide and Prestrud 2005, Heide 2011).

Once established, endodormancy ensures that growth will not be resumed during warm spells in winter. In tree species adapted to cool climates, endodormancy is generally released after sufficiently long exposure to cool, non-freezing temperatures ('chilling'; Perry 1971, Sarvas 1974). Yet, the actual range of effective temperatures for chilling are only vaguely known for forest trees, and cool, non-freezing temperatures up to 10 °C, most likely between 2 and 4 °C are expected to be most effective (Battey 2000). Higher temperatures may even negate previous chilling (Perry 1971), while lower (sub-zero) temperatures are generally considered to be ineffective for the fulfilment of the chilling requirement, presumably because very low temperatures prevent a physiological integration of signals (too low metabolic activity). Once the chilling requirement is fulfilled, metabolic activity increases, hydrolytic enzymes are activated and carbohydrate reserves gradually become mobilized. As a first visually identifiable clue, the onset of bud swelling indicates that the transition from endodormancy to ecodormancy has occurred (Saure 1985, Pallardy 2008). The bud water content rises (Essiamah and Eschrich 1986) and the buds are becoming increasingly susceptible to freezing. The subsequent release of ecodormancy is modulated by favourable environmental conditions. Bud burst of many short lived and pioneer species is then mediated by warm temperatures only and buds burst occurs, when the accumulated temperature sum exceeds a genotype-specific threshold (forcing requirement; degree days; Nienstaedt 1967, Perry 1971). Photoperiod sensitivity is most pronounced in *Fagus sylvatica* L. (Klebs 1914, Wareing 1953, Heide 1993b), but was also observed in other tree species (Heide 1993a,

Partanen et al. 1998, Caffarra et al. 2011, Basler and Körner 2012). Already 120 years ago, Jost (1894) observed a failure or major delay of bud burst in *Fagus* on twigs subjected to complete darkness in-situ. Photoperiod controls of spring phenology were adopted mainly by long-lived, late successional tree species (Caffarra and Donnelly 2010, Körner and Basler 2010). Photoperiod may interact at different stages of dormancy release, e.g., long photoperiods are likely to substitute for a lack of chilling (Downs and Borthwick 1956, Wareing 1969, Heide 1993a) and a decrease the thermal requirement for bud burst (Myking and Heide 1995, Caffarra et al. 2011). However, photoperiodic responses in spring phenology are highly species dependent and still not widely acknowledged, mostly due to the fact that species commonly operate within a photoperiod ‘window’ in which temperature has an overwhelming effect, particularly in cool years (Körner 2007). In a nutshell, the three potential environmental drivers (chilling, photoperiod and temperature) of spring phenology interact in complex, species-specific ways, that await to be clearly disentangled.

In photoperiod-sensitive species, a delayed bud burst under short photoperiods may relate to a later onset of bud development or to slower rates of bud development. In this study, we assessed the responses of early spring phenological phases (bud swelling and bud burst) to photoperiod at different temperatures during release from endodormancy in three photoperiod-sensitive species (European beech *Fagus sylvatica* L., sessile oak *Quercus petraea* (Mattuschka) Liebl. and Norway spruce *Picea abies* (L.) H.Karst.) and an assumingly photoperiod-insensitive species (sycamore maple *Acer pseudoplatanus* L.). We conducted growth chamber experiments using cuttings from mature trees after three consecutive sampling dates (presumed states of dormancy release) in late winter and early spring. To account for possible ecotypic differentiation we sampled each species in populations from two elevations and across two regions. We expected an earlier dormancy release and bud burst in warm temperatures and a distinct delay of bud burst under shorter photoperiods in the late successional species (*Fagus*, *Quercus*, *Picea*) assessed here.

## Methods

### *Sampling Region*

High and a low elevation sampling sites were defined along two elevational gradients in the region of Chur (46.51°N/9.51°E, hereafter named ‘eastern transect’) and Lavey (46.12°N/7.02°E, ‘western transect’), Switzerland (for further details see Basler and Körner 2012). The elevational difference between the high and low sampling sites was around 500 m, which corresponds to a mean temperature difference of around 3 K on both transects. In the year preceding the experiment, temperature loggers (TidBit v2, Onset Computer Corporation, Bourne MA, USA) were placed at the sampling sites inside the forest in order to track the local air temperatures (2 m above ground, shaded). The two regions were treated as replicates of the elevational sampling for all further analysis (no climatic contrast based on our records). In order to avoid confusion between temperatures (°C) and temperature differences, we join other authors in adopting K (for Kelvin) for all differences in temperature.

### *Sampling*

During late winter/early spring 2010 cuttings of four species (*Acer pseudoplatanus* L., *Fagus sylvatica* L., *Quercus petraea* (Mattuschka) Liebl. and *Picea abies* (L.) H.Karst.; in the following we refer to species by their genus name) were sampled three times (26/27 January, 1/2 March, 30/31 March) on each of the four sampling sites. On each site, dormant twigs of five individual trees per species were sampled from the lower canopy (5 to 6 meters above ground, 5 twigs per tree) using a 4 m tree pruner (Fiskars, Helsinki, Finland). The twigs were immediately labelled, watered, and transported to the Institute of Botany within 6 hours where they were stored at 2 °C in the dark until sample preparation on the following day (28 January, 3 March, 1 April).

### *Sample treatment*

Sample preparation was done according to the method described in Basler and Körner (2012): the twigs were cut to a length of 30–40 cm, the lower part dipped into a disinfectant chlorine solution, re-cut a second time underwater at a steep angle using sterile branch scissors, and placed into 0.5 l glass bottles filled with 0.4 l cool, chlorine free tap water. During the experiment, the water was changed weekly and at the same time twigs were re-cut another 1–3 cm in order to assure good water supply.

### *Treatments*

The treatments consisted of a fully reciprocal design of two temperatures treatments (6 °C versus 9 °C daily mean temperature with a diurnal cycle with a 10 K amplitude,  $\pm 5$  K day cycle) and photoperiods (initially 9.2 h (SD) versus 10.8 h (LD), increased daily by the natural daily increase of photoperiod at 46.5° N) assigned to four computer controlled growth cabinets (each 253 cm  $\times$  120 cm  $\times$  195 cm, Weiss Klimatechnik GmbH, Germany). The initial photoperiod resembled the natural photoperiod of 25 January (SD) and 25 February (LD) at the sampling sites. The photoperiod in all treatments consisted of 8 hours high intensity light from metal halide lamps (MF400LS/U, EYE Iwasaki Electric Co., Japan) providing  $506 \pm 30 \mu\text{mol m}^{-2}\text{s}^{-1}$  PFD (Red:FarRed 4.2) at plant level and a low PFD intensity extension period using incandescent lamps (Classic A 100W, Osram AG, Munich, Germany) providing  $42 \pm 6 \mu\text{mol m}^{-2}\text{s}^{-1}$  PFD (Red:FarRed 0.8). This low PDF extension to the desired photoperiod should prevent a confounding between photoperiod and the dose of photon flux received (Wareing 1953). An additional set of cuttings was placed in a warm greenhouse ( $> 21$  °C) with long daylength (16 h) provided by metal halide lamps to determine the time to bud burst under warm forcing conditions

### ***Visual bud census***

Bud development was observed in two day intervals, using a four-stage scale for bud development (dormant, swollen, bud burst, leaf unfolding). Bud burst was defined by the appearance of the first green leaf tip. Due to the large number of samples (1200 twigs after the third sampling), we used a customized barcode system to efficiently accomplish the bud observations: The samples were identified by a barcode-label and the visually determined bud status was stored directly into a spreadsheet using a bar-coded scale for the state of bud development.

### ***Tracking bud swelling and image analysis***

Bud development until bud burst was tracked using image time series made by scanning the twigs every 3 to 4 days using a commercial flatbed scanner (CanoScan LiDe 200, Canon, Tokyo, Japan; scanned at 300dpi resolution). Bud width, -length and -projected area were then extracted from the individual bud images using custom designed semi-automatic software (by the author, written in python; [www.python.org](http://www.python.org)). A total of 960 time series ( $\sim 10^5$  images) was assembled; however, 234 time series had to be excluded from further analysis, as no continuous observation of the same bud on the twig was possible or the observed bud failed to burst.

The onset of bud swelling ( $\lambda$ ) and thus, the duration of the bud swelling period under our controlled temperature treatments, were determined as the nonlinear least-squares estimates of a partially linear model fitted to the individual bud measurement time series  $y(t)$ :

$$\log(y(t)) = \begin{cases} y_o & (t < \lambda) \\ \mu_{\max}(t - \lambda) + a & (t \geq \lambda) \end{cases} \quad (3.1)$$

where  $t$  denotes time,  $y_o$  is the fitted size of the dormant bud and  $\mu_{\max}$  the fitted maximal bud growth rate. The parameter  $a$  was given as

$$a = y_{\max} - \log(e^{\mu_{\max}(t-\lambda)} + e^{y_{\max}-y_o} - 1) \quad (3.2)$$

where  $y_{max}$  is a theoretical asymptote fitted to allow decelerated growth prior to bud burst. In cases where growth deceleration before bud burst was very low or absent, the fitting procedure failed to converge using Eq. (3.2) in Eq. (3.1). Therefore, a reduced model with parameter  $a$  set to  $a = y_o$  (as Eq. (3.2) approaches to  $y_o$  for large values of  $y_{max}$ ), was additionally fitted to the bud measurement time series and the model resulting in a lower residual sum of squares was used for the determination of  $\lambda$  (see also Supplementary Fig. S3.1). The model derived from bacterial growth models with lag phase (e.g. Buchanan et al. 1997, Baty and Delignette-Muller 2004). Our adaptation of the lag-exponential model was found to be more versatile to fit our data and provide more stable estimates of onset of bud swelling ( $\lambda$ ) than a logistic- or Gompertz model with lag phase. Time series consisting of less than four valid bud measurements or where bud swelling was assumed to have started before the first measurement of the time series (where a simple exponential model, without the initial linear period, provided a better fit) were excluded from further analysis. The bud size at bud burst was determined using the parameters derived from the above equations. The scanned bud images were also used to double-check the visually determined bud burst dates assessed during the experiment.

## *Statistics*

Effects of sampling date (prolonged exposure to in situ temperatures) and elevation of origin on bud size at sampling and depth of dormancy (days to bud burst during warm  $> 21$  °C, long day conditions 16 h) were tested using ANOVAs, followed by post-hoc (TukeyHSD) to test for individual differences between treatments.

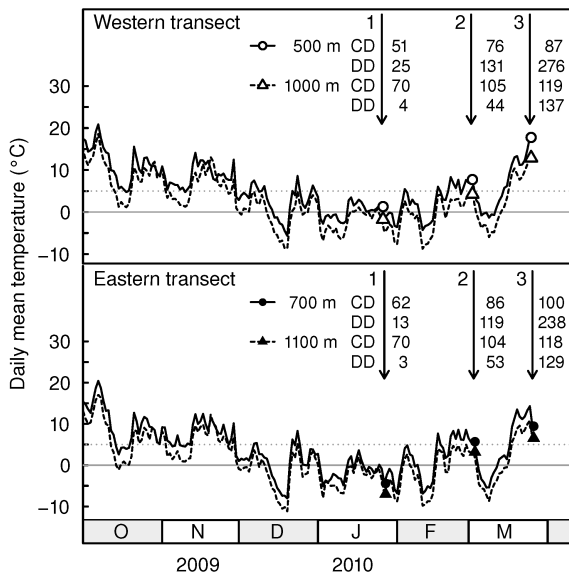
Effects of temperature, photoperiod and, for bud burst only, elevation of origin, including all their interactive effects on the onset of bud swelling, bud growth rate and bud burst were tested by fitting linear models with restricted maximum likelihood (REML) using sampling date nested in site as random factor.

All statistical analyses and figures were done using R 2.15.0 (R Development Core Team 2010)

## Results

### *Field conditions/Treatments*

The local winter temperatures 2009/2010 on both sampling sites before sampling corresponded closely to the long term average, with the exception of a warm spell in November 2009. The temperatures on the eastern transect were slightly warmer than on the western transect, while the temperature difference between the high and low samplings sites was around 3 K on both transects. The period between the first and second sampling date was dominated by cool temperatures with daily means well below 5 °C and occasional freezing spells. Between the second and third sampling, the trees experienced an additional period of sub-zero temperatures



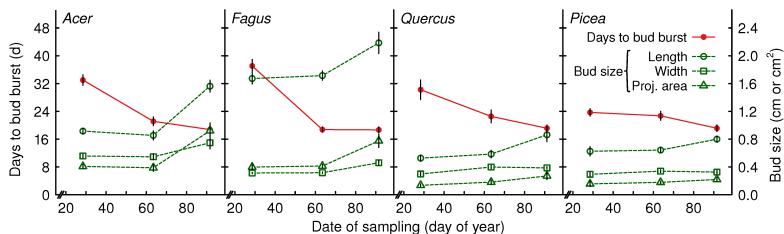
**Fig. 3.1:** Daily mean temperatures at the sampling sites in the winter before the experiment and between the three consecutive sampling dates. CD indicate number of chilling days since 1 November with daily mean temperature <5 °C and DD degree-days >0 °C since 1 January until sampling.

before the temperatures started to rise with daily means above 5 °C in second half of March (Fig. 3.1).

In the growth chambers, the treatment temperatures could be maintained to near the set points with an overall mean temperature of  $8.7 \pm 2.3$  °C (short daylength, SD) and  $8.7 \pm 2.6$  °C (long daylength, LD) in the warm treatments and the  $5.6 \pm 2.2$  °C (SD) and  $5.7 \pm 2.1$  °C (LD) in the cool treatments. During the whole experiment (96 days), temperature sums were 829 degree-days (SD) and 822 degree-days (LD) in the warm treatments, and 530 (SD) and 548 (LD) degree-days in the cool treatments. In the warm greenhouse, the mean temperature of the additional long day forcing treatment was  $22.6 \pm 2.8$  °C (2158 degree-days).

### *Development of bud size and dormancy on sampling sites*

In situ bud size during late winter and early spring was influenced by elevation of origin and sampling date (26/27 January, 1/2 March, 30/31 March). At the first sampling date end of January, all sampled buds appeared to be fully dormant. Significantly larger buds were observed only at the last sampling end of March, indicating that bud swelling has already started under the local weather conditions at the sampling sites (Fig. 3.2). A significant increase of bud length, -width, and -projected area at the last sampling was found in all four species, although in absence of an increase of bud width in *Quercus*. Against expectation, we observed also



**Fig. 3.2:** Mean ( $\pm$  SE) days to bud burst under forcing conditions ( $> 21$  °C/16 h photoperiod) and bud size in four species at three consecutive sampling dates during winter/spring 2010. The figure shows pooled data over four sampling sites.



elevational differences in two species: Buds from higher elevation were consistently larger than from lower elevations at all three samplings dates in *Acer*, while *Picea* showed the opposite pattern.

The assessment of dormancy state at sampling as measured by the number of days to budburst under a warm, long day treatment ( $> 21^{\circ}\text{C}/16\text{ h}$  photoperiod), revealed that the initial dormancy at the first sampling in January was deeper in the broad-leaved species (*Acer*, *Fagus*, *Quercus*) than in the needle-leaved *Picea* (Fig. 3.2). In all four species, depth of dormancy decreased with later sampling dates, until only ca. 16 forcing days were required (at  $> 21^{\circ}\text{C}$ ) for bud burst after the last sampling by the end of March (Fig. 3.2). In *Fagus* and *Quercus*, elevation of origin influenced the decrease of dormancy with later sampling dates. In *Fagus*, high elevation cuttings exhibited a deeper initial dormancy than low elevation cuttings, while no distinct elevational pattern in dormancy state across the three sampling dates was present in *Quercus* (Table 3.1). In all species, days to bud break shortened despite no sign of bud swelling.

### ***The influence of temperature and photoperiod on bud development***

Photoperiod and temperature treatments resulted in species specific responses of bud swelling and bud burst.

#### **Bud Swelling**

As the projected bud area measurement is likely to provide the best general representation of bud size and correlates well with bud length (coefficients of determination  $R^2$  *Acer* 0.96, *Fagus* 0.83, *Quercus* 0.88, *Picea* 0.89) and, to a lesser extent, with bud width (*Acer*  $R^2 = 0.76$ , *Fagus* 0.83, *Quercus* 0.61, *Picea* 0.67) we report projected bud area data only. No a priori difference in initial (dormant) bud size was present between the individual treatments (Table 3.2). Bud swelling started 27 to 61 days after sampling for the first sampling cohort, 21 to 39 days after sampling for the second sampling cohort (Fig. 3.3) and has already started under in situ conditions before the third sampling date (hence, this sampling cohort was excluded

from the analysis of bud swelling). Bud swelling was mainly influenced by temperature and, to a lesser extent, by photoperiod (Fig. 3.3). Thereby, warm temperatures significantly advanced the onset of bud swelling and increased the maximum rate of bud growth in all four species (Table 3.2). Long photoperiod also advanced the onset of bud swelling and increased maximum bud growth rates, but only in *Fagus*. However, the total duration of bud swelling until bud burst was affected by temperature and photoperiods in all species, with the exception of *Acer*, where it was controlled by temperature only. In all species, bud swelling started earlier under our treatment conditions in the second sampling cohort, while the time of sampling had no effect on maximum bud growth rates or on the duration of bud swelling until bud burst. Still, later sampling reduced the temperature effect on the onset of bud swelling in *Acer* and the photoperiod effect on the onset of bud swelling in *Picea*. Later sampling also decreased the in-

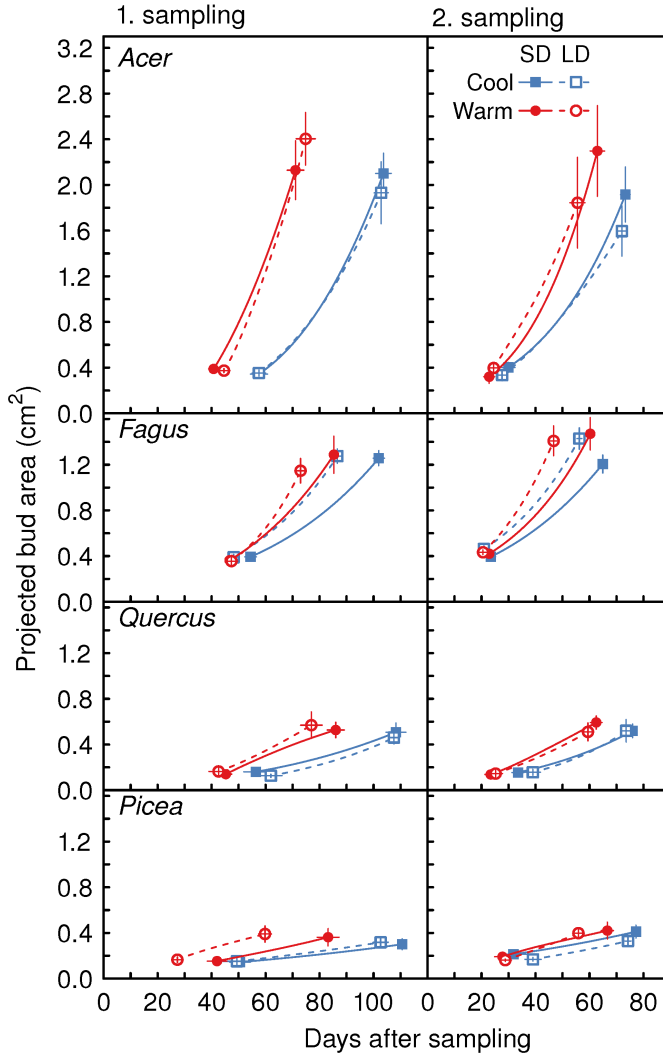
**Table 3.1:** Effects of date of sampling (26/27 January, 1/2 March, 30/31 March) and elevation of origin on bud size at the time of sampling and the days to bud burst after a subsequent transfer to forcing conditions ( $>21^{\circ}\text{C}/16\text{ h}$  photoperiod) in four species. The table shows results of ANOVA as  $p$ -values of  $F$ -tests, bold values are statistically significant ( $p < 0.05$ )

	Bud dimensions			Dormancy
	Length	Width	Proj. area	Days to bud burst
<i>Acer pseudoplatanus</i>				
Sampling	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Elevation	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.503
$S \times E$	0.126	0.73	0.185	0.081
<i>Fagus sylvatica</i>				
Sampling	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Elevation	0.500	0.393	0.53	<b>0.019</b>
$S \times E$	0.23	<b>0.003</b>	<b>0.021</b>	<b>0.019</b>
<i>Quercus petraea</i>				
Sampling	<b>&lt;0.001</b>	0.336	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Elevation	0.461	0.462	0.274	0.967
$S \times E$	0.840	<b>0.042</b>	0.531	<b>0.002</b>
<i>Picea abies</i>				
Sampling	<b>0.018</b>	0.069	0.122	<b>0.028</b>
Elevation	<b>&lt;0.001</b>	<b>0.003</b>	<b>&lt;0.001</b>	0.503
$S \times E$	0.368	<b>0.017</b>	0.109	0.456

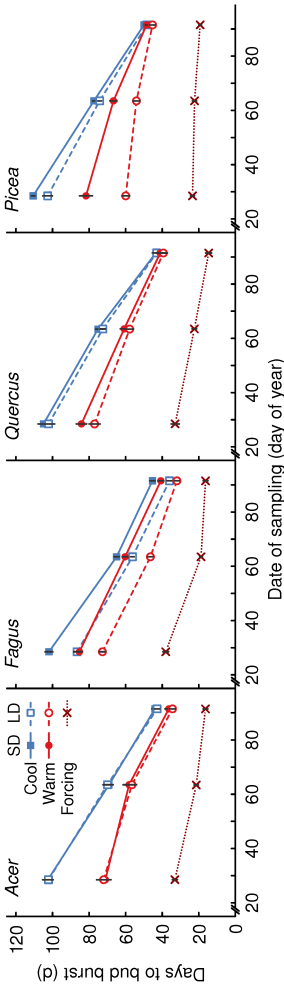
fluence of temperature on the duration of bud swelling in all species but *Fagus*. No significant differences were observed for the bud size at bud burst in neither species.

**Table 3.2:** Effects of temperature, photoperiod and sampling date (26/27 January, 1/2 March; reflecting increased natural chilling) on the initial bud size (projected area;  $BS_i$ ), bud size before bud burst ( $BS_{BB}$ ), onset ( $\lambda$ ) and duration ( $t_{BS}$ ) of bud swelling and maximum growth rate ( $\mu_{max}$ ). The table shows results of ANOVA as  $p$ -values of  $F$ -tests, bold values are statistically significant ( $p < 0.05$ )

	Bud swelling				
	$BS_i$	$\lambda$	$\mu_{max}$	$BS_{BB}$	$t_{BS}$
<i>Acer pseudoplatanus</i>					
Temperature	0.605	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.109	<b>&lt;0.001</b>
Photoperiod	0.971	0.690	0.201	0.308	0.265
Sampling	0.516	<b>0.004</b>	0.232	0.328	0.375
$T \times P$	0.520	0.165	0.600	0.689	0.237
$T \times S$	0.314	<b>0.002</b>	0.154	0.835	<b>0.019</b>
$P \times S$	0.699	0.450	0.411	0.250	<b>0.266</b>
<i>Fagus sylvatica</i>					
Temperature	0.338	<b>0.017</b>	<b>&lt;0.001</b>	0.543	<b>&lt;0.001</b>
Photoperiod	0.309	<b>0.013</b>	<b>0.001</b>	0.921	<b>&lt;0.001</b>
Sampling	0.052	<b>&lt;0.001</b>	0.938	0.303	0.225
$T \times P$	0.370	0.083	0.216	0.086	<b>0.050</b>
$T \times S$	0.269	0.098	0.734	0.175	0.065
$P \times S$	0.164	0.943	0.901	0.266	0.316
<i>Quercus petraea</i>					
Temperature	0.373	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.076	<b>0.001</b>
Photoperiod	0.869	0.235	0.320	0.682	<b>0.001</b>
Sampling	0.911	<b>0.005</b>	0.728	0.693	0.061
$T \times P$	0.144	0.131	0.117	0.959	0.703
$T \times S$	0.238	0.332	0.442	0.750	<b>0.013</b>
$P \times S$	0.621	0.595	0.525	0.728	0.993
<i>Picea abies</i>					
Temperature	0.830	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.059	<b>&lt;0.001</b>
Photoperiod	0.353	0.240	0.079	0.683	<b>&lt;0.001</b>
Sampling	0.282	<b>0.029</b>	0.445	0.411	<b>0.029</b>
$T \times P$	0.856	0.067	0.685	0.680	0.637
$T \times S$	0.363	0.110	0.808	0.626	<b>0.013</b>
$P \times S$	0.143	<b>0.024</b>	0.301	0.356	0.540



**Fig. 3.3:** Average bud growth from the onset of bud swelling until bud burst under different temperature and photoperiod conditions of two consecutive sampling dates (26/27 January, 1/2 March). The underlying parameters were obtained by fitting a lag-exponential function to the individual bud size time series (see *Methods*). Error bars represent  $\pm$  SE. The figure shows pooled data over four sampling sites.



**Fig. 3.4:** Mean days ( $\pm$ SE) to bud burst under different temperatures ( $6^\circ\text{C}$  vs.  $9^\circ\text{C}$ ) and photoperiods (initially 9.2 h (SD) versus 10.8 h (LD), increased daily by the natural daily increase of photoperiod at  $46.5^\circ\text{N}$ ) at three consecutive sampling dates during winter/spring 2010. Additionally, the bud burst under the forcing conditions ( $> 21^\circ\text{C}/16\text{ h}$  photoperiod, Fig. 3.2) is shown. The figure shows pooled data over four sampling sites.

**Table 3.3:** Mean differences ( $\pm$ SD) in the days to bud burst between the temperature treatments ( $\Delta\text{BB}(T)$ ; cold-warm) and photoperiod treatments ( $\Delta\text{BB}(P)$ ; short-long) as influenced by elevation of origin and date of sampling.

Sampling	<i>Acer pseudoplatanus</i>		<i>Fagus sylvatica</i>		<i>Quercus petraea</i>		<i>Picea abies</i>	
	Elevation	$\Delta\text{BB}(P)$	$\Delta\text{BB}(T)$	$\Delta\text{BB}(P)$	$\Delta\text{BB}(T)$	$\Delta\text{BB}(P)$	$\Delta\text{BB}(T)$	$\Delta\text{BB}(P)$
January 26/27	high	$31.6 \pm 14.5$	$-2.0 \pm 27.1$	$17.4 \pm 13.9$	$13.5 \pm 16.0$	$19.9 \pm 17.7$	$2.0 \pm 22.7$	$38.1 \pm 19.4$
	low	$29.9 \pm 23.2$	$1.6 \pm 31.8$	$13.2 \pm 15.8$	$14.5 \pm 15.2$	$27.4 \pm 14.6$	$6.7 \pm 24.3$	$32.9 \pm 20.4$
March 1/2	high	$16.8 \pm 14.4$	$0.4 \pm 18.8$	$9.3 \pm 10.7$	$10.0 \pm 10.4$	$15.1 \pm 11.2$	$3.6 \pm 15.6$	$16.5 \pm 13.1$
	low	$7.4 \pm 17.3$	$0.0 \pm 18.0$	$5.1 \pm 12.1$	$12.8 \pm 8.6$	$14.3 \pm 8.1$	$0.5 \pm 13.4$	$14.3 \pm 10.1$
March 30/31	high	$9.8 \pm 10.5$	$3.2 \pm 12.4$	$4.0 \pm 11.9$	$8.0 \pm 10.8$	$2.0 \pm 11.6$	$0.8 \pm 11.7$	$2.5 \pm 7.4$
	low	$6.1 \pm 10.5$	$0.2 \pm 11.4$	$4.1 \pm 9.4$	$9.9 \pm 6.8$	$4.8 \pm 12.7$	$1.4 \pm 13.7$	$2.5 \pm 5.1$

## Bud burst

In all treatments and for all three sampling dates, some buds randomly failed to burst and desiccated before the end of the experiment. In general, we observed higher bud burst percentages in the diffuse porous species *Acer* (92%) and *Fagus* (98%), and a lower percentage in the ring porous *Quercus* (79 %) and the coniferous *Picea* (80%). In the latter species bud burst failed most likely due to conduit failure in cuttings' xylem during the experiment, despite our precautional re-cutting and exchange of water.

The time of bud burst, the most striking phenological event in spring, was significantly influenced by date of sampling, temperature and photoperiod. Here again, later sampling dates, warmer temperatures and longer photoperiods consistently decreased the time to bud burst under our treatment conditions (Fig. 3.4).

With later sampling dates, and thus shorter exposition to the contrasting treatment conditions the treatment effects generally weakened (Fig. 3.4, Table 3.3). The strongest temperature effect on bud burst was found in *Picea*, although the effect was only slightly weaker in *Acer* and *Quercus*. *Fagus* was least responsive to temperature (Table 3.4). The photoperiod effect on the time of bud burst was strongest in *Fagus* and *Picea*, only relatively weak in *Quercus* and absent in *Acer*.

In all species except *Picea*, the time of bud burst was additionally influenced by the cutting's elevation of origin on at least one of the three sampling dates (Table 3.3 and 3.4): in *Acer* and *Fagus*, we observed earlier bud burst on low elevation cuttings compared to high elevation cuttings, irrespective of temperature, when sampled at the first sampling date. With later sampling dates, this effect disappeared in *Acer* and was reversed in *Fagus*. A reversed pattern that is, bud burst of high-elevation-cuttings preceding those of low-elevation-cuttings, was observed in *Quercus*, but only for the first two sampling dates, while no such effect of elevation of origin was found for the third sampling date.

Furthermore, a significantly larger photoperiod effect was found under warm temperatures than under cool temperatures for the first two sampling dates in *Picea* and for the second sampling in *Fagus*, whereas no significant photoperiod  $\times$  temperature interaction was found in the other species. Finally, in *Picea* cuttings of the second sampling date, the photoperiod effect was significantly stronger in cuttings from high elevations than in those from low elevations and we also observed a slightly stronger

response to temperature in the low elevation samples than in those from high elevations.

**Table 3.4:** Analysis of variance of date of bud burst in response to temperature and photoperiod and elevation of origin in four species after three consecutive sampling dates (26/27 January, 1/2 March, 30/31 March) during winter/spring 2010.

	1. sampling		2. sampling		3. sampling	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
<i>Acer pseudoplatanus</i>	$F_{1,68}$		$F_{1,69}$		$F_{1,65}$	
Temperature (T)	79.90	<b>&lt;0.001</b>	21.87	<b>&lt;0.001</b>	19.61	<b>&lt;0.001</b>
Photoperiod (P)	0.00	0.980	0.00	0.979	0.44	0.510
Elevation (E)	3.43	0.068	3.49	0.066	0.28	0.600
<i>T</i> × <i>P</i>	0.00	0.988	0.08	0.775	0.09	0.766
<i>T</i> × <i>E</i>	0.01	0.943	3.23	0.077	0.99	0.324
<i>P</i> × <i>E</i>	0.60	0.442	0.04	0.838	0.99	0.323
<i>Fagus sylvatica</i>	$F_{1,72}$		$F_{1,70}$		$F_{1,70}$	
Temperature (T)	78.42	<b>&lt;0.001</b>	34.78	<b>&lt;0.001</b>	9.38	<b>0.003</b>
Photoperiod (P)	66.65	<b>&lt;0.001</b>	79.57	<b>&lt;0.001</b>	44.02	<b>&lt;0.001</b>
Elevation (E)	9.63	<b>0.003</b>	4.49	<b>0.038</b>	9.33	<b>0.003</b>
<i>T</i> × <i>P</i>	0.00	0.957	6.80	<b>0.011</b>	0.02	0.897
<i>T</i> × <i>E</i>	0.71	0.403	2.53	0.116	0.07	0.787
<i>P</i> × <i>E</i>	0.40	0.531	0.79	0.376	0.03	0.862
<i>Quercus petraea</i>	$F_{1,58}$		$F_{1,53}$		$F_{1,55}$	
Temperature (T)	114.97	<b>&lt;0.001</b>	165.66	<b>&lt;0.001</b>	15.35	<b>&lt;0.001</b>
Photoperiod (P)	8.84	<b>0.004</b>	7.00	<b>0.011</b>	2.20	0.144
Elevation (E)	22.40	<b>&lt;0.001</b>	17.14	<b>&lt;0.001</b>	0.63	0.431
<i>T</i> × <i>P</i>	0.67	0.416	1.19	0.280	0.03	0.869
<i>T</i> × <i>E</i>	2.23	0.141	0.00	0.974	6.38	<b>0.014</b>
<i>P</i> × <i>E</i>	0.48	0.492	1.02	0.317	0.60	0.441
<i>Picea abies</i>	$F_{1,57}$		$F_{1,59}$		$F_{1,52}$	
Temperature (T)	141.21	<b>&lt;0.001</b>	81.08	<b>&lt;0.001</b>	4.16	<b>0.046</b>
Photoperiod (P)	30.32	<b>&lt;0.001</b>	27.91	<b>&lt;0.001</b>	5.07	<b>0.029</b>
Elevation (E)	0.01	0.905	0.85	0.360	2.66	0.109
<i>T</i> × <i>P</i>	9.14	<b>0.004</b>	9.60	<b>0.003</b>	0.67	0.418
<i>T</i> × <i>E</i>	0.96	0.331	0.90	0.346	0.01	0.929
<i>P</i> × <i>E</i>	0.28	0.601	10.80	<b>0.002</b>	1.27	0.265

Significance level  $p < 0.05$  (significant values in bold)

## Discussion

The results show that bud burst of cuttings collected from mature trees during late winter and early spring respond differently to temperature and photoperiod, and these differences depended on minute differences in in situ stages of bud development. The origin of elevation as well as species identity influence responsiveness. The more advanced endo- or ecodormancy release was the less did the treatments conditions affected further bud development and the timing of bud burst. Hence, very early advances of bud development preset later phenology.

## Chilling

In temperate climates, the release of endodormancy requires the exposure to chilling temperatures before buds resume growth under warm temperatures. Insufficient chilling may delay bud burst or decrease bud burst percentage under warm forcing conditions (Samish 1954). Here, the initial dormancy in February was strongest in *Fagus*, intermediate in *Acer* and *Quercus* and surprisingly low in *Picea*. The remarkably low dormancy in *Picea* may arise from the fact that this species, in contrast to the other species, seems to require less stringent chilling experience (Nienstaedt 1967, Worrall and Mergen 1967, Sogaard et al. 2008). The significant reduction in dormancy state observed between the first and second sampling cohorts (despite the lack of warm days at the field sites) may reflect the fulfilment of chilling requirements. The chill days as a measure for the degree of chilling as used here (days with mean temperature  $< 5^{\circ}\text{C}$ ) is only a rough approximation for the actual (unknown) dose-response to cool temperatures. Yet, there are no known physiological or molecular markers that indicate the fulfilment of the chilling requirement (Cooke et al. 2012). However, under our temperature and photoperiod test conditions buds can be assumed to have experienced sufficient chilling either by the cool in situ winter temperatures before sampling or later, under our rather moderate temperature treatments, with low night temperatures likely to have added to the fulfilment of chilling requests, if there was a need. The high bud burst percentage as well as, the low thermal requirement under forcing conditions observed support this assumption. Since the effective



ranges of chilling and growth promoting temperatures may overlap, low temperatures within the upper range of potential chilling temperatures are also able to promote bud growth and induce bud burst, as was observed for example in experiments with *Betula pendula* Roth and *B. pubescens* Ehrh. (Myking and Heide 1995) and *Sorbus aucuparia* L. (Heide 2011).

High resolution data for actual field temperatures inside buds combined with histological observations in cut buds (Sutinen et al. 2009, Sutinen et al. 2012) or in situ automatic dendrometer-type assessments of buds would help to identify the threshold temperatures for tissue growth in buds. From results of studies in other tissues in cold adapted plants, we expect a threshold near 5 °C (Alvarez-Uria and Körner 2007, Körner 2008, Rossi et al. 2008)

### ***Bud swelling***

Using individual bud image time series permitted us to accurately monitor the bud swelling period, which closely follows the transition from endo- to ecodormancy under our controlled conditions, together with an increase in metabolic activity, rise in bud water content and mobilization of storage reserves following dormancy release (Saure 1985, Pallardy 2008). Our data, for the first time, permitted to assess the responses of the onset of bud swelling to temperature and photoperiod, but also the estimation of bud growth curves of individual buds. Although, the phenological phase of bud swelling is often part of observation protocols such as the widely used BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie; Meier 2001), the precise start of bud swelling is very difficult to ascertain over a multitude of buds in regular intervals. Thus, previous studies largely reported with more striking, later phenophases, such as bud burst or leaf unfolding, which also mark the start of the new growing season. Our results show, that the onset and duration of bud growth are co-controlled by temperature and photoperiod in *Fagus* and *Picea* and to some extent in *Quercus*, but not in *Acer*, which appears to be temperature controlled only. These responses suggest further, that although maximum bud growth rate is mainly driven by concurrent temperatures (with the exception of *Fagus*, where long photoperiods increase maximum growth

rates), the duration of bud swelling is modulated by photoperiod in photoperiod sensitive species.

The onset of bud swelling advanced in all species and treatment conditions with later sampling, which may be related to decreasing endodormancy in the later sampled cohorts and thus, likely linked to the additional natural chilling received in situ. Significant photoperiod effects on the onset of bud swelling, despite the sufficiently fulfilled chilling requirement (as indicated by the stable, low thermal requirement under forcing conditions) were present in *Fagus*. Still, with later sampling, and thus, advanced dormancy release, these photoperiodic effects decreased. Using calculated bud growth indices, chilling has been reported to affect the subsequent temperature response of bud growth in several species (Campbell and Sugano 1975, Cannell 1989, Battey 2000). We did not observe significantly different maximum bud growth rates or bud swelling duration with later sampling dates, suggesting again, that the species were sufficiently chilled before bud swelling started or that such effects on bud growth rates may only be detected in stronger, less realistic temperature contrasts. Furthermore, our decreased duration of the bud swelling period in *Fagus*, *Quercus* and *Picea* under long photoperiods supports the conclusion that photoperiod may affect bud development also during ecodormancy release, as was reported for *Betula pubescens* (Myking and Heide 1995, Caffarra et al. 2011), rather than substituting for a lack of chilling only (Downs and Borthwick 1956, Vegis 1964, Nienstaedt 1967, Cannell and Smith 1983).

Although the underlying physiological drivers of bud dormancy release are not yet completely understood, plant hormones, e.g. abscisic Acid (ABA), gibberellic acid (GA), auxins (IAA) and cytokinins, are strongly involved in regulating bud dormancy (Wareing and Saunders 1971, Arora et al. 2003, Welling and Palva 2006, Chao et al. 2007, Meier et al. 2012). Among these, levels of ABA and GA and IAA are affected by phytochrome, the plants sensory apparatus for perceiving daylength (Olsen et al. 1997b, Sawada et al. 2008). While short-day induced ABA is mostly involved in dormancy induction (Rinne et al. 1994, Welling et al. 2002), a phytochrome mediated increase in GA levels has been observed in *Salix pentandra* L. during dormancy release (Olsen et al. 1997a). Thus, the photoperiod responses observed here may reflect such plant hormonal effects, and these hormones may be candidates for further physiological research on plant dormancy release in photoperiod sensitive late successional trees.

## ***Bud burst***

As expected, warmer temperatures accelerated the final stages of bud development and resulted in earlier bud burst. With later sampling in the field, the temperature effects on bud burst diminished, indicating the gradually advancing dormancy release during late winter. While the buds of *Acer*, *Quercus* and *Picea* were about equally sensitive to temperature, generally lower temperature sensitivity was observed in *Fagus*. This finding is consistent with the generally lower temperature sensitivity of *Fagus* compared to other co-occurring species observed along elevational gradients (Dittmar et al. 2006, Migliavacca et al. 2008, Vitasse et al. 2009), and the findings of a common garden experiment along the same elevational gradients as used in this study, where a sensitivity to the mean temperature of the month of leaf unfolding of  $-2.6 \pm 0.2$  days  $K^{-1}$  in *Fagus*, and  $-4.0 \pm 0.3$  days  $K^{-1}$  in *Acer* was found (Vitasse et al. 2013). However, *Fagus* is the most photoperiod sensitive species, and photoperiod responses may have influenced these in situ ‘temperature sensitivities’ as well. By controlling for both, temperature and photoperiod conditions, we obtained similar low temperature sensitivities in *Fagus* compared to the other species. Low temperature sensitivity and strong photoperiod sensitivity of bud development may lead to the low inter-annual variation reported for leaf unfolding in *Fagus* (Menzel et al. 2001, Studer et al. 2005, Vitasse and Basler 2013). The large delay of bud burst in *Picea* under our low temperature treatments, despite the low depth of dormancy, is likely to be related to the fact that this species exhibits a low, early fulfilled, chilling requirement and these low temperatures may be close to the threshold temperature for bud development.

The observed photoperiod responses in the four species are in line with the results from a previous screening of 14 species for photoperiod influences on bud burst on the same elevational gradients (Basler and Körner 2012). For *Fagus*, the photoperiod effect on bud burst observed here confirms previous findings (Klebs 1914, Wareing 1953, Heide 1993b, Caffarra and Donnelly 2010).

The observed diminishing of photoperiod effects with later sampling dates may either have been influenced by longer exposure to chilling temperatures in situ or by the threshold nature of the photoperiod effect once the critical photoperiod has been passed and developmental barriers are released, photoperiod may not exert any further influence. A diminishing

photoperiod effect with increasing chilling has been described for several species (Worrall and Mergen 1967, Heide 1993a, Myking and Heide 1995, Partanen et al. 2005, Caffarra and Donnelly 2010) and these authors concluded that photoperiod was effective in insufficiently chilled buds only. Hence, following their reasoning, photoperiod could substitute chilling effects. Most of these studies, assessed the effect of photoperiod after a controlled amount of chilling followed by rather high forcing temperatures ( $> 20\text{ }^{\circ}\text{C}$ ), much warmer than the temperature ever would be in field and much higher than the temperatures employed in our experiments.

### ***Methodical considerations***

Experiments, such as the ones presented here, may suffer from effects of related to the disconnection of cuttings, from potential whole-tree signals (e.g. hormonal signals produced outside the branchlet/bud), even though the phenology of adult trees seems to be well represented by that of cuttings (Vitasse and Basler 2014). For obvious reasons, however, in situ manipulation of photoperiod on adult trees was not an option and seedlings are not a good substitute to study phenology of mature trees, due to ontogenic differences in phenology (Ununger et al. 1988, Besford et al. 1996, Partanen et al. 2001, Vitasse 2013). We assume that the use of cuttings data lead to a conservative picture of photoperiod signals compared to whole tree responses (see Basler and Körner 2012). Real-scale photoperiod reduction in mature forests is perhaps the most challenging types of any manipulation in global change research. It is the shortening of photoperiod in a warmer world (earlier thermal forcing) that matters, and no such experiment has ever been attempted on mature trees, and we doubt its feasibility, given the complete darkness (night extension) needed day-by-day. In-situ shortening of photoperiod was anyhow conducted with saplings, e.g. Hänninen (1995) used 10–15 years old, 1.5 to 2 m tall saplings of *Pinus sylvestris* L. and found only a negligible effect of photoperiod on the timing of bud burst. Latitudinal transplant experiments seem more feasible, but in this case the temperature regime comes into play. Planting low elevation northern provenances at high elevations in the south maybe a possibility. Unfortunately, arboreta (or park trees) commonly hold no record of seed/plant origin.

## Conclusions

We showed that photoperiod is involved in the release of bud dormancy in three out of four late successional tree species and we evidenced species specific photoperiod effects on the onset of bud swelling and bud growth rates during the forcing period linked. Although recent climate warming caused a shift of spring phenology towards an earlier onset of the growing season in many plant species (Parmesan and Yohe 2003, Menzel et al. 2006), late successional, photoperiod sensitive species are thus, unlikely to fully track future warming at current rates, as photoperiodic clues become increasingly important. Warm temperatures in autumn (Heide 2003) or less chilling during winter (Morin et al. 2010) may delay bud burst even further in certain climates (e.g. mild costal climates). In consequence, the photoperiod sensitive species may not profit from a substantially extended growing season in a warmer climate, especially as the autumn phenological events (growth cessation and bud set) are unlikely to become considerably delayed in a warming climate due to their strong photoperiodic control (Kramer 1936, Wareing 1956, Vaartaja 1959, Thomas and Vince-Prue 1997). Contrary, in many tree species warm night temperatures in autumn have even been found to hasten growth cessation, except for photoperiod insensitive species and a few northern ecotypes where low temperature alone has been found to induce growth cessation in autumn (as reviewed in Hänninen and Tanino 2011). Photoperiod thresholds are elevation specific (ecotypic) and photoperiod sensitivity commonly found in late successional tree species, is a strategy to escape fatal freezing damage in immature, less robust tissue by delaying bud burst into low risk periods, irrespective of actual weather.

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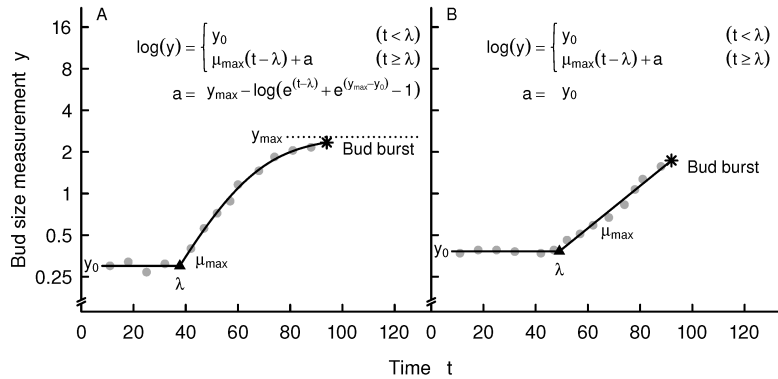
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## Supplementary material



**Fig. S3.1:** Example for the lag-exponential model used to determine the onset of bud swelling ( $\lambda$ ) from bud measurement time series. A. An example of the model fitted to a projected area time series of an individual bud. B. An example where the reduced model (without deceleration) allowed the fitting procedure to converge and provided a better fit. The model resulting in a lower residual sum of squares was used for the determination of  $\lambda$ . The parameter  $y_0$  denotes the size of the dormant bud,  $\mu_{\max}$  the maximal bud growth rate (slope) and  $y_{\max}$  is a theoretical asymptote for growth deceleration prior to bud burst. Asterisks indicate the time of bud burst of the specific bud. Note the logarithmic scale on the y-axis.



## Chapter 4

# Evaluating phenological models for the prediction of leaf-out dates in six temperate tree species across central Europe

David Basler

**Abstract** Inter-annual variation in climate is reflected by changes in the timing of phenology. Over the last decades a considerable number of models have been developed in order to explain the inter-annual variation of spring phenology in trees. Contrary to empirical models, ‘process-based’ models aim at simulating physiological processes in order to yield more realistic predictions of growing season onset dates. Despite the increasing knowledge on the environmental controls of seasonal dormancy in trees, the detailed action and interaction of the involved environmental drivers (chilling, photoperiod and warm temperature) remains to be elucidated. This study aims at a uniform comparison of a wide range of existing models (and new recombinations), on a multitude of long-term observation series in six tree species across central Europe, using extensive cross-validation. Even though the assessed models differ in the phases of dormancy and environmental drivers accounted for, they yielded a surprisingly similar quality of prediction of leaf unfolding dates. Depending on the species, the lowest average prediction errors for leaf unfolding (RMSE) ranged from 7 to 9 days for the dataset pooled across sites and years and from 4 to 6 days for site-specific predictions, in absence of any obvious geographical pattern. Simple models, that feature ecodormancy release only, performed similar or better than more complex models, which additionally include endodormancy release through

chilling temperatures. Model parameterisation tended to converge towards similar behaviour and models with many parameters tended to overfit on the 40 year time-series of leaf unfolding. Additionally, all models tended to underestimate the inter-annual variation of leaf unfolding and failed to predict very early or late dates of leaf unfolding in certain years. The transfer of site-specific parameters to other sites was associated with an almost doubling of the average prediction error, independent of distance and climatic similarity between the calibration and validation sites. The findings challenge the accurate implementation of the physiological processes controlling spring phenology in the models and highlight shortcomings associated with model parameterisation on observational time-series only.

**Key words:** Bud burst, Chilling, Temperature, Spring phenology, Process-based, Photoperiod

## Introduction

Plant phenology studies the seasonal and visible phenomena of plant development related to weather. The timing of phenological events reflects a combination of internal (genetic) settings and environmental influences. Given its significance for avoiding late spring freezing damage and impacts of early autumnal cold events, a well-timed phenology is crucial for plant survival. In addition, the control of synchronous flowering among individuals assures sexual reproduction. The phenological events defining the onset and end of the growing season are of special interest, since they are setting the length of the growing season, and thereby controlling range limits of species (Chuine and Beaubien 2001). During the dormant period, buds pass through three distinct states of dormancy (Lang et al. 1987): (1) paradormancy, a state of specific bud dormancy maintained due to physiological factors outside the bud but inside the plant (e.g., apical dominance), (2) endodormancy, state of inactivity mediated by factor inside the bud and (3) ecodormancy, a state of inactivity imposed by unfavourable environmental conditions at otherwise full preparedness for advancing seasonal development. The transitions between the different phases of dormancy are gradual and species-specific (Perry 1971). In tree species adapted to cool climates, dormancy is induced by the shortening of

day-length in autumn, perceived in leaves, and modulated by concurrent temperatures. Moderate sub-zero temperatures are then inducing endodormancy, which is generally released in late winter or early spring, after sufficiently long exposure to cool, but non-freezing temperatures in the range of 2–7 °C ('chilling'; Coville 1920, Doorenbos 1953, Battey 2000). Many species need an additional weather independent photoperiod signal to effectively advance the transition from endodormancy to the following ecodormancy. During this stage, actual weather (largely temperature) controls bud development and bud burst (environmental 'forcing').

Phenology gained much attention during the last decades, once its implications in the climate change discussion became acknowledged. Ever since phenological data was collected, the phenological events were related to climate and simple models were built to calculate the timing of phenological phases, especially in agro-ecosystems. Nowadays, the applications of phenological models range from reconstruction and quality assessment of phenological time-series, spatial extrapolation of observations and even to species-specific predictions of phenology, and thus species performance, in future climate. Thus, phenology plays an important role in species distribution models (e.g., Chuine and Beaubien 2001) or dynamic global vegetation models (e.g., Krinner et al. 2005). Through the intimate linkage with the length of the growing season and thus, net primary production, phenology plays also an important role in carbon cycle models at ecosystem and global scale (Richardson et al. 2013). For temperate and boreal tree species, numerous models have been developed to simulate the events of spring phenology, such as bud burst or leaf unfolding, whereas only few models attempted to simulate the autumnal phases of phenology, such as leaf colouration and leaf fall (White et al. 1997, Delpierre et al. 2009). The more mechanistic models commonly outperform simple correlative statistical models for phenology, which often use linear correlations to spring temperature only (but see Olsson and Jönsson 2014). These 'process-based' models are also able to reflect the non-linear responses of phenology to the various environmental drivers. In simple phenological models, the bud development towards bud-burst is basically defined as a response to concurrent temperature, mostly by adopting the concept of accumulated temperature over a certain threshold (degree days). However, the shortcomings of this simple approach (accounting for the release of ecodormancy only) and the increasing knowledge of the underlying physiological processes motivated the development of numerous

advanced models for spring phenology, which account also for chilling and photoperiod influences. Most recently, even the complex interactions of all three drivers of spring phenology, chilling signals, photoperiod, and actual thermal forcing were integrated into a single model (Caffarra et al. 2011). However, with the increasing number of factors, complexity of models increases dramatically and parameterisation becomes increasingly difficult. At first, the statistical fitting of parameters was difficult and often led to unstable parameter estimates (Kramer 1994), thus Hänninen (1995) compared 96 model formulations using parameters derived from literature. Later, efficient optimisation methods (Chuine et al. 1998) and appropriate methods for the statistical estimation of prediction errors, such as bootstrapping (Häkkinen 1999) or cross-validation (Chuine et al. 1999) led to further improvement of model parameterisation and evaluation. No single model structure was found to predict spring phenology across different species, so the best predictive models are still species-specific (Hunter and Lechowicz 1992, Chuine et al. 1998, Schaber and Badeck 2003) and different model structures may perform equally well for a given species (Schaber and Badeck 2003). A recent uncertainty analysis for a set of phenological models using data from Harvard forest revealed, that prediction errors are largely a result of the uncertain nature and strength of the actual drivers (model structure), and to a lesser extent due to model parameterisation (Migliavacca et al. 2012). The more recently developed models have been tested on rather limited datasets for only a few species. The current study collectively analyses the performance of current process-based phenology model structures for three aspects (1) generalisation, (2) site-specific accuracy and (3) spatial transferability, using a large and consistent phenology data set covering 40 year of observation on a multitude of sites throughout Europe for 6 temperate tree species. The assessed models differ with respect to the mechanisms they account for (dormancy induction, endodormancy release and ecodormancy release), the employed drivers (forcing temperature, chilling temperature and photoperiod), and the specific responses for different species. This study aims at improving the understanding of the capabilities and uncertainties of these models, and disclosing some pitfalls in modelling the spring phenology of temperate and boreal forest trees.



## Material and methods

### *Models types*

The ‘process-based’ phenology-models published so far, and included here, simulate the environmental influence on bud development, until a critical developmental threshold for bud burst or leaf unfolding is reached. Parameters common to most models are a starting date, after which the specific environmental drivers affect bud development, and one or more parameters controlling the rate of response to environmental drivers.

The models are grouped according to their scope of operation into three categories: (1) models explaining ecodormancy release only, (2) models explaining the release of endo- and ecodormancy and (3) models explaining the whole transition from dormancy induction until bud burst. Further, I classified models by the environmental drivers they are accounting for: chilling temperature, photoperiod and forcing temperature (Table 4.1). Models were implemented according to the original publication (Table 4.1, supplementary Table S4.1); however, I fitted the starting date rather than using an arbitrary date (such as 1 January). For each parameter, upper and lower limit was defined within a wide, but (biologically) reasonable range (see supplementary Table S4.2).

### **Models accounting for ecodormancy release only**

These are the oldest models, dating back to de Réaumur (1735), accounting for thermal forcing in spring only. These ‘Thermal Time’ models (Wang 1960, Cannell and Smith 1983, Hunter and Lechowicz 1992, Chuine et al. 1999) are using degree days as forcing units. A modification of this model type, hereafter named the ‘sigmoid Thermal Time model’ (Hänninen 1990, Kramer 1994) uses a sigmoid, rather than linear, forcing function (see supplementary Table S4.1). Although photoperiod is well known to influence phenology of crops (e.g., Masle et al. 1989, Siebert and Ewert 2012) and late successional tree species (Caffarra and Donnelly 2010, Körner and Basler 2010, Basler and Körner 2014), few models yet include photoperiod as explicit driver of spring phenology (the fixed starting date of most models may, however, imply a strong photoperiod

**Table 4.1:** Overview of the phenological models for leaf unfolding included in this study. The models are grouped by implemented processes and drivers: chilling temperatures (*C*), forcing temperatures (*F*) and photoperiod (*P*). Temperature responses not separable in chilling or forcing are indicated with *T*.

Model name	Drivers	No. Parameters	Comments/References
<b>Empirical</b>			
NULL		1	mean date of leaf unfolding
Linear	<i>T</i>	2	linear correlation with mean spring temperatures
<b>Ecodormancy release only</b>			
Thermal Time <sup>a</sup>	<i>F</i>	3 (4)	de Réaumur (1735), Wang (1960), Cannell and Smith (1983), Chuine et al. (1999), Hunter and Lechowicz (1992), Hänninen (1990), Kramer (1994)
Photothermal-time <sup>a</sup>	<i>PF</i>	3 (4)	Masle et al. (1989), Črepinšek et al. (2006)
M1 <sup>a</sup>	<i>PF</i>	4 (5)	Blümel and Chmielewski (2012)
<b>Endo- and eco dormancy release</b>			
Alternating	<i>CF</i>	5	Cannell Smith (1983), Murray et al. (1989)
Sequential <sup>b</sup>	<i>CF</i>	8	Hänninen (1990), Kramer (1994)
Parallel <sup>b</sup>	<i>CF</i>	9	Landsberg (1974), Hänninen (1990), Kramer (1994)
Unified	<i>CF</i>	9	Chuine (2000)
Sequential <sup>b</sup>	<i>CPF</i>	9	combination of Sequential model with M1 model
Parallel M1 <sup>b</sup>	<i>CPF</i>	10	combination of Parallel model with M1 model
Unified M1	<i>CPF</i>	10	combination of Unified model with M1 model
DormPhot spring	<i>CPF</i>	8	DormPhot model without dormancy induction
PIM 1 - 12	<i>PT</i>	10	Schaber and Badeck (2003)
<b>Dormancy induction, endo- and ecodormancy release</b>			
Four-Phase <sup>b</sup>	<i>CF</i>	12	Vegis 1964, Hänninen (1990)
Deepening-Rest <sup>b</sup>	<i>CF</i>	10	Kobayashi and Fuchigami (1983)
DormPhot	<i>CPF</i>	11	Caffarra et al. (2011)

<sup>a</sup> These models were also calibrated using a sigmoid temperature response (Hänninen 1990, Kramer 1994), adding one parameter;

<sup>b</sup> These models were also calibrated using a bell-shaped chilling response (Chuine 2000)

threshold). In the Photothermal-time model developed for crops (Masle et al. 1989) and successfully applied to trees by Črepinšek et al. (2006), photoperiod has been included as an additional factor influencing the rate of forcing. Recently, a study investigating the shortcomings of the classic Thermal Time model again suggested the inclusion of photoperiod as explicit driver and thereby proposed an extension of the Photothermal-time model by an additional exponential constant (M1 model; Blümel and Chmielewski 2012).

### **Models accounting for endo- and ecodormancy release**

The chilling requirement, indicating to the plant that winter has passed, plays an additional role in dormancy release of temperate and boreal trees. In current models, the response to chilling temperatures is implemented either as a triangular function of temperature (defined by minimal chilling temperature, optimal chilling temperature and maximal chilling temperature; Hänninen 1990, Kramer 1994) or a bell shaped curve (Chuine 2000; see equation in supplementary Table S4.1). Depending on the model's assumption, the chilling requirement has to be fulfilled either before forcing temperatures are accumulating, such as in the Sequential model (Hänninen 1990, Kramer 1994), or chilling and forcing may act simultaneously, as implemented in the Parallel model (Landsberg 1974, Hänninen 1990, Kramer 1994). The either sequential or parallel integrations of chilling into models have later been combined in the Unified Model (Chuine 2000). However, the flexible structure of this model has a cost in terms of higher number of parameters to be defined. For this study, the three formerly mentioned models were extended with the addition of a photoperiod response, as photoperiod is known to control the phenology of certain species: In the Parallel-, Sequential- and Unified model, the (sigmoid) response to forcing temperature was extended by the photoperiod response factor of the M1 model (see equation in supplementary Table S4.1).

Again another formulation for including chilling fulfilment has been introduced by Murray et al. (1989) as the Alternating model, where a specific day may either add to the accumulation of forcing or to the accumulation of chilling, depending on the actual daily mean temperature. I did not include further chilling models designed mainly for specific fruit trees, such as the ChillHours Model (summed hours with  $T < 7.2$  °C; Wein-

berger 1950), the Utah Model (temperature-weighted accumulation of chilling hours; Richardson et al. 1974), the Positive Utah Model (Lindsley-Noakes et al. 1995), and the very complex ‘Dynamic model’ for chilling accumulation (Fishman et al. 1987a,b), as these models would require hourly input data not available for this study.

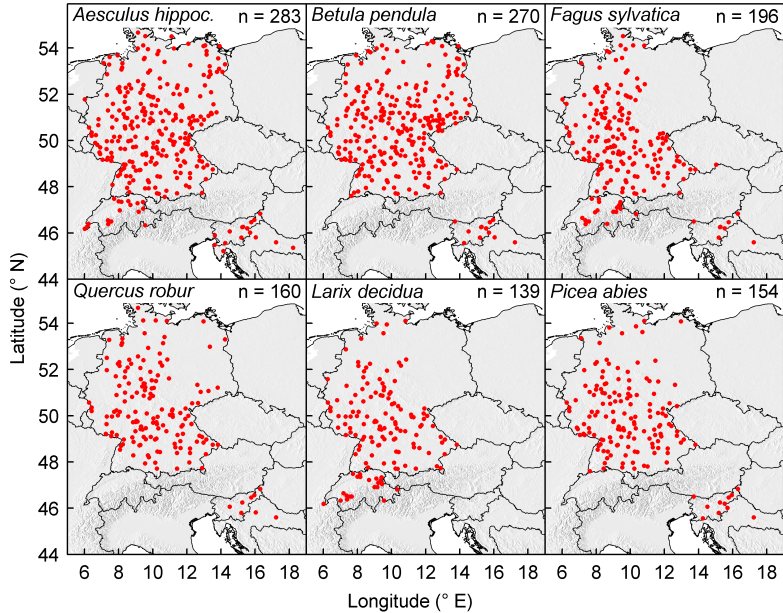
A different approach at phenological modelling accounts for molecular (e.g., hormonal) regulation of bud dormancy and was introduced by Schaber and Badeck (2003) as the ‘Promoter–Inhibitor models’ (PIM): these models do not explicitly separate endodormancy and ecodormancy but rather describe dormancy release as a continuum under control of the balance of (virtual) promoters and inhibitors. Promoter-accumulation and inhibitor-decomposition rates are modulated by different combinations of temperature and/or photoperiod.

### **Models for the whole dormant period**

Some more sophisticated models include (temperature-induced) dormancy induction prior to the release of dormancy through chilling and warm temperatures, such as the Four-Phase Model (Vegis 1964, Hänninen 1990) or the Deepening-Rest model (Kobayashi and Fuchigami 1983). The most complex model of this type, the DormPhot model (Caffarra et al. 2011) designed for *Betula*, integrates even the complex interactions of photoperiod with dormancy induction, chilling and thermal forcing.

### **NULL model and linear regression**

All models were compared to the NULL model that assumes a fixed mean date of leaf unfolding over all years. Additionally, I also provide the results of a simple linear regression model of leaf unfolding dates against mean spring temperature: the relevant period was determined by choosing the best linear correlation of leaf unfolding dates with mean temperatures of either a single month (January–May) or of a continuous combination of these months.



**Fig. 4.1:** Locations of the phenological observation sites used in this study (leaf unfolding data 1970–2009). Data was provided by the Pan European Phenological Network (PEP).

### *Phenological data*

The phenological time-series used for this study was extracted from the PEP725 Pan European Phenology Database (Data set accessed 2013-01-07 at <http://www.zamg.ac.at/pep725/>). Out of the many tree species covered by this dataset, I selected 6 species with abundant temporal and spatial coverage: *Aesculus hippocastanum* L., *Betula pendula* Roth, *Fagus sylvatica* L., *Quercus robur* L., *Larix decidua* Mill. and *Picea abies* (L.) H. Karst. (in the following species are referred to by their genus). These species represent contrasting characteristics in terms of successional status (early vs. late successional), leaf traits (broad leaved vs. coniferous) and canopy duration (deciduous vs. evergreen). As phenological spring phase, leaf unfolding (BBCH code 11) was selected for the broad-leaved species

and needle elongation (BBCH 10) for conifers. In each species, all data covering the observation period of 1970–2009 was used to apply a robust mean outlier detection using the 30-day rule (Schaber and Badeck 2002) to exclude the most obvious flaws. Only stations where the full 40 years were covered after outlier removal were used in the study. Time-series fulfilling these requirements were located in Germany, Switzerland, Austria, Slovenia and Croatia (Fig. 4.1), spanning an elevation range from sea level to 1440 m a.s.l. Mean leaf unfolding was earliest in *Larix*, followed by *Aesculus* and *Betula*, while a later mean leaf unfolding was found in the late successional species *Fagus*, *Quercus* and *Picea* (Table 4.2). The inter-annual range of the overall spring phenology per site was around 5 weeks.

**Table 4.2:** Properties of the phenological datasets used in this study. Mean ( $\pm$  SD) day of leaf unfolding (BBCH 11), inter-annual range and trend during the observed period 1970–2009.

Species	Sites	Data points	Mean leaf unfolding (DOY)	Maximum inter-annual range	Mean trend 1970-2009 ( $dy^{-1}$ )	Mean $R^2$ 1970-2009
<i>A. hippocastanum</i>	283	11320	109.8 $\pm$ 7.5	37.3 $\pm$ 6.4	-0.32 $\pm$ 0.20	0.19 $\pm$ 0.2
<i>B. pendula</i>	270	10800	109.9 $\pm$ 6.5	38.0 $\pm$ 6.1	-0.29 $\pm$ 0.16	0.14 $\pm$ 0.1
<i>F. sylvatica</i>	196	7840	118.4 $\pm$ 6.1	33.2 $\pm$ 6.4	-0.29 $\pm$ 0.17	0.23 $\pm$ 0.2
<i>Q. robur</i>	160	6400	124.6 $\pm$ 6.7	35.8 $\pm$ 6.5	-0.38 $\pm$ 0.18	0.29 $\pm$ 0.2
<i>L. decidua</i> <sup>a</sup>	139	5560	105.7 $\pm$ 8.3	43.9 $\pm$ 7.3	-0.31 $\pm$ 0.22	0.13 $\pm$ 0.1
<i>P. abies</i> <sup>a</sup>	154	6160	128.0 $\pm$ 6.7	36.1 $\pm$ 8.0	-0.36 $\pm$ 0.19	0.27 $\pm$ 0.2

<sup>a</sup> needle elongation (BBCH 10)

## Climate data

As temperature reference, the gridded daily mean temperatures provided in the E-OBS Dataset version 7.0 (Haylock et al. 2008) was used. The dataset provides European daily mean temperatures since 1950 on a 0.25° regular grid ( $\sim$ 28 km). Temperatures at the phenological observation sites were calculated by using the corresponding grid cell's temperature, cor-

rected for the observation site's elevation by a linear lapse rate. The lapse rates were calculated daily, using a linear regression of the temperatures of the 25 surrounding grid cells against their elevation. The applied method provides reasonable estimates of local temperature when tested against actual weather station data (obtained from 157 weather stations across Switzerland and Germany during the 40 years period: mean  $R^2 = 0.98 \pm 0.06$ , RMSE  $1.0 \pm 0.6$  °C; Data provided by the German Weather Service DWD and MeteoSwiss) and outperformed the use of monthly- or annual averaged lapse rates. Photoperiod was calculated as a function of latitude and day-of-year using basic trigonometry (sunrise to sunset, without twilight; [http://www.gandraxa.com/length\\_of\\_day.xml](http://www.gandraxa.com/length_of_day.xml)).

### ***Model calibration and validation***

First, to assess the general performance of the models, I fitted the models for each species to the pooled dataset of all years and sites, and used a 10-fold cross-validation (the resulting errors are hereafter referred to as 'pooled calibration' and 'pooled validation'). Second, to assess the site-specific performance of the models, each model was fitted over the whole 40 years ('site-specific calibration') as well as evaluated using leave-one-out cross-validation over the 40 year observation period per individual site ('site-specific validation'). Finally, to characterise the spatial transferability of site-specific model parameters, I used the parameters calibrated on each site ('site specific calibration', see above) to predict the phenology of all remaining sites ('external site validation') for each model.

### ***Software***

The model evaluation framework used here was implemented using the programming language C. Best parameters were estimated by minimising the root mean squared error (RMSE) between observations and prediction, applying an adaption of the Adaptive Simulated Annealing code (ASA; Ingber 1993). Simulated annealing (Metropolis et al. 1953) has been used previously for the parameterisation of phenology models and

has been shown to reliably find the global minimum error (Chuine et al. 1998, Schaber and Badeck 2003, Caffarra et al. 2011). For a single calibration of a model, the simulated-annealing procedure was repeated 20 times each (using random initial parameters) to find the best fitting parameters. Given that the parameter estimation is computationally intensive, I parallelised the code using OpenMPI ([www.openmpi.com](http://www.openmpi.com)) and the calculations were done on the high-performance computing (HPC)-cluster of the University of Basel, using up to 40 processors in parallel. Including the extensive cross- and spatial-validation of the models, a total approximately 1.8 million model calibrations and 54 million validations on different (sub-) datasets were calculated for the 36 models (excluding the NULL model).

## *Statistics*

Model root mean squared errors (RMSE) were tested for normal distribution using the Shapiro–Wilk test. Model performance was compared using an ANOVA on the log transformed RMSE of the individual sites using site as random factor. A post-hoc Tukey HSD test was then used to identify performance differences between models. As a measure for climatic similarity between sites, the mean Euclidian distances between the daily mean temperatures from September up to and including May over the 40 years observation period was calculated. Model residuals of all sites and years were checked for correlation with basic geographical variables (latitude, longitude and elevation), mean spring temperature (January–May) and the timing of leaf phenology (mean and variation).

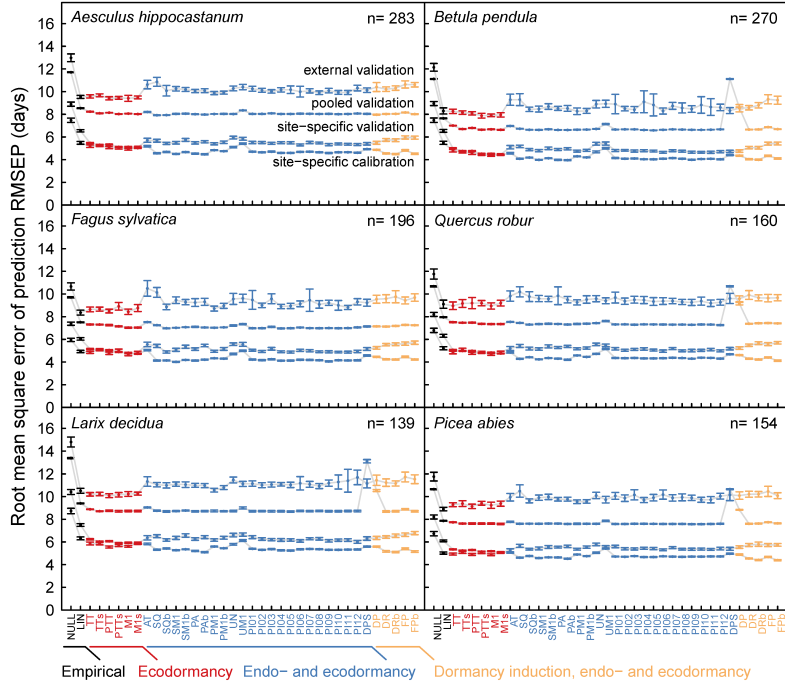
All statistical analyses and figures were done using R 2.15.0 (R Development Core Team 2010)

## **Results**

### *Overall phenology*

The estimation of a single, best-fit set of parameters for each model to match phenology across all sites resulted in a mean pooled calibration





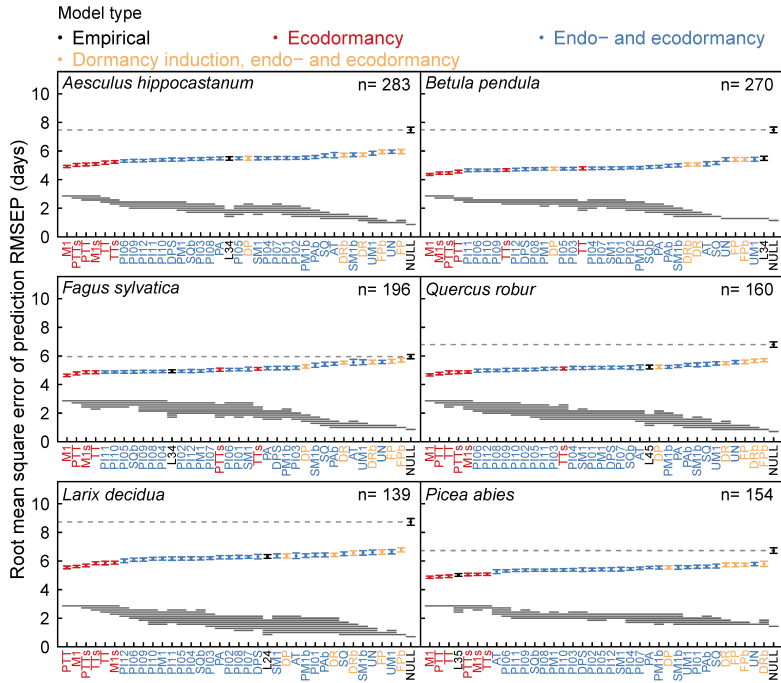
**Fig. 4.2:** Mean ( $\pm$  SE) RMSE of predicted leaf unfolding dates of 37 models in 6 species for pooled validation across all years and sites (40 years  $\times$   $n$  sites; 10-fold cross validation), site-specific calibration/validation (40 years per site; leave-one-out cross-validation) and, external validation of site-specific parameters. Model abbreviations: NULL: Mean date of leaf unfolding, LIN: Simple linear regression against mean temperature of specified months (*Aesculus*, *Betula* and *Fagus*: March and April; *Quercus*: April and May; *Larix*: February to April; *Picea*: March to May), TT: Thermal Time, PTT: Photothermal-time, M1: M1 model, AT: Alternating, SQ: Sequential, SM1: Sequential M1, PA: Parallel, PM1: Parallel M1, UN: Unified, UM1: Unified M1, PM01-PM12: Promotor-Inhibitor, DPS: DormPhot spring, DP: DormPhot, DR: Deepening-Rest FP: Four-Phase; 's' denotes the variant using a sigmoid, rather than linear forcing function; 'b' denotes the variant using a bell-shaped, rather than triangular, chilling function.

error (RMSE) of around  $7 \pm 9$  days, depending on species: lowest calibration errors were obtained in *Betula* (6.6 days), highest in *Larix* (8.6 days) while *Fagus*, *Quercus*, *Picea* and *Aesculus* took intermediate positions (6.9, 7.2, 7.5 and 7.9 days, respectively). Due to the large size of the dataset, the pooled validation error (as estimated by a 10-fold cross-validation) was very similar to the pooled calibration error and varied only slightly across the individual cross-validation folds. As expected, all models outperformed the NULL model ( $9.9 \pm 13.6$  days); however no single model was distinctly superior. In contrast, no significantly different performance (RMSE) was observed between the majorities of the models (Fig. 4.2, ‘pooled validation’). The residuals of the different models were highly correlated (mean  $r = 0.97 \pm 0.02$ ), except for the null models (mean  $r = 0.67 \pm 0.05$ ).

Species-specific differences were found for the time period providing the lowest calibration and validation error in the simple linear regression of leaf unfolding dates with mean temperatures: the mean of March and April temperatures was best for *Aesculus*, *Betula* and *Fagus*, whereas the mean of April and May temperatures was best for *Quercus*, the mean of February–April temperatures for *Larix* and finally the mean of March–May temperatures for *Picea*.

### ***Site-specific phenology***

Fitting the models to the individual 40 year time-series of each site resulted in a decreased mean RMSE across sites in all models compared to pooled validation error (Fig. 4.2). The lowest mean site-specific validation RMSEs ranged from 4 to 5 days, with only minor differences among species (Fig. 4.3). While the models accounted for up to 70% of the variation in leaf unfolding during model calibration, only up to 40% of the variation was explained by the models in the cross-validation. Site-specific model calibration excludes the variation between sites (between ecotypes or between individual trees), at the cost of less stable predictions, as indicated by the considerable difference between calibration and validation error, which was roughly 12% larger than the calibration error across species and models (Fig. 4.2). Interestingly, models with only few parameters, based on forcing temperature and photoperiod (M1, Photothermal-



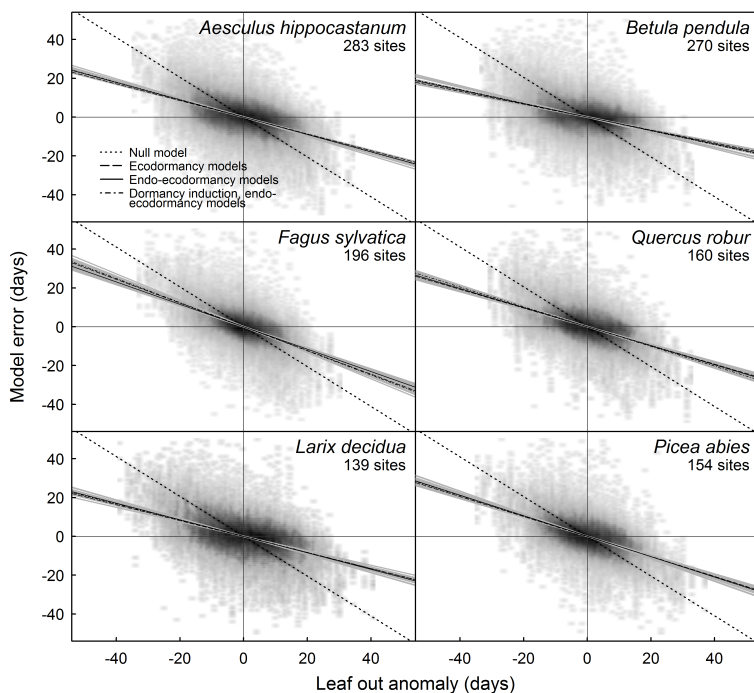
**Fig. 4.3:** Mean ( $\pm$ SE) RMSE of the predicted leaf unfolding date from the site-specific leave-one-out cross-validation of 37 models on 40 years of leaf unfolding observations in 6 species. Horizontal grey lines under the bars connect non-significantly different models (ANOVA followed by Tukey HSD;  $p < 0.05$ ). The relative distance between model RMSE and RMSE of the NULL model (= total variation, dashed line) represents the explained variance of the models ( $R^2$ ). Models were sorted by increasing RMSE. For model abbreviations, see Fig. 4.2.

time) or on forcing temperature alone (Thermal Time model), resulted in a smaller within-site cross-validation error than models including a chilling requirement (Fig. 4.3). Contrary, the models including the chilling requirement achieved a smaller calibration error when fitted over the full 40 year time-series, likely due to overfitting of these highly parameterised models. No model achieved outstanding performance at all sites, as indicated by the ranking of the models according to their mean cross-validation error (Fig. 4.3). Most models fit reasonably well on the majority of sites and

worse on only a few sites, resulting in a positively skewed, log-normal distribution of the RMSE across sites. Within a site, the residuals were normally distributed. No tight correlation of the model performance with geographical parameters of the sites (latitude, longitude and elevation) or site mean spring temperature (January–May) was present in any model ( $R^2$  always  $< 0.06$ , *slope* always  $< 0.3$ ). However, the prediction error of all models was correlated with the yearly anomaly of phenology i.e. models predictions were worse in years where the timing of leaf-out diverged further from the long-term mean (Fig. 4.4). This trend was strongest in *Fagus* (mean *slope* =  $-0.58 \pm 0.05$ , mean  $R^2 = 0.37 \pm 0.05$ ; excluding the NULL model) and *Picea* (*slope* =  $-0.52 \pm 0.02$ ,  $R^2 = 0.35 \pm 0.04$ ), intermediate in *Quercus* (*slope* =  $-0.49 \pm 0.02$ ,  $R^2 = 0.33 \pm 0.03$ ) and *Aesculus* (*slope* =  $-0.45 \pm 0.02$ ,  $R^2 = 0.29 \pm 0.03$ ), and slightly less pronounced in *Larix* (*slope* =  $-0.42 \pm 0.02$ ,  $R^2 = 0.27 \pm 0.03$ ) and *Betula* (*slope* =  $-0.35 \pm 0.02$ ,  $R^2 = 0.21 \pm 0.03$ ).

No significant differences (significance level 0.05) were found between model using the different chilling (triangular vs. bell shaped) and forcing functions (linear above base temperature vs. sigmoid temperature response), except in *Fagus* and *Picea*, where the Sequential model applying the bell shaped chilling functions achieved a significantly lower error than the variant applying the triangular response function. The addition of photoperiod to the classical endodormancy release models (Parallel, Sequential and Unified) led to slightly (not significantly) lower mean prediction errors for leaf unfolding in all species, especially in the case of the Parallel M1 model. Of the models describing the whole dormancy period, the DormPhot model always yielded the lowest prediction errors and was significantly different from other models of the same category in *Aesculus* and *Betula*, but not in the four other species.

Interestingly, the start day of the models simulating the ecodormancy phase only (without chilling) was always fitted best between mid-January and mid-February, indicating that most variation of the actual bud burst dates under current climate may be (statistically) related to the temperature and photoperiod conditions during early spring. Similarly, the calibration of the chilling response in endodormancy-release models often yielded rather late starting dates for chilling accumulation (early to mid-winter).



**Fig. 4.4:** Prediction errors (residuals) of 36 phenological models in site-specific cross-validations ( $40 \text{ year} \times n \text{ sites}$ ) as influenced by the phenological anomaly per site in 6 tree species. Black lines indicate the mean trends across models categories. Individual model trends are indicated with light grey lines. Due to the large number of data points a smoothed grayscale density representation of the scatterplot is shown.

### *External validation*

Applying the best site-specific parameter estimates of the different models to predict phenology of the remaining sites resulted in an almost two-fold increase of the overall validation error compared to the site-specific calibration error (1.8-fold increase compared to the site-specific validation error, 1.25-fold compared to the pooled validation error; Fig. 4.2). On some sites however, the specific parameters resulted even in a lower RMSE than on the site used for parameter calibration. The lowest mean

RMSE of the external-validation across sites ranged from  $7.9 \pm 15$  days in *Betula* (M1 model),  $8.3 \pm 1.8$  days in *Fagus* (M1 model),  $9.3 \pm 1.9$  days in *Aesculus* (M1 model),  $9 \pm 1.9$  days in *Picea* (Photothermal-time model) to  $10 \pm 12.0$  days in *Larix* (Photothermal-time model). However, the best models performed only 29% (*Fagus*) to 35% (*Betula*) better than the NULL model ( $10.8 \pm 14.2$  days; Fig. 4.5). Again, the mean external-validation RMSEs of the different models were almost similar, as was formerly found for the site-specific cross-validation RMSE. The external-validation performance was neither reasonably correlated with the geographical distance from the calibration site to the validation site in any model ( $R^2$  always  $< 0.03$ , *slope* always  $< 0.01$ ) nor with the mean Euclidean distance value of climate similarity among observation sites ( $R^2$  always  $< 0.05$ , *slope* always  $< 0.02$ ).

The model evaluation error increased with the increasing deviation of the evaluations site's mean phenology from the overall mean phenology. Surprisingly, the simple linear regression was among the models resulting in the lowest transfer error in 4 out of the 6 species and yielded even the lowest absolute mean validation error in *Fagus* and *Picea*.

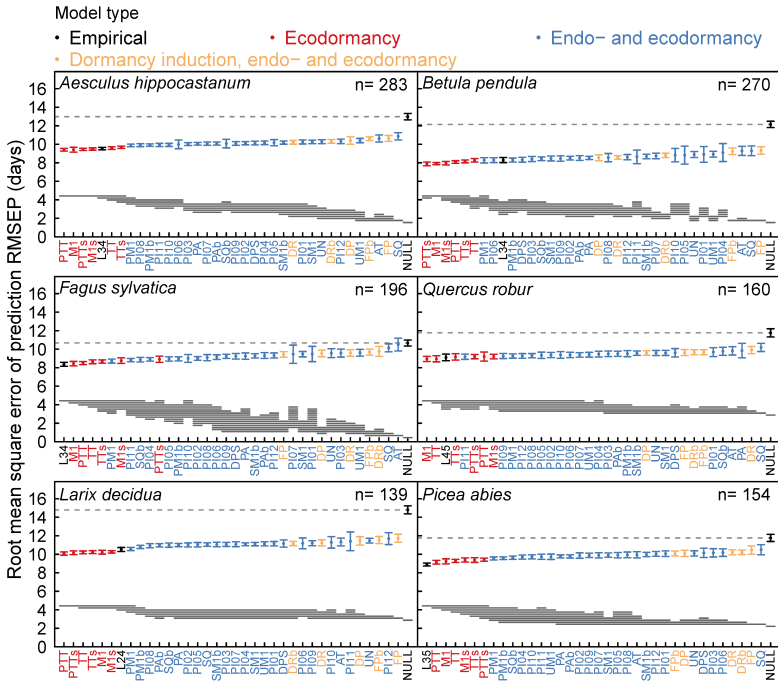
## ***Discussion***

The comparison of 35 'process-based' phenological models for leaf unfolding in 6 tree species using a long-term observation dataset (40 years) revealed a surprisingly small effect of model structure on the quality of prediction under a current climate. In the following the observed patterns and their implications for phenology modelling are discussed.

### ***Model performance and structure***

#### **Model prediction errors**

In general, no model could accurately predict the phenology of leaf unfolding with a single set of parameters calibrated across all years and sites. Instead, a broad range of models yielded an equally large error with highly



**Fig. 4.5:** Mean ( $\pm$ SE) RMSE of the predicted leaf unfolding date of 37 models in 6 species from the external site validation based on parameters calibrated on 40 years leaf unfolding observations per site. Horizontal grey lines under bars connect non-significantly different models (ANOVA followed by Tukey HSD;  $p < 0.05$ ). The relative distance between model RMSE and RMSE of the NULL model (= total variation, dashed line) represents the explained variance of the models ( $R^2$ ). Models were sorted by increasing RMSE. For model abbreviations, see Fig. 4.2.

correlated residuals. A mean error for leaf unfolding dates of more than a week is rather insufficient for most applications, as, for example, a variation of leaf unfolding of  $\sim 10$  days has been suggested to induce a variation of  $\sim 5.0\%$  of annual GPP and  $\sim 2\%$  annual ET using global circulation models (Migliavacca et al. 2012). Site-specific calibration decreased the mean prediction error to around  $4 \pm 5$  days, similar to that of previous studies using fewer models and data (e.g., Schaber and Badeck 2003).

## Spatial extrapolation

The increased mean prediction error present when applying site-specific parameter sets to predict the phenology of other sites (external validation; Fig. 4.2), indicates a limited spatial extrapolation potential of process based models. Indeed, for all 6 species assessed here, the prediction quality of a simple linear regression model was almost similar to that of the process-based models, a pattern also observed for two species by Olsson and Jönsson (2014). The generally high prediction error for the external validation support the conclusions of a recent study investigating leaf unfolding of *Betula* along urbanisation gradients in Germany (using the DormPhot model), that the possibility to use a space-for-time substitution in phenological time-series is limited (Jochner et al. 2013). This claim is especially true as the increase in the error of prediction on the validation site was neither correlated with the distance from the calibration site nor with climate similarity of the sites (in terms of temperature). Thus, the observed increase of the prediction error is likely based on several confounded factors: (1) the individual variation of phenology among trees, (2) possible ecotypic/provenance differentiation of trees across sites or (3) the overfitting of a highly parameterised model on the small site-specific dataset (40 observations of leaf unfolding per site).

Leaf phenology is known to exhibit considerable variation even between individuals of a given population. For example, most of the variation of *Pinus sylvestris* L. in Scotland occurs within, and not between populations (Salmela et al. 2013). As most of the time-series used here, represent the phenology of individual trees (depending on the observation protocol of the original collector/weather service), it is unknown to which extent the individual tree is representing the mean phenology of a site.

A provenance differentiation of phenology, often related to climatic gradients, has been evidenced in common gardens for the timing of bud burst (e.g., Kriebel and Wang 1962, von Wuehlisch et al. 1995) and has been attributed to different chilling and thermal forcing requirement among provenances, for example, a variation in the required temperature sums for bud burst (Gunderson et al. 2012, Vitasse et al. 2013). Some ecotypic differentiation has also been evidenced for the photoperiodic induction of bud set (Wareing 1956, Vaartaja 1959, Kriebel and Wang 1962, von Wuehlisch et al. 1995, Thomas and Vince-Prue 1997) and bud burst in spring (Linkosalo and Lechowicz 2006, Basler and Körner 2012, 2014).



However, the direction of the response of such genetic clines depends on species and the genetic effect on the timing of bud burst is often smaller than the environmental effects *in situ* (Vitasse et al. 2013). Conducting ‘hypothetical transplants’, several previous modelling studies, concluded that local adaptation plays a subordinate role in predicting the flowering phenology of several, early successional lowland tree species (Chuine et al. 1999, 2000a,b).

### **Modelling endodormancy release**

The similar performance of different models in all validation procedures conducted here might have been caused by the general spring-temperature accumulation principle common to all these models (even though the temperature-response functions differ among models). An analysis of the calibrated parameters and the high correlation among predictions of different models indicate, that most models conform to a model dominated by the accumulation of thermal forcing, while the additional parameters (e.g., for chilling accumulation) modulated the residual variation only. Indeed, certain parameter combinations may even prune some features from the complex models, reducing them to simple thermal time models (Linkosalo et al. 2008).

The finding that simple ecodormancy release models produce slightly better estimates of leaf phenology under current conditions than models with additional endodormancy release through chilling, is in line with several previous modelling studies (Häkkinen et al. 1998, Hannerz 1999, Linkosalo et al. 2000, 2008, Leinonen and Kramer 2002, Hänninen and Kramer 2007, Granhus et al. 2009). A valid calibration of the chilling responses during winter is limited to sites and species where the chilling fulfilment is currently an issue and where a lack of chilling is also reflected in delayed leaf unfolding: on sites where the chilling requirement is fulfilled early in winter, buds remain in a state of ecodormancy for much of the remaining winter (as growth is suspended due to low temperature or short photoperiods). Hence, the chilling parameters are unlikely to be estimated unambiguously using fitting procedures, especially since the actual chilling requirements and effective ranges of chilling temperatures are yet scarcely known for forest tree species (Battay 2000) and thus wide parameter ranges have to be used for model calibration.

The calibration of models which include dormancy induction (Dorm-Phot, Deepening-Rest and Four-Phase model), suffer from the sparsely available observational data on the relevant autumnal phases (bud set and dormancy induction). The bulk of autumnal observations consist of partially coincidental, but more striking events such as leaf colouring and leaf fall which have also been used as starting dates in phenological modelling studies (Schaber and Badeck 2003). However, these events do not represent the physiological state of bud dormancy induction and are strongly modified by a few cold nights in autumn (but see Delpierre et al. 2009). Statistical calibration of these parameters restricted to previously fixed parameter ranges is thus often the only solution to calibrate such models.

### **Photoperiod responses during ecodormancy release**

Models that included photoperiod during the ecodormancy release phase were generally among the models with the best performance in all 6 species. Linkosalo et al. (2006) speculated that the lower performance of models including chilling, compared to simple forcing models, may be caused by a yet inadequately formulated temperature response during the early phase of ecodormancy in late winter, soon after the fulfilment of the chilling requirement. The general trend to late starting dates in phenological models suggests either a negligible effect of mid-winter temperatures on the date of leaf unfolding under current conditions (as is also indicated by the good fit of the simple linear regressions), indicating either that the state of dormancy may affect the shape of the temperature response to chilling (Campbell and Sugano 1975, Hänninen 1990) or forcing temperatures (Junttila and Hänninen 2012), or the presence of a strong external threshold, such as a possible photoperiod threshold. The trend to late starting dates may further result from the intrinsic model structure, such as analysed for the Thermal Time model (Blümel and Chmielewski 2012). The inclusion of photoperiod as driver during ecodormancy release, as in the Photothermal-time or M1 model or also in combination with precedent endodormancy release, may slightly indeed improve estimates of leaf unfolding dates and lead to more realistic parameters for the 6 tree species included here, and thus, should be considered as a reliable starting point for further model development.

### **Underestimated inter-annual variation**

All evaluated models underestimated the inter-annual variation of leaf unfolding dates. The significant decrease of predictions quality for very early/late years indicates that models do not fully represent the true response to the environmental drivers. The underestimation of inter-annual variation was slightly less pronounced in *Betula* and *Aesculus* (Fig. 4.4), the two species, where the inter-annual range of leaf-out dates is larger (Table 4.1), suggesting that the sparse coverage of extreme responses in long-term observational data may bias model calibration and prevent the generalisation of the resulting parameter set. Consequently, either a dataset containing a wider range of responses or a model calibration scheme that puts more weight to extreme responses might be needed to improve model parameterisation. These results support Hänninen's (1995) conclusions, based on a comparison of leaf unfolding dates in field and in warming experiments, that to predict phenology in a future climate, the use of complementary data from experiments should be considered to increase data coverage for the calibration of the model parameters. However, in most cases the amount of data resulting from experiments does not allow for direct calibration of complex models and should rather be used to complement existing observational time-series or to restrict parameter ranges, also because data from experiments may not be representative for observations in situ (Wolkovich et al. 2012). For example, the phenology of tree seedlings, as often used in experiments, does not represent the responses of adult trees due to ontogenic differences (Vitasse 2013). Thus, to study dormancy release in warming and photoperiod experiments, cuttings from adult trees should preferably be used (Vitasse and Basler 2014, Primack et al. 2015), whereas rooted cuttings or grafts from adult trees may further be used to investigate the induction of dormancy or responses to chilling temperatures in mid-winter.

Another possibility to extend the range of observed responses is to assess phenological data of transplant gardens e.g. International Phenological Gardens (Schnelle and Volkert 1974, Chmielewski and Rötzer 2001, Primack and Miller-Rushing 2009), where identical clones of trees were transplanted to different climates and long time-series of phenological observations are available. In fact, such data has been used, for example to show that the transferability of model parameter estimates within a genotype is high (Chuine et al. 2000a), and to calibrate the very complex Dorm-

Phot model for *Betula* (Caffarra et al. 2011). Unfortunately, most data from experiments is limited to only few provenances and may thus, even be less suited for spatial extrapolation than in-situ observations.

## ***Methodical considerations***

### **Temperature data**

The quality of the driving temperature data may have influenced model calibration and thus, the validation among sites. The gridded climate dataset used for this study is missing the exact local temperature conditions actually perceived by trees (despite the lapse-rate correction applied). Yet, even precise weather station data from the vicinity of the phenological observation site may not reflect the actual temperature trees or buds experience, yet the situation is certainly better for tall trees than for low stature vegetation (Kollas et al. 2014). Furthermore, for practical reasons, e.g. data availability, data handling and computation time, only daily mean temperature, rather than higher resolution temperature data, have been applied to calibrate and run the models. This neglects the influence of the diurnal amplitude of the temperature, known to affect phenology in several species (Campbell and Sugano 1979, Erez and Couvillon 1987, Myking 1997, Partanen et al. 1998, Saxe et al. 2001).

### **Phenological time-series**

The phenological data used for the parameterisation and validation of the models were obtained from several national phenological networks across Europe, which used historically different observation protocols. The data collected by many different observers are likely not free from flaws, although the robust error detection scheme (Schaber and Badeck 2002) applied to the time-series, should have excluded the most unlikely data from the dataset. Unfortunately, no measure of the accuracy of the remaining individual observations is available.

## ***Conclusions***

The results obtained here show that it is not possible to prioritise a single mechanistic model as possible candidate for the actual mechanism of dormancy release. Yet, the relatively similar performance of all 35 process based model assessed here, indicates that the model have the structural flexibility to reproduce the weather-induced inter-annual variation of spring phenology, even though their intrinsic mechanistic assumption or parameterisation may not reflect actual physiological responses that control dormancy release. The transition from an empirically fitted model to a model that accurately describes the physiological processes is gradual and largely depends on the level of prior knowledge to reasonably restrict parameter ranges. There are yet no clear physiological or molecular markers that would allow to clearly separate and model each phase separately, e.g., the fulfilment of the chilling requirement (Cooke et al. 2012). For most time-series in central Europe, simple models (simulating the ecodormancy release only) yield reasonable prediction for the concurrent climate when calibrated with long phenological time-series. The simulation of endodormancy release or even of the whole period of dormancy in a phenological model is challenged by at least three, partially linked aspects: first, the accurate mechanistic understanding of dormancy induction and release, second their correct representation in models, and third, model calibration (overfitting) and data availability. The understanding of the underlying physiological mechanisms is essential, when making predictions for the future climates beyond current observational data coverage. Thus, for making such predictions I suggest running an ensemble of selected simple (ecodormancy) and more complex (endo-ecodormancy) models, until the underlining processes are fully revealed and accurately represented in a model.

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## Supplementary material

**Table S4.1:** Temperature response functions and structures of chilling/forcing -based and promoter/inhibitor-based spring phenology models used in this study. The models are driven by daily mean temperature ( $T_i$ ) and daylength ( $L_i$ ) after a starting date to. Most models using the growing degree day temperature response ( $r_g$ ) were also tested using the sigmoid temperature response ( $r_s$ ). Bold face indicates parameters to be fitted.

Temp. responses	Triangular temperature response	$r_t(T_i) = \begin{cases} T_{\min} \leq T_i < T_{\text{opt}} & : \frac{T_i - T_{\min}}{T_{\text{opt}} - T_{\min}} \\ T_{\text{opt}} \leq T_i < T_{\max} & : \frac{T_i - T_{\text{opt}}}{T_{\max} - T_{\text{opt}}} \\ T_i < T_{\min} \vee T_i > T_{\max} & : 0 \end{cases}$	Growing-degree-day temperature response	$r_g(T_i) = \begin{cases} T_i \leq T_{\text{base}} & : 0 \\ T_i > T_{\text{base}} & : (T_i - T_{\text{base}}) \end{cases}$		
Temp. responses	Bell-shaped temperature response	$r_b(T_i) = \frac{1}{1 + e^{a(T_i - T_i - c)^2 + b(T_i - c)}}$	sigmoid temperature response	$r_s(T_i) = \frac{1}{1 + e^{-b(T_i - c)}}$		
	State of chilling	$S_{\text{chl}} = \sum_{i=t_0}^n R_{\text{chl}}$	State of forcing	$S_{\text{frc}} = \sum_{i=t_0}^n R_{\text{frc}}$	Criteria for bud burst	$S_{\text{frc}} \geq F_{\text{crit}}$
	Thermal Time model <sup>1</sup>	$R_{\text{frc}} = r_g$	sigmoid T. Time model <sup>2</sup>	$R_{\text{frc}} = r_c$		
	Photo Thermal Time model <sup>3</sup>	$R_{\text{frc}} = \frac{L_i}{24} r_g$	M1 model <sup>4</sup>	$R_{\text{frc}} = \left(\frac{L_i}{24}\right)^k r_g$		
	Sequential model <sup>5</sup>	$R_{\text{chl}} = r_t$ $R_{\text{frc}} = k r_r^k$ $k = \begin{cases} S_{\text{chl}} < C_{\text{req}} & : 0 \\ S_{\text{chl}} \geq C_{\text{req}} & : 1 \end{cases}$	Parallel model <sup>5</sup>	$R_{\text{chl}} = r_t$ $R_{\text{frc}} = k r_r^k$ $k = \begin{cases} S_{\text{chl}} < C_{\text{req}} & : C_{\text{ini}} + S_{\text{chl}} \frac{1 - C_{\text{ini}}}{C_{\text{req}}} \\ S_{\text{chl}} \geq C_{\text{req}} & : 1 \end{cases}$		
	Unified model <sup>6</sup>	$R_{\text{chl}} = r_t$ $k = \begin{cases} S_{\text{chl}} < C_{\text{req}} & : 0 \\ S_{\text{chl}} \geq C_{\text{req}} & : 1 \end{cases}$ $R_{\text{frc}} = k r_r^k$ $F_{\text{crit}} = \mathbf{w} e^{F_{\text{Schl}}}$	Alternating model <sup>7</sup>	$R_{\text{chl}} = \begin{cases} 0 & T_i < T_{\text{base}} \\ 1 & T_i \geq T_{\text{base}} \end{cases}$ $R_{\text{frc}} = r_g$ $F_{\text{crit}} = \mathbf{a} + \mathbf{b} e^{S_{\text{chl}}}$		
	Deepening Rest <sup>8</sup>	$R_{\text{chl}} = r_t$ $R_{\text{frc}} = k F_c$ $k = \begin{cases} S_{\text{chl}} < C_{\text{dr}} & : \frac{1 - S_{\text{chl}}}{C_{\text{dr}}} \frac{1 - C_{\text{ini}}}{C_{\text{dr}}} \\ C_{\text{dr}} \geq S_{\text{chl}} < C_{\text{req}} & : C_{\text{ini}} + \frac{(1 - C_{\text{ini}})(S_{\text{chl}} - C_{\text{dr}})}{C_{\text{req}} - C_{\text{dr}}} \\ S_{\text{chl}} \geq C_{\text{req}} & : 1 \end{cases}$	Four Phase model <sup>9</sup>	$R_{\text{chl}} = r_t$ $T_{\text{trb}} = \begin{cases} S_{\text{chl}} < C_{\text{dr}} & : T_1 + S_{\text{chl}} \frac{T_2 - T_1}{C_{\text{dr}}} \\ C_{\text{dr}} \leq S_{\text{chl}} < C_{\text{req}} & : T_1 + S_{\text{chl}} \frac{(T_2 - T_1)(S_{\text{chl}} - C_{\text{dr}})}{C_{\text{req}} - C_{\text{dr}}} \\ S_{\text{chl}} < C_{\text{tr}} \wedge T_i > T_{\text{trb}} & : 1 \\ S_{\text{chl}} > C_{\text{req}} & : 1 \\ C_{\text{pr}} \leq S_{\text{chl}} < C_{\text{req}} \wedge T_i > T_{\text{th}} & : 1 \\ \text{else} & : 0 \end{cases}$ $k = \begin{cases} S_{\text{chl}} < C_{\text{dr}} & : \frac{1 - S_{\text{chl}}}{C_{\text{dr}}} \frac{1 - C_{\text{ini}}}{C_{\text{dr}}} \\ C_{\text{dr}} \geq S_{\text{chl}} < C_{\text{req}} & : C_{\text{ini}} + \frac{(1 - C_{\text{ini}})(S_{\text{chl}} - C_{\text{dr}})}{C_{\text{req}} - C_{\text{dr}}} \\ S_{\text{chl}} \geq C_{\text{req}} & : 1 \end{cases}$		
	DormlPhot model <sup>10</sup>	$S_L = \sum_{i=t_0}^n \frac{1}{1 + e^{a_1(T_i - b_1)}} \frac{1}{1 + e^{10(L_i - L_{\text{crit}})}}$ $R_{\text{chl}} = \begin{cases} S_L < L_{\text{crit}} & : 0 \\ S_L \geq L_{\text{crit}} & : r_t \end{cases}$		$d_{50} = \frac{24}{1 + e^{a_2(S_{\text{chl}} - C_{\text{req}})}}$ $t_{50} = \frac{60}{1 + e^{a_3(T_i - b_{50})}}$ $R_{\text{frc}} = \begin{cases} S_L < L_{\text{crit}} & : 0 \\ S_L \geq L_{\text{crit}} & : \frac{1}{1 + e^{-a_4(T_i - b_{50})}} \end{cases}$		
	Initial state	$I_{t_0} = 1$ $F_{t_0} = 0$	State of promoter / inhibitor	$I_{t+1} = I_t + \Delta I$ $F_{t+1} = F_t + \Delta P$	Criteria for bud burst	$P \geq 1$
	PIM 1	$\Delta I = -a_2 r_{t2} I$	$\Delta P = -a_3 r_{t3} (1 - I) - a_4 P$	PIM 7	$\Delta I = -a_2 r_{t2} I \frac{1}{24}$	$\Delta P = -a_3 r_{t3} (1 - I) - a_4 P$
	PIM 2	$\Delta I = -a_2 r_{t2} I$	$\Delta P = -a_3 r_{t3} (1 - I) - a_4 P \frac{24 - L_i}{24}$	PIM 8	$\Delta I = -a_2 r_{t2} I \frac{1}{24}$	$\Delta P = -a_3 r_{t3} (1 - I) - a_4 P \frac{24 - L_i}{24}$
	PIM 3	$\Delta I = -a_2 \frac{24 - L_i}{24} - a_2 r_{t2} I$	$\Delta P = -a_3 r_{t3} (1 - I)$	PIM 9	$\Delta I = a_1 \frac{24 - L_i}{24} - a_2 r_{t2} I \frac{1}{24}$	$\Delta P = -a_3 r_{t3} (1 - I)$
	PIM 4	$\Delta I = -a_2 r_{t2} I$	$\Delta P = -a_3 r_{t3} (1 - I) \frac{L_i}{24} - a_4 P$	PIM 10	$\Delta I = -a_2 r_{t2} I \frac{1}{24}$	$\Delta P = -a_3 r_{t3} (1 - I) \frac{L_i}{24} - a_4 P$
	PIM 5	$\Delta I = -a_2 r_{t2} I$	$\Delta P = -a_3 r_{t3} (1 - I) \frac{L_i}{24} - a_4 P \frac{24 - L_i}{24}$	PIM 11	$\Delta I = -a_2 r_{t2} I \frac{1}{24}$	$\Delta P = -a_3 r_{t3} (1 - I) \frac{L_i}{24} - a_4 P \frac{24 - L_i}{24}$
	PIM 6	$\Delta I = a_1 \frac{24 - L_i}{24} - a_2 r_{t2} I$	$\Delta P = -a_3 r_{t3} (1 - I) \frac{L_i}{24}$	PIM 12	$\Delta I = a_1 \frac{24 - L_i}{24} - a_2 r_{t2} I \frac{1}{24}$	$\Delta P = -a_3 r_{t3} (1 - I) \frac{L_i}{24}$

<sup>1</sup> Réaumur 1735; Wang 1960, Cannell and Smith 1983, Chuine et al. 1999, Hunter and Lechowicz 1992; <sup>2</sup> Hanninen 1990; Kramer 1994; <sup>3</sup> Masle et al. 1989, Crepinsek et al. 2006; <sup>4</sup> Blümel and Chmielewski 2012; <sup>5</sup> Landsberg 1974; Hanninen 1990; Kramer 1994; <sup>6</sup> Chuine 2000; <sup>7</sup> Cannell Smith 1983, Murray et al. 1989; <sup>8</sup> Kobayashi and Fuchigami 1983; <sup>9</sup> Vegis 1964; <sup>10</sup> Caffarra et al. 2011; <sup>11</sup> Schaber and Badeck 2003

**Table S4.2:** Parameter ranges used to calibrate the models.

<b>General parameters for several models</b>	
Startday <sup>a</sup>	$-168 \leq t_o \leq 182$
Growing degree day response	Sigmoid temperature response
$-5 \leq T_{base} \leq 10$	$0 \leq F_{crit} \leq 350$
$0 \leq F_{crit} \leq 2000$	$0 \leq b \leq 100$
	$0 \leq c \leq 100$
Triangular chilling response	Bell-shaped chilling response
$-5 \leq T_{opt} \leq 10$	$0 \leq C_a \leq 10$
$-5 \leq T_{min} \leq 10$	$-20 \leq C_b \leq 20$
$0 \leq T_{max} \leq 15$	$-10 \leq C_c \leq 10$
$0 \leq C_{req} \leq 350$	
<b>Model-specific parameter</b>	
Parallel	Alternating
$0 \leq C_{ini} \leq 1$	$-168 \leq t_{o_{chill}} \leq 182$
	$0 \leq a \leq 500$
DeepeningRest	$0 \leq b \leq 1000$
$0 \leq C_{ini} \leq 1$	$0 \leq c \leq 5$
$0 \leq C_{dr} \leq 350$	$0 \leq T_{base} \leq 8$
Unified	FourPhase
$0 \leq F_b \leq 100$	$0 \leq C_{tr} \leq 350$
$0 \leq F_c \leq 15$	$0 \leq C_{pr} \leq 350$
$0 \leq w \leq 1000$	$0 \leq T_1 \leq 15$
$-100 \leq k \leq 0$	$0 \leq T_2 \leq 20$
	$0 \leq b \leq 50$
PIM 1-12	$-5 \leq c \leq 10$
$-25 \leq I_{Tmin} \leq 10$	
$-15 \leq I_{Topt} \leq 20$	DormPhot
$0 \leq I_{Tmax} \leq 35$	$8 \leq L_{crit} \leq 14$
$-20 \leq P_{Tmin} \leq 15$	$0 \leq D_{crit} \leq 100$
$0 \leq P_{Topt} \leq 40$	$0 \leq C_{crit} \leq 100$
$5 \leq P_{Tmax} \leq 45$	$0 \leq F_{crit} \leq 100$
$0 \leq a_1 \leq 1$	$-5 \leq a_L \leq 5$
$0 \leq a_2 \leq 1$	$0 \leq b_L \leq 20$
$0 \leq a_3 \leq 1$	$0 \leq a_C \leq 5$
$0 \leq a_4 \leq 1$	$0 \leq cC \leq 20$
	$0 \leq h_{DL} \leq 20$

<sup>a</sup>startday refers to the start day of forcing for ecodormancy models, to the startday of chilling or dormancy induction for all other models. Startday -182 refers to day 183 (July 1) of the previous year

**Part II**  
**Further studies and reviews**



## Chapter 5

# Phenology under global warming

Christian Körner, David Basler

*In most temperate tree species, phenological events such as flowering and autumnal cessation of growth are not primarily controlled by temperature.*

Phenological events such as bud burst, flowering, and senescence have received increased interest in the light of global warming (Cleland et al. 2007, Khanduri et al. 2008, Morin et al. 2009). Spring events at temperate latitudes have advanced by 2.5 days per decade since 1971 (Menzel et al. 2006). As global warming progresses, how will it affect the arrival of spring and the length of the growing season?

In humid extratropical areas, the three most important factors controlling phenology in dominant forest tree species are the degree of winter chilling, photoperiod (day length relative to night length), and temperature (Hay 1990, Chuine and Cour 1999, Körner 2007; see the Fig. 5.1). Because the seasonal course of temperature varies strongly from year to year, sensitivity to photoperiod protects plants from the potentially fatal consequences of simply tracking temperatures at the ‘wrong’ time of the year. Photoperiod controls the induction (formation of winter buds, leaf abscission meristems, and freezing resistance; Wareing 1956, Li et al. 2003, Keskkitalo et al. 2005) and release from dormancy, the onset of growth, and reproductive events, including synchronous flowering (Keller and Körner 2003, Jackson 2009). Temperature plays a modulating role and triggers the visible progress of phenology, such as leaf coloration, in many species.

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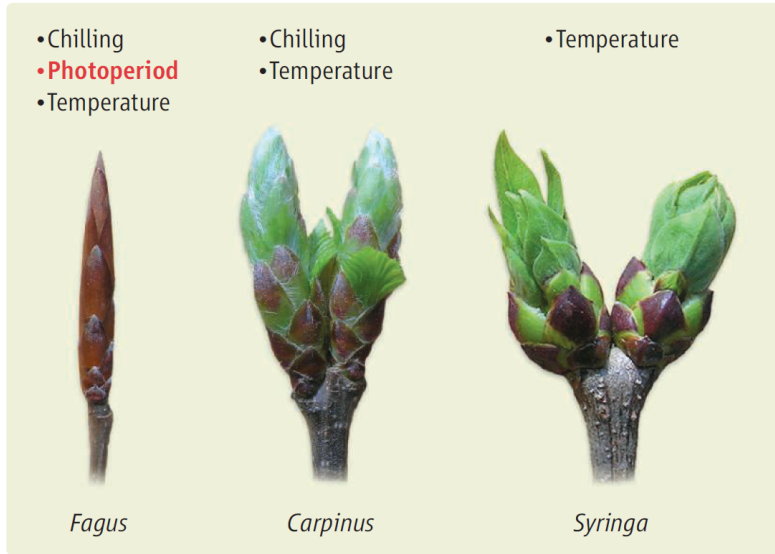
Because the photoperiod is equally long in autumn and spring, dormancy release in spring requires the information that winter has passed, obtained from the dose of low temperatures experienced by the plant. When this chilling requirement is fulfilled, plants become receptive to photoperiod signals. Once a critical photoperiod has passed, actual bud break is a matter of concurrent temperature. A lack of sufficient chilling in mild winters delays bud break (Murray et al. 1989) but may be partially replaced by long photoperiods and/or very high temperatures (Heide 1993).

Not all tree species are sensitive to photoperiod, but the long-lived, late successional species that become dominant in mature forests commonly are. The genetic controls of plant development by photoperiod even remain in action when these temperate tree species are transplanted to subtropical parks, where bud break in hackberry (*Celtis*), beech (*Fagus*), and oak (*Quercus*) species was never found to occur before early March, despite exceptionally high temperatures in this exotic environment (Borchert et al. 2005). It is thus a misconception to linearly extrapolate a few days advance of leafing during warm years into a proportional lengthening of the growing season in climate warming scenarios (Penuelas 2009, Moser et al. 2010).

Shorter-lived, early successional species adopt a more risky life strategy (Körner 2007). Many phenological observations in the literature come from such pioneer species as hazel, poplars, or birch, which are opportunistic (photoperiod-insensitive in spring). Other opportunistic species include weeds, as well as ornamental plants from warmer climates.

For instance, the famous phenological time series for horse chestnut in the streets of Geneva (Defila and Clot 2001), showing clear advances in leafing, is for an exotic species from a sub-Mediterranean setting. Another prominent time series shows early flowering of domestic cherry trees (Defila and Clot 2001), which exhibit adaptive traits from central Asia, from where the cultivars originate. In these continental regions, the advent of spring is rather invariable, presumably due to the great distance from the sea, and phenological tracking of temperature bears no risk. In fact, trees in these regions should be more likely to keep tracking climatic warming than those in climates with more unpredictable weather systems, an interesting question to be explored in future work. Many ornamental plants in temperate gardens are photoperiod-insensitive, and their spring phenology





**Fig. 5.1:** Not just temperature. Spring development in many ornamental plants from warm regions, such as lilac (*Syringa*), is primarily controlled by temperature, whereas early successional species native to temperate latitudes, such as hornbeam (*Carpinus*), only become temperature-sensitive once their chilling demand has been fulfilled. Late successional taxa, such as beech (*Fagus*), are photoperiod controlled, with temperature only exerting a limited modulating effect once the critical day length has passed. This mechanism prevents such taxa from sprouting at the ‘wrong’ time.

tracks temperature with only very minor chilling requirements, as exemplified by lilac (*Syringa*; Larcher 2007).

Phenology in late successional species will thus not continue to track climatic warming (the lengthening of the potential growing season) but will increasingly become constrained by internal controls, as the photoperiod threshold (set by genes) is approached. For most extratropical trees, seasons will not become substantially longer until new genotypes emerge, which will take a few tree generations (a few hundred years)<sup>1</sup>.

<sup>1</sup> This estimate is based on what is known for weeds, which need about five generations to evolve new latitude-specific photoperiod genotypes (Langlet 1971).

Opportunistic taxa may profit from a warmer climate and may thus gain a competitive advantage over photoperiod-sensitive taxa. Rapid climatic warming may also drive current tree genotypes into a disparity between their insurance against ‘misleading’ (too early in the season) warm temperatures and concurrent temperature-sensitive soil processes such as mineralization. Ecosystem nutrient losses are a potential consequence of trees getting out of phase with the climate system. Climatic warming should thus not be seen as a self-evident cause for more tree growth.

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## Chapter 6

# What role for photoperiod in the bud burst phenology of European beech

Yann Vitasse, David Basler

**Abstract** A considerable number of studies have investigated the phenology of European beech using models, experimental controlled conditions, or descriptive surveys of patterns in situ. In spite of this interest, there is no consensus about the environmental factors controlling bud burst in beech, especially about the role of photoperiod and chilling temperature (cold temperature effective to release bud dormancy). However, recent experimental and modelling studies provide new insights into the means by which these environmental factors control beech phenology. This present contribution aims to reconcile contradictory hypotheses about the main environmental factors controlling bud burst date of European beech. First, we review the main published results on the environmental control of beech phenology both in controlled and in natural conditions. Second, supported by the findings of recent studies, we propose a new theory for the role of photoperiod during the chilling phase for explaining spatial and temporal variations in bud burst phenology of European beech. Examples using long-term data from the Swiss Alps and Germany are presented to support this theory. The possible impacts of future and ongoing climate warming on beech phenology are discussed. Finally, due to interactions between chilling, forcing temperature, and photoperiod, we assert that beech phenology follows a nonlinear trend across biogeographical gradients such as changes in elevation or latitude and that the bud burst

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date of beech is expected not to undergo significant changes in response to global warming, especially in warmer climates.

**Key words:** *Fagus sylvatica*, Spring phenology, Bud burst, Chilling, Photoperiod, Temperature, Climate change

## Introduction

The European beech (*Fagus sylvatica* L.) is one of the most dominant forest tree species in Europe. Its distribution area is mainly concentrated in the Centre and West of Europe and covers various habitats ranging from mountainous regions in southern and Eastern Europe to lowlands in central Europe (Bolte et al. 2007). That said, and despite the considerable importance of the beginning of the growing season for tree growth (Rötzer et al. 2004, Churkina et al. 2005, Gomory and Paule 2011), tree fitness and tree species distribution area (Chuine 2010), it is surprising that the way environmental factors act on bud burst phenology of beech is still poorly understood. Indeed, among temperate tree species, European beech has always occupied a special place for scientists studying the phenology of trees but nevertheless the accurate prediction of beech bud burst date is still problematic because of various unresolved issues (Kramer 1994). The “apparent” contradictory results found in the literature led to different assumptions about the main environmental factors controlling spatial and temporal variations in bud burst dates of beech. Here, through our critical appraisal of recent findings, we were able to reconcile the conflicting results and propose a new theory for the role of photoperiod during the chilling phase to explain spatial and temporal variations in bud burst dates of European beech. We first review the different results reported from observational studies, from experimental manipulations with particular reference to biogeographical gradients, and from modelling studies. Then, we finally test our theory through a new analysis of data from different elevations.

## Spatial and temporal patterns of bud burst in nature

The spring phenological pattern of European beech differs to that of other temperate tree species in two main aspects. First, the bud burst date of beech shows less temporal and spatial variations than most of the other deciduous tree species in Europe. For example, along elevational gradients, beech exhibits a slight delay in leaf unfolding dates, generally less than 20 days  $1000\text{ m}^{-1}$ , whereas other co-occurring tree species, such as *Quercus petraea* or *Larix decidua*, delay more than 30 days  $1000\text{ m}^{-1}$  (Dittmar and Elling 2006, Migliavacca et al. 2008, Vitasse et al. 2009b, Davi et al. 2011, Jochner et al. 2012). During the last decades, the bud burst date of beech shows only low year-to-year variation, especially in mild climates. For instance, beech exhibits the lowest variability in leaf unfolding dates from year to year among the six forest tree species monitored in the same 107 sites in Germany during the period 1980–2009 (Table 6.1). Furthermore, although climatic warming over the last decades has significantly advanced spring phenology in most deciduous tree species (reviewed by Bertin 2008), European beech has exhibited little or no spring phenological shift during this period (Menzel et al. 2001, Studer et al. 2005, Vitasse et al. 2009a). Accordingly, both spatial and temporal studies have reported relatively little sensitivity of beech bud burst date to spring temperature, with an average advance of 2 days for every  $1\text{ }^{\circ}\text{C}$  increase (Kramer 1995, Vitasse et al. 2009a, Lebourgeois et al. 2010, Kreyling et al. 2012). Second, European beech is considered to be a late-flushing species, compared with most of the co-occurring broad-leaved tree species under mild and warm climates in Europe (Kramer 1995, Gordo and Sanz 2009, Vitasse et al. 2009a). Nevertheless, since tree species can respond to different environmental cues, e.g., photoperiod-sensitive against photoperiod-insensitive species (Körner and Basler 2010, Polgar and Primack 2011), and can have different sensitivity to temperature (Vitasse et al. 2009a), the species ranking for the timing of flushing can also change with climate. For instance, Vitasse et al. (2009b) reported that in the French Pyrenees Mountains, bud burst of beech commenced 20 days later than sessile oak and about one week later than sycamore and European ash at low elevation (below 500 m), whereas it commenced one week earlier than those species at high elevation (above 1500 m). In summary, beech can be considered as a late-flushing species under warm or mild climates, but not necessarily under colder climates within its range.

**Table 6.1:** Mean leaf unfolding date (day of the year) and pooled standard deviation (BBCH 11) across 107 sites in Germany from 1980 to 2009.

Species	Mean date of leaf unfolding	Pooled SD
<i>Alnus glutinosa</i>	107.0	11.95
<i>Betula pendula</i>	107.1	9.42
<i>Fagus sylvatica</i>	116.6	7.46
<i>Fraxinus excelsior</i>	126.3	9.03
<i>Picea abies</i> <sup>a</sup>	126.4	8.63
<i>Quercus robur</i>	122.1	8.06

Only sites where all species are present were selected. Data were provided by the members of the PEP725 Pan European Phenology Data project (Accessed 2011-04-1 at <http://www.zamg.ac.at/pep725/>)

<sup>a</sup> Leaf separation (BBCH10)

## What can we learn from experimental studies?

Many experiments have been conducted using European beech in an attempt to elucidate the biological mechanisms involved in bud dormancy release. This knowledge is crucial for us to understand and predict how trees will respond in different climates. So far, the diversity of often contradictory results from these experiments has proven hard to unify, leaving the environmental controls of dormancy release and beech bud burst poorly understood. However, due to the renewed enthusiasm of researchers to assess the future shift of tree phenology in response to climate warming, several new experimental studies have been conducted, providing a better insight into the different factors involved and their interactions in the mediation of bud burst of European beech.

### *Evidence of high chilling requirement for dormancy release*

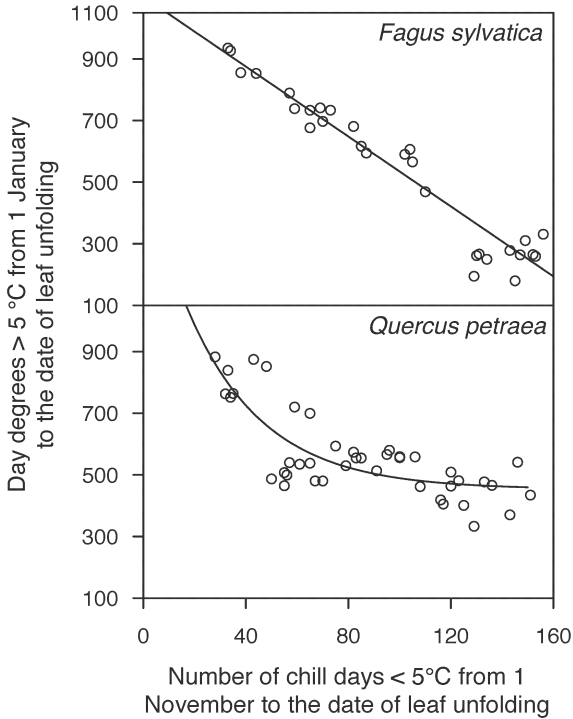
Before the 1990s, experimental studies using tree seedlings under controlled conditions generally highlighted the role of chilling temperatures and/or the photoperiod in determining the date of bud burst of some late-leaving species included European beech. For instance, Murray et al.



(1989) demonstrated for a set of common European broad-leaved tree species that an increased duration of chilling temperatures led to a decrease in the heating requirement for bud burst. They pointed out that, of this set, beech was unique because it was the only species to never reach its minimal thermal time requirement as long as the previous chilling temperature increased. Based on this result, they suggested that European beech has a very high chilling requirement to fully release bud dormancy, something which was confirmed later by Falusi and Calamassi (1990) and by Caffarra and Donnelly (2011). We applied the same calculation method as Murray et al. (1989) to beech phenology data recorded in situ from large elevational gradients and published in Vitasse et al. (2009b), which confirmed that beech seems to have a high chilling requirement (Fig. 6.1). The increase of the chilling duration exponentially reduces the thermal time to bud burst in sessile oak, among many other tree species (e.g. Murray et al. 1989, Harrington et al. 2010); however, in European beech, there was a linear relationship in our data between thermal time requirement to bud burst and the duration of chilling (Fig. 6.1). This result would indicate that most of the beech populations monitored in Vitasse et al. (2009b) currently inhabit environments which do not allow the full satisfaction of their chilling requirement, except perhaps for the highest populations which experienced more than 120 days of chilling days over winter (Fig. 6.1). However, this result should be interpreted with caution, because photoperiod is a confounding factor in situ as it may affect the relationship between forcing (warm temperatures) and chilling requirement (see next section). In addition, the temperature ranges where chilling and forcing temperature are effective on bud dormancy are likely overlapping and are still unclear for most species (Harrington et al. 2010, Cooke et al. 2012).

### ***Evidence of photoperiodic mediation in late winter and early spring***

Although it is commonly assumed that chilling temperatures play a crucial role in regulation of bud burst dates for European beech, the role of photoperiod remains more equivocal. There are conflicting reports on the influence of photoperiod during chilling, forcing and both phases of bud development. Some studies have claimed that photoperiod may modulate



**Fig. 6.1:** Relationship between the thermal time required to bud burst, calculated as the sum of day degrees  $> 5^{\circ}\text{C}$  from 1st January to the date of leaf unfolding, and the accumulated number of chill days for populations of *Fagus sylvatica* and *Quercus petraea* monitored across two elevational gradients in Pyrenees mountains during 2005–2007 period (dataset from Vitasse et al. 2009b). A linear regression model was fitted to *Fagus sylvatica* ( $y = -3.22x + 591$ ) and a nonlinear model was fitted to *Quercus petraea* ( $y = 220 + 594 e^{-0.037x}$ ). The studied sites covered 10 populations of *Fagus sylvatica* and 14 populations of *Quercus petraea* at elevations ranging from 100 to 1600 m a.s.l.. Air temperature at 2 m height was recorded hourly in each site, whereas leaf development was assessed every 10 days (see Vitasse et al. 2009a, b for further information)

the amount of accumulated forcing temperature required to initiate bud burst of beech even after the buds were assumed to be fully chilled (Wareing 1953, Heide 1993), while Falusi and Calamassi (1990) found only a negligible effect of photoperiod. Heide (1993) reported that both photoperiod and chilling temperature together control the timing of bud burst. In particular, he found that nonchilled buds sampled in November and December are unable to develop until they have received a substantial period of chilling, even under long day conditions. Finally, by controlling both the amount of chilling and the photoperiod on beech seedlings, Falusi and Calamassi (1996) reported that long days could partially substitute winter chilling. In other words, a longer photoperiod may reduce the thermal time requirement for bud burst when chilling temperatures are insufficient to fully release the buds from dormancy. More recently, Caffarra and Donnelly (2011) reconfirmed these earlier results that photoperiod only has a strong effect on buds when they are not fully chilled. This study clearly shows a decrease in the photoperiod effect with increasing exposure to chilling temperatures.

### ***Interactions between photoperiod and chilling/forcing temperatures***

Since beech is assumed to have a very large chilling requirement, which tends to be reached only in the coldest parts of its current distribution (see previous section), it is likely that overwintered buds collected in previous experimental studies were not all fully chilled (e.g. in Wareing 1953, Heide 1993). Thus, the equivocal results of these studies on the role of photoperiod are controversial because they are likely to be based on a mixture of fully or partially chilled buds. The contradictory results could also arise from the way experiments are conducted. For instance, some studies used different fixed photoperiod (e.g. Heide 1993, Caffarra and Donnelly 2011) rather than gradually lengthening photoperiods (Basler and Körner 2012), or used cutting twigs (Heide 1993) rather than the whole plant (e.g. Falusi and Calamassi 1996, Caffarra and Donnelly 2011). Finally, all experiments manipulating photoperiod have been conducted on seedlings or twig cuttings which may not mirror the phenology of adult trees growing in situ (Basler and Körner 2012, Vitasse Unpublished data).

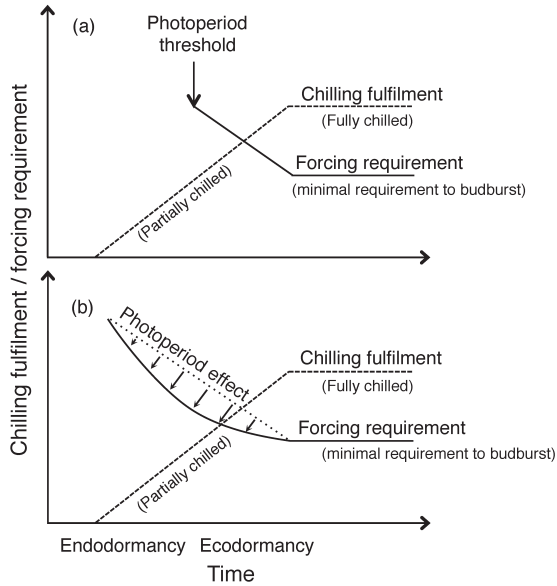
The main challenge today is to quantify how the photoperiod and chilling temperatures interact together to influence the timing of beech bud burst as well as the physiological and molecular modes of action of these processes (Falusi and Calamassi 2003, Cooke et al. 2012). Indeed, there are two main different ways in which photoperiod, sensed by the plants phytochrome system, may interact with chilling and forcing temperatures: (1) A fixed photoperiod threshold might be required to trigger dormancy release, subsequently allowing buds to respond to forcing temperature with a forcing requirement depending on the chilling fulfilment (Fig. 6.2a), (2) The forcing requirement for bud burst might decrease towards its minimal value when increases in the photoperiod are detected (Fig. 6.2b), or the accumulation rate of forcing temperature could be accelerated by increasing bud sensitivity to forcing as photoperiod increases, or after passing a certain threshold of photoperiod (not shown).

A recent experimental study conducted on *Betula pubescens* combined with a new phenological model that accounts for the effects and interactions of temperature and photoperiod supports the last hypothesis, suggesting that photoperiod affects the rate of forcing accumulation (Caffarra et al. 2011). These authors also demonstrate that the photoperiod effect is greater when there is a deficit in the amount of chilling that bud experiences.

## **What can we learn from modelling studies?**

### ***Phenological models underline the importance of chilling temperatures in the prediction of bud burst dates for European beech***

Until recently, two classes of process-based models were classically used to simulate spring phenological phases of trees. The first class of models, called hereafter the ‘1-phase models’, considers only forcing temperature, assuming that bud burst occurs after a fixed sum of forcing units has been reached. This kind of model implicitly assumes that dormancy is fully released before the starting date of forcing accumulation. The second class of models, called hereafter the ‘2-phase models’, considers the ac-



**Fig. 6.2:** Conceptual scheme on the two hypotheses for the role of photoperiod on forcing requirement in the bud burst phenology of European beech. Fig. 6.2a. A fixed photoperiod threshold triggers dormancy release, subsequently allowing buds to respond to forcing temperature; Fig. 6.2b. The forcing requirement for bud burst decreases towards its minimal value when increases in the photoperiod are detected. Note that, alternatively, the accumulation rate of forcing temperature could be accelerated by increasing bud sensitivity to forcing as photoperiod increases (not drawn).

tion of chilling temperatures during the endodormancy phase (winter deep dormancy caused by plant endogenous factors) and forcing temperatures during the ecodormancy phase (dormancy maintained by environmental factors, see Lang et al. 1987). The 2-phase models assume that the accumulation of forcing units starts and/or evolves according to the state of bud development during endodormancy (Chuine 2000, Hänninen and Kramer 2007, Vitasse et al. 2011) and that the critical sum of forcing units may be related to the amount of chilling units previously received (Cannell and Smith 1983, Murray et al. 1989). For most tree species, the 1-phase models have been shown to perform similarly or better than the 2-phase models (e.g. Hunter and Lechowicz 1992), suggesting that under current

and past climate, the chilling requirement of trees seems to be fully met. In contrast, for beech, or in general for late-leafing species, the 2-phase models tend to outperform the 1-phase models (Kramer 1994, Thompson and Clark 2008, Vitasse et al. 2011). This is in agreement with the assumption that beech has a high chilling requirement to release dormancy and may not always saturate its chilling phase, especially in the mild winter experienced in southern and central parts of its distribution area. However, outputs from phenological models should be interpreted with caution since the state of chilling can be year to year correlated with the state of forcing (controlled by spring temperature). This correlation would give equivalent performance between 1- and 2-phase models without proving that chilling requirement is fully met. The advantage of 2-phase models would appear when the amount of chilling strongly varies from year to year and if this variation is not correlated with spring temperature. Towards new models integrating photoperiodic effect In spite of the improvement of predictions gained by using 2-phase models, the accuracy of these predictions is generally lower for European beech than for the other cooccurring tree species (Vitasse et al. 2011). This is likely due to the additional photoperiod sensitivity of this species besides the chilling and forcing temperature effects, as demonstrated by the experimental studies presented in the previous section. Although the influence of photoperiod in beech phenology has been previously tested in models, the results were inconsistent: Schaber and Badeck (2003) suggested strong photoperiodic control with chilling playing only a subordinate role, whereas Kramer (1994) found lower model efficiency when photoperiod was incorporated into the model. However, these two previous studies included photoperiod only as a function affecting the rate of chilling. Yet, experimental studies suggest that the photoperiod effect acts more on forcing rate via interaction with chilling requirement (Falusi and Calamassi 1996), as it was also demonstrated for *Betula pubescens* (Myking and Heide 1995, Caffarra et al. 2011). Hence, an original moresophisticated type of model which integrates some components of those models previously applied to beech (Kramer 1994, Chuine 2000, Hänninen and Kramer 2007) to address the effect of photoperiod on the rate of forcing accumulation and requirement, depending on prior chilling temperature, is currently being developed and performs better than classical 2-phase models on *Betula pubescens* (Caffarra et al. 2011). This type of model shows promise for testing our assumptions about the main environmental factors driving spring phenology in European beech.

***How to explain the temporal and spatial phenological patterns of European beech: towards concordance between observations and results from experiments and models?***

As mentioned in the first section, beech exhibits low variation in bud burst date from year to year or along environmental gradients in comparison with other species. This is especially true at low latitudes or elevations, i.e., in milder winter conditions. According to our theory, one would expect weak variations in the date of bud burst in warm conditions such as at low elevation in a maritime climate and higher variations in cooler winter conditions, such as at high elevation, in the northern part of Europe or with a continental climate. Indeed, under mild winter conditions, buds are likely not to reach their optimal chilling and subsequently require a higher degree of forcing before bud burst, which could potentially delay the date of bud burst. However, as photoperiod lengthens through spring, it might compensate for this delay, either by increasing the forcing accumulation rate or by decreasing the amount of forcing required to bud burst (Fig. 6.2b). Consequently, in such warmer climates, the effect of photoperiod should counterbalance the lack of chilling that occurs during warmer winters and early spring, leading to weak variations in bud burst dates from year to year. Similarly, buds are chilled more (or fully chilled) during cooler years, so require both a reduced amount of forcing temperature and lessened importance of photoperiod before bud burst, resulting in a more-modest delay of bud burst compared with warmer years than might otherwise be expected, i.e., a feedback loop is created that tends to stabilise bud burst date. In contrast, in cooler winter conditions as occur at high elevation, buds tend to be fully chilled, removing the possible interaction between chilling temperature and photoperiod. The bud burst dates would thus mostly depend on forcing temperatures, increasing the potential interannual variation of these dates and the correlation with spring temperatures.

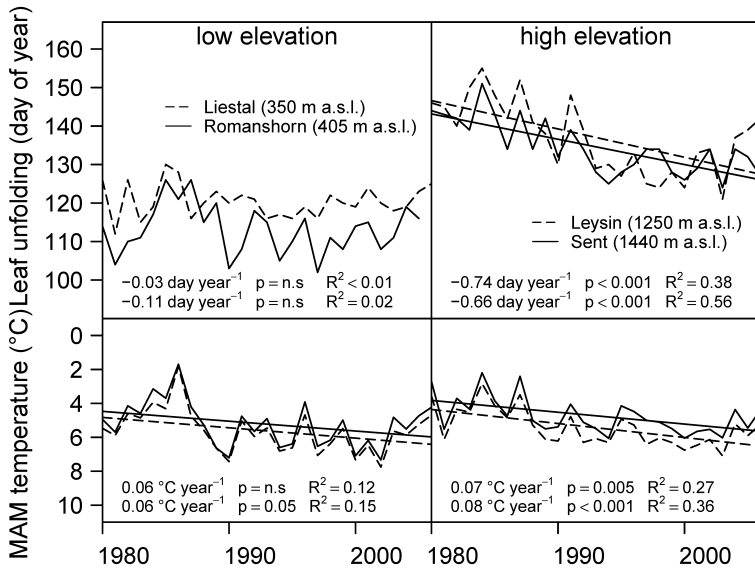
This representation of the relative contributions of the three factors according to climate conditions is in line with the suggestion of Wareing (1953), 60 years ago (!), that beech bud burst dates are mainly controlled by photoperiod for southern populations and by thermic conditions for the northernmost populations. This assertion is also supported by the recorded temporal variation of bud burst dates from low and high elevation beech

populations in the Swiss Alps during the three last decades. The leaf unfolding date of beech exhibits greater variability from year to year at high elevation than at low elevation over this period (Fig. 6.3). In addition, although the mean spring temperature has significantly increased during this period at both low and high elevations, a significant trend towards an earlier flushing was detected only for higher elevation (Fig. 6.3). These temporal trends strengthen the hypothesis that forcing temperatures predominately drive phenological variations in beech growing in climates with cold winters, whereas photoperiod and chilling reduce phenological variation at sites with mild winter conditions.

## **Implications for climate warming**

Based on results from the different approaches cited above, the bud burst date of beech is expected not to undergo significant changes in response to global warming, in particular in the warmer part of its distribution area. The number of years with insufficient chilling temperatures to fully break dormancy is likely to increase under climate change, especially at lower latitude or elevation. As a result, the amount of forcing temperature required for bud burst may increase and offset the predicted advance of flushing in response to increasing spring temperatures. However, photoperiodic control in spring may counterbalance the lack of chilling, by decreasing the amount of forcing required or by increasing bud sensitivity to forcing temperature, leading to more conserved bud burst dates from year to year. Thus, the advance of the date of bud burst of this species in response to global warming, related to winter and spring temperatures, may be limited. However, the bud burst date of beech is expected to be more sensitive in the cold boundary of its distribution area such as at high elevation, since in such climates forcing temperature will remain the main limiting factor. These expectations are in agreement with long series of phenological observations of European beech available in Europe. For instance, in Slovenia, Cufar et al. (2012) reported earlier leaf unfolding date at high elevation over the last decades but no significant trend at low elevation. In Switzerland, observations of beech phenology along an elevational range of 200–1440 m a.s.l. show the same consistent pattern: beech populations inhabiting colder climates (high elevation) exhibited a greater

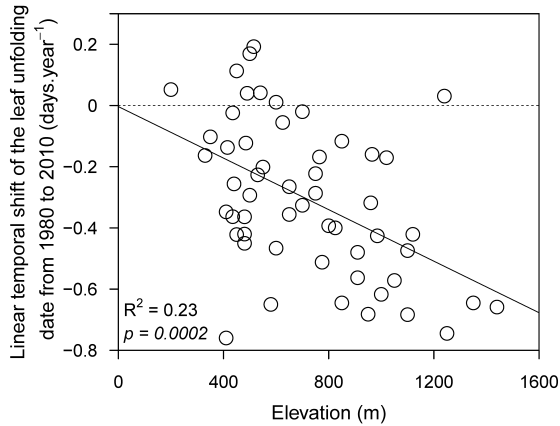




**Fig. 6.3:** Year-to-year variation of the bud burst date of beech in four sites located at low and high elevation in Switzerland for the period 1980–2006 (upper graphs) and the corresponding mean spring temperature from March to May (MAM temperature) during the same period (lower graphs). Note the reversed temperature scale. Phenological time series were provided by the Swiss Phenological Network, MeteoSwiss. Temperature in the four sites was calculated at a  $100 \times 100$  m resolution by interpolating daily values of MeteoSwiss weather stations using the daymet method of Thornton et al. (1997). Data source: Federal Office of Meteorology and Climatology, MeteoSwiss, Switzerland; Swiss Federal Institute for Forest, Snow and Landscape Research, WSL.

advance than populations inhabiting warmer climates (lower elevation) for the three last decades (Fig. 6.4).

The variation in bud burst/leaf unfolding dates of European beech, and subsequently in flowering dates, is therefore expected to decrease along elevational gradients with ongoing climate warming. A smaller discrepancy in the date of flowering and bud burst between populations inhabiting different elevations could have implications for genetic diversity, enhancing the possibilities of gene crossing among populations. Finally, the growing



**Fig. 6.4:** Linear temporal trends of the bud burst date of beech (days year<sup>-1</sup>, 1980–2010) observed in 54 sites in Switzerland according to their elevation. Phenological time series were provided by the Swiss Phenological Network, MeteoSwiss. Only continuous time series with full coverage of the time period 1980–2010 were used.

season length may be extended less in response to the increasing temperatures for beech populations than for other photoperiod-insensitive tree species (Körner and Basler 2010). On the other hand, photoperiod-insensitive tree species, which closely track the warming spring temperatures, may be more exposed to late frost events in the future, while for beech, the dual role of chilling and forcing temperatures in combination with photoperiod could serve to protect this species against such damage (Gu et al. 2008).

## Conclusions

New insights into European beech phenology have been documented from experimental and modelling studies providing robust evidence for the roles of photoperiod and temperature during winter and early spring. Thus, the bud burst date of beech is likely to be driven by both chilling and forcing temperatures with an interaction effect of the photoperiod on forc-

ing rate (or forcing requirement) more pronounced when the chilling requirement is partially satisfied, rather than when buds are fully chilled. Finally, this review also underlines that phenology of beech follows a non-linear trend across biogeographical gradients such as elevational gradients (Fig. 6.4), due to a change of the relative importance of the three main environmental factors according to climate conditions. New models that include photoperiod effect are being developed (Caffarra et al. 2011) and seem promising to fit phenological data of European beech. Fitted to bud burst data acquired from the warmest margin of beech distribution area (South Europe and low elevation) where chilling requirement of buds to fully release dormancy is likely to be only partially fulfilled, these models could be particularly relevant to examine whether their parameterization matches with the assumptions presented here. However, there are still some ‘black boxes’ in the environmental mechanisms affecting beech phenology. First, we are not able to distinguish whether the advance of bud burst date in response to increasing chilling exposure is due to a lower requirement in forcing temperature to bud burst, an advance of the endodormancy release or both. It remains unclear whether, when buds are not fully chilled, a longer photoperiod decreases forcing requirement or increases the sensitivity of beech to forcing temperatures (higher forcing rate). Then, we are uncertain whether forcing and chilling can occur simultaneously. Finally, the last and most challenging knowledge gap is to quantify at what temperature range and temperature threshold forcing and chilling accumulation occur, and whether these temperature thresholds/ranges are fixed or change as the photoperiod increases.

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## Chapter 7

# Is the use of cuttings a good proxy to explore phenological responses of temperate forests in warming and photoperiod experiments?

Yann Vitasse, David Basler

**Abstract** For obvious practical reasons, tree phenological data obtained in warming and photoperiod experiments are generally conducted on juvenile trees (saplings and seedlings) or on watered or rooted cuttings collected from adult trees. As juvenile trees differ from adult trees in their phenological response to environmental conditions, they represent inappropriate plant material to experimentally assess the phenological responses of forests to seasonality. Cuttings are physiologically closer to adult trees, but cutting itself and the disruption of hormonal signals may create artefacts. This study aimed to investigate the potential deviation between phenological responses of cuttings vs donor trees. We hypothesized that, once dormant, buds may respond autonomously to environmental influences such as chilling, photoperiod and warming, and, thus, cuttings may exhibit similar phenological responses to mature trees. We compared bud development of seedlings, saplings and mature trees of three deciduous tree species with bud development of cuttings that were excised from both saplings and adults and positioned in situ in the vicinity of adult trees within a mature mixed forest in the foothills of the Swiss Jura Mountains. No significant difference was detected in the timing of bud burst between cuttings and donor trees for the three studied tree species when the vertical thermal profile was accounted for. However, a significant difference in the timing of flushing was found between seedlings, saplings and adults, with earlier flushing during the juvenile stage. At least for the three studied

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species, this study clearly demonstrates that cuttings are better surrogates than juvenile trees to assess potential phenological responses of temperate forests to climate change in warming and photoperiod experiments

**Key words:** Adult trees, Bud burst, Phenology, Saplings, Seedlings

## Introduction

Numerous long time-series of phenological observations are now available worldwide in temperate climates and show that temperate deciduous trees are leafing-out earlier as a result of rising temperature (e.g., in North America: Beaubien and Freeland 2000; in Europe: Menzel 2000; in East Asia: Ibanez et al. 2010). Earlier leaf-out of trees has a considerable impact on ecosystem processes, because it directly affects the water balance and may influence productivity (Richardson et al. 2010), biotic interactions through, for instance, the synchrony of host parasites or herbivores (e.g., van Asch and Visser 2007) and may feed back on the climate system (Richardson et al. 2013). Long time-series of phenological observations are valuable for the calibration of phenological models that aim at projecting tree responses to a future warmer climate. However, the confidence in phenological simulations derived from such data for the future remains limited because projections are made into unexplored ranges of interaction between temperature and photoperiod. Indeed, temperate forests are expected to face warmer temperatures earlier in the season in the coming decades, so in other words at a shorter photoperiod. In addition, temperate trees exhibit a species-specific chilling requirement for dormancy release, that is, they require a minimum duration of cold hours before warm temperature (or thermal time) can lead to bud burst (Murray et al. 1989). In milder regions, the chilling requirement might not be fulfilled in coming decades under continued climate warming, especially for populations growing in the warmest parts of the species range (Morin et al. 2009, Vitasse et al. 2011) and in tree species having a high chilling requirement such as *Fagus sylvatica* L. (Vitasse and Basler 2013). Thus, phenological responses of trees to temperature increase might be mitigated in the future by a larger influence of photoperiod and chilling temperatures (Körner and Basler 2010). The scientific community is currently debating whether



spring phenology will continue to advance in the forthcoming decades with the continued rise in temperatures (Chuine et al. 2010).

To overcome this major issue, numerous warming and photoperiod experiments have recently been conducted on temperate trees using either seedlings, saplings or cuttings. Although these experimental studies confirmed that warmer temperature does indeed advance bud burst in a non-linear way (e.g., Ghelardini et al. 2010, Morin et al. 2010, Fu et al. 2012), the magnitude of the phenological response to temperature contrasts with the one deduced from long series of phenological observations (Wolkovich et al. 2012). Similarly, in photoperiod manipulation experiments using watered cuttings, short photoperiods have been found to delay the timing of bud burst in some tree species (Heide 1993, Basler and Körner 2012), especially under lower chilling conditions (Caffarra and Donnelly 2011, Laube et al. 2014). For obvious practical reasons, phenological data obtained in warming and photoperiod experiments are generally conducted on juvenile 'trees' or on watered/rooted cuttings from adult trees. Hence, a crucial issue arises: are these appropriate proxies to assess the phenological responses of adult trees to environmental cues?

Understory trees of temperate forests generally begin their growing season earlier than conspecific canopy trees (Seiwa 1999a, Richardson and O'Keefe 2009), allowing them to benefit from light before canopy closure (Augspurger 2008). This phenological discrepancy between canopy trees and understory trees has recently been assigned to ontogenic rather than micro-environmental effects (Vitasse 2013). Therefore, phenological data obtained from juvenile trees appear inappropriate to infer phenological responses of forests to climate change. Here we asked whether cuttings from adult trees would be a better substitute than juvenile trees in warming and photoperiod experiments to unravel the mechanisms of phenology in temperate forest trees.

Dormancy in temperate trees involves an interrelated series of physiological processes regulated by internal and external factors (Lang 1994, Horvath et al. 2003). According to Lang (1994), bud dormancy can be categorized into three types in temperate climate. Buds are in paradormancy when growth inhibition is induced by distant organs (during autumn), in endodormancy when growth inhibition is induced by internal bud signals (late autumn and early winter) and in ecodormancy when growth inhibition is induced by unfavourable external conditions (early and late spring). However, the different dormancy phases are not strictly separated

in time and are known to interact with each other (Cooke et al. 2012). Cuttings are disconnected from whole-tree growth-promoting/inhibiting signals that can potentially affect bud burst such as sugars or phytohormones. Among the phytohormones, abscisic acid, gibberellic acid (GA) and cytokinins are known to interact with bud burst, with the former maintaining and the other two releasing bud endodormancy (Wareing and Saunders 1971, Arora et al. 2003, Chao et al. 2007, Cooke et al. 2012). However, in contrast to paradormancy and endodormancy induction in autumn, the maintenance and the release of endodormancy in winter might reside solely within the buds, which could then autonomously respond to specific combinations of low and moderate temperatures or photoperiod (Lang 1994). Indeed, after the initiation of endodormancy, meristem cells of apical buds are likely to be insulated from growth-promoting signals, such as GA (Lang 1994, Rinne et al. 2001, Rohde and Bhalerao 2007). One study demonstrated that meristem cells in dormant buds of *Betula pubescens* Ehrh. would recover their connection between each other, or with adjacent tissue, via plasmodesmal channels that are progressively restored after they have undergone sufficient chilling temperatures (Rinne et al. 2001). In addition, recent studies identified genes in temperate fruit trees that are suppressed by exposure to chilling conditions, acting therefore as quantitative repressors of bud development in spring (Jiménez et al. 2010, Barros et al. 2012, Saito et al. 2013). Although the role of phytohormones has been reasonably well identified for bud set and endodormancy induction, the molecular mechanisms controlling endodormancy release and the ecodormancy phase after exposure to chilling temperatures have not been clarified (Horvath et al. 2003, Cooke et al. 2012). Here we hypothesized that cuttings harvested after endodormancy induction would constitute a better proxy than juvenile trees to infer the phenological response of forests to climate change.

## Materials and methods

### *Study site and study species*

The experiment was conducted in a mature mixed forest stand (~110 years old) near the village of Hofstetten (47° 28' N, 7° 30' E, 570–580 m above

sea level) in the surroundings of the Swiss Canopy Crane (Körner et al. 2005), located 12 km south-west of Basel, Switzerland. Soils are of the rendzina type on calcareous bedrock. The dominant tree species are *Fagus sylvatica* L. and *Picea abies* L., while *Acer campestre* L., *Acer pseudoplatanus* L., *Carpinus betulus* L., *Fraxinus excelsior* L., *Prunus avium* L. and *Tilia platyphyllos* Scop. occur as companion species. The site is situated on a north-facing slope with no access to the ground water table and has essentially rocky subsoil at 40–90 cm below the surface. The mean annual air temperature recorded on a long term series at the nearest climate station was 10.3 °C and the mean annual precipitation was 810 mm (1970–2011 recorded at Binningen, 316 m a.s.l. ~10 km away from the study site). Using the same temperature dataset, the mean air temperature over the ~6-month growing season from April to October was 15.6 °C, with a mean temperature for the warmest month (July) of 19.3 °C. The winters are mild with the mean temperature of the coldest month (January) being around 1.5 °C. At the study site there are usually only a few weeks of slight snow cover during mid-winter.

We selected three tree species having contrasting spring phenology in the study site: an early flushing species, *Carpinus betulus*; an intermediate flushing species, *Fagus sylvatica*; and a late flushing species, *Acer pseudoplatanus*, based on the phenological data recorded on adults in the previous year (Vitasse 2013). For clarity and brevity, hereafter we will refer to each species by its genus.

## ***Experimental design***

In November 2012, we selected and tagged 10 mature trees for each species, having a low branch (approximately between 6 and 9 m height), so that cuttings could be collected from the ground by using a pole pruner and so that observations of bud development within the same branch would be accurate from the ground. In the vicinity of each selected mature tree, one sapling (~2.9–3.5 m height; Table 7.1) and three seedlings (~0.24–0.54 m height; Table 7.1) were also marked and assigned to the corresponding adult tree. The average distance between adult trees and the assigned seedlings and saplings was from 3 to 15 m depending on the species (see details in Table 7.1). The experiment was set

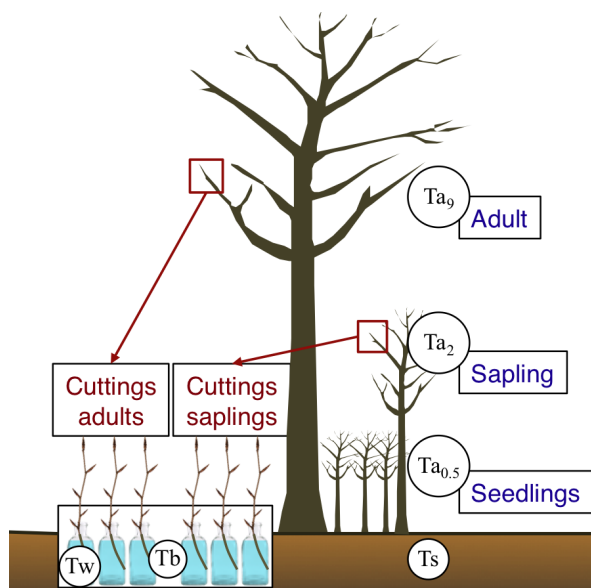
up on 1 March 2013, immediately after a substantial cold period. Three twigs ( $\sim 30$  cm in length) were sampled from each selected adult tree (at  $\sim 6$ – $9$  m in height) and sapling and placed individually into 0.5 l glass bottles filled with chlorine-free tap water. The bottles were then placed into plastic boxes ( $30 \times 20 \times 22$  cm; with drainage holes) beneath the canopy of the corresponding mature tree ( $< 5$  m distant, Table 7.1). Thus, a plastic box comprising six cuttings (three cuttings from the adult tree and three cuttings from the corresponding sapling) was positioned at each selected adult tree (Fig. 7.1). The boxes were buried into the soil and a 2-cm-thick foam plastic sheet was used to cover the top of each box to prevent the water from freezing. Every 2 weeks, the water in all bottles was replaced with local (untreated) tap water and, at the same time, the base of cuttings was recut (by  $\sim 1$ – $2$  cm) to prevent vessel occlusion.

**Table 7.1:** Height  $\pm$  SE of the selected adults, saplings and seedlings and distance  $\pm$  SE from an adult of each experimental group.

Species	Adult	Seedlings		Saplings		Cuttings
	Height (m)	Height (m)	Distance from adult (m)	Height (m)	Distance from adult (m)	Distance from adult (m)
<i>Carpinus</i>	$19.7 \pm 1.8$	$0.54 \pm 0.04$	$6.9 \pm 0.6$	$2.9 \pm 0.6$	$5.8 \pm 1.3$	$2.4 \pm 0.4$
<i>Acer</i>	$23.2 \pm 1.2$	$0.25 \pm 0.02$	$9.9 \pm 0.9$	$3.3 \pm 0.5$	$14.9 \pm 2.4$	$4.1 \pm 1.2$
<i>Fagus</i>	$31.1 \pm 0.4$	$0.24 \pm 0.01$	$3.4 \pm 0.4$	$3.5 \pm 0.5$	$4.6 \pm 0.7$	$2.1 \pm 0.1$

### *Temperature data*

The air temperature was recorded at 30-min intervals using data loggers (TidBit v2 UTBI-001, Onset Computer Corporation, Bourne, MA, USA) at 0.5 m above the ground in the understory (approximate seedling height, denoted hereafter as  $T_{a0.5}$ ), at 2 m above the ground (approximate sapling height, denoted as  $T_{a2}$ ) and at 9 m above the ground (approximately the first branches of the adult tree where phenology was monitored, denoted as  $T_{a9}$ ; see Fig. 7.1). All loggers were positioned under a white double-



**Fig. 7.1:** The experimental design used for one experimental plot to compare the phenology of seedlings, saplings, adults and cuttings from saplings and adults. This study design was replicated 10 times for each of the three species.  $T_{a9}$ ,  $T_{a2}$  and  $T_{a0.5}$  correspond to the air temperatures recorded at 9, 2 and 0.5 m during the experiment;  $T_s$  corresponds to the soil temperature recorded at a depth of 10 cm;  $T_w$  and  $T_b$  correspond to water temperature recorded inside a glass bottle used for cuttings and the temperature recorded at the bottom of the box outside the bottles.

layered, aerated plastic shelter to prevent any exposure to rain or to direct sunlight. Additional loggers recorded temperatures inside the experimental boxes: two loggers were placed into water inside bottles containing cuttings (denoted as  $T_w$ ) and another two were placed at the bottom of the box (denoted as  $T_b$ ). Soil temperature was also recorded at a depth of 10 cm (denoted as  $T_s$ ; see Fig. 7.1). Two loggers were used for each height position and the average of the two was used in all further results, except for  $T_9$ , because only one logger was found after the experiment. To compare cumulative degree hours among the different height positions, we accumulated the temperature difference for every hour above a  $5^\circ\text{C}$  threshold, starting on 1 March 2013 (when cuttings were sampled and

placed into the experimental boxes) until the end of the flushing period (10 May 2013). After the study period, all loggers were immersed 2 h in an ice-water bath for 0 °C calibration and cross-checking the sensors for identical readings. Deviations never exceeded 0.17 K among the different loggers, meeting the manufacturer's specifications.

### ***Phenological observations***

For each experimental group (i.e., seedlings, saplings, adult trees, cuttings from saplings and cuttings from adult trees), bud development was monitored twice a week from 1 March 2013 to the end of the flushing period (10 May 2013). We used a four-stage categorical scale according to Vitasse (2013). Stage 0 (dormant bud) is characterized by the absence of any visible bud development; at stage 1 (bud swelling), buds were swollen and/or elongating; at stage 2 (bud burst), bud scales were open and leaves were partially visible; at stage 3 (leaf-out), leaves had fully emerged from the buds but were still folded, crinkled or pendant, depending on species; and at stage 4 (leaf unfolded), at least one leaf was fully unfolded. For seedlings and cuttings, we considered the apical bud only. For saplings, phenology was monitored on the apical bud of each of the three previously tagged branches. For adult trees, phenology was monitored using binoculars (Canon 10 × 30 Image Stabilization Binoculars) on the lowest branch of the canopy that was used to harvest cuttings (approximately between 6 and 9 m in height) and the phenological score was assigned as an average score assessed over three terminal buds of this selected branch.

The bud burst and leaf-out dates were reached for each selected individual, branch or cutting when the apical bud reached stages 2 and 3, respectively, which was estimated by linear interpolation when necessary (i.e., when this stage occurred in between two monitoring dates).

### ***Data analysis***

While cuttings are obviously dependent on the donor trees, natural seedlings, saplings and adult trees could have been considered as independent

and treated using a one-way analysis of variance followed by Tukey's HSD tests. However, since natural seedlings and saplings have been selected as close as possible to each of the 10 adult trees, we decided to consider them dependent on each other in terms of microclimate (same temperature). In other words, one replicate consists of one adult tree, one sapling, three seedlings and three cuttings excised from both the adult tree and the sapling. In consequence, we conducted multiple paired *t*-tests within each species to compare the dates of bud burst and leaf-out among the five experimental groups. The difference between pairs was tested for assumptions of normal distribution for each of the 120 paired *t*-tests performed using Shapiro—Wilk normality tests. It was found to follow a normal distribution for >80% of the tests. However, after a visual examination of residuals (Q–Q plot) we assumed that the other 20% did not excessively deviate from a normal distribution. All *p*-values were adjusted using Bonferroni corrections, and details of the degree of freedom and *t*-statistic values are provided in Supplementary Data (see Table S7.1 and S7.2). Comparisons between the five categories are shown for bud burst and leaf-out stages (stages 2 and 3) only because stage 1 (the onset of bud swelling) is rather difficult to observe in adult trees and the mobile carbon pool might be limited in cuttings for further development, once the leaves are out (stage 3). Some cuttings did not reach phenological stage 3 (*Acer*: one replicate of adult cuttings, *Carpinus*: three replicates of adult cuttings), probably because the conductive vessels became plugged and/or reserves were exhausted. These data were dismissed from analyses regarding stage 3. To avoid confusion between temperatures (°C) and temperature differences, we join other authors in adopting K (for Kelvin) for all differences in temperature.

All analyses were performed using R 2.12.2 (R Development Core Team 2011).

## Results

### *Temperature data*

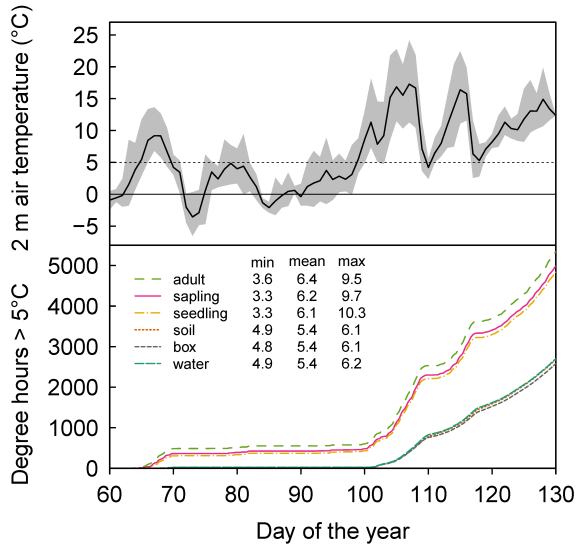
Early spring 2013 was particularly cool until mid-April. During this period, daily mean temperatures were mostly <5 °C (Fig. 7.2). A gradual

increase in temperature was found from the ground to a height of 9 m above ground (approximately the height where the lowest buds of adults have been monitored). The mean  $T_{a9}$  during the experiment was 0.1 and 0.3 K warmer than those recorded at sapling ( $T_{a2}$ ) and seedling height ( $T_{a0.5}$ ), respectively. On 1 May 2013, i.e., 2 months after the beginning of the experiment, the cumulative degree hours above 5 °C reached 3774 °C h at a height of 9 m, whereas it reached 3485 and 3377 °C h at sapling and seedling height, respectively (Fig. 7.2). The boxes used to place the cuttings were well isolated from the air temperature as there was negligible difference between the temperature recorded inside the boxes (into water in the bottles and outside of water) and soil temperature at a depth of 10 cm (Fig. 7.2). According to the temperature recorded in our experimental boxes, no freezing of water occurred within the buried boxes.

### ***Phenological differences among species***

The three study species exhibited different timings of bud development, nevertheless to a lower extent than expected (and known from normal spring weather) due to the prolonged cool weather in early spring (Fig. 7.2). Such cold weather prevents flushing despite stepwise dormancy release. Regarding adult trees, *Carpinus* was the earliest flushing species and *Acer* the latest, though, beyond phenological stage 2 (bud burst), the discrepancy of bud development among species was considerably reduced (Fig. 7.3). The average bud-burst date (stage 2) of adult trees occurred on the day of year  $104.0 \pm 0.7$  for *Carpinus*,  $110.4 \pm 0.8$  for *Fagus* and  $113.0 \pm 0.8$  for *Acer*. Interestingly the ranking across species was not conserved at the seedling stage: bud-burst date of *Acer* seedlings occurred 5 days earlier than in *Fagus* (Fig. 7.3). Phenological differences among seedlings, saplings and adults under natural conditions A clear phenological discrepancy was found between young and adult trees for *Carpinus* and *Acer* (Fig. 7.3, Table 7.2). For *Carpinus*, bud burst of saplings and seedlings occurred 9.6 and 13.8 days earlier (paired *t*-test,  $p < 0.05$  and  $p < 0.001$ , respectively) than in adult trees (Table 7.2). For *Acer*, bud burst of seedlings occurred 7.8 days earlier ( $p < 0.0001$ ) than adult trees, whereas saplings tended to exhibit earlier bud burst and leaf-out than adult trees, but this was only marginally significant ( $p < 0.10$ , Table 7.2). For



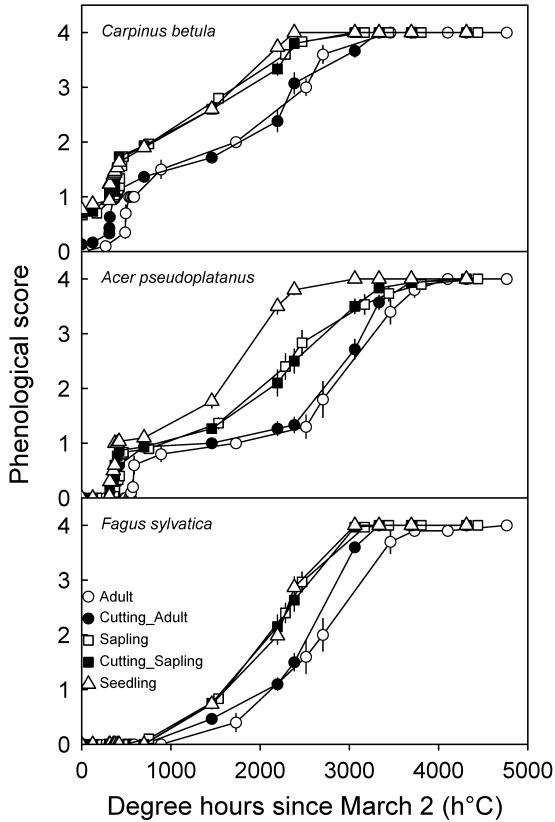


**Fig. 7.2:** Air temperatures at various heights above the ground. The upper panel shows the air temperature at 2 m recorded from 1 March 2013 to the end of the flushing period. The thick line corresponds to the daily mean temperatures surrounded in a grey area by the daily minimum and daily maximum temperatures. The lower panel shows cumulative degree hours above 5 °C from the beginning of the experiment (1 March 2013) to the date of the last individual flushing (mid-May 2013) at seedling height (0.5 m height), at sapling height (2 m height), at a height of 9 m (approximately the height where buds of adults were monitored), at a depth of 10 cm in the soil, inside the experimental boxes in water and inside the experimental boxes out of water. The inset table shows the mean of daily minimum, mean and maximum air temperatures during the same period for each logger.

*Fagus*, both saplings and seedlings exhibited slightly earlier phenology than adults, but this was not significant (Table 7.2).

When the microclimate due to the difference in height between seedlings, saplings and adults was taken into account (mean temperature increase with height above ground; Fig. 7.2), the phenological discrepancy among the three lifestage categories became enhanced (Fig. 7.4). Thus, in all three species, bud burst and leaf-out of seedlings and saplings occurred after significantly fewer degree hours than in adult trees (Fig. 7.4,

Table 7.3). In addition, a clear phenological discrepancy was detected among the three life-stage categories for *Acer*, with seedlings requiring 689 and 1312 °C h less than saplings and adult trees, respectively, to bud burst ( $p < 0.05$ ; Table 7.3). No difference of heat requirement for bud burst was detected between seedlings and saplings for the other two species.

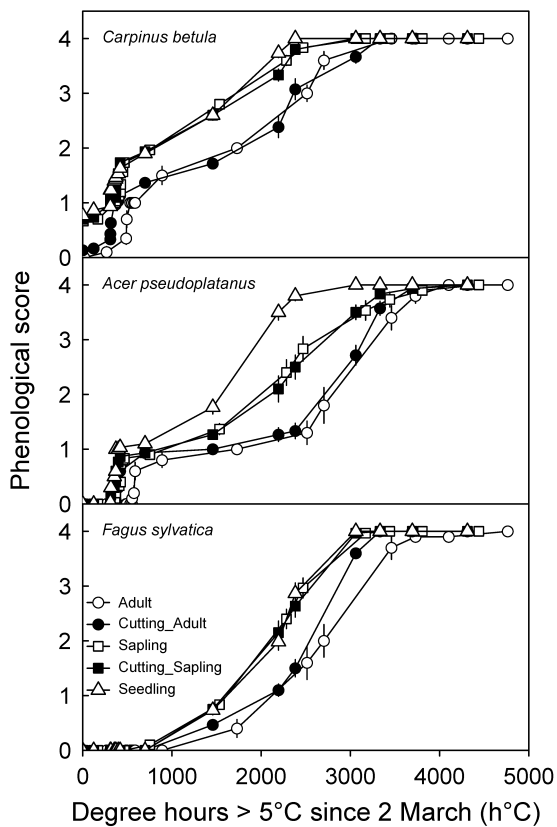


**Fig. 7.3:** Bud and leaf development progression in spring for each experimental group. The time on the x-axis is expressed as day of the year. Bars represent standard errors.

**Table 7.2:** Mean phenological differences expressed in days among each experimental group at phenological stages 2 (bud burst) and 3 (leaf-out) tested by a paired t-test.

	Adult			Adult cutting			Sapling			Sapling cutting		
	Stage 2	Stage 3	Stage 3	Stage 2	Stage 3	Stage 3	Stage 2	Stage 3	Stage 3	Stage 2	Stage 3	Stage 3
<i>C. betulus</i>												
Adult cutting	1.2 <sup>ns</sup>	1.5 <sup>ns</sup>										
Sapling	-9.6*	-2.7**		-10.8***	-4.2 <sup>ns</sup>							
Sapling cutting	-8.7 <sup>ns</sup>	-1.7 <sup>ns</sup>		-9.9*	-3.0 <sup>ns</sup>		0.9 <sup>ns</sup>	1.1 <sup>ns</sup>				
Seedling	-13.8**	-2.5*		-15.1***	-4.4 <sup>ns</sup>		-4.2 <sup>ns</sup>	0.2 <sup>ns</sup>		-5.2 <sup>ns</sup>	-0.8 <sup>ns</sup>	
<i>A. pseudoplatanus</i>												
Adult cutting	1.1*	2.1 <sup>ns</sup>										
Sapling	-2.8 <sup>ns</sup>	-3.0 <sup>ns</sup>		-3.9 <sup>ns</sup>	-5.0 <sup>ns</sup>							
Sapling cutting	-2.3 <sup>ns</sup>	-2.1 <sup>ns</sup>		-3.4*	-4.2*		0.4 <sup>ns</sup>	0.9 <sup>ns</sup>				
Seedling	-7.8***	-8.2***		-8.9***	-10.1**		-5.0 <sup>ns</sup>	-5.2 <sup>ns</sup>		-5.4**	-6.2***	
<i>F. sylvatica</i>												
Adult cutting	2.5 <sup>ns</sup>	0.8 <sup>ns</sup>										
Sapling	-1.2 <sup>ns</sup>	-2.9 <sup>ns</sup>		-3.7*	-3.7*							
Sapling cutting	-0.3 <sup>ns</sup>	-2.3 <sup>ns</sup>		-2.8 <sup>ns</sup>	-3.0 <sup>ns</sup>		0.9 <sup>ns</sup>	0.7 <sup>ns</sup>				
Seedling	-0.5 <sup>ns</sup>	-1.8 <sup>ns</sup>		-2.9*	-2.6**		0.7 <sup>ns</sup>	1.1 <sup>ns</sup>		-0.2 <sup>ns</sup>	0.4 <sup>ns</sup>	

<sup>ns</sup>  $p > 0.05$ , \*  $p \leq 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .  $p$ -values were adjusted using Bonferroni corrections. Values in bold correspond to the comparison of bud development between cuttings and donor trees.



**Fig. 7.4:** Bud and leaf development progression in spring for each experimental group. The time on the x-axis is expressed as thermal time, i.e., accumulation of degrees above 5 °C at an hourly scale since the beginning of the experiment (1 March 2013). Bars represent standard errors.

**Table 7.3:** Mean phenological differences expressed in degree hours ( $^{\circ}\text{Ch}$ ) among each experimental group at phenological stages 2 (bud burst) and 3 (leaf-out) tested by a paired t-test.

	Adult			Adult cutting			Sapling			Sapling cutting		
	Stage 2	Stage 3		Stage 2	Stage 3		Stage 2	Stage 3		Stage 2	Stage 3	
<i>C. betulus</i>												
Adult cutting	68 <sup>ns</sup>	-142 <sup>ns</sup>										
Sapling	-698**	-875***		-766**	-695 <sup>ns</sup>		-29 <sup>ns</sup>	118 <sup>ns</sup>				
Sapling cutting	-727**	-757**		-795**	-572 <sup>ns</sup>		-26 <sup>ns</sup>	-11.9 <sup>ns</sup>	2 <sup>ns</sup>			-130 <sup>ns</sup>
Seedling	-724**	-887***		-792*	-791 <sup>ns</sup>							
<i>A. pseudoplatanus</i>												
Adult cutting	-83 <sup>ns</sup>	-71 <sup>ns</sup>										
Sapling	-622 <sup>ns</sup>	-672*		-539 <sup>ns</sup>	-602 <sup>ns</sup>		-5 <sup>ns</sup>	36 <sup>ns</sup>				
Sapling cutting	-628**	-625**		-545**	-575**							
Seedling	-1312***	-1373***		-1229***	-1290***		-689*	-712*				-684** -748**
<i>F. sylvatica</i>												
Adult cutting	-165 <sup>ns</sup>	-188 <sup>ns</sup>										
Sapling	-530*	-493*		-365*	-306 <sup>ns</sup>							
Sapling cutting	-526*	-525*		-362 <sup>ns</sup>	-338 <sup>ns</sup>		3 <sup>ns</sup>	-32 <sup>ns</sup>				
Seedling	-460*	-513*		-295*	-326*		70 <sup>ns</sup>	-20 <sup>ns</sup>	67 <sup>ns</sup>			12 <sup>ns</sup>

<sup>ns</sup>  $p > 0.05$ , \*  $p \leq 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .  $p$ -values were adjusted using Bonferroni corrections. Values in bold correspond to the comparison of bud development between cuttings and donor trees.

### ***Phenological differences between cuttings and donor trees***

Overall, bud burst of cuttings tightly paralleled bud burst of donor trees (Fig. 7.3). However, the phenological discrepancy between cuttings and donor trees was always positive, indicating a slight phenological delay of cuttings. This slightly later phenology of cuttings was found to be significant within the adult category only for *Acer* at stage 2 (1.1 days delay; Table 7.2).

Interestingly, when comparing the thermal time to bud burst between cuttings and the whole tree, no significant difference was found for any species, phenological stage or life stage (Table 7.3, Fig. 7.4). This is because the air temperature at sapling height and at the height where adult branches were monitored was slightly warmer than the air temperature recorded at seedling height, i.e., at the height where also our cuttings were positioned in the plastic boxes (Fig. 7.1 and 7.2). In contrast, for all species cuttings of saplings exhibited significantly fewer degree hours to bud burst than adult trees (from  $-525$  to  $-757$  °C h, for *Fagus* and *Carpinus*, respectively; Table 7.3, Fig. 7.4).

## **Discussion**

Unravelling the mechanisms that drive tree phenology in response to environmental cues in experiments is challenging due to the size of adult individuals, constraining researchers to use either young trees or cuttings as substitutes. The present study confirms that young trees do exhibit different phenology from conspecific adult trees, as previously reported (Seiwa 1999a, 1999b, Richardson and O'Keefe 2009, Vitasse 2013). Therefore, phenological data obtained from juvenile trees (seedlings and saplings) should not be considered as surrogates for phenological responses of adult trees (Vitasse 2013). In contrast, the phenology of watered cuttings held under field conditions was found to mirror the phenology of donor trees both in saplings (earlier) and adult trees (later), and would therefore constitute a better surrogate for adult trees than juvenile trees. The findings also suggest that there is no whole-tree influence on bud development in spring, e.g., long-distance hormonal signals, which differs from that in cuttings for these three species (branch autonomy).

### ***Buds may respond autonomously to environmental cues***

The physiological mechanism of dormancy release remains poorly understood. Once endodormancy is released, the remaining cascade of responses appears to be intrinsic to the bud itself (e.g., the gradual reconstitution of symplasmic channels among apical meristem cells in response to chilling temperatures; Rinne et al. 2001). Nevertheless, local transport of hormones from tissues located close to, but outside the buds is not excluded in cuttings, though cuttings lack internal sap pressure. The role of hormones in dormancy release is still controversial (reviewed in Rohde and Bhalerao 2007 and references therein; see also Cooke et al. 2012). An increase in GA was generally reported prior to and during bud burst in forest trees, and even in cuttings. However, the origin of GA is unclear and could be located in buds or in adjacent tissues with a local transfer to bud cells rather than from distant tissues (Hewett and Wareing 1973). Similarly the concentration of cytokinins increases during dormancy release and prior to bud burst (e.g., in Scots pine according to Qamaruddin et al. 1990). For *Salix babylonica* L., Staden (1979) suggested that buds themselves do not have the ability to synthesize cytokines but they can hydrolyse storage forms with the resumption of extension growth.

Although the timing of bud burst in rooted cuttings or cuttings bearing several buds has been shown to be parallel to that on mature trees in fruit tree species (Arias and Crabbé 1975, Couvillon et al. 1975), to our knowledge, it has never been shown in forest tree species.

### ***Importance of recording temperature at a micro-scale***

Here, we attributed the slightly later phenology of cuttings compared with donor trees to micro-environmental gradients, given that the temperature recorded at the monitored branch level of adult trees was slightly warmer than the one measured at seedling height (the mean difference during the experiment 0.3 K,  $\sim 500^\circ\text{C h}$ ). Indeed, no significant phenological discrepancy was found between cuttings and donor trees when comparing the thermal time to bud burst. The daily course of the mean temperature averaged over the period of the experiment showed that the daily maximal temperature is reached later at a height of 9 m (and probably even later

in the canopy) than in the understorey, which seems to affect the cooling during late evening and night in a way that the air temperature at a height of 9 m remains slightly warmer than in the understorey (supplementary Fig. S7.1). This pattern is likely the result of the site topography (north-facing slope) and its effect on the incoming radiation. In contrast, the phenological discrepancy between juvenile and adult trees is clearly the result of ontogenic effects rather than micro-environmental effects, since juvenile trees flushed earlier than adult trees in all three species, in spite of the cooler temperature recorded at seedling height.

### ***Comparison among species***

Overall, the phenological discrepancy observed between seedlings and adults was much smaller during spring 2013 than the one recorded on the same site and species in the previous year (Vitasse 2013). This could be explained by the exceptionally cool early spring that occurred during the present experiment. Indeed, if considering that juvenile trees exhibit a shallower level of endodormancy than adult trees (Vitasse 2013), and so are ‘programmed’ for earlier leaf-out than canopy trees, but cannot proceed because of cold weather, the seedling–adult phenology gap is reduced. Thus, under warm early spring conditions, such as occurred in 2012 (Vitasse 2013), the gap widens, while it gets narrower under cooler early spring conditions, as occurred in the present study.

Interestingly, the ranking across species was not conserved between adult and seedling stages: bud burst of seedlings occurred 5 days earlier in *Acer* compared with *Fagus*, whereas in adult trees, bud burst of *Acer* occurred 3 days later than for *Fagus*. The same change in the sequence of species phenology between seedling and adult stages was reported in the previous year at the same site (Vitasse 2013). We attributed this sequence change between young and mature trees to different environmental requirement for a full dormancy release in juvenile trees, such as a lower chilling requirement, which is especially distinct in *Acer*. In agreement with this hypothesis, rooted cuttings from adult trees were shown to exhibit greater chilling requirement than juvenile plant material in *Acer saccharinum* L. (Ashby et al. 1991), strengthening the evidence that cuttings excised from adults are better at mirroring adult trees than seedlings.



A shallower endodormancy in young trees enables them to start their development before canopy closure and therefore to enhance their growth and competitive abilities, which is crucial under dense understory conditions (Gill et al. 1998, Augspurger 2008). In contrast, adult trees reaching the canopy adopt a safer strategy in relation to late spring freezing events by exhibiting a deeper endodormancy (higher requirement of chilling and longer photoperiod for a full dormancy release), allowing them to reduce the likelihood of freezing damage, particularly in reproductive organs. Thus, the species and age-specific relationship between the different environmental requirements may lead to changes in the phenological sequence among species between young and adult trees under different spring conditions. Accordingly, the sequence of bud burst timing on watered cuttings among 36 woody species was shown to significantly change under low chilling conditions (Laube et al. 2014).

### ***Methodological considerations***

The use of watered cuttings to assess spring phenological responses has some shortcomings: only part of the whole bud dormancy cycle can be manipulated, as watered cuttings have a restricted lifetime, and are thus most appropriate to investigate dormancy release and bud burst. Here, we sampled the cuttings on 1 March 2013, assuming that the chilling requirements have been met before, and the buds were therefore in the ecodormant phase. Yet, we are confident that sampling during the endodormancy phase would not have influenced the general response patterns observed here. Ring-porous species such as *Quercus* and *Fraxinus* may give rise to conduit failure when used as cuttings, which is less likely to occur in diffuse-porous species as examined here. Rooted cuttings excised from adult trees may overcome the issue of short experimental period, and it needs to be explored whether such rooted cuttings retain adult stage developmental physiology. The use of either single node cuttings or cuttings bearing several buds may also influence the results. Here we used cuttings bearing several buds instead of single node cuttings in order to maintain correlative inhibition that may influence bud burst timing (Ghelardini et al. 2010), but only apical buds were monitored here to minimize bias among the different experimental groups of test plants.

## Conclusion

Our study reveals that bud burst of watered cuttings respond to environmental cues similarly to adult trees in the three studied species and, thus, can be used as a substitute for mature trees in warming and photoperiod experiments. In contrast, our study highlights that neither cuttings extracted from saplings nor intact saplings or seedlings in natural conditions constitute a good surrogate for adult phenology, since they all exhibited earlier phenology than canopy trees. Hence, due to strong ontogenetic effects acting on phenology, experiments conducted on young trees are not appropriate to infer phenological responses of forests to climate warming, and cuttings excised from adults should be preferred instead, but this treatment may affect species differently depending on their xylem structure. Finally, actual spring weather may narrow or widen the phenology gap between juvenile and adult trees.

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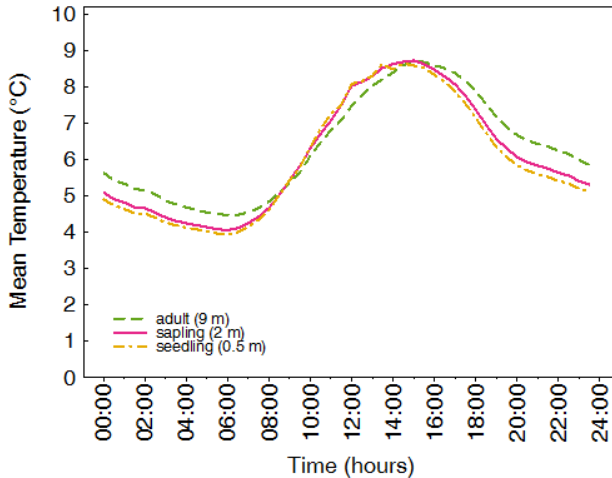
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## Supplementary material



**Fig. S7.1:** Daily course of mean temperature at different height levels (seedling, sapling and adult height) in the study site during the experiment (Mean across 70 days, from 1 March 2013 to 10 May 2013, 30 minutes logging interval)



**Table S7.2:** Details of the multiple paired *t*-tests performed on phenological differences expressed in degree hours (°Ch) among each experimental group at the phenological stages 2 (bud burst) and 3 (leaf-out) the data. The *t*-statistic value and the DF is reported.

	Adult			Adult Cutting			Sapling			Sapling Cutting						
	Stage 2 DF	Stage 2 <i>t</i>	Stage 3 DF	Stage 2 DF	Stage 2 <i>t</i>	Stage 3 DF	Stage 2 DF	Stage 2 <i>t</i>	Stage 3 DF	Stage 2 DF	Stage 2 <i>t</i>	Stage 3 DF	Stage 3 <i>t</i>			
<i>Carpinus betulus</i>																
Adult Cutting	9	0.6	6	-1												
Sapling	9	-6.2	9	-10	9	-6	6	-3.6								
Sapling cutting	9	-6.5	9	-5.3	9	-6.2	6	-2.4	9	2	9	2				
Seedling	9	-5.6	9	-9.4	9	-4.6	6	-3.5	9	-0.4	9	-0.2	9	0	9	-1.2
<i>Acer pseudoplatanus</i>																
Adult Cutting	9	-1.3	8	-0.9												
Sapling	9	-3.4	9	-3.8	9	-3.1	8	-3.6								
Sapling cutting	9	-5.6	9	-5.1	9	-5.2	8	-5.6	9	-0.1	9	0.4				
Seedling	9	-12.5	9	-9.6	9	-10	8	-8.3	9	-4.2	9	-4.4	9	-5.2	9	-5.8
<i>Fagus sylvatica</i>																
Adult Cutting	9	-2.2	9	-1.9												
Sapling	9	-4.1	9	-3.7	9	-3.7	9	-3.2								
Sapling cutting	9	-3.9	9	-3.8	9	-3.4	9	-3.2	9	0.1	9	-0.8				
Seedling	9	-4.3	9	-4.5	9	-4.3	9	-4.7	9	0.6	9	-0.2	9	0.6	9	0.1





**Part III**  
**Summary and conclusions**



# **Chapter 8**

## **Summary and general conclusions**

David Basler

### **Aim**

The aim of this thesis was to arrive at an understanding of how the temperate tree species respond and adjust their phenology to temperature and photoperiod in order to escape the risk of fatal freezing damage in spring. The following key questions were addressed:

1. Is the timing of bud burst of temperate forest trees related to photoperiod and if so, how strong is photoperiod control?
2. How is the phenology and development before bud burst (during dormancy release) affected by the interaction of photoperiod and temperature?
3. Are the mechanisms of current 'process-based' phenological models able to reflect the interacting nature of the underlying drivers?

### **Summary**

Using both, experimental and modeling approaches, I investigated the environmental controls of spring phenology in temperate forest trees. In the following, I provide a general summary of the work conducted, as well as a short outlook, highlighting the prospects for possible follow-up studies.

### **Photoperiod sensitivity of bud burst in 14 temperate forest tree species (Chapter 2)**

Depending on the successional status and life strategy, the spring phenology of temperate tree species is affected by daylength: while early successional species are thought to take a rather 'opportunistic' approach and their development follows (potentially misleading) warm temperatures only, late successional species are thought to respond rather 'conservative' and avoid the risk of possible freezing damage. We tested these hypotheses in 14 species by exposing cuttings to controlled photoperiod conditions (gradually adjusted with time according to the natural daylength expansion during spring). We observed a distinct photoperiod sensitivity in the late successional *Fagus sylvatica*, and a weaker, but still significant delay of bud burst under shorter photoperiod in *Tilia cordata*, *Quercus petraea*, *Abies alba* and *Picea abies*, but not in the other nine species. Elevation of and region of origin further affected photoperiod sensitivity in three out of the five photoperiod sensitive species. We concluded that photoperiodic constraints may keep these species from tracking the rising temperatures at the current rate.

### **Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species (Chapter 3)**

A delayed bud swelling under short photoperiod may be the result of a later start of bud development, due to a photoperiod threshold, or may result from slower rates of bud development under short photoperiods. In four species, we investigated the response of bud swelling (a clear marker that dormancy has been released) under contrasting, controlled temperature and photoperiod conditions during the period of natural dormancy release in spring. We observed that, the depth of dormancy (reflected by days to bud burst under warm, long day conditions) decreased already before any sign of bud swelling became evident. We found a clear effect of photoperiod on the duration of bud swelling in *Picea*, *Quercus* and *Fagus*, but not in *Acer*, while the onset of bud swelling was affected by the photoperiod treatment in *Fagus* only. We thus concluded that photoperiod has a quantitative effect on bud growth and development, rather than just exerting a simple threshold function.

### **Evaluating phenological models for the prediction of leaf-out dates in six temperate tree species across central Europe (Chapter 4)**

In an attempt at exploring whether the photoperiodic responses observed in our experiments could help to improve the currently existing, widely used phenological models when applied beyond the range currently covered by observational data, I assessed the performance of a large number of different published models (and recombinations thereof) on a large long-time phenological data set. Surprisingly, even quite contrasting models structures yielded quite similar predictions for the timing of leaf unfolding. An in-depth analysis revealed, that the highly parameterized models most likely overfit to the data, and that responses taking place during winter are not reflected in these long-term observation data sets and thus remain unaccounted for in models. The low prediction error of most models actually results from the temperature effect shortly before bud burst. Thus, arrive at realistic projections of phenology in future climates, models need to be calibrated against trustworthy experimental data and their statistical validation should not be assumed to reflect the actual mechanisms of environmental control of trees phenology.

### **Phenology under global warming (Chapter 5)**

In this ‘Perspectives’, we summarized the concept of photoperiod control of spring phenology in trees, based on past research. We divide tree species into three basic categories, based on the environmental control of spring phenology employed: Species responding to temperature only, species responding to warm temperatures only after a certain chilling requirement has been fulfilled and finally species, in which the response to warm temperature is facilitated by the opening of a photoperiod ‘window’, after the chilling requirements have been fulfilled. Late successional tree species are thought to belong to the last category. This basic classification served as working hypothesis for this thesis. Originally thought as a reminder of a seemingly ignored environmental driver, this article has received a lot of attention and initiated a revival of the debate on the influence of photoperiod on phenology.

### **What role for photoperiod in the bud burst phenology of European beech (Chapter 6)**

As one of the most dominant tree species in Europe, data for *Fagus sylvatica* is well represented in the scientific literature, and yet the basic physiology is poorly understood. The temporal variation of phenology of *Fagus* is characterized by a remarkably low inter-annual variation, compared to other species. Indeed, many studies (also the ones in this thesis) have found a distinct photoperiod sensitivity of this species. Other authors suggested that *Fagus* exhibits a large chilling requirement. From this assumption we merged results obtained from experimental studies, modeling studies, and long term-observations to compose a conceptual model of the interactions of chilling and photoperiod, accounting for the very stable timing of bud burst at low elevation sites, as well as for the earlier of bud burst observed at higher elevations during the last decades due to climate warming. We conclude that photoperiod may exert a stabilizing effect on bud burst in *Fagus*, by delaying bud burst in regions where the chilling requirement is fulfilled early in winter, and by substituting the chilling signal, and accelerating bud burst in mild regions where the chilling requirement may not be fulfilled, such as at the southern range limits of the distribution.

### **Is the use of cuttings a good proxy to explore phenological responses of temperate forests in warming and photoperiod experiments? (Chapter 7)**

Facing the problem that mature trees do not fit into growth chambers, and that seedling and sampling differ in their responses from adult trees, the use of cuttings from adult trees seems like a pragmatic solution. Indeed cuttings are often used to assess phenological responses to temperature and photoperiod under controlled conditions (e.g., also in Chapter 2 and 3). However, cuttings are per definition disconnected from possible whole-tree signals that facilitate dormancy release and bud burst. In this study, we assessed the phenology of mature trees, saplings and seedling, as well as from cuttings from mature trees and from saplings in three species in-situ. We found that the response of cuttings reflects the phenology of the donor tree and reflects also the distinct difference in the timing of bud

burst between the young life stages and mature trees. Thus, we concluded that cuttings may serve as proxy for the responses of mature trees in the examined species.

## Outlook

Based on our findings, (controlled) experiments should be undertaken using multiple photoperiod treatments to quantify the interactions of photoperiod and temperature and also to elucidate the nature of ecotypic differentiation. An ambitious follow-up project should first establish a set of visual, anatomical, physiological or molecular markers for the bud dormancy state and then advance towards a real-scale photoperiod reduction in mature forest trees, the perhaps most challenging type of any manipulation in global change research. This would require the day/ night extension by complete darkness (because photoperiod signal act dose-independent, with a very low threshold). Once such an experiment is setup, the additional manipulation of chilling and/or forcing would then seem to be straightforward.

## General Conclusions

The timing of spring phenology is the evolutionary result of minimizing the risk of late frost damage in spring. The results obtained in this thesis suggest that species have adopted different strategies to optimize the trade-off between maximizing the growing season and minimizing the risk of freezing damage. Distinct photoperiod sensitivity, as exhibited by *Fagus*, seems to be the safest strategy to avoid freezing damage in climates with unpredictable weather in spring. However, the additional safety is costly in terms of the missed opportunity of a longer growing season in warm years. Nevertheless, *Fagus* has successfully competed across large parts of Europe. Given the different degrees of photoperiod sensitivity exhibited by late successional trees, together with their interaction with possible chilling and forcing requirements, the response to warming will be non-linear. The phenology of most late successional species will thus

not continue to track climatic warming at the current rate. We are confident that the responses derived from cuttings are representative for mature trees at least for the species examined. Yet, the disruption from the whole tree signals could still lead to rather conservative estimates of the photoperiod responses. Opportunistic taxa may increasingly profit from a warmer climate and may thus gain a competitive advantage over photoperiod-sensitive taxa.