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Research

Perception of photoperiod in individual buds of mature trees regulates leaf-out

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Summary

- Experimental data on the perception of day length and temperature in dormant temperate zone trees are surprisingly scarce.
- In order to investigate when and where these environmental signals are perceived, we carried out bagging experiments in which buds on branches of *Fagus sylvatica*, *Aesculus hippocastanum* and *Picea abies* trees were exposed to natural light increase or kept at constant 8-h days from December until June. Parallel experiments used twigs cut from the same trees, harvesting treated and control twigs seven times and then exposing them to 8- or 16-h days in a glasshouse.
- Under 8-h days, budburst in *Fagus* outdoors was delayed by 41 d and in *Aesculus* by 4 d; in *Picea*, day length had no effect. Buds on nearby branches reacted autonomously, and leaf primordia only reacted to light cues in late dormancy after accumulating warm days. Experiments applying different wavelength spectra and high-resolution spectrometry to buds indicate a phytochrome-mediated photoperiod control.
- By demonstrating local photoperiodic control of buds, revealing the time when these signals are perceived, and showing the interplay between photoperiod and chilling, this study contributes to improved modelling of the impact of climate warming on photosensitive species.

Introduction

In temperate zone trees and shrubs, winter dormancy release and budburst are mediated by temperature and photoperiod (Heide, 1993a,b; Körner & Basler, 2010; Polgar & Primack, 2011; Basler & Körner, 2012; Laube *et al.*, 2014). Although leaf senescence in autumn is usually regulated by photoperiod (Cooke *et al.*, 2012), the role of photoperiod in the regulation of bud burst varies among species (Basler & Körner, 2012; Laube *et al.*, 2014; Zohner & Renner, 2014). Of the 44 temperate zone tree species investigated, spring leaf-out is influenced by photoperiod in 18, whereas in the remaining species, winter and spring temperatures alone regulate bud burst (Heide, 1993b; Basler & Körner, 2012; Laube *et al.*, 2014). The species-specific importance of photoperiod as a leaf-out cue probably arises from the trade-off between frost prevention and selection for early photosynthesis: photoperiod tracking protects species against leafing out during brief warming periods and thus reduces the risk of frost damage. By contrast, a day length-independent leaf-out strategy allows species to use early warm days, but exposes them to damage from late frosts (Körner & Basler, 2010; Zohner & Renner, 2014).

Experimental studies focusing on the impact of day length on dormancy release in trees have used seedlings cultivated indoors (Falusi & Calamassi, 1990; Caffarra & Donnelly, 2011) or buds on cut twigs brought indoors at different times during winter/spring (Heide, 1993a,b; Ghelardini *et al.*, 2010; Basler & Körner, 2012; Laube *et al.*, 2014). A problem with these experiments is that twigs cut later experience longer chilling and longer, continuously increasing photoperiods than those cut earlier (Table 1). The change in day length between 14 December and 14 March in the temperate zone is considerable; for example, in Munich it is 3.5 h, and buds on twigs cut on these two dates and moved to an 8-h light regime indoors therefore experience vastly different jumps in photoperiod. The failure to control for this, and also for possible effects of gradual vs sudden day length increase, may have led to an under-appreciation of the effects of photoperiod on the timing of budburst (Laube *et al.*, 2014; Polgar *et al.*, 2014).

Here we experimentally study the effects of day length and chilling on leaf-out in three large, temperate tree species – *Fagus sylvatica*, *Aesculus hippocastanum* and *Picea abies*. For *Fagus sylvatica*, studies based on cut twigs or seedlings all report a day length-dependent leaf-out strategy (Heide, 1993a; Basler & Körner, 2012; Caffarra & Donnelly, 2011; Vitasse & Basler, 2013; Laube *et al.*, 2014). Evidence for the other two species is equivocal. Although Basler & Körner (2012) find a day length-dependent flushing strategy in *Picea* and no photoperiod requirements in *Aesculus*, Laube *et al.* (2014) conclude the opposite, with *Aesculus* in their study being the species with the highest photoperiod threshold of 36 species analysed.

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Table 1 Experimental set-up of the experiments on leaf-out in cut twigs of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*

Start of experiment (Collection date)	21 Dec	29 Jan	11 Feb	24 Feb	10 March	21 March	4 April
Day length outside at start of experiment (h)	8	9.4	10	10.6	11.6	12	13
Chilling status: Chill days (<5°)	38	64	75	83	92	95	101
Day-degrees (> 5°C) at start of experiment	0	24	29	43	64	119	195

Different collection dates of twigs equate with different degrees of chilling. Chill days were calculated as days with mean temperature below 5°C since November 1 (following Murray *et al.*, 1989; Laube *et al.*, 2014). Photoperiod treatments for cut twigs were 8 or 16 h of light per day.

Knowledge about the underlying molecular mechanisms of photoperiodic dormancy regulation in trees is fragmentary. Phytochromes and the clock system (*LHY* and *TOC* genes) interact with the CO/FT signalling network to regulate flowering, and this pathway likely is also involved in regulating dormancy release (Cooke *et al.*, 2012). Photoreceptors and clock genes are found in all (living) plant cells, and their action can differ between organs (James *et al.*, 2008; *Arabidopsis*; Cooke *et al.*, 2012: review). In tobacco, Thain *et al.* (2000) showed that parts of single leaves can independently reset their clock systems in reaction to different light cues and that circadian rhythms in one leaf are independent of entrainment in other leaves. Cooke *et al.* (2012) therefore suggest that buds also might independently entrain to light (and/or temperature) cues. Such a mechanism would enable each bud to react autonomously to environmental cues. To our knowledge, this hypothesis has never been tested in trees.

In order to address the twin questions of the extent of bud autonomy and of the interaction between chilling and photoperiod, we conducted experiments in mature individuals of the three species mentioned above. These are the first reported *in situ* experiments on how photoperiod affects bud burst and leaf-out in adult trees. We kept some buds under constant day length, while letting others (on the same tree) experience the natural increase in day length during spring. Still using the same trees, we cut treated and untreated twigs seven times during the winter and spring and exposed them to 8- or 16-h light regimes indoors to test at which time the photoperiod signal becomes relevant as a leaf-out trigger, and to what extent photoperiod interacts with chilling status and warming temperatures. Combining the *in situ* experiment with the twig-cutting approach also allowed us to address effects of sudden vs gradual day length changes given different chilling status.

Materials and Methods

Experiments on buds on outdoor trees and buds on cut twigs brought indoors

The study took place in the botanical garden of Munich between 21 December 2013 and 1 June 2014. Cutting and bagging experiments were conducted on *Aesculus hippocastanum* L., *Fagus sylvatica* L. and *Picea abies* L. (H.Karst.) trees growing permanently outdoors. Leaf-out of individual buds was defined as the date when the bud scales had broken and the leaf had pushed out all the way to the petiole. For the bagging experiments, which ran from 1 January 2014 until the day of leaf-out in the respective species, we covered 10 branches per species with 1 m-long light-tight bags placed around the twigs every day at 17:00 h and removed the next morning at 09:00 h (Supporting Information Fig. S1). This ensured an 8-h photoperiod. Simultaneously, translucent bags of the same size and plastic thickness were placed on another 10 twigs on the same tree individuals. Climate data were obtained from Hobo data loggers (Onset Computer Corp., Bourne, MA, USA), placed inside each type of bag for each treatment, on openly exposed control twigs, and in the glasshouse (below). The percentage of leaf-out under both types of bags as well as on naturally exposed twigs was monitored every 3 d (100% leaf-out was achieved when all buds on the observed 10 branches per treatment had leafed out; Fig. 1).

For the cutting experiments, we sampled 30 replicate twigs per species on seven dates during winter/spring 2013/14 (cutting dates: 21 December, 29 January, 11 February, 24 February, 10 March, 21 March and 4 April; see Table 1). After cutting, twigs were disinfected with sodium hypochlorite solution (200 ppm active chlorine), re-cut a second time to *c.* 40 cm, and then placed in 0.5-l glass bottles filled with 0.4 l cool tap water enriched with the broad-spectrum antibiotic gentamicin sulfate (40 $\mu g\,l^{-1}$; Sigma-Aldrich; Basler & Körner, 2012; Larcher *et al.*, 2010). Twigs were subsequently kept under short day (8 h) or long day (16 h) conditions. Temperatures in the glasshouse ranged from 18°C during the day to 14°C at night. Water was changed twice a week, and twigs were trimmed weekly by *c.* 2 cm. Additionally, on 11 February and 21 March, 16 twigs per species from each of the bagging treatments (translucent and light-tight bag) were cut

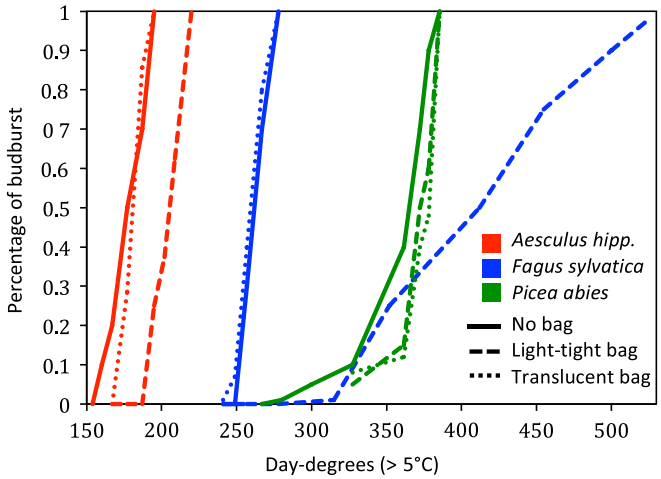

Fig. 1 Percentage of budburst per day-degree under 8-h day length (light-tight bag) and naturally increasing day length (translucent bag and without bag) for *Aesculus hippocastanum* (red), *Fagus sylvatica* (blue) and *Picea abies* (green).

and transferred to a glasshouse chamber, where they were exposed to experimental photoperiods as described above (see Fig. 2a for a scheme of treatment conditions). For all three species and all treatments, bud development was monitored every second day, and the leaf-out dates of the first 10 twigs (without bagging) or six (with bagging) that leafed out were recorded. A twig was scored as having leafed out when three buds had their leaves pushed out all the way to the petiole.

We conducted repeated-measures ANOVA to test for effects of naturally increasing day length vs constant short-day treatment

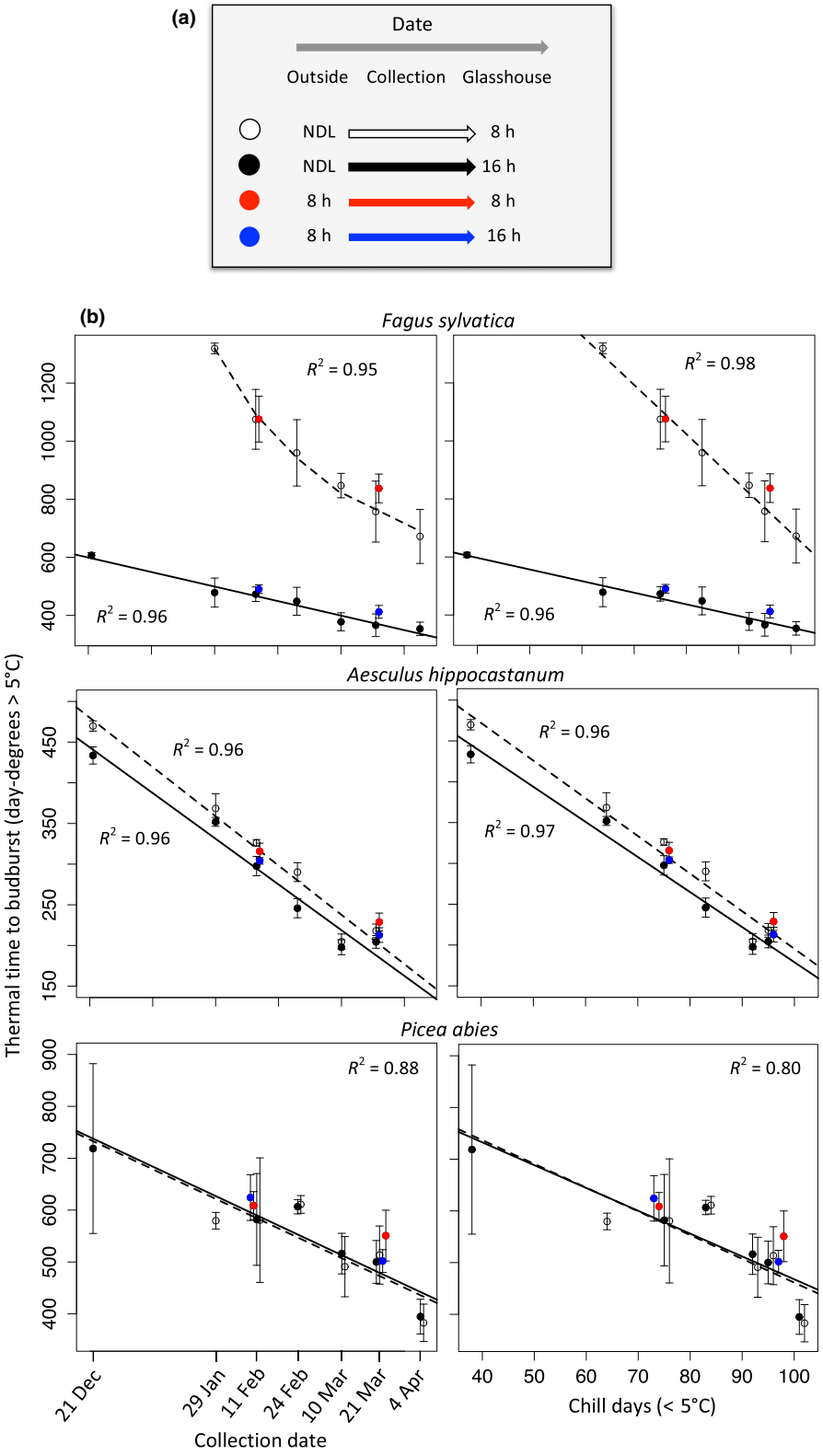

Fig. 2 (a) Explanation of treatment conditions for twig cuttings. Colour coding refers to (b). Outside, natural increase in day length (NDL) until collection date vs constant 8-h day length via bag treatment (8 h); Collection, twig collection (transfer from field to glasshouse at seven different times; see Table 1); Glasshouse, fixed day length (8 or 16 h) in glasshouse chambers. (b) Correlation between collection date (left panels), chilling (right panels) and thermal time to budburst (day-degrees > 5°C) under 8 and 16 h day length for twig cuttings of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*. For explanation of treatment conditions see (a). For statistical analysis see Table 2. Points and error bars represent the mean ± SE of thermal time to budburst. Twigs of *Fagus* and *Picea* were collected seven times during winter/spring 2013/2014; those of *Aesculus* were collected only six times because leaf-out of *Aesculus* in the field had preceded the last cutting date on 4 April. Thermal time to budburst did not increase when twigs were kept under a constant day length of 8 h in the field (light-tight bags) before collection (repeated measures ANOVA: $P > 0.1$; see coloured points).

before twigs were cut and brought indoors. ANCOVA was used to test for interactions between chilling and photoperiod treatments. Accumulated day-degrees (> 5°C) until leaf-out (= sum of day-degrees accumulated outside after 1 January and in the climate chamber) were used as response variable. All statistical analyses relied on R (R Core Team, 2014).

Light perception and transmission through buds

In order to test for the light spectrum that plants use to regulate budburst, we exposed twigs of the photosensitive species *A. hippocastanum* and *F. sylvatica* to: the entire light spectrum; red light (> 575 nm); and far-red light (> 700 nm) (Fig. 3). Fifteen twigs were collected per species and treatment, using the same cutting procedure as above and the leaf-out dates of the first 10 twigs were recorded. The cutting date was 5 March 2015, and twigs were exposed to 16 h of light per day. Additional twigs were kept under 8- or 12-h day length (and exposed to the entire light spectrum) to test their photoperiod sensitivity. A Tukey-Kramer test was conducted to test for differences in thermal time to budburst among the treatments.

Bud scales consist of thick cuticle-like material and hardly allow for transmission of light that might be sensed by subjacent leaf tissue. To test for the quantity and quality of light they transmit, we carried out transmission analyses, using the HR4000 high-Resolution Spectrometer (Ocean Optics, Dunedin, FL, USA), which is responsive from 200 to 1100 nm. We therefore bisected the buds and removed leaf primordial tissue inside the buds of *A. hippocastanum*, *F. sylvatica* and *P. abies* and measured light transmission through all remaining bud scales (Fig. 4), and through a single bud scale (Fig. S3). For each species, we calculated the mean of the transmission spectra of 10 buds. We also measured the light transmission of the bags used in our *in situ* experiment to ensure that translucent bags transmitted across the entire spectrum while light-tight bags efficiently filtered out light across the spectrum.

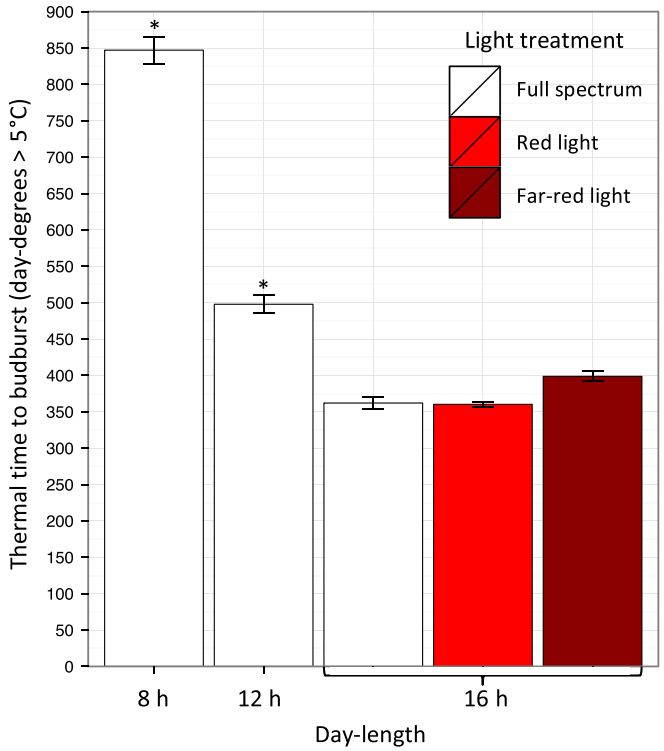

Fig. 3 Thermal time (day-degrees > 5°C) to budburst under different light spectra for *Fagus sylvatica*. Twigs were collected on 5 March 2015 and exposed to 8-, 12- and 16-h day length under: the entire light spectrum; red light (> 575 nm); or far-red light (> 700 nm). Buds exposed to red or far-red light reacted no differently from those exposed to the entire light spectrum. Treatments differed significantly from the 16 h, full light spectrum treatment: *, $P < 0.05$. Error bars represent the mean ± SE of thermal time to budburst.

Results

Effects of photoperiod on buds outdoors and on cut twigs brought indoors

Buds of *F. sylvatica* kept under constant 8-h day length (achieved by bagging twigs of outdoor trees every evening and unbagging them every morning) achieved 100% budburst 41 days later than those that experienced the natural day length increase (Figs 4, 5, S2). The same conditions delayed budburst in *A. hippocastanum* by four days and had no effect on budburst in *P. abies*. Twigs that had experienced constant 8-h days or naturally increasing day length were harvested at seven different times (Table 1) and brought into the glasshouse where they received the experimental treatments summarized in Fig. 2(a). Later cutting dates equate with plants having reached a higher chilling status and having accumulated more day-degrees (Table 1).

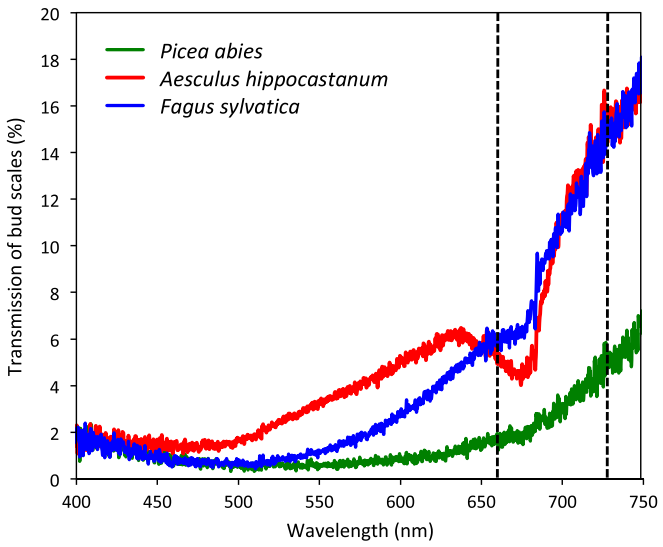

Fig. 4 Transmission spectra of buds of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*. Buds were bisected and leaf primordial tissue was removed before measurements, thus the graph reflects the quality and quantity of light that could be sensed by photoreceptors located in leaf primordia. Dashed lines indicate the absorption maxima for Phytochrome a (P_r and P_{fr}).

Fig. 5 Development of *Fagus sylvatica* buds kept under translucent (upper twig) or light-tight bags (lower twig) on 25 April 2014.

Leaf-out date in all the species was unaffected by whether twigs experienced a *gradual* (natural) day length increase (up to 12 h days) or *constant* 8-h short days (bag treatment) before being brought indoors (repeated measures ANOVA: $P > 0.1$ and Fig. 2(b), compare the blue and red dots to the black and white dots, respectively): Buds on twigs cut in February or late March, when they had already experienced quite long days outdoors, and brought into 8- or 16-h glasshouse conditions underwent budburst at the same time as buds on twigs kept under a constant 8-h day until then (see Fig. 2b).

In *F. sylvatica*, the day-degrees until leaf-out accumulated by buds kept under 8-h day length were correlated exponentially with collection date (Fig. 2b, left top panel: curve fitting white and red dots), whereas the association between day-degrees and accumulated chill days was linear (Fig. 2b, right top panel). For buds on twigs kept under 16-h day length, collection date and chill days were linearly and negatively correlated with accumulated day-degrees (Fig. 2b, top panels: curves fitting black and blue dots). The effect of day length treatment on forcing requirements was highly significant, and there was also a highly significant interaction between chilling status and day length treatment, with higher chilling reducing day length requirements and longer days reducing chilling requirements (see Table 2; Fig. 2b).

In *Aesculus*, day length barely affected forcing requirements (Table 2, $P = 0.09$), and chilling status did not affect photoperiod requirements. Collection date and chilling status were linearly correlated with required day-degrees until leaf-out (Table 2; Fig. 2b, middle panels). In *Picea,* day length had no significant effect on forcing requirements (Table 2), and collection date and chilling status were linearly correlated with day-degrees until leaf-out (Fig. 2b, lower panels).

Light perception and transmission analyses of buds

In *A. hippocastanum* and *F. sylvatica*, leaf-out date under 16-h days did not differ regardless of whether buds were exposed to the full light spectrum, only red light or only far-red light ($P > 0.15$; see Fig. 3 for *F. sylvatica*), even though in both species, under far-red conditions, leaves appeared pale due to lack of chlorophyll. Day lengths of 8 or 12 h delayed budburst in *Fagus* by 42 or 15 d and in *Aesculus* by 3 or 1 d.

Table 2 Results of ANCOVA to test for the effect of day length on species' forcing requirements, while controlling for the effect of chilling status

Explanatory factor	*Fagus* ($n = 13$)	*Aesculus* ($n = 12$)	*Picea* ($n = 12$)
Chilling	$F(1,12) = 91.3$ **$P < 0.001$**	$F(1,11) = 320.8$ **$P < 0.001$**	$F(1,11) = 25.7$ **$P < 0.001$**
Photoperiod	$F(1,12) = 1440.2$ **$P < 0.001$**	$F(1,11) = 3.8$ **$P = 0.09$**	$F(1,11) = 0.05$ $P = 0.83$
Interaction Chilling × Photoperiod	$F(1,12) = 140.6$ **$P < 0.001$**	$F(1,11) = 0.9$ $P = 0.39$	$F(1,11) = 0.03$ $P < 0.87$

n, refers to the number of treatments (Chilling (number of collection dates) × Photoperiod (8 or 16 h)); see also Fig. 2(b). *P* values < 0.1 are shown in bold.

Transmission spectra of the entire bud scale tissue were similar among species, but the relative amplitudes of transmission bands differed (Fig. 4). In the range between 600 and 800 nm, bud scales of *Aesculus* and *Fagus* transmitted two to three times more light than those of *Picea*. In all three species, light transmission increased with longer wavelengths. Between 400 and 500 nm, transmission was < 2%, whereas above 500 nm it steeply increased, reaching 100% at 900 nm. In *Aesculus*, the transmission spectrum shows a local minimum *c.* 670 nm, likely due to chlorophylls located in the inner surface of bud scales in this species, whereas *Fagus* and *Picea* bud scales are dead and do not contain any chlorophyll. For transmission spectrum analysis of single bud scales, see Fig. S3.

Discussion

Photoperiod signal perceived at the local bud level

Animals have central circadian pacemakers in the brain that entrain peripheral clocks (Liu & Reppert, 2000). This leads to a close coupling between the circadian clocks of individual cells and increases the precision of timing *in vivo* (Thain *et al.*, 2000). Sessile organisms, such as most plants, by contrast have largely autonomous or weakly coupled circadian clocks that allow for independence among a plant's modules in the entrained phases of circadian rhythms. Using *in vivo* reporter gene imaging in tobacco, Thain *et al.* (2000) found that the clock systems even of sections within leaves are functionally independent. Our experiments on the effect of photoperiod on bud break on nearby twigs of single individuals of *F. sylvatica*, *A. hippocastanum* and *P. abies* provide evidence for the extent of local control (Fig. 5). The light signal likely is perceived by receptors just below the bud scales, and the genetic system involved in leaf-out regulation must therefore be located in the young leaf primordial cells. This allows each bud to react autonomously to cues by maintaining an independent circadian clock system during winter and to respond to day length increase in

spring (Thain *et al.*, 2000; tobacco; James *et al.*, 2008; *Arabidopsis*; our Fig. 5 for *F. sylvatica*).

Compared to light perception, even less is known about the mechanisms of temperature sensing during bud dormancy release, although experiments on one-node cuttings prove that bud autonomy also exists for forcing and chilling requirements (Vitasse & Basler, 2014), and there is evidence that circadian clocks are involved (Rensing & Ruoff, 2002; Cooke *et al.*, 2012). Findings in *Populus* of an upregulation of the clock gene *LHY* under cold conditions and of low *LHY* expression causing delayed budburst (Ibáñez *et al.*, 2010) point to a connection between the circadian clock and chilling fulfilment. This would permit extremely fine-scale leaf-out regulation and acclimation to the microclimate differences commonly experienced by large, perennial individuals (Augspurger, 2004; Vitasse & Basler, 2013).

Interplay between chilling and photoperiod

The three tree species studied here behaved differently in terms of the extent to which chilling status and warming temperatures (degree day) interacted with day length. In *A. hippocastanum*, delayed budburst under short days probably is merely a consequence of slower growth as a result of lower light availability. By contrast, in *F. sylvatica,* day length had a huge effect on forcing requirements, and leaf-out was not possible under short days and low chilling (Fig. 2b, top panel). The correlation between cutting time and thermal time to budburst has a different slope for 8- and 16-h day length treatments (Fig. 2b). This demonstrates that the extent of chilling fulfilment influences photoperiod requirements and *vice versa*, with chilling partially substituting for unmet photoperiod requirements (see also Laube *et al.*, 2014) and increasing day length substituting for a lack of chilling. That exposure of buds to natural day length (12 h day length on 21 March) or 8-h days (bag treatment) before 21 March did not affect the leaf-out dates on twigs brought to the glasshouse (see Fig. 2b) indicates that photoperiod signals do not cause irreversible molecular responses in buds and that day length influences only the late phase of dormancy, when substantial forcing has accumulated. Long days occurring during cold periods with little accumulation of warm days therefore have no effect on subsequent forcing requirements. This can be seen in Fig. 2(b), where there is no difference in thermal requirements between buds that had experienced a gradual day length increase (up to 12 h light per day) and buds that were kept under constant 8-h day length until 21 March (compare the red or blue to the white or black points, respectively).

Our experiments also reveal that for *F. sylvatica* there is a linear, negative relationship between accumulated chill days and forcing requirements (day-degrees required), whereas collection date under short days (8 h) was nonlinearly correlated with day-degrees (Fig. 2b, top panel), probably because late in spring the number of predictably cold days varies greatly. This implies that using chill-days in leaf-out models will more accurately forecast leaf-out behaviour than will day-of-year models, although the exact temperature threshold and the precise physiological and molecular mechanisms that lead to chilling fulfilment are not yet understood (Cooke *et al.*, 2012).

For *F. sylvatica*, Vitasse & Basler (2013) put forward two hypotheses for how photoperiod may modulate the relationship between chilling status and thermal time (day-degrees) to budburst: Either, a fixed photoperiod threshold has to be reached to allow for perception of thermal time or else forcing requirements continuously decrease with increasing photoperiod. Our experiments suggest that both hypotheses are partially correct. On the one hand, insufficiently chilled buds require that a certain photoperiod threshold be exceeded before bud development (buds on twigs did not leaf-out under low chilling and 8-h day length; see Fig. 2b). Buds that had passed their chilling threshold, on the other hand, leafed out under short days, but even in these buds, longer days significantly reduced the thermal time required for budburst.

In short, *Fagus* obligatorily requires a minimal day length to allow for budburst when chilling requirements are not met, and long days partially substitute for unmet chilling requirements. *Aesculus* shows a constant delay in leaf-out under short days, does not obligatorily require a certain day length, and shows no modulating effect of day length on chilling requirements or *vice versa.* In *Picea* we found no effect of photoperiod on budburst. Our results for *Aesculus* and *Picea* are in agreement with those of Laube *et al.* (2014), but contradict Basler & Körner (2012) who found day length-dependent flushing in *Picea* and day length-independent budburst in *Aesculus*. Laube *et al.* (2014) and the present study used only individuals at low elevation, whereas Basler & Körner (2012) analysed trees along an elevational gradient of 1000 m and found that low-elevation *Picea* were less sensitive to photoperiod than high-elevation individuals. This points to ecotypic differentiation of photoperiod requirements. Intraspecific phenological plasticity or ecotypes deserve further study.

Experimental implications

Our experiments control for a possible artefact in previous studies that used buds on twigs transferred to vases in glasshouses: Twigs cut later during the winter experience increasing day lengths and higher chilling than those cut earlier (Table 1). Twigs cut at different times are thus not strictly comparable in chilling status because the photoperiod effect is not controlled for. Our experiments (in all three species), however, revealed that buds on twigs cut on 21 March and brought into a glasshouse for 16- or 8-h light treatments behaved no different regardless whether they had experienced naturally increasing day lengths (up to 12-h day length) or had been kept under a constant 8-h day (by the outdoor bagging experiments; Fig. 2b). This indirectly validates the results of earlier studies in which twigs were cut early or late in spring to study the effects of chilling, but without controlling for the day length increase experienced before they were cut (Laube *et al.*, 2014; Polgar *et al.*, 2014). That buds *in situ* and on cut twigs react similarly to similar treatments as shown here (see also Vitasse & Basler, 2014) underlines the utility of the twig cutting method for inferring woody species' responses to photoperiod.

Red light induces responses to photoperiod and bud scales filter-out nonred light

In this study we show that red light is sufficient to induce budburst as a response to day length increase (Fig. 3) and find that leaf primordial cells receive sufficient red light in spite of being tightly covered by dead bud scale tissue (Fig. 4). These data strongly suggest that phytochromes mediate the day length response of buds. Between 400 and 600 nm, bud scales filtered out light efficiently, but between 600 and 800 nm, they transmitted 2–20% of the incoming light, with far-red light transmitted three-times more than red light (Fig. 4). Bud scales thus function as optical filters, modulating the phytochrome system (Pukacki & Giertych, 1982: *P. abies* and *Pinus sylvestris*; Solymosi & Böddi, 2006: 37 woody species).

Picea abies buds transmitted the least light (Fig. 4) because of the numerous scales per bud, whereas individual *Picea* scales let through more light than those of *Aesculus* and *Fagus*; Fig. S3). Being photoperiod insensitive (Table 2, Fig. 2b), *Picea* can probably afford a higher number of bud scales, perhaps providing increased frost protection, whereas in photo-sensitive species like *Fagus* and *Aesculus* there could be a trade-off between frost resistance (more bud scales) and sufficient light transmittance (fewer bud scales).

Conclusion

This study investigated bud responses to photoperiod in adult trees growing outside, whereas earlier studies on woody species all extrapolated from bud responses on cut twigs or seedlings. We found that: dormancy release is controlled at the bud level, with light sensing (and probably also temperature sensing) occurring inside buds; leaf primordia only react to light cues during the late phase of dormancy release when they have begun accumulating warm days; in *Fagus*, but not the other species, photoperiod can partially substitute for a lack of chilling and *vice versa*; and red light triggers the day length response, with bud scales filtering-out most of the remaining light spectrum received by the primordia.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Treatment of twigs with light-tight bags, to ensure an 8-h photoperiod and translucent bags as control.

Fig. S2 Bud development of *Aesculus hippocastanum* (on 3 April 2014) and *Fagus sylvatica* (on 22 April 2014) on mature trees kept under 8-h day length and naturally increasing day length.

Fig. S3 Transmission spectra of single bud scales of *Aesculus hippocastanum*, *Fagus sylvatica* and *Picea abies*.

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