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ORIGINAL PAPER

The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst

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Abstract The process of adaptation is the result of stabilising selection caused by two opposite forces: protection against an unfavourable season (survival adaptation), and effective use of growing resources (capacity adaptation). As plant species have evolved different life strategies based on different trade offs between survival and capacity adaptations, different phenological responses are also expected among species. The aim of this study was to compare budburst responses of two opportunistic species (*Betula pubescens*, and *Salix x smithiana*) with that of two long-lived, late successional species (*Fagus sylvatica* and *Tilia cordata*) and consider their ecological significance. Thus, we performed a series of experiments whereby temperature and photoperiod were manipulated during dormancy. *T. cordata* and *F. sylvatica* showed low rates of budburst, high chilling requirements and responsiveness to light intensity, while *B. pubescens* and *S. x smithiana* had high rates of budburst, low chilling requirements and were not affected by light intensity. In addition, budburst in *B. pubescens* and *S. x smithiana* was more responsive to high forcing temperatures than in *T. cordata* and *F. sylvatica*. These results suggest that the timing of growth onset in *B. pubescens* and *S. x smithiana* (opportunistic) is regulated through a less conservative mechanism than in *T. cordata* and *F. sylvatica* (long-lived, late successional), and that these species trade a higher risk of frost damage for the opportunity of vigorous growth at the beginning of spring, before canopy closure. This information should be considered when assessing the impacts of climate change on vegetation or developing phenological models.

Keywords Budburst · Life strategy · Photoperiod · Temperature · *Fagus sylvatica* · *Salix x smithiana* · *Tilia cordata* · *Betula pubescens*

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Introduction

In temperate and seasonal climates, woody perennials have evolved control mechanisms of their growth cycle that synchronise them with annual variations in temperature and precipitation. An important adaptation allowing temperate trees to survive the harshness of the winter season is dormancy, whereby vegetative activity is suspended until favourable growth condition resume in spring (Leopold 1996; Viémont and Crabbé 2000). In late summer, trees enter dormancy and develop dormant buds, mainly as a response to an extension in night length, corresponding to a shortening photoperiod (Hänninen 1990; Battey 2000). During this state of "suspended growth" (known as "endo-dormancy") growth is inhibited even in favourable environmental conditions (such as warm temperatures and long photoperiods). Endo-dormancy is released during the winter by the exposure to cold or chilling temperatures (Sarvas 1974; Battey 2000). Chilling is a vaguely defined concept, typically referring to temperatures below about 10°C that act by breaking dormancy in trees (Battey 2000). After endo-dormancy release, exposure to warm temperatures (a period known

as forcing) is the main trigger leading from environmentally imposed dormancy ("eco-dormancy") to budburst (Sarvas 1974; Cannell and Smith 1983; Hänninen 1990; Battey 2000; Arora et al. 2003), even though in some species long days have been shown to reduce the time to budburst (Heide 1993a; Myking and Heide 1995). There are many exceptions to this general pattern. For example, apple and pear trees are not influenced by day-length, but only by temperature (Heide and Prestrud 2005). On the other hand, *Fagus sylvatica* has been reported to require a relatively long photoperiod in addition to chilling in order to resume growth in spring (Heide 1993a; Borchert et al. 2005; Korner and Basler 2010), whereas peach trees can resume growth even without receiving any chilling, provided that a combination of other conditions (such as high temperatures in conjunction with long photoperiod) is present (Erez and Lavee 1971).

These differences are likely to have developed as adaptations in response to the particular environmental conditions to which plants were exposed. In fact the regulation of the timing of growth onset and cessation is a fundamental feature allowing the syncronisation of the plant growth cycle with favourable seasonal conditions, and, similar to other characteristics, has been driven by selective pressure (Leopold 1996; Bennie et al. 2010; Korner and Basler 2010). This process of adaptation has been seen as the result of stabilising selection caused by two opposite forces: protection against an unfavourable season (survival adaptation), and effective use of growing resources (capacity adaptation) (Hänninen and Hari 1996; Leinonen and Hänninen 2002). The optimal timing of budburst can then be considered the result of a specific combination of these driving forces. However, as different plant species have evolved different life strategies based on different trade offs between survival and capacity adaptations, different phenological responses are also expected among species. To date, few experimental studies have compared the environmental control of phenology in different species (Downs and Borthwick 1956; Murray et al. 1989; Myking and Heide 1995; Rinne et al 1997; Heide 2008), focussing mostly on single species (see, for example, Heide 1974, 1993b; Mahmood et al. 2000; Li et al. 2003). However, the study of these differences and their implications is particularly important if the impacts of climate change are to be evaluated at the ecosystem level. Do phenological responses to changing environmental conditions reflect the different ecological role of plant species? The aim of this study was to compare budburst responses of two opportunistic species (*Betula pubescens*, and *Salix x smithiana*) with that of two long-lived, late successional species (*Fagus sylvatica* and *Tilia cordata*) in different environmental conditions, and to consider their ecological significance.

Materials and methods

Selection and vegetative propagation of experimental material

The experiments were conducted on the *B. pubescens* , *F. sylvatica, S. x smithiana* and *T. cordata* clones grown in the International Phenological Gardens (IPGs) network, which comprises 51 garden sites throughout Europe (Chmielewski 1996). The clones under study, originally from Germany, were propagated vegetatively in order to obtain experimental material with homogenous genotype, ensuring that any observed differences could be attributed to environmental conditions rather than genotypical differences among plants. The IPG clones of the above species were propagated vegetatively for 2 years following different procedures. *S. x smithiana* was propagated by winter cuttings. At the beginning of March 2003, hardwood shoots were cut from the parent plant growing in the IPG located at Valentia in County Kerry, Ireland (IPG No. 13) and immersed in water contained in glass beakers. These shoots developed roots after 2 months and were planted subsequently into 1 l pots. *F. sylvatica* was propagated by grafting. At the beginning of March 2003, hardwood shoots were cut from the parent plant growing in the IPG at Valentia and grafted onto 2-year-old seedlings of *F. sylvatica* purchased from a local nursery. The grafted rootstocks were planted into 2 l pots. *T. cordata* and *B. pubescens* were propagated by summer cuttings taken from the soft, new growth of the tree, as described by Hartmann et al. (1997). In June 2003 we took softwood cuttings from the *B. pubescens* IPG clone growing at the John Fitzgerald Kennedy Arboretum in Wexford (Ireland, IPG no.14). After collection, the softwood cuttings were treated with rooting powder (0.4% NAA) and placed in compost-filled trays before being transferred to a mist unit until they rooted approximately 6 weeks later. The rooted cuttings were potted up in 1-l pots.

Experimental design

During the course of 2003 and 2004, three experiments were performed on the propagated clones of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata* (Table 1). The experiments were designed to investigate the primary effects and interactions of temperature and photoperiod during the different phases of bud development and growth on the timing and percentage of budburst.

Observations were recorded every day following the initiation of forcing conditions, for a period of 80 days. The experiments were divided into two sequential phases, referred to as *chilling* and *forcing*. A summarised description of the chilling and forcing treatments applied is shown in Table 1. The experiments were conducted either on sets

Table 1 Forcing and chilling treatments received in each of the experiments performed during the study. "Ambient" refers to non-controlled temperature (received outdoors or in a glasshouse), which was recorded daily or obtained from a nearby weather station as daily max and min

Experiment	Species	Experimental material	Chilling treatment			Forcing Treatment		Additional factors tested
			Temperature (°C)	Photoperiod (hours)	Duration (days)	Temperature (°C)	Photoperiod (hours)	
1	*Betula pubescens* *Fagus sylvatica* *Salix x smithiana* *Tilia cordata*	Budsticks	Ambient	Natural	NA[a]	22	16	High and low light intensity[b]
2	*F. sylvatica* *S. x smithiana* *T. cordata*	Budsticks	Ambient	Natural	NA	-3, 6, 12, 18, 24, 32[b]	16	NA
3	*B. pubescens* *F. sylvatica* *S. x smithiana* *T. cordata*	Potted trees	3	0 (complete darkness)	0 (no chilling), 11, 30, 55, 105[b]	18	8, 16[b]	NA

[a] Not applicable

[b] Factors varied within each experiment

of rooted shoots or on sets of cuttings, each containing one bud, taken from the central portion of the branches of the clones and cut into segments 3–5 cm in length. These one-node cuttings were protected with Parafilm at their distal end to prevent water loss and desiccation, and placed in compost-filled trays before being transferred to the experimental conditions. The experimental material was watered regularly and the compost remained moist at all times. All treatments in growth cabinets (Sanyo Fitotron 600 L, Sanyo-Gallenkamp, Loughborough, UK, and Fisons Fitotron 600 H, Fisons Scientific, Loughborough, UK) received a photon flux density of 170±10 μmol m^{-2} s^{-1} with a red/far red ratio varying between 7 and 9, (quantum sensor: Li-Cor, Lincoln, NE) unless otherwise indicated. Treatments in the heated glasshouse received natural day length and an average temperature of about 15°C but never falling below 12°C. Temperature was recorded daily with a datalogger or manually (max and min). Daily maximum and minimum temperatures in outdoor treatments were recorded by a nearby weather station.

Experiments

Experiment 1. Effect of light intensity

On 28 February 2004, dormant twigs were removed from adult IPG clones of *B. pubescens*, *F. sylvatica*, *T. cordata* and *S. x smithiana* growing outdoors in IPG 14, Wexford (Ireland). From these twigs we obtained one-node cuttings. The cuttings of each species were split into two groups, which were placed in trays filled with peat compost (ten cuttings in each of the two trays). The two trays were transferred to a growth cabinet set at 24°C, with a 16-h photoperiod. Inside the cabinet, one of the trays was shaded with a white cotton fabric screen, which decreased the light intensity without altering the red/far red ratio. The other was openly exposed to the light. The photon flux densities in the two light intensity treatments were 250 μmol m^{-2} s^{-1} and 75 μmol m^{-2} s^{-1}, respectively. In both cases the red/far red ratio was 7±1. Ten one-node cuttings for each species were tested in each of the two light intensity treatments.

Experiment 2. Effects of forcing temperature

On 28 February 2004, dormant twigs were taken from trees of *F. sylvatica*, *T. cordata* and *S. x smithiana* growing outdoors in IPG 14. Sixty twigs for each species were stored in the dark at 3°C for 16 days, until the start of the experiment. By this time the plant's chilling requirement was assumed to have been met. Each twig was cut into a one-node cutting. The 60 one-node cuttings were split into groups of ten and transferred into five growth cabinets set at 6, 12, 18, 24 and 32°C and an incubator (described by Clifton-Brown and Jones 1999) set at −3°C, maintained on a 16-h photoperiod. As information on the response to temperature and photoperiod of *B. pubescens* was already available from an analogous experiment (Caffarra et al., in review), this species was not included.

Experiment 3. Effect of chilling duration and forcing photoperiod

We investigated the main effects and interactions between chilling duration and photoperiod in recently propagated *F. sylvatica*, *T. cordata*, *S. x smithiana* and *B. pubescens* plants. After being potted up in early October 2003, the rooted cuttings were kept in a temperature-controlled glasshouse until November. While the temperature in this glasshouse was maintained above 12°C, prior to transfer the plants experienced a few days (less than 2 weeks) where mean daily temperatures went below 12°C. Forty plants for each species were transferred to a cold room, permanently dark, at a constant temperature of 3°C, where they were exposed to chilling for varying lengths of time: 0, 11, 30, 55 and 105 days. Batches of eight plants per species were selected randomly and removed from the cold room at the end of each period before being transferred into two growth cabinets. These cabinets were set at 22°C during the light period and 14°C during the dark period, with a photoperiod of either 16 h (long day treatments) or 8 h (short day treatments). Each of the ten treatments, i.e. five chilling durations × two forcing photoperiods, was applied to sets of four plants per species.

Statistical analyses

We analysed the response of budburst timing and percentage of budburst to the environmental factors that were varied in each experiment. When rooted shoots were used in the experiments, we analysed their mean time to budburst, which was obtained by averaging the number of days to budburst of each bud (arithmetic mean). When budsticks were used, the number of days to budburst of each budstick was considered. The count of the number of days to budburst was started from the moment of transfer into the forcing treatment. Budburst percentage in rooted shoots was defined as the percentage of buds flushing against the total number of buds on the shoots. For one-node budsticks, budburst percentage was either 0 (non-flushing) or 100% (flushing). When budburst percentage was affected by partial desiccation, only the data for budburst timing of healthy buds were used. The rate of forcing (FR), used in phenological models (Chuine 2000; Caffarra and Eccel 2010) was calculated to facilitate the comparison of the budburst rates of different species. FR was obtained by normalising the mean budburst rates measured in Experiment 1 (1/mean time to budburst) to 1 at 32°C and fitting these data with a sigmoidal curve of the following equation:

$$FR(t) = \frac{1}{1 + e^{a(Tt-b)}} \quad (1)$$

where T_t stands for temperature and a and b are parameters expressing the slope and inflection point of the curve, respectively. The significance of estimated parameters was checked with a t-test.

The data were subjected to one-way and factorial analysis of variance (ANOVA) to check for significant differences among the treatment means. Data from the experiments were tested for deviance from normality and for the homogeneity of variance using Fligner-Killeen's and Levene's tests (Quinn and Keough 2002). Percentage of budburst was arc-sin transformed prior to statistical analysis, to stabilize variance. Experimental designs with unequal sample size were analysed with Type III sum of squares as suggested by Quinn and Keough (2002). Unbalanced designs with empty cells were analysed by selecting subsets of the data with observations in all cells in order to test "balanced" hypotheses for lower-order effects (Quinn and Keough 2002) and by grouping factors together in order to avoid the calculation of interaction effects among factors with incomplete levels.

Results

Effect of temperature

Chilling

Chilling duration was an important factor for the percentage and timing of budburst, significantly affecting both responses in all the species under investigation. Long chilling duration generally advanced budburst and increased percentage budburst (Experiment 3; Figs. 1, 2). In fact, the ANOVAs indicated that this factor had significant effects on both percentage budburst and timing of budburst in all species under study ($P<0.001$; Table 2). In general, the effects of chilling duration were most evident at the initial stages of chilling, with the decrease in time to budburst being sharpest as chilling duration increased from 11–30 to 55 days, depending on the species and forcing photoperiod (Fig. 1a–d). Similarly, the percentage of budburst showed the sharpest increase between 11 and 55 days of chilling for *S. x smithiana* and *T. cordata* (Fig. 2b,c) and between 0 and 55 days of chilling for *B. pubescens* (Fig. 2d), while the response of *F. sylvatica* was strongly affected by photoperiod in all treatments (Fig. 2a).

Forcing

Forcing temperature had a significant effect on the time to budburst in all species in Experiment 2 (Table 3), with higher forcing temperatures advancing budburst. There was a general decrease in number of days to budburst at increasing temperatures, but while *F. sylvatica* and *T. cordata* showed a

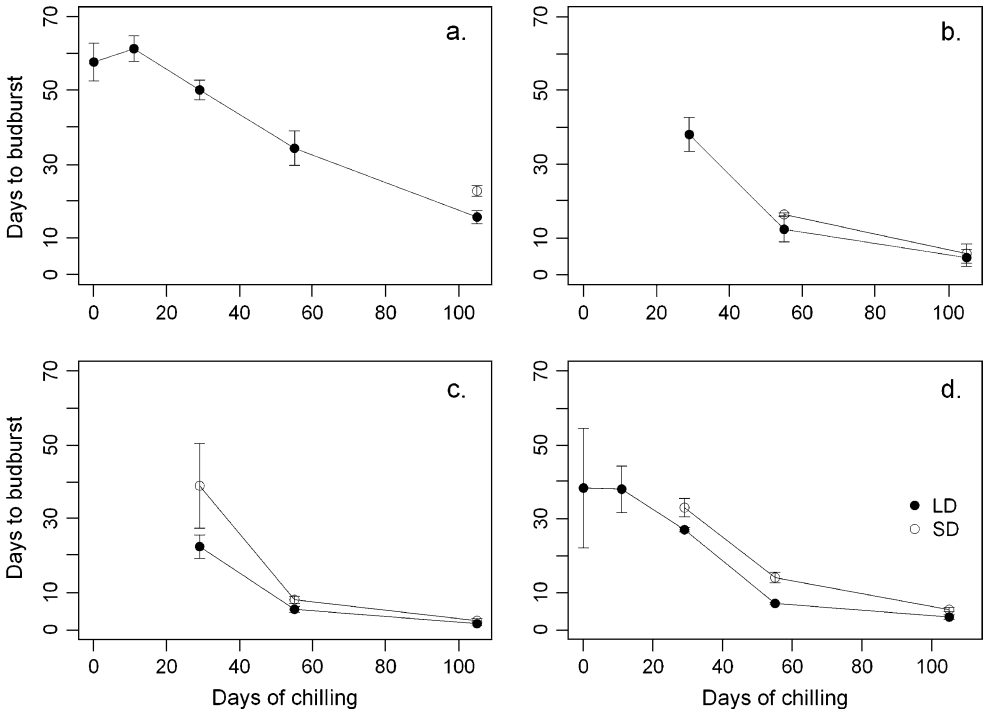

Fig. 1 Relationship between number of days to budburst and chilling duration for **a** *Fagus sylvatica*, **b** *Tilia cordata*, **c** *Salix x smithiana* and **d** *Betula pubescens* grouped by forcing photoperiod. *Filled symbols* 16 h, *open symbols* 8 h. Data are from Experiment 3 (effect of chilling duration and forcing photoperiod). *Error bars* Standard error of the mean

generally longer time to budburst, *S. x smithiana* had a faster budburst. The results from an analogous experiment (Caffarra et al., in review) showed that the budburst rate of *B. pubescens* was quite similar to that found for *S. x smithiana* in the current study, especially at temperatures above 12°C. The number of days to budburst and rates of forcing of *B. pubescens* from Caffarra et al. (in review) were plotted with the present results, for comparative purposes (Fig. 3a,b). The relationship between the rates of forcing and temperature in these four species showed highly significant

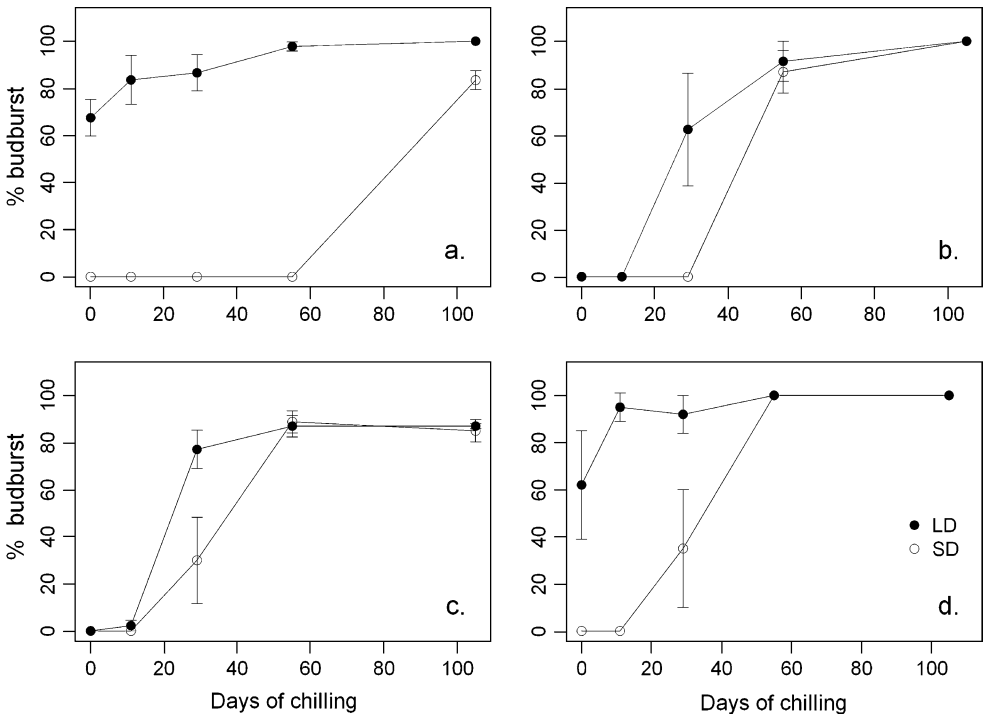

Fig. 2 Relationship between percentage of budburst and chilling duration for **a** *F. sylvatica*, **b** *T. cordata*, **c** *S. x smithiana*, and **d** *B. pubescens* grouped by forcing photoperiod. *Filled symbols* 16 h, *open symbols* 8 h. Data are from Experiment 3 (effect of chilling duration and forcing photoperiod). *Error bars* Standard error of the mean

Table 2 Results of analysis of variance (ANOVA). *P*-values for the effect of chilling duration and photoperiod on the time to budburst and percentage of budburst of *F. sylvatica*, *S. x smithiana*, *T. cordata* and *B. pubescens*. Data are from Experiment 3. Percentage budburst: all species analysed for main and interaction effects of chilling duration (0, 11, 30, 55 and 105 days) × photoperiod [long day (LD) and short day (SD)]. Time to budburst: due to missing blocks caused by absence of budburst in treatments receiving short chilling durations, subsets of data with observations in all cells were selected. *ChD* Chilling duration, *Pht* photoperiod

		Time to budburst	Percentage of budburst
F. sylvatica	ChD	<0.001	<0.001
	Pht	0.001	<0.001
	ChD*Pht	na	<0.001
S. x smithiana	ChD	<0.001	<0.001
	Pht	0.001	0.002
	ChD*Pht	0.002	0.002
T. cordata	ChD	<0.001	<0.001
	Pht	0.018	0.187
	ChD*Pht	0.079	0.648
B. pubescens	ChD	<0.001	<0.001
	Pht	<0.001	<0.001
	ChD*Pht	<0.001	<0.01

sigmoidal patterns (the t-test on model parameters yielded a significance of $P<0.05$, and the R^2 values of the fitted functions were≥0.90). The comparison between these response functions showed similarities between *F. sylvatica* and *T. cordata*, with rates of forcing increasing rapidly at low temperatures but slowly at the optimum. On the other hand, *B. pubescens* and *S. x smithiana* had rates of forcing that rose slowly at low temperatures and more rapidly as temperature neared 32°C. These similarities were confirmed by the similarity of the fitted parameters (Table 4). An F-test performed on model residuals confirmed that the models fitted to the data pertaining to different species were significantly different ($P<0.001$).

Effect of photoperiod

Forcing photoperiod significantly affected the timing of budburst in all the species tested ($P<0.05$), and percentage of budburst in *F. sylvatica* and *S. x smithiana* ($P<0.05$) but not in *T. cordata* ($P=0.187$) (Experiment 3, Table 2). Long days during the forcing period increased the percentage of budburst (Fig. 2) and decreased the time to budburst, especially after short chilling periods. This effect was confirmed by the significant interaction between chilling duration and photoperiod on budburst percentage (Table 2). The effect of photoperiod was evident especially for short chilling durations. In particular, *F. sylvatica* did not flush (0% budburst) under SD conditions unless it received at least 105 days of chilling, (Fig. 2a) while for *T. cordata* and *S. x smithiana* day length had little or no effect after 55 chilling days (Fig. 2b,c) . On the other hand, *F. sylvatica* plants that had received little or no chilling were able to flush in LD forcing conditions with percentages of budburst of 50% or more, while *S. x smithiana* and *T. cordata* showed a larger requirement for chilling, regardless of the forcing photoperiod. Similar to *F. sylvatica*, *B. pubescens* showed higher percentages of budburst in LD forcing conditions even after little or no chilling, but in accordance with the other two species, this effect was evident only after short chilling durations (Fig. 2d).

Table 3 Results of analysis of variance (ANOVA) showing *P*-values for the effect of forcing temperature (Experiment 2) and light intensity (Experiment 1) on the time to budburst of fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana* and *T. cordata*

Species	Forcing temperature	Light intensity
B. pubescens	<0.001[a]	0.071
F. sylvatica	<0.001	<0.001
S. x smithiana	<0.001	0.347
T. cordata	<0.001	0.008

[a] The effect of forcing temperature on *B. pubescens* was tested by Caffarra et al. (in review) and is reported for comparative purposes

Effect of light intensity

High light intensity advanced budburst by about 1 day in *B. pubescens* and *T. cordata* (from 2.8 to 1.8 days, and from 4.8 to 3.8 days, respectively). This effect was statistically significant for *T. cordata* ($P<0.01$) and almost significant for *B. pubescens* ($P=0.07$). High light intensity markedly advanced budburst (4.1 days on average) in *F. sylvatica* (from 11.3 to 7.2 days; $P<0.001$). On the other hand ,light intensity did not affect the timing of budburst in *S. x smithiana* cuttings ($P=0.35$), which flushed all at the same

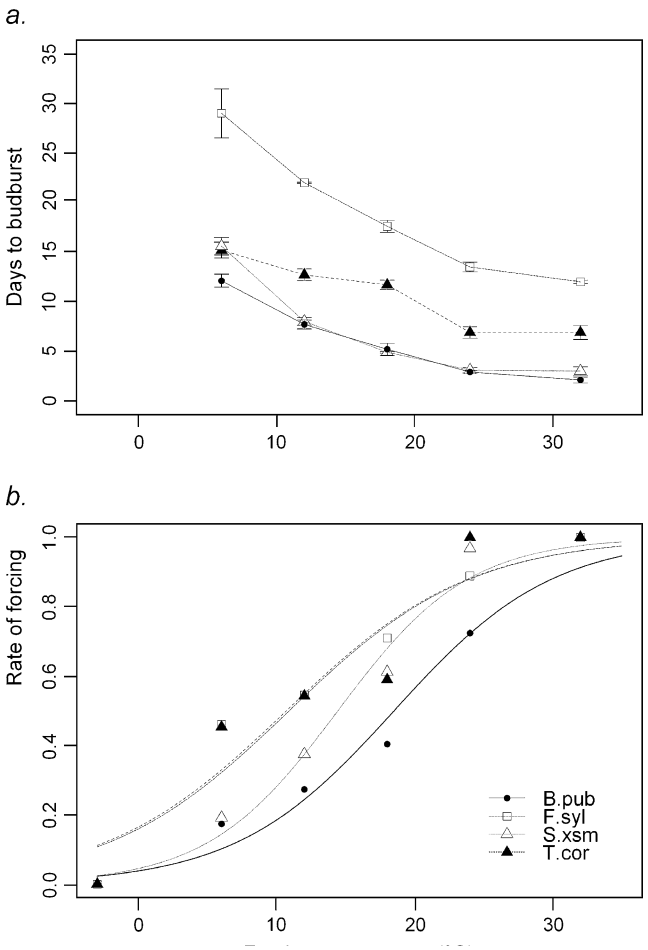

Fig. 3 **a** Days to budburst, and **b** rates of forcing in fully chilled one-node budsticks of *B. pubescens* (B.pub), *F. sylvatica* (F.syl), *S. x smithiana* (S.xsm) and *T. cordata* (T.cor). The data, from Experiment 2 (effect of forcing temperature), have been fitted with sigmoidal curves (*P*-values<0.01). *Vertical bars* Standard errors of the mean

time (4 days after the transfer into forcing conditions) regardless of the light intensity treatment (Table 3). The times to budburst of the four species in different light intensities are shown in Fig. 4.

Discussion

Effects and interactions of temperature and photoperiod during dormancy

It appeared evident that while, in all species studied, the release from dormancy was controlled by both photoperiod and chilling, the degree to which the two triggers contributed to this process varied among species. All species responded to increasing chilling duration or increasing photoperiod by advancing their time to budburst, with the largest effects in the early stages of chilling, in agreement with Cannell and Smith (1983), Murray et al. (1989) and Myking and Heide (1995). However, whereas in *F. sylvatica* LD was a complete or almost complete substitute for chilling, *S. x smithiana* and *T. cordata* had a more substantial chilling requirement for growth resumption. The current results are in contrast with the conclusions of a previous study by Heide (1993b), that both a substantial period of chilling and long photoperiod were required by *F. sylvatica* for release from endo-dormancy. In fact, in our case photoperiod was able to promote budburst despite the fact that the plants had been exposed to only a very short chilling period before the start of the experiment. In addition, we found that *F. sylvatica* exposed to SD was able to flush after receiving 105 days of artificial chilling, thus disproving the theory that *F. sylvatica* had a LD requirement for budburst. The contrast between our findings and those of Heide (1993b) could be due to the fact that while we tested whole plants he used cut twigs, which, as pointed out by Heide (1993b), use up their carbohydrate reserves at a fast rate and thus cannot be monitored reliably for long periods. Photoperiod has been shown to have a strong control on budbreak of *F. sylvatica* even in subtropical environments, where it never occurs before March despite the high winter temperatures (Borchert et al. 2005).

On the other hand, our results demonstrated clearly that both *T. cordata* and *S. x smithiana* need a more substantial chilling exposure to break endo-dormancy. Considering the 10-day exposure to temperatures below 12°C before the start of the experiment, such a requirement would correspond to a 21–40 chilling day period followed by LD forcing conditions for both species. Plants of *T. cordata* and *S. x smithiana* that did not receive sufficient chilling did not flush for the full 80-day period of monitoring, showing that their chilling requirements could not be substituted by LD or a long exposure to forcing conditions. In particular, if chilling was followed by SD forcing conditions, *T. cordata* required a longer chilling duration for growth resumption to take place compared to *S. x smithiana* (40–65 as opposed to 21–40 days of chilling). Various studies have shown an interaction between photoperiod and duration of exposure to chilling conditions on budburst timing also for *B. pubescens*, in which LD promoted growth after partial chilling exposure (Heide 1993a; Myking and Heide 1995; Partanen et al 2005). Our data confirm that *B. pubescens* is very sensitive to photoperiod. Similarly to *F. sylvatica* its budburst was promoted by LD even after short chilling durations but, unlike *F. sylvatica* and similar to *S. x smithiana*, it resumed growth in SD after relatively short chilling durations. Whereas the results relating to *T. cordata* and *B. pubescens* should be interpreted with some caution, as these two species were the most recently propagated (the depth of their endo-dormancy could have been affected by

Table 4 Coefficients and R^2 values of the sigmoidal curves (see Eq. 1) fitted to the rates of forcing of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*

	B. pubescens	*F. sylvatica*	*S. x smithiana*	*T. cordata*
R^2	0.97	0.96	0.98	0.90
a	-0.174	-0.152	-0.211	-0.151
b	18.5	10.87	14.43	10.76

propagation procedures), our results clearly indicate a comparatively higher chilling requirement and a lower photoperiod sensitivity in *T. cordata* than in *B. pubescens*.

Effect of forcing temperature

The response of the rate of forcing to increasing temperature can be described by a sigmoidal curve, as shown by previous studies (Caffarra and Eccel 2010; Sarvas 1974). The comparison between the fitted curve parameters revealed affinities between *T. cordata* and *F. sylvatica* on the one hand and between *B. pubescens* and *S. x smithiana* on the other. In fact, the rate of forcing of *T. cordata* and *F. sylvatica* showed a rapid increase at low temperatures, with inflection points of the fitted logistic curves around 11°C for both species, while those of *B. pubescens* and *S. x smithiana* rose slowly at low temperatures and more rapidly above 15°C, reaching their optima at higher temperatures. These response patterns suggested a higher sensitivity to high temperature in *S. x smithiana* and *B. pubescens*, which are the faster developing and earlier flushing species. Likewise, the responses of *F. sylvatica* and *T. cordata* suggested a higher sensitivity to temperatures below 15°C and a smaller response to heat waves at the time of growth onset. In addition, the number of days to budburst after transfer into forcing conditions was always lower in *S. x smithiana* and *B. pubescens* compared to *T. cordata* and *F. sylvatica*.

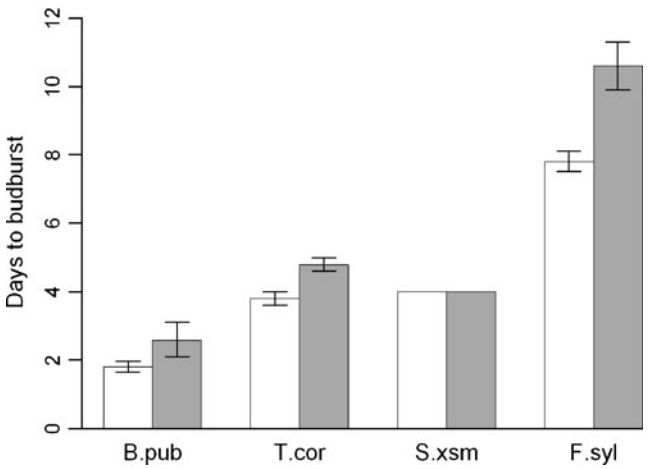

Fig. 4 Days to budburst in fully chilled one-node budsticks of *B. pubescens* (B.pub), *T. cordata* (T.cor), *S. x smithiana* (S.xsm) and *F. sylvatica* (F.syl) exposed to high and low light intensity (Experiment 1). *White bars* high light intensity, *grey bars* low light intensity, *error bars* standard errors of the mean

Effect of light intensity

Whereas the photon flux density applied in the high light intensity treatment was considerably lower than that observed on a sunny day, the present results showed that the response of phenology to even small variations in the level of light intensity differed in different tree species. Partanen et al. (2001) hypothesised that the promoting effect of light intensity on budburst was caused by the absorbed radiation energy during clear, sunny days. The present results contest this hypothesis, as the observed advance in budburst timing in *F. sylvatica*, *T. cordata* and *B. pubescens* was too large to be attributed to increased bud temperature alone. In fact our growth cabinets were ventilated, and the radiation energy produced by the applied light intensity treatments was far weaker than outdoors on a sunny day. In addition, the results from Experiment 2, where an increase in air temperature from 24 to 32°C produced only minimal or no advances in the budburst timings of *F. sylvatica* and *T. cordata*, suggest that, for these two species, the observed budburst advance in high light intensity was too consistent to be due only to increased bud temperature. Instead it might be related to a higher photosynthetic rate in the buds of *F. sylvatica* and *T. cordata*, two notably shade-tolerant species that can take advantage of very low light irradiances (Harbinson and Woodward 1984; Ellenberg 1988; Kazda et al. 2000). This hypothesis finds support in a study by Larcher and Nagele (1992), who observed that photosynthetic activity in *F. sylvatica* buds and twigs was resumed quickly by warm temperatures throughout the winter.

Ecological significance of phenology

The present analysis confirms the adaptive value of mechanisms that, in various ways, regulate dormancy and budburst in trees. For example, photoperiod sensitivity was particularly well developed in *B. pubescens*, which typically grows at northern latitudes where the differences in the light signal are strong and reliable indicators of the time of year. However, the current study shows that phenological adaptations are also related to the particular life strategy of each species, in agreement with Kramer et al. (2000), and Korner and Basler (2010) who recognised different types of phenological responses among temperate tree species to variation in temperature and photoperiod.

The response of *F. sylvatica* and *T. cordata* to environmental triggers that release them from dormancy confirms their conservative life strategy. While *F. sylvatica* counts on

a dual regulating system that minimises frost risk while ensuring budburst, both species respond to temperature "conservatively", with comparatively smaller increases in the rate of forcing at high temperatures and moderate to large chilling requirements for budburst in SD forcing conditions. This system makes budburst less sensitive to early spring heatwaves, which might be followed by late frosts and may be advantageous in a global warming scenario with extreme climate events during spring. On the other hand, opportunistic pioneer species, such as *B. pubescens* and *S. x smithiana*, showed generally smaller chilling requirements for budburst in SD forcing conditions, and a higher sensitivity to forcing temperature, as shown by their rates of forcing. These characteristics agree with their ecological role of rapidly colonising species of open and disturbed environments (Verwijst 2001; Sennerby-Forsse et al. 1992; Atkinson 1992). While budburst was promoted by LD even after minimal (< 2 weeks) chilling in *B. pubescens*, in *S. x smithiana* the photoperiod effect was more limited. These mechanisms might allow seedlings and juvenile trees of these shade-intolerant species to trade a higher risk of frost damage for the opportunity of vigorous growth at the beginning of spring, before canopy closure. Similarly, the marked response of *F. sylvatica* and *T. cordata* to increased light intensity could be due to the similarities in their ecology and their common successional role of dominant, shade-tolerant tree species being able to take advantage of very low light intensities such as those filtering through the bud scales. These results suggested that the sensitivity to environmental cues could have different implications in different species, depending on the interplay and the duration of these effects. For example, it is evident that both *B. pubescens* and *F. sylvatica* are affected by photoperiod, but while in *F. sylvatica* photoperiod seems to act as a limiting factor during the winter months, in *B. pubescens* short photoperiod prevents budburst only during a short period of time at the beginning of winter.

According to Korner and Basler (2010), long-lived late successional species might be less responsive to changes in climate due to their multiple, conservative controls of phenology, while opportunistic species may profit from a warmer climate by extending their growing season. The results from the current study support this latter view. The analysis of phenological series from the IPGs shows that the timing of budburst of *F. sylvatica* and *T. cordata* is already less sensitive to climatic variability than that of *B. pubescens*, with a smaller variation in budburst timing. In fact, over the decade 1981–1990 the mean standard deviation of budburst date for *B. pubescens* (averaged across the series from IPGs 14 in Ireland, 19 in Belgium, 23 in Germany and 46 in Switzerland) was 12 days, as opposed to 6 and 7 days for *F. sylvatica* and *T. cordata*. A meta-analysis of phenological series from all over Europe (Menzel et al. 2006) has shown a general advance in spring events of trees, presumably triggered by the recent increase in temperature, more pronounced for earlier species. As many early successional species such as *Betula*, *Salix* and *Prunus* sp. typically show an early budburst, this study could offer further support to the hypothesis that climate change affects the phenology of opportunistic tree species more than that of dominant species. However, due to the high number of factors affecting the ecology and phenology of plant species and to the preliminary nature of this work, more studies comparing different species and genotypes should verify this view.

Summary

The aim of this study was to compare budburst responses of different tree species under different environmental conditions, and to consider their ecological significance. Our results suggest that phenology is regulated by a complex interplay of factors whose effects are related to the life strategy and distribution of each species. In addition, environmental cues can have different effects in different species, depending on their interaction and duration. Dominant, long-lived species such as *T. cordata* and *F. sylvatica* showed lower rates of budburst (longer times to budburst), high chilling requirements and responsiveness to light intensity, while opportunistic, pioneer species such as *B. pubescens* and *S. x smithiana* had high rates of budburst (shorter times to budburst), low chilling requirements and were not affected by light intensity. In addition, budburst in *B. pubescens* and *S. x smithiana* was more responsive to high forcing temperatures than in *T. cordata* and *F. sylvatica*. It appeared evident that while in all species studied dormancy release was controlled by both photoperiod and chilling, the degree to which the two triggers contributed to the progress of dormancy varied among species. In particular, while in *F. sylvatica* and *T. cordata* short photoperiod was used as a signal to prevent growth until a moderate-large chilling exposure had been fulfilled, in *B. pubescens* short photoperiod did not prevent growth even after 30–40 days of chilling. On the other hand, in *S. x smithiana* the effect of photoperiod was more limited. These results suggest that the timing of growth onset in *B. pubescens* and *S. x smithiana* was regulated through a less conservative mechanism than in *T. cordata* and *F. sylvatica*, and that these species trade a higher risk of frost damage for the opportunity of vigorous growth at the beginning of spring, when more light is available. Nonetheless, the use of one-node cuttings and newly rooted cuttings (*B. pubescens* and *T. cordata*) as opposed to whole trees suggests that these results should be interpreted with caution. Whereas rooted cuttings and trees grown from

seeds show a similar phenology and growth rate in a number of tree species (Ritchie et al. 1992; Sasse and Sands 1996; Jurásek 2007), one-node cuttings might show shorter times to budburst compared to whole trees due to the lack of correlative inhibition (Chao et al. 2007). In addition, as these experiments used one specific tree clone for each species, intra-specific variability in phenology was not considered, so the present findings need further validation before they can be generalised. Thus, more studies evaluating different species and genotypes are needed. A more precise picture of the evolutionary drivers of phenology is important to assess potential responses of vegetation to climate change.

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