Forest Ecology and Management 485 (2021) 118813

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Chilling accumulation and photoperiod regulate rest break and bud burst in five subtropical tree species

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ARTICLE INFO

Keywords:
Annual cycle
Climatic adaptation
Climatic change
Endodormancy
False spring
Frost damage
Overwintering
Process-based modelling
Seasonality
Spring phenology

ABSTRACT

The environmental regulation of spring phenology in boreal and temperate trees is generally well-understood, but little is known about the regulation in subtropical trees. It has been shown recently that similarly to the more northern trees, subtropical trees also exhibit rest (endodormancy) and chilling requirement of rest break (chilling requirement of endodormancy release), but the effects of photoperiod remain largely unexplored. Here we did an experimental study of the effects of chilling accumulation and photoperiod on the occurrence (bud burst percentage, BB%) and timing (days to bud burst, DBB) of bud burst in five subtropical tree species growing commonly in subtropical China. In all of the five species examined, both chilling accumulation and photoperiod showed a significant effect on DBB, and several significant effects were found for BB%. The responses to chilling accumulation and photoperiod we found are thought to be adaptive to the conditions of relatively short and warm subtropical winters: first, an independent effect of photoperiod would reduce the risk of frost damage caused by a premature bud burst in the case of false springs, which are especially common in subtropical conditions. Second, an interaction of photoperiod with chilling accumulation would facilitate a timely bud burst in spring after an exceptionally warm winter with reduced chilling accumulation. On the basis of our findings, we put forward a conceptual model for the various effects of chilling accumulation and photoperiod on rest break and bud burst in subtropical trees. The model facilitates future efforts towards developing process-based spring phenology models for subtropical tree species. Our limited but novel results show that 1) the modelling needs to address the effects of photoperiod; 2) because of the large differences found in the responses among the five species examined, the model development needs to be based on species-specific experimental data.

1. Introduction

The timing of spring phenological events in trees plays a major ecological role in many forested ecosystems. It affects the ecosystems' carbon balance (Richardson et al., 2009; Keenan et al., 2014), the distribution patterns of species (Chuine, 2010; Dantec et al., 2014), and plant-animal interactions (Cohen et al., 2018). Numerous studies have shown that climate warming has accelerated the bud burst and growth onset of trees in spring (Chuine et al., 2000; Zheng et al., 2016), leading to an extended growing season and increased carbon uptake. However, other studies suggest that the response of bud burst and leaf-out to climate warming is conservative (Fu et al., 2015; Laskin et al., 2019) and that in some cases climate warming may even delay spring bud burst (Heide, 2003; Ford et al., 2016; Chen et al., 2017). A better understanding of the effects of environmental cues on phenological timing in spring would improve our ability to assess and project the effects of climate warming on the length of the growing season and the carbon sequestration potential of forests (Way, 2011).

In boreal and temperate trees, air temperature and, less clearly, photoperiod, are regarded as the main factors influencing spring phenological dates (Perry, 1971; Fuchigami et al., 1982; Cooke et al., 2012; Hänninen, 2016; Hänninen et al., 2019). Autumn and winter chilling temperatures cause rest break (endodormancy release), and high spring forcing temperatures cause the microscopic ontogenetic development of the buds towards bud burst. It has been known for a long time that in some boreal and temperate tree species, photoperiod interacts with temperature, so that long photoperiods compensate for any lack of chilling in rest break (Worrall and Mergen, 1967; Nienstaedt, 1967; Myking and Heide, 1995; Caffarra and Donnelly, 2011), but in other respects photoperiod has traditionally been considered to have a small, if any, role in regulating the rest break and subsequent bud burst of most boreal and temperate trees (Hänninen, 2016). More recently,

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https://doi.org/10.1016/j.foreco.2020.118813
Received 22 September 2020; Received in revised form 21 November 2020; Accepted 23 November 2020

however, increasing evidence has accumulated for a greater role of photoperiod (Basler and Körner, 2012; Fu et al., 2019a,b; Wang et al., 2020), but contrary to the role of chilling, there is no consensus on the effects of photoperiod on rest break and bud burst in boreal and temperate trees (Körner and Basler, 2010a,b; Chuine et al., 2010; Vitasse and Basler, 2013; Zohner et al., 2016).

The central role of chilling in the rest break of boreal and temperate trees is explained by its adaptive function in these northern conditions (Sarvas, 1974; Hänninen, 2016). Boreal and northern temperate regions generally have a long and cold autumn and winter period. In these conditions, chilling accumulation takes place from year to year, starting from autumn and continuing till the air temperatures rise again to the level causing ontogenetic development towards bud burst (forcing temperatures; Hänninen, 1990; Chuine et al., 1998). Thus the chilling requirement of rest completion protects trees from a phenomenon recently referred to as 'false spring' (Marino et al., 2011; Chamberlain et al., 2019), that is, a premature bud burst in a mild spell during the overwintering, leading to heavy frost damage in subsequent freezing periods (Cannell, 1985; Hänninen, 1991; Augspurger, 2013).

In contrast with the large number of studies addressing the environmental regulation of phenology in boreal and temperate trees, the roles of environmental cues on spring phenology in subtropical trees remains largely unexplored (Hänninen et al., 2019). With the exception of a few previous studies documenting the chilling requirement of rest break in subtropical horticultural fruit trees and fruit trees grown in subtropical areas (Erez, 2000; Lyrene, 2004; Sun et al., 2012), the chilling requirement in subtropical trees had not been studied experimentally until Du et al. (2019) and Song et al. (2020) recently demonstrated that native subtropical tree species also exhibit rest and a chilling requirement of rest break. They also found that the chilling requirement is lower in subtropical than in boreal and temperate trees.

Contrary to the boreal and northern temperate regions, winter is generally short and relatively warm in the southern temperate and especially in subtropical regions, but freezing winter temperatures nevertheless occur in these regions, too. This gives rise to the question whether the chilling requirement alone is sufficient to optimally synchronize bud burst and leaf-out with the seasonality of the climate in subtropical conditions. If not, what kind of role could photoperiod have in subtropical trees?

To examine the roles of chilling and photoperiod in the bud burst of subtropical trees, we subjected seedlings/twigs of five subtropical tree species to varying durations of natural chilling, then transferred them into forcing conditions, where a high temperature was combined with either short (SD) or long (LD) photoperiod. In the forcing conditions, the occurrence and timing of bud burst were observed, and the experimental results were used for testing the following four hypotheses: (1) Chilling is the driving force of rest break in subtropical trees, too. (2) Subtropical trees have a relatively low chilling requirement of rest completion. (3) Bud burst is regulated by photoperiod in subtropical trees, too, so that in comparison with LD, SD decreases the occurrence and/or delays the timing of bud burst. (4) Photoperiod interacts with chilling in subtropical trees, so that LD has a large effect on bud burst after short durations of chilling but after long durations of chilling its effect is minor, indicating that LD compensates for any lack of chilling and accelerates rest break in insufficiently chilled trees. The effects of chilling and photoperiod on bud burst in subtropical trees have only rarely been examined earlier, so that testing these four hypotheses will provide novel information that facilitates the efforts towards process-based modelling of the timing of bud burst in subtropical trees under climate warming (Chen et al., 2017; Hänninen et al., 2019).

2. Materials and methods

2.1. Experimental site and plant materials

The experiment was conducted in 2019–2020 on the Zhejiang A&F University campus (30°14′N, 119°42′E) in Hangzhou, southeastern China. The location has a subtropical, monsoonal climate. The mean annual precipitation is 1614 mm, the mean annual air temperature +15.6 °C, and the mean monthly air temperature +4.5 °C in January and +28.9 °C in July (Zhang, 2015). The experiments were carried out with five subtropical tree species growing commonly in subtropical southeastern China: *Cinnamomum chekiangense*, *Liriodendron chinense*, *Metasequoia glyptostroboides*, *Phoebe chekiangensis*, and *Torreya grandis*. Since it is practically impossible to conduct chamber experiments with adult trees, phenological experiments are generally conducted with seedlings or with twigs detached from adult trees (Vitasse and Basler, 2014; Partanen et al., 2016; Zohner et al., 2016). In the present study, seedlings were used with three tree species and twigs with two tree species.

One-year-old *Cinnamomum*, *Phoebe* and *Torreya* seedlings were transferred to the university campus from the nearby nursery of Tianmushan National Forest Station on 1 November 2019. All seedlings were propagated by seeds from native trees and were cultivated by standard nursery management practices (Zhang et al., 2015). After initial cultivation, the seedlings were transplanted into 3.8 L polyethylene pots filled with soil substrate containing 5 peat : 2 vermiculite : 1 perlite : 2 organic matter by volume (Universal potting soil, Hangzhou, China). The seedlings were kept in natural outdoor conditions on the campus until the beginning of the experiment.

Twigs of *Liriodendron* and *Metasequoia* were collected from 19-year-old mature trees grown on the campus. For both species, five sample trees were selected. In each sampling, an approximately 20-cm-long twig was randomly sampled and detached with scissors from the southward side of the middle part of the crown of each tree. After detaching, the twigs were disinfected with sodium hypochlorite solution (200 ppm active chlorine). The twigs were cut under water and were put in test tubes (3 cm in diameter and 20 cm in height) filled with tap water, in which 40 μg L^{-1} of the broad-spectrum antibiotic gentamicin sulfate was added (Zohner et al., 2016).

2.2. Experimental design

The rest break is a gradual process, requiring prolonged chilling. Accordingly, in order to examine the progress of rest break in the chilling conditions, groups of seedlings/twigs in the chilling conditions are randomly sampled after varying durations of chilling. Despite the recent advances in understanding the molecular and physiological basis of the rest (endodormancy) status of the buds (Cooke et al., 2012; Tylewicz et al., 2018; Zhang et al., 2018), no measurable marker is available yet for directly examining the rest status after chilling. That is why the rest status is examined indirectly by transferring the experimental seedlings/twigs after the chilling treatment to standard growth-promoting high temperature forcing conditions, where a regrowth test is carried out by observing the occurrence and timing of bud burst. In order to examine the effects of photoperiod on rest break, the regrowth test in the forcing conditions is carried out under both LD and SD.

The rest condition of the bud in the forcing conditions is manifested in one of two possible ways: either the bud will show no bud burst at all, despite prolonged forcing; or the bud burst takes longer than that of the buds of seedlings/twigs where rest has already been completed. Accordingly, two indices were calculated for each treatment group transferred from the chilling conditions to the forcing conditions at a given time: 1) Bud burst percentage, BB%, which indicates the percentage of seedlings/twigs showing bud burst out of the total number of twigs/seedlings in the treatment group. BB% addresses the variation in the chilling requirement among the experimental seedlings/twigs. 2) Days to Bud Burst (DBB), which indicates the number of days in the forcing conditions required for bud burst. It determines the rest status in quantitative terms, as the time required for bud burst (DBB) is often higher after partial rest break than after rest completion (Hänninen, 2016).

In the analysis of the results, the values of BB% and mean DBB are plotted against the duration of chilling. The progress of the rest break with successively longer durations of chilling is manifested as 1) increasing values of BB% and 2) decreasing values of mean DBB (Hänninen et al., 2019). The potential effects of photoperiod are seen as higher values of BB% and/or lower values of mean DBB under LD than SD forcing.

2.3. Experimental conditions

The seedlings and twigs were first exposed to varying durations of chilling in natural conditions and were then transferred into forcing conditions in growth chambers, where a regrowth test was carried out (see above). Air temperature was recorded hourly in both outdoor natural conditions and the growth chambers with iButton Data Loggers (Model DS1912L, Embedded Data Systems Co., Ltd, KY, USA). The average daily temperature at the experimental site was still relatively high (>10 °C) in early November (see Fig. S1), and leaf senescence in the deciduous species *Liriodendron* and *Metasequoia* generally occurred in mid to late November. Therefore we started the experiment on 9 December 2019, when the average daily temperature had dropped below 10 °C, so that chilling accumulation had started.

Forcing took place in four growth chambers (Model MGC-450HP, Yiheng Technology Co., Shanghai, China), under either a short day (SD, 10 h) or a long day (LD, 14 h) treatment, which approximated the annual minimum and maximum day lengths at the experimental site on the winter (10.2 h) and the summer (14.1 h) solstice (Du et al., 2019; Song et al., 2020). Two replicate chambers were used for both photoperiodic treatments. In all chambers, air temperature was set at a constant 20 °C, RH at 70%, atmospheric concentration of CO_2 at 300–400 ppm, and photon flux density during the light periods at 400 μmol m^{-2} s^{-1}. The forcing temperature in the four chambers throughout the experiments showed no statistically significant difference between the two LD chambers (19.98 ± 0.99, 20.03 ± 1.02 °C) or the two SD chambers (20.01 ± 0.98, 19.99 ± 1.01 °C).

The seedlings and the twigs were transferred from natural outdoor conditions to LD and SD forcing conditions on 9 Dec, 16 Dec, 23 Dec 2019 and on 6 Jan, 20 Jan, 2 Feb and 18 Feb 2020, thus creating seven durations of chilling: 0, 1, 2, 4, 6, 8 and 10 weeks. In each transfer, 10 replicated seedlings/twigs were transferred to both SD and LD forcing chambers. The seedlings were watered every third day to keep the growth medium moist. For the twigs, we changed the water in the test tubes every fourth day, and each time this was done we also cut the cut end of the twig under water once more. Observations of bud burst in the forcing conditions were stopped on 20 May 2020.

2.4. Determination of bud burst

For each species, we identified four phenological stages in the development of the buds: bud closed, bud swelling, leaf emergence, and leaf unfolding. Bud burst was determined on the basis of attaining the last stage (leaf unfolding), but the earlier three stages were also observed in order to improve the accuracy of identifying the fourth stage. Bud development was observed every third day. In *Torreya* seedlings, the terminal bud and its adjacent lateral buds were observed, and bud burst was determined as the first day when 50% of the buds had reached the fourth stage. For *Cinnamomum* and *Phoebe* seedlings, only the terminal bud was observed since no lateral buds are formed in these seedlings. For *Liriodendron* twigs, both terminal and lateral buds were observed, but bud burst was determined on the basis of observing the terminal bud alone, since in most cases only the terminal buds showed bud burst as identified with the aid of the fourth developmental stage.

Metasequoia twigs have an exceptionally large number of buds. In the sampling the twigs, our purpose was to cut each twig so that it had exactly ten healthy buds. In a few twigs we noticed 1–3 additional small and shriveled buds after the sampling. They were carefully removed before starting the experimental treatments. Bud burst in *Metasequoia* twigs was determined in terms of the first day when three buds on the twig had reached the last stage (leaf unfolding). For the determination of bud burst in *Metasequoia* twigs, then, the percentage of observed buds required to show bud burst (30%, or 3 out of the 10 observed buds) was lower than the corresponding percentage for *Torreya* seedlings (50%, see above). This was motivated by the exceptionally high number of buds in the *Metasequoia* twigs and their peculiar developmental pattern observed in the forcing conditions: the buds bursting first developed into the leaf expansion phase rapidly, while the buds bursting later developed much more slowly. Accordingly, the first buds already had well-developed leaves at the time when the later buds attained the phase of leaf unfolding used as the criterion of bud burst. At the twig level, then, the percentage of 30% used for *Metasequoia* was a better criterion than the percentage of 50% for the early phase of vegetative development observed with the other tree species.

2.5. Data analysis

For each seedling/twig showing bud burst, the number of days to bud burst (DBB) in the forcing conditions was counted to quantify the timing of bud burst, and the bud burst percentage, BB%, was calculated to quantify the occurrence of bud burst in the treatment (see Experimental design above). In addition, because of the exceptionally high number of buds in the *Metasequoia* twigs, we counted the number of burst buds on each twig and then calculated the average for the twigs in each treatment.

In order to examine the effects of chilling we determined, for each treatment, the chilling accumulation at the time of the transfer from natural chilling conditions to forcing conditions. Chilling accumulation was calculated from 9 December (the date of the first transfer) to the date of transferring the seedlings/twigs from natural chilling conditions to forcing conditions. The unit used was the number of hours with the hourly temperature falling within the rest breaking temperature range. For temperate trees, the range of 0 to +5 °C has often been regarded as effective in rest break (Ma et al., 2018; Chen et al., 2018, 2019; Fu et al., 2019a,b), but for subtropical trees, higher temperatures have also been found effective, with +10 °C showing an effect similar to that of the lower temperatures (Zhang et al., 2021). Accordingly, chilling accumulation was calculated for the ranges of both 0 to 5 °C and 0 to 10 °C. As similar results were obtained for both of the two ranges, only the results obtained for the range of 0–10 °C are reported.

The forcing requirement of bud burst is usually determined by calculating the accumulation of degree hours or some other type of forcing unit for the time required for bud burst in the forcing conditions (Sarvas, 1974; Murray et al., 1989; Hänninen, 1990). However, as we had only one constant forcing temperature in our study, we determined the forcing requirement simply as the number of days to bud burst, DBB, in the forcing conditions.

In each of the five species, differences in BB% among the treatments were analyzed by logistic regression with a binary response (bud burst/no bud burst; Partanen et al., 2016). The explanatory variables were the duration of chilling and the forcing photoperiod. Interactions among the explanatory variables were also examined. Differences in DBB among the treatments were analyzed in each of the five species with a two-way analysis of variance (ANOVA) (Partanen et al., 2016). For the response variable, a log transformation of DBB was used, and the explanatory variables were the duration of chilling and the forcing photoperiod here as well. In addition, all interactions between the two explanatory variables were examined. All statistical analyses were carried out with the SPSS (Version 16.0, SPSS Inc., Chicago, USA).

3. Results

3.1. Effects of chilling and photoperiod on the occurrence of bud burst, BB %

The effects of chilling and photoperiod on bud burst percentage (BB %) showed considerable differences among the five subtropical tree species examined (Table 1; Fig. 1a). In *Phoebe* seedlings, BB% was 100% in all treatments, showing no sensitivity to either chilling or photoperiod. In *Metasequoia* twigs and *Cinnamomum* and *Torreya* seedlings, BB% was significantly affected by chilling, so that BB% generally increased with prolonged duration of chilling (Fig. 1a). Notably, most of the examined species achieved high values of BB% even after short durations of chilling, especially under LD conditions, in which *Metasequoia* twigs and *Cinnamomum* and *Phoebe* seedlings even reached BB% values over 90% even after short durations of chilling (Fig. 1a). These findings suggest that subtropical trees generally have a low chilling requirement for the occurrence of bud burst.

The effects of photoperiod and/or its interaction with chilling on BB % were significant in three cases: *Liriodendron* twigs and *Cinnamomum* and *Torreya* seedlings (Table 1). Whenever there was a difference between the BB% values observed under the two photoperiods, the value was higher under LD than under SD (Fig. 1a). In *Liriodendron* twigs, BB% was highly sensitive to photoperiod. Regardless of the duration of chilling, BB% under LD conditions was always higher than 50% in this species, but under SD conditions it was lower than 20% (Fig. 1a; Fig. 2ab). In *Cinnamomum* seedlings, too, BB% was independently affected by photoperiod, being generally higher under LD than under SD. In *Torreya* seedlings, BB% was affected by a significant interaction of photoperiod with chilling. In *Metasequoia* twigs, BB% was not significantly affected by photoperiod, but the value of BB% was reduced under SD with short durations of chilling (0 and 1 weeks; Fig. 1a). In comparison with LD, SD also significantly reduced the number of bursting buds in each individual twig of *Metasequoia* (Fig. 3).

3.2. Effects of chilling and photoperiod on the timing of bud burst, DBB

In all of the five species examined, DBB was significantly affected by both chilling and photoperiod (Table 2; Fig. 1b). Prolonged chilling generally decreased DBB in all species, and lower values of DBB were observed under LD than under SD (Fig. 1b). The interaction of chilling and photoperiod on DBB was significant in *Cinnamomum* seedlings and *Metasequoia* twigs, where the difference in DBB between LD and SD treatment became smaller with prolonged chilling (Table 2; Fig. 1b), suggesting that the role of photoperiod became less important when the seedlings/twigs underwent sufficient chilling. In *Metasequoia* twigs, for instance, SD largely delayed bud burst after one week of chilling (Fig. 2c), but after 8 weeks of chilling its effect was minor (Fig. 2d). Contrary to *Cinnamomum* seedlings and *Metasequoia* twigs, the effects of chilling and photoperiod on DBB in *Liriodendron* twigs and *Phoebe* and *Torreya* seedlings were independent of each other (Table 2), so that regardless of the duration of chilling, higher DBB was observed under SD than under LD (Fig. 1b). The analysis of relationships among the forcing requirement, chilling accumulation and photoperiod brought further evidence of the important role of photoperiod in regulating bud burst, as in most cases photoperiod affected the forcing required at a given chilling accumulation (Fig. 4).

Table 1
A logistic regression analysis with a binary response of the factors affecting the bud burst percentage, BB%, in seedlings of *Cinnamomum chekiangense* and *Torreya grandis*; and twigs of *Liriodendron chinense* and *Metasequoia glyptostroboides*, in forcing conditions after chilling. The analysis was not applied to *Phoebe chekiangensis*, as the value of BB% in that species was 100% in all treatments.

Species	Chilling *P*	Photoperiod *P*	Chilling × Photoperiod *P*
Cinnamomum	**0.002**	**0.021**	0.805
Liriodendron	0.66	**0.018**	**0.041**
Metasequoia	**0.035**	0.998	0.999
Torreya	**<0.0001**	0.556	**0.02**

4. Discussion

4.1. Quantifying the effects of chilling and photoperiod on rest break and bud burst

Our aim was to examine the effects of chilling and photoperiod on rest break and subsequent bud burst in five subtropical tree species. Understanding these effects is crucial for projecting the effects of climate warming on the spring phenology of subtropical forested ecosystems. Our results show that both chilling and photoperiod can, either individually or interactively, affect rest break and bud burst in subtropical trees, but the roles of chilling and photoperiod showed differences among the five species examined.

To get a comprehensive picture of the roles of chilling and photoperiod, we examined their effects on two indices (Hänninen, 1990, 2016; Hänninen et al., 2019). First, the occurrence of bud burst as quantified with the index bud burst percentage, BB%, indicates the proportion of the buds (seedlings/twigs) in which rest break has taken place sufficiently to allow the buds to burst during the relatively long experimental forcing period applied in the study. After this first chilling requirement is met for a given bud, further chilling may still accelerate its rate of ontogenetic development towards bud burst, shortening the time to bud burst in the forcing conditions until the rest is fully completed (timing of bud burst as quantified with days to bud burst, DBB). Similarly to prolonged chilling, long photoperiods may facilitate rest break as manifested by higher BB% values and/or lower DBB values under LD than under SD.

4.2. Effects of chilling

Our results showed, in support of our first hypothesis, that increases in the duration of chilling decreased DBB in all of the five subtropical tree species examined (Fig. 1; Table 2), indicating that all of them have a chilling requirement of rest break (Hänninen et al., 2019). This is in line with recent findings for other subtropical tree species (Du et al., 2019; Song et al., 2020). In most cases, BB% also showed a response to chilling, generally increasing with prolonged chilling (Fig. 1; Table 1). In *Phoebe* seedlings, however, BB% was 100 in all treatments, showing that buds of this species are able to burst even without any chilling. Furthermore, in the LD treatment of *Liriodendron* twigs and the SD treatment of *Torreya* seedlings, a transient drop of BB% was observed after 4 and 6 weeks of chilling. A similar pattern has been found earlier for *Picea abies* by Partanen et al. (2005). They suggested that after the initial rest completion there is a secondary rest period, which is broken by additional chilling later on. Our results, however, do not support this hypothesis, for in our study the values of DBB decreased monotonously with increased durations of chilling (Fig. 1). This suggests that the transient drops in the BB% values in our study may have been caused by unnoticed shortcomings in implementing the experiment (Partanen et al., 2020).

In the present study, relatively short durations of chilling were sufficient to cause subsequent bud burst in the forcing conditions for a large part of the seedlings, as indicated by the rapid increase in the BB% values obtained with 0 to 2 weeks of chilling already (Fig. 1). This accords with our second hypothesis, which posited a relatively low chilling requirement of rest completion for subtropical trees. Previous work has shown that boreal trees generally require longer chilling for increases in the BB% values (Hänninen, 1990; Hänninen and Pelkonen, 1989; Partanen et al., 2020). This also accords with the findings of Du et al. (2019). However, considerably longer chilling periods were required in the present study for the levelling off of the DBB curves (Fig. 1), which is

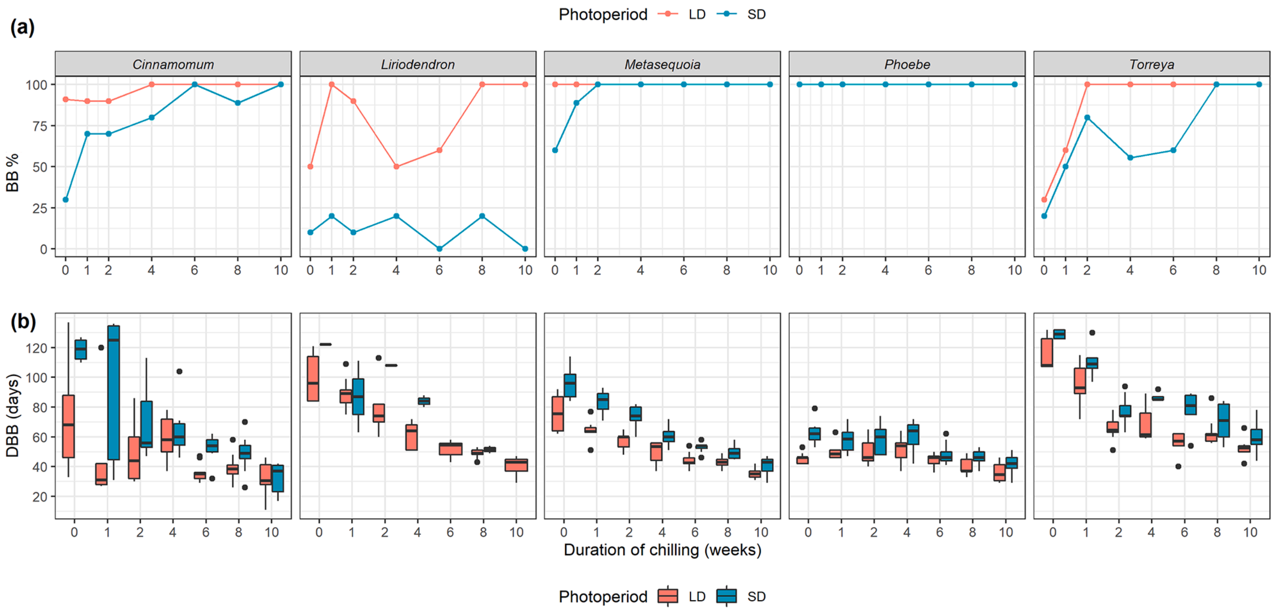

Fig. 1. (a) Bud burst percentage, BB%, and (b) days to bud burst, DBB (mean ± SE), of seedlings of *Cinnamomum chekiangense*, *Phoebe chekiangensis*, and *Torreya grandis*; and twigs of *Liriodendron chinense* and *Metasequoia glyptostroboides* in an experiment where the seedlings/twigs were transferred to forcing conditions with 20 °C for either a long day (LD, 14 h day length) or a short day (SD, 10 h day length) treatment after different durations of chilling in natural conditions. The LD and SD forcing treatments were designed with reference to the natural seasonal variation of day length at the subtropical growing sites of the tree species examined. In the forcing conditions, bud burst was determined on the basis of the occurrence of leaf unfolding. For details, see Material and methods.

a prerequisite for full rest completion (Worrall and Mergen, 1967; Leinonen et al., 1995; Leinonen, 1996; Myking, 1997, 1998; Hänninen et al., 2019). Thus our results concerning DBB do not provide clear support for our second hypothesis of the smaller chilling requirement in subtropical than in more northern trees. Furthermore, any comparison of the chilling requirements of trees from different climatic zones is complicated by the differences in the effective chilling temperature ranges of the tree species (Zhang et al., 2021).

4.3. *Effects of photoperiod*

Our results showed, in support of our third hypothesis, that in four out of the five subtropical tree species examined, photoperiod played an important role in the occurrence of bud burst as quantified by BB%. This was especially true for *Liriodendron* twigs, for regardless of the chilling duration, very few twigs of that species achieved bud burst under the SD treatments, which suggests a strong photoperiodic control of bud burst. In the other species, too, BB% was generally lower under SD conditions than under LD conditions (Fig. 1). *Phoebe* seedlings, however, were an exception in that the occurrence of bud burst was not affected by either chilling or photoperiod. Our third hypothesis was further supported by the finding that long photoperiod significantly decreased DBB in all the species examined (Fig. 1; Table 2). These findings suggest that LD can either promote rest break or otherwise accelerate ontogenetic development in subtropical trees. Finally, our third hypothesis was also supported by the higher number of bursting buds in *Metasequoia* twigs under LD than under SD (Fig. 3).

In contrast to our results, Du et al. (2019), using detached twigs in their experiment, found sensitivity of rest break and bud burst to photoperiod in only 2 of the 33 subtropical tree species they examined. They did not study the same species as we did, but this will hardly explain the difference. Rather, if the effect of photoperiod is as common in subtropical tree species as suggested by our results based on a sample of five species, then it should also have been seen in the larger sample of species included in Du et al.'s (2019) study. One can only speculate on the reason for this difference, but the high mortality in Du et al.'s (2019) study, as well as their relatively limited number of chilling treatments, may have interfered with their results. Our experiment, on the contrary, was conducted with both seedlings and twigs, and little or no mortality in either set of experimental material was observed. In all, then, despite the disagreement with the results of Du et al. (2019), we suggest that photoperiod is an important cue for bud burst in subtropical trees. This is in line with Zohner et al. (2016), who suggested that trees growing at relatively low latitudes are sensitive to photoperiod.

For a long time, chilling has been regarded as the main driving force of rest break in boreal and temperate trees (Perry, 1971; Sarvas, 1974; Fuchigami et al., 1982; Hänninen, 1990). In line with that notion, our results suggest that the role of photoperiod in regulating rest break and bud burst is greater in subtropical than the boreal and temperate trees. These results are also supported by our discussion of the climatic adaptation of subtropical trees below. Recently, however, more evidence for the role of photoperiod in these more northern trees has accumulated (Basler and Körner, 2012; Fu et al., 2019a,b; Wang et al. 2020), but contrary to the role of chilling, there is no consensus on the effects of photoperiod on rest break and bud burst in boreal and temperate trees (Körner and Basler, 2010a,b; Chuine et al., 2010; Vitasse and Basler, 2013; Zohner et al., 2016). In order to get conclusive results on the potential difference between the subtropical and the more northern trees in regard to the role of photoperiod in regulating rest break and bud burst, comparative experimental studies, including species from the various climatic zones in the same experiments, are called for.

4.4. *Interactive effects of chilling and photoperiod*

For *Cinnamomum* seedlings and *Metasequoia* twigs, our experimental results provided support for our fourth hypothesis that photoperiod interacts with chilling in regulating bud burst. In these two species, BB% was generally higher and DBB generally lower in LD than in SD forcing after short durations of chilling, but with longer chilling durations these differences largely disappeared (Fig. 1). Earlier on, interactions of this kind have been found for many boreal and temperate tree species (Worrall and Mergen, 1967; Nienstaedt, 1967; Myking and Heide, 1995; Caffarra and Donnelly, 2011). For DBB the interaction was significant

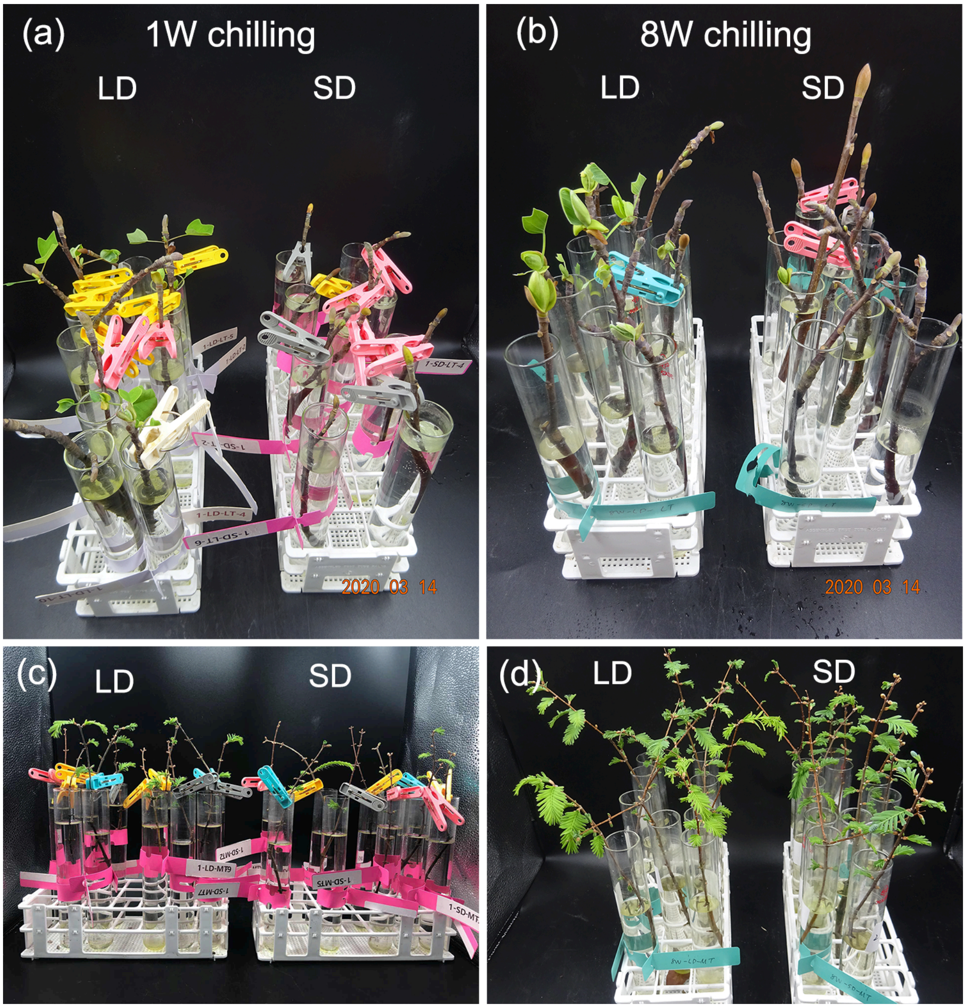

Fig. 2. Examples of two degrees of photoperiod sensitivity of rest break and bud burst in subtropical tree species. The long day (LD = 14 h) and short day (SD = 10 h) forcing treatments were designed with reference to the natural seasonal variation of day length at the subtropical growing sites of the tree species examined. In (a, b) *Liriodendron chinense* twigs, very few bud bursts were observed under SD forcing, no matter whether the experimental twigs were exposed to a short (a, one week) or a long (b, eight weeks) chilling before the forcing. Thus *Liriodendron chinense* showed high sensitivity to photoperiod. In (c, d) *Metasequoia glyptostroboides* twigs, the effect of forcing photoperiod on bud burst was large after short (c, one week) chilling but minor after long (d, eight weeks) chilling. *Metasequoia glyptostroboides*, then, showed moderate sensitivity to photoperiod. The photos were taken on 14 March 2020.

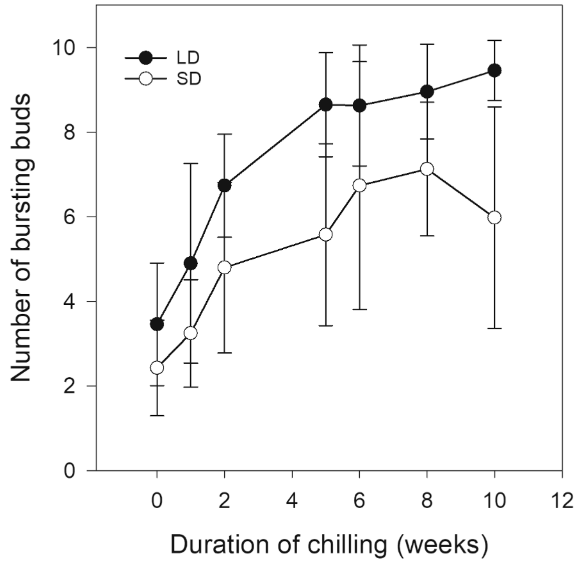

Fig. 3. Effects of the duration of chilling and photoperiod on the average number of bursting buds in each individual *Metasequoia glyptostroboides* twig. The long day (LD = 14 h) and short day (SD = 10 h) forcing treatments were designed with reference to the natural seasonal variation of day length at the subtropical growing sites of the tree species examined. The number of bursting buds was counted at the end of the experiment on 20 May 2020.

(Table 2), but for BB% it was not (Table 1). The interactive pattern was also seen clearly in the BB% curves for both species, as the two curves representing LD and SD converged with long durations of chilling (Fig. 1). However, the interaction in BB% was not significant for either species because the convergence took place either after a long (*Cinnamomum*) or short (*Metasequoia*) duration of chilling, so that the BB% value for the two photoperiods was in most cases different (*Metasequoia*) or the same (*Cinnamomum*) (Fig. 1).

The fourth hypothesis was not supported for the other tree species because the interaction of photoperiod and chilling assumed by the hypothesis was not found in them. A different type of significant interaction was found in the BB% values of *Liriodendron* twigs and *Torreya* seedlings (Fig. 1; Table 1), but as stated above, the transient drops of BB % causing the interaction in these species were probably caused by some unnoticed shortcomings in implementing the experiment (Partanen et al., 2020).

4.5. *Climatic adaptation of subtropical trees – a conceptual model*

Synchronization of the annual cycle of growth and dormancy with the annual climatic cycle is essential in the climatic adaptation of all tree species growing in locations where air temperature shows strong seasonal variation with freezing temperatures occurring in winter (Sarvas, 1972, 1974; Fuchigami et al., 1982; Cooke et al., 2012; Hänninen, 2016). As compared with the more northern conditions, the winters in the subtropical zone are relatively shorth and warm. This has two important implications for the overwintering of subtropical trees: First,

Table 2
A two-way analysis of variance of the factors affecting the days to bud burst, DBB, of seedlings of *Cinnamomum chekiangense*, *Phoebe chekiangensis*, and *Torreya grandis*; and twigs of *Liriodendron chinense* and *Metasequoia glyptostroboides*, in forcing conditions after chilling.

Species	Chilling		Photoperiod		Chilling × Photoperiod	
	F	*P*	*F*	*P*	*F*	*P*
Cinnamomum	**11.777**	**<0.0001**	**25.265**	**<0.0001**	**2.874**	**0.013**
Liriodendron	**35.615**	**<0.0001**	**16.502**	**<0.0001**	1.236	0.311
Metasequoia	**106.981**	**<0.0001**	**106.139**	**<0.0001**	**3.46**	**0.003**
Phoebe	**16.796**	**<0.0001**	**52.588**	**<0.0001**	1.876	0.09
Torreya	**48.366**	**<0.0001**	**41.722**	**<0.0001**	1.473	0.196

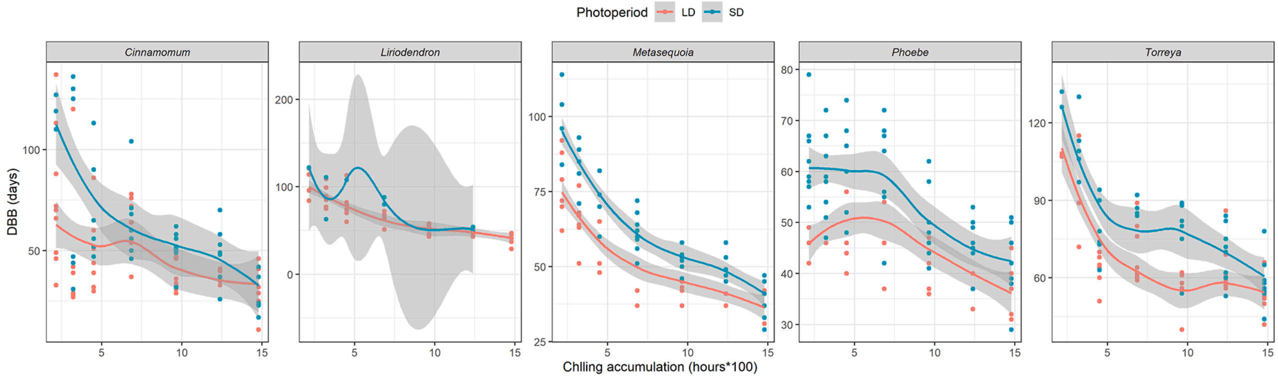

Fig. 4. Effects of chilling accumulation and photoperiod on the forcing requirement of bud burst in seedlings of *Cinnamomum chekiangense*, *Phoebe chekiangensis*, and *Torreya grandis*; and twigs of *Liriodendron chinense* and *Metasequoia glyptostroboides* in an experiment where the seedlings/twigs were transferred to forcing conditions with 20 °C for either a long day (LD, 14 h day length) or a short day (SD, 10 h day length) treatment after different durations of chilling in natural conditions. The forcing requirement is represented here by the vertical axis variable days to bud burst, DBB. The long day (LD = 14 h) and short day (SD = 10 h) forcing treatments were designed with reference to the natural seasonal variation of day length at the subtropical growing sites of the tree species examined. Chilling accumulation was calculated as the number of hours with air temperature in the range 0–10 °C. The grey shading indicates the 95% confidence interval. For details, see Material and methods.

as both high temperatures of up to +20 °C and freezing temperatures occur regularly during the overwintering period, the risk of false springs causing a premature bud burst and subsequent frost damage is especially pronounced in the subtropical conditions. Second, due to the generally warm and short winters, there is a high risk of insufficient chilling in subtropical conditions, causing the delay, or even the prevention, of bud burst during spring.

Our results, even though being based so far on relatively limited experimental data, highlight several potentially important aspects of climatic adaptation of subtropical tree species to their particular overwintering conditions. The small chilling requirement for the occurrence of bud burst (BB%) is an obvious adaptation to the relatively small chilling accumulation in the subtropical conditions. Accordingly, limited chilling will not prevent bud burst, even though it may delay it, as suggested by the higher chilling requirement for DBB found in the present study.

In order to facilitate further experimental studies addressing this relatively unexplored topic, based on our results we put forward a conceptual model for the effects of chilling and photoperiod on rest break and bud burst in subtropical trees (Fig. 5). For both occurrence (BB%) and timing (DBB) of bud burst, photoperiod may have either an independent effect (Körner and Basler, 2010; Basler and Körner, 2012), or it may interact with chilling (Worrall and Mergen, 1967; Nienstaedt, 1967; Myking and Heide, 1995; Caffarra and Donnelly, 2011). The independent effect prevents (BB%), or delays (DBB), bud burst regardless of the amount of accumulated chilling; whereas the interactive effect disappears with chilling accumulation. This is why we refer to the independent and interactive effects with the concepts of high and moderate photoperiod sensitivity, respectively (Fig. 5).

In our previous study, we found that chilling requirement is generally fully met in Mid-January in most subtropical tree species (Zhang et al., 2021). However, false springs frequently occur in subtropical areas after that. The high sensitivity to photoperiod would prevent (high sensitivity of BB%, *Liriodendron* twigs) or delay (high sensitivity of DBB, *Liriodendron* twigs and *Phoebe* and *Torreya* seedlings) bud burst during warm winters (Fig. 1), thus reducing the risk of frost damage caused by false springs. The moderate sensitivity, on the contrary, makes timely bud burst in spring possible also when there has been insufficient chilling accumulation (Campbell, 1978; Kramer, 1994). Based on our results, this mechanism works in *Cinnamomum* seedlings and *Metasequoia* twigs (Fig. 1). In brief, the high sensitivity to photoperiod prevents a too early and the moderate sensitivity a too late, bud burst, respectively, under the conditions of short and relatively warm subtropical winters.

In *Phoebe* seedlings, we found no sensitivity of BB% to either chilling or photoperiod, and also the decrease of DBB caused by chilling, or by LD, as compared with SD, was relatively minor in them (Fig. 1). This implies that the rest in *Phoebe* seedlings is exceptionally shallow. This would make *Phoebe* as exceptionally susceptible to frost damage as a result of false springs. Indeed, Li et al. (2005) reported that freezing injury frequently occurs in *Phoebe* in Nanjing, a location in subtropical China.

Process-based tree phenology models are currently often used in climatic change impact assessments for boreal and subtropical trees (Hänninen and Kramer, 2007; Chuine et al., 2013; Hänninen et al., 2019). Our results suggest that when the scope of the process-based modelling is broadened to cover subtropical trees (Chen et al., 2017), then the effect of photoperiod needs to be included in the models. Our conceptual model provides a good starting point for such modelling studies. However, more experimental studies are needed before the effects of photoperiod can be introduced into the process-based tree phenology models. This is because the models include quantitative

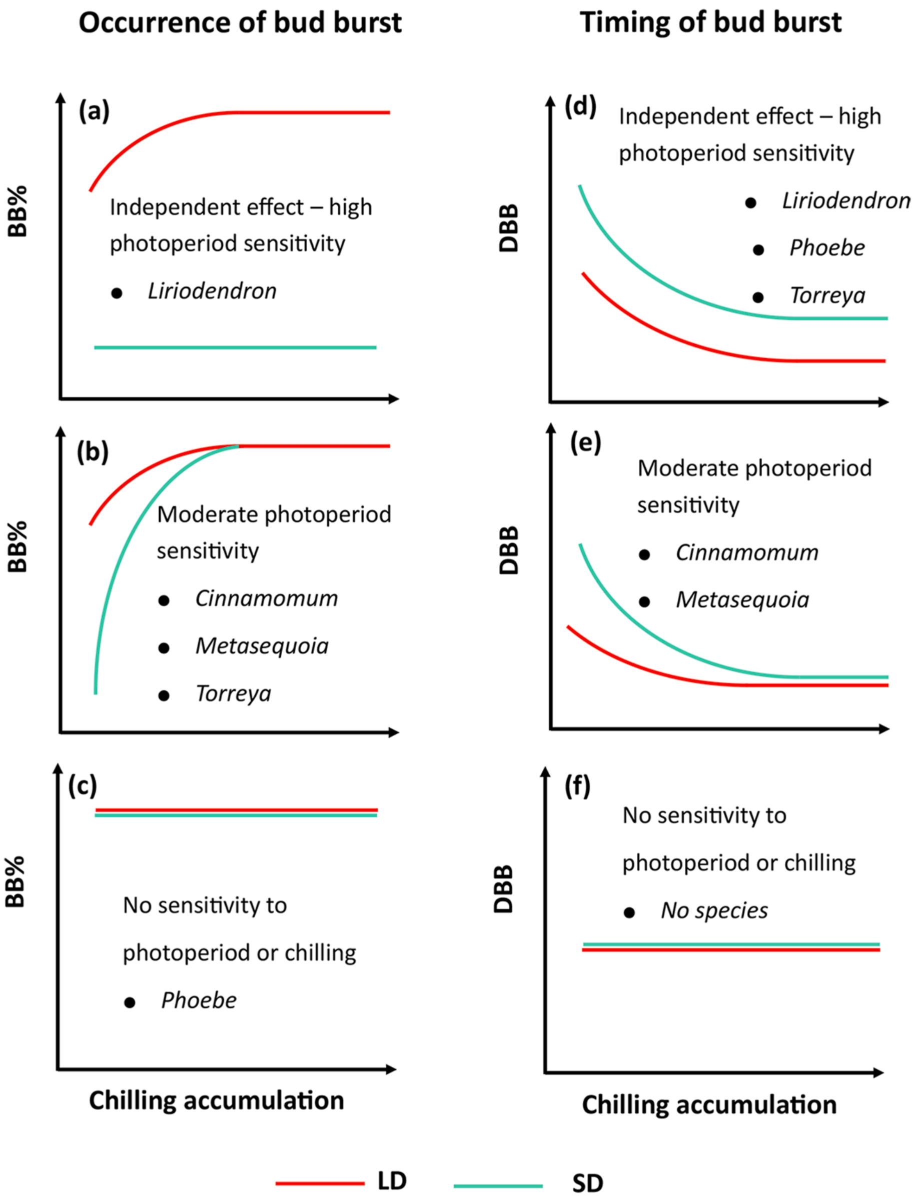

Fig. 5. A conceptual model summarizing the effects of photoperiod and chilling accumulation on rest break and bud burst in subtropical trees as manifested in the present study. The effects on both (a-c) the occurrence (bud burst percentage, BB%) and (d-f) the timing of bud burst (days to bud burst, DBB) are addressed in the model. An independent effect of photoperiod shows up regardless of the year-to-year variation in chilling accumulation and is therefore designated as a high photoperiod sensitivity (a, d). An interactive effect is realized only when there is insufficient chilling accumulation and is therefore designated as a moderate photoperiod sensitivity (b, e). Both degrees of photoperiod sensitivity facilitate adaptation to the relatively warm subtropical winters with the air temperature often varying from freezing temperatures to forcing temperatures of up to +20 °C. High photoperiod sensitivity reduces the risk of frost damage caused by false springs, whereas moderate photoperiod sensitivity facilitates timely bud burst in springs following an exceptionally warm winter with little chilling accumulation. If both BB% (c) and DBB (f) are unaffected by either photoperiod or chilling accumulation, then the trees have no rest (endodormancy), and bud burst is regulated by the forcing temperature alone. Such species were not found in the present study. For details and previous references to studies of temperate and boreal tree species, see Discussion.

responses to the climatic factors addressed (Hänninen and Kramer, 2007; Chuine et al., 2013; Hänninen et al., 2019). Thus, in order to include the effect of photoperiod in the models, the classical dichotomy of LD and SD is not sufficient. Rather, one needs to have experimental data for the responses to a range of different photoperiods.

Due to practical limitations in our experiments, three species (*Cinnamomum*, *Phoebe*, and *Torreya*) were represented by seedlings only and two species (*Liriodendron* and *Metasequoia*) by twigs detached from adult trees only. No consistent patterns based on whether the species was represented by seedlings or twigs were found among the five species examined. For instance, the two species represented by twigs showed drastically different sensitivities to photoperiod (Fig. 2). This suggests that the differences found among the five species are true differences among the species, not between the seedlings and the adult trees. However, on the basis of our results one cannot exclude the possibility that there may also be differences in the responses between seedlings and adult trees of any one species.

5. Conclusions

Our experimental results provide a first-time evaluation of the effects of chilling and photoperiod on rest break and bud burst in five subtropical tree species. The results suggest that despite the differences among the species, bud burst in all the five subtropical tree species examined is affected by both chilling and photoperiod, either independently or interactively. This contrasts with many boreal and temperate trees, in which the role of photoperiod in regulating spring phenology remains controversial. The difference is probably due to the climatic differences among the respective geographical zones. In the subtropical zone, winter is generally warm, so that the chilling requirement alone cannot guarantee an optimal timing of bud burst in all species in the spring. In subtropical conditions, then, photoperiod helps to avoid premature bud burst and subsequent frost damage in winter and facilitates a timely bud burst in spring following an exceptionally warm winter with reduced chilling accumulation. On the basis of our limited but novel findings, we put forward a conceptual model of the effects of chilling accumulation and photoperiod on rest break and bud burst in subtropical trees. The conceptual model will facilitate the development of quantitative process-based three phenology models as more extensive experimental results come out. The big differences in responses to photoperiod and chilling found among the five tree species examined also indicate that rather than generalizing results obtained with one subtropical tree species to other species, the model development should be based on specific experiments carried out separately with each species. Our study will facilitate the long-term development of process-based tree phenology models for use in impact assessments of the climate change at the stand and ecosystem levels.

Author contributions

The study was designed by R.Z., J.W. and H.H. The experiment was carried out by F.W., J.Z. and J.L. The data were analysed by F.W., R.Z. and J.Z. The manuscript was written by R.Z. with inputs from H.H. All authors approved the final manuscript.

CRediT authorship contribution statement

Rui Zhang: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Fucheng Wang:** Formal analysis, Investigation, Visualization. **Jinbin Zheng:** Formal analysis, Investigation, Visualization. **Jianhong Lin:** Investigation. **Heikki Hänninen:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision. **Jiasheng Wu:** Conceptualization, Methodology, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Pekka Hirvonen (www.toisinsanoen.eu) for revising the language of the manuscript.

Funding

The study was financed by The Chinese National Natural Science Foundation [31800579], The National Forestry and Grassland Technological Innovation Program for Young TopNotch Talents [2020132604], Zhejiang Provincial Natural Science Foundation of China [LQ18C160001], the Key Research Program of Zhejiang Province [2018C02004], and the Major Project for Agricultural Breeding of Zhejiang Province [2016C02052-12].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118813.

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