

Research Article

Chilling rather than photoperiod controls budburst for gymnosperm species in subtropical China

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Handling Editor: Shucun Sun

Received: 2 December 2020, **First Decision:** 6 February 2021, **Accepted:** 6 June 2021, **Online Publication:** 12 July 2021

Abstract

The mechanisms regulating spring phenology have been extensively studied in angiosperm species. However, given that gymnosperms and angiosperms diverged 300 million years ago, phenology may be triggered by different cues in gymnosperm species. The regulatory mechanisms of phenology in subtropical regions remain largely unknown. In combination, it remains untested whether subtropical gymnosperm species have chilling requirements and are photosensitive. We conducted a climate chamber experiment with three chilling and three photoperiod treatments to investigate budburst during an 8-week forcing period. We tested whether budburst of eight gymnosperm species (*Cryptomeria japonica*, *Cunninghamia lanceolata*, *Cupressus funebris*, *Ginkgo biloba*, *Metasequoia glyptostroboides*, *Pinus massoniana*, *Pseudolarix amabilis* and *Podocarpus macrophyllus*) was photoperiod sensitive or has strong chilling requirements and whether photoperiod or chilling was more important for advancing budburst. Chilling advanced budburst and increased the percentage of budburst for gymnosperm species. Gymnosperm species required moderate chilling days to advance budburst. Interestingly, the forcing requirement for gymnosperm species was higher than that for angiosperms in the same forest, suggesting that gymnosperms may need more cumulative forcing to initiate budburst than do angiosperms. Compared with temperate gymnosperm species in Germany (194–600 °C days), the subtropical species studied here had a much higher forcing requirement (814–1150 °C days). The effects of photoperiod were minor, suggesting that chilling outweighs photoperiod in advancing budburst of gymnosperm species in this subtropical region. These results reveal that increased winter temperatures with continued global warming may impact not only angiosperms but also gymnosperms, leading to their delayed spring budburst.

Keywords chilling requirement, climate change, forcing requirement, Gutianshan, spring phenology, twig cutting experiment

低温而不是光周期调控中国亚热带裸子植物的出芽物候

摘要: 被子植物春季物候的调控机制已经得到了广泛的研究。然而, 裸子植物和被子植物在3亿年前就产生分化, 裸子植物与被子植物的物候可能是受不同的因素所调控。亚热带植物物候的调节机制在

很大程度上尚不明确，亚热带裸子植物物候是否由冷激需求和光照调控仍未得到验证。本研究在人工气候箱中设置了3个冷激处理和3个光周期处理，并对切枝的出芽期进行了为期8周的研究。实验中我们测试了8种裸子植物：柳杉(*Cryptomeria japonica*)、杉木(*Cunninghamia lanceolata*)、柏树(*Cupressus funebris*)、银杏(*Ginkgo biloba*)、水杉(*Metasequoia glyptostroboides*)、马尾松(*Pinus massoniana*)、金钱松(*Pseudolarix amabilis*)和罗汉松(*Podocarpus macrophyllus*)，检验其出芽物候是否对光周期敏感或者是否具有较强的冷激需求，以及这两种因素哪个对促进出芽更为重要。研究结果表明，对于裸子植物，冷激促进了出芽并提高了出芽率，而且裸子植物需要适度的低温天数来实现出芽。有趣的是，在同一森林中裸子植物比被子植物对积温的需求更高。与德国温带裸子植物(194–600 d · °C)相比，亚热带裸子植物(814–1150 d · °C)对积温的需求更高。光周期对裸子植物出芽的影响较小，说明冷激对裸子植物出芽的促进作用大于光周期。这些结果表明，随着全球气候持续变暖，冬季气温的升高不仅会影响亚热带被子植物也会影响裸子植物的物候，从而可能导致春季出芽期的延迟。

关键词：冷激需求，气候变化，积温需求，古田山，春季物候，切枝实验

INTRODUCTION

Phenology, the timing of recurrent life-history events such as budburst, leaf-out, flowering and leaf senescence, relates to environmental conditions and species interactions (Cleland *et al.* 2007; Menzel *et al.* 2020). Plant phenology is very sensitive to environmental change, so it is a clear display of the effects of climate change (Ahas *et al.* 2002; Du *et al.* 2017; Fu *et al.* 2015; Guo *et al.* 2019; Parmesan 2006; Zohner and Renner 2018). Plant phenology underpins a variety of aspects of ecosystem function (Piao *et al.* 2007; Richardson *et al.* 2010; Scheffers *et al.* 2016) and could influence the structure of plant communities (Du *et al.* 2019b; Wolf *et al.* 2017) and species distributions (Chuine 2010). Plant phenology can also change climate systems by altering the biophysical and biochemical feedback processes between the atmosphere and the land surface (Piao *et al.* 2019; Richardson *et al.* 2010).

Temperature and photoperiod are the two most important factors controlling plant phenology (Körner and Basler 2010). In temperate regions, temperature is the main factor determining spring phenology of woody plants (Shi *et al.* 2017; Walther *et al.* 2002). The effect of temperature can be applied both through winter chilling and through forcing (spring warming temperature). To break endodormancy, plants require the cool temperatures of a chilling period and then they enter the phase of ecodormancy, during which the rate of ontogenetic development increases with increasing temperature (Chuine 2000). A chilling requirement is important because if plants tracked only warm temperatures for phenological events such as budburst and leaf-out

in early spring, they could suffer fatal damage if the freezing weather returned (Körner and Basler 2010; Rinne and Hanninen 2008). Photoperiod sensitivity of budburst may prevent some species from tracking the increasingly early onset of warm weather in spring i.e. associated with climate warming (Keskitalo *et al.* 2005). Under future global warming scenarios, insufficient chilling temperature accumulation during the dormancy period could result in delayed leafing and flowering dates (Chen *et al.* 2017; Yu *et al.* 2010; Zhai *et al.* 2019).

One method for testing the effects of different environmental cues on spring phenology is using twig cuttings as substitutes for adult individuals (Flynn and Wolkovich 2018; Polgar *et al.* 2014; Vitasse and Basler 2014; Wang *et al.* 2020; Zohner *et al.* 2016). Studies focusing on temperate woody plants have found that spring forcing and winter chilling were more important than photoperiod in driving the spring phenology of temperate angiosperm species (Laube *et al.* 2014a; Polgar *et al.* 2014; Zohner *et al.* 2016) and that rising air humidity during the spring did not trigger leaf-out (Laube *et al.* 2014b; Zohner *et al.* 2019). However, the phenological regulation mechanisms of spring phenology (e.g. chilling requirements, photoperiod, spring warming) remain largely unknown in subtropical regions (but see Du *et al.* 2019a), particularly for gymnosperm species. To our knowledge, only one study has explored the chilling requirements of gymnosperms (Laube *et al.* 2014a), but they only considered five genera in a temperate forest. The environmental drivers of spring phenology for other gymnosperm species, particularly for subtropical gymnosperms, need to be tested.

Gymnosperm plants are economically important for timber and resin production because of their straight grain (Kumar and Sati 2016). Gymnosperms are also pollution resistant so they play a critical role in the preservation of the environment (Sahni 1986). Although the mechanisms regulating spring phenology have been extensively studied in angiosperm woody species, phenology may be triggered by different cues in gymnosperm species (Nose *et al.* 2020), given that the most recent common ancestor of gymnosperms and angiosperms lived 300 million years ago (Bowe *et al.* 2000).

In our study, we present a climate chamber experiment with eight gymnosperm species from subtropical China. We studied the effects of chilling and photoperiod on spring forcing requirements for budburst. We tested: (i) whether the budburst of gymnosperm species was sensitive to photoperiod or whether it had strong chilling requirements; (ii) which of these two factors was more important in influencing spring budburst.

MATERIALS AND METHODS

Sampling region

We selected adults from eight gymnosperm species (*Cryptomeria japonica*, *Cunninghamia lanceolata*, *Cupressus funebris*, *Ginkgo biloba*, *Metasequoia glyptostroboides*, *Pinus massoniana*, *Pseudolarix amabilis* and *Podocarpus macrophyllus*) growing in the Gutianshan Nature Reserve (29°10'–29°17' N, 118°03'–118°11' E), Kaihua County, Zhejiang Province in eastern China. These species were selected because they are common in subtropical regions of China and were available for sampling. The Nature Reserve is characterized by a subtropical monsoon climate. The mean annual temperature is 15.3 °C. The highest monthly mean temperature (27.9 °C) occurs in July. The lowest monthly mean temperature (4.3 °C) occurs in January. The mean annual precipitation is 1787 mm, with obvious seasonal variation throughout the year. The topography is relatively undulating (Lin *et al.* 2013). The parent rock of the mountain range is granite, with soil pH ranging from 5.5 to 6.5 (Hu *et al.* 2008). A large portion of the broad-leaved forests in the Nature Reserve has reached an advanced successional stage (Legendre *et al.* 2009). The dominant vegetation type in Gutianshan Nature Reserve is typical subtropical evergreen broad-leaved forest, and the dominant

species are *Castanopsis eyrei* (Fagaceae), *Schima superba* (Theaceae) and *P. massoniana* (Pinaceae) (Zhang *et al.* 2011). Both flowering and fruiting patterns show significant seasonality in this forest, with flowering dates clustered from April to June and fruiting dates clustered from September to October (Pan *et al.* 2020).

Experiment

In this climate chamber experiment we implemented three chilling and three photoperiod treatments to investigate budburst during an 8-week forcing period.

Different chilling treatments (C1–C3, low to high) were represented by different lengths of exposure to outdoor chilling conditions (for rough estimates of chilling conditions, see Table 1). We collected twigs on 31 December (C1), 5 February (C2) and 28 February (C3). The second cutting day was a few days late because a heavy snow on 1 February prevented us from climbing the mountain to collect twigs until 5 February. We defined three photoperiod treatments in climate chambers, with daily photoperiods of 10, 12 and 14 h (P10, P12 and P14), which roughly covers the annual range of day length at this latitude (from 10.1 to 13.9 h). The temperature in all chambers for photoperiod and chilling treatments was set to 25 °C. Photon flux in the chambers was approximately 13.2 k Lux. Relative humidity was held constant at around 80%.

Sample preparation was done according to the method describe in Basler and Körner (2012). We collected 18 twigs of each species from at least 3 adults, and all twigs were cut to a length of 30–40 cm long. We sampled a total of 432 individuals over the three cutting dates. Twigs were cleaned and disinfected with commercial hypochlorite solution before they were placed into 250 mL plastic bottles that were filled with 200 mL tap water. For each species, we kept six replicate twigs (originating from three donor trees) per chamber (fully randomized). Twigs were recut about 1 cm so the twigs could continue to take up water, and the water in each bottle was changed weekly. The developmental stage of buds was recorded three times per week. Twigs that had not reached budburst by the end of week 8 were removed from the chambers.

Weather data

Weather data were obtained from an adjacent meteorological station (<2 km from the field site) run by the local meteorological service.

Table 1: Description of the three chilling treatments

Treatment	Start of experiment (dd.mm.yyyy)	End of experiment (dd.mm.yyyy)	Chilling days (days)	Thermal time (°C days)	Day length at the start of experiment (h:min)
Chilling 1 (low)	02.01.2018	27.02.2018	15	7	10:10
Chilling 2 (intermediate)	07.02.2018	04.04.2018	38	127	10:42
Chilling 3 (high)	01.03.2018	26.04.2018	45	296	11:18

Chilling days were defined as the number of days with mean temperature below 5 °C between 1 November and the date of twig collection. Because an accumulation of forcing units (high temperatures forcing development) also occurred in the field, the thermal time (in degree days above 0 °C since 1 January) at each cutting date is also given.

Statistical analysis

Twigs that did not achieve budburst within 8 weeks were considered dead and were removed from analysis. This left 272 out of 432 twigs (63%) representing 8 species that could be used for analysis.

We measured the number of days between collection and budburst for each twig. Chilling days were calculated as the number of days after 1 November on which the mean temperature was below 5 °C (Murray *et al.* 1989; Polgar and Primack 2011). The unit of thermal forcing was the sum of degree days, starting 1 January, with a base threshold of 0 °C, which encompasses forcing units received temperatures during the field and temperatures during the chamber experiment.

We used binomial generalized linear models to assess the effects of chilling and photoperiod on budburst. The short experimental duration of 8 weeks may have resulted in an abbreviated response, or a reduced forcing sum. Survival analyses were performed using the Survival package (Therneau 2013). The ‘survival’ in our analysis is equivalent to ‘no budburst’; while medians are Kaplan–Meier estimates of the forcing sum at which 50% of the twigs reached budburst (Du *et al.* 2019a; Laube *et al.* 2014a). Statistical significance of the treatment effect on the forcing sums was assessed using nonparametric log-rank tests, which are based on differences in survival curves (Harrington and Fleming 1982).

All calculations were performed in R version 3.5.2 statistical software (R Core Team 2018).

RESULTS

Effects of chilling and photoperiod on ability to achieve budburst

The eight species all achieved budburst in the experiment (Fig. 1), indicating that the twig cutting method was effective at assessing budburst in gymnosperms. Sixty-three percent of twigs achieved budburst for these eight species (272/432). In all three treatments, both chilling and photoperiod significantly affected budburst ($P < 0.001$, Fig. 2). The percentage of cuttings that achieved budburst was lowest in C1 (low chilling) at 45.1% ($N = 144$), followed by C2 (intermediate chilling) at 70.1% ($N = 144$) and C3 (high chilling) at 73.6% ($N = 144$). Generally, cuttings in the moderate day length treatment had a higher percentage of budburst than the short and long photoperiods

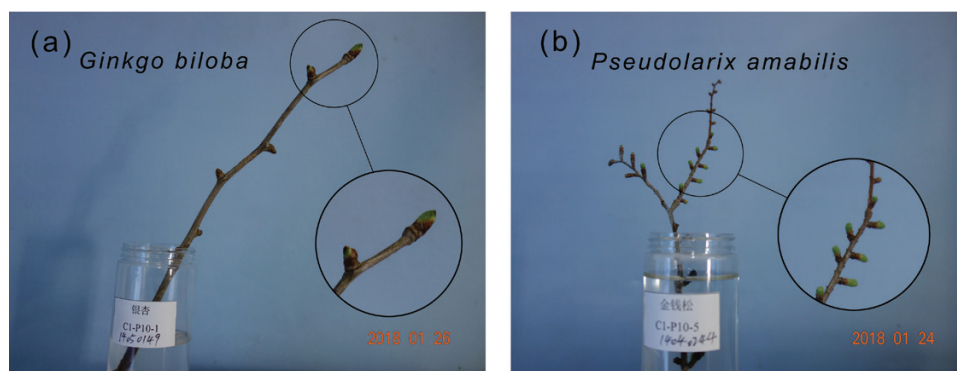


Figure 1: Examples of twigs sampled for budburst experiment, demonstrating that twigs can be used to study the influence of chilling and photoperiod on gymnosperm species phenology. (a) *Ginkgo biloba*; (b) *Pseudolarix amabilis*.

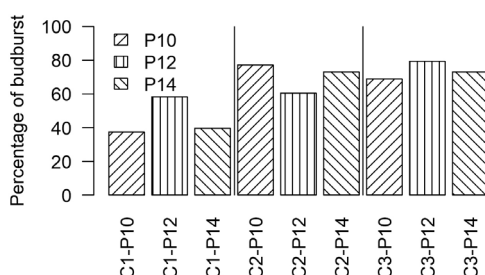


Figure 2: The percentage of twigs that achieved budburst in chilling and photoperiod treatments. Chilling C1: low chilling, C2: intermediate chilling, C3: high chilling. Photoperiod P10: 10 h, P12: 12 h, P14: 14 h daily photoperiod.

(Fig. 2). Similarly, the percentage of cuttings that achieved budburst was highest in P12 (moderate photoperiod) at 66.0% ($N = 144$), followed by P10 (short photoperiods) at 61.1% ($N = 144$) and P14 (long photoperiods) at 61.8% ($N = 144$). The differences in budburst among photoperiod treatments were 18.8% for C1 (between C1-P12 and C1-P14), 12.5% for C2 (between C2-P12 and C2-P14) and 4.2% for C3 (between C3-P10 and C3-P14) treatment (Fig. 2).

Increased chilling significantly enhanced budburst for four of the eight species, *P. massoniana*, *C. lanceolata*, *M. glyptostroboideis* and *G. biloba* (Table 2). Both the C2 and C3 treatments showed significant, positive effects on budburst for two species (*P. massoniana* and *Cunninghamia lanceolata*), while another two species showed significantly increased budburst ability in either C2 or C3 (*G. biloba* and *M. glyptostroboideis*).

Day length significantly, though marginally, influenced budburst rate for one species only (*G. biloba*; Table 2). Day length significantly lowered budburst rate in P14, compared with P10 and P12 for *G. biloba* (P10: 83%, P12: 83%, P14: 61%).

Effects of chilling and photoperiod treatments on forcing requirements

Increased chilling advanced budburst, with median days until budburst counting 17 (C2) and 13 days (C3) (Fig. 3a). The C3 treatment significantly advanced budburst compared with C2 ($P < 0.001$), and C2 advanced budburst significantly compared with C1 ($P < 0.001$). For photoperiod (Fig. 3b), no significant advances were found between P10 (median = 22 days) and P12 (median = 22 days, $P = 0.41$); between P10 and P14 treatments (median = 21 days, $P = 0.28$) or between P12 and P14 treatments ($P = 0.09$).

For the total amount of forcing (given in degree days until budburst), plants in the C1 treatment required more degree days to reach budburst (Fig. 4a). Median forcing requirements were considerably higher for C1 (1150 °C days) than for C2 (814 °C days) and C3 (815 °C days) ($P < 0.001$). In comparison, differences in median forcing requirements among the photoperiod treatments were considerably smaller (10 h: 940 °C days; photoperiod 12 h: 918 °C days; photoperiod 14 h: 951 °C days; Fig. 4b). Longer photoperiods reduced forcing requirements significantly in the C1 ($P = 0.042$) treatment, but not in the C2 ($P = 0.057$) and C3 ($P = 0.78$) treatments. The reduction in forcing requirements was also significant between C1 and C2 ($P = 0.006$).

DISCUSSION

Effects of chilling

We found that long chilling duration generally advanced budburst and increased percentage of budburst for gymnosperm species, which is in agreement with one study on nine gymnosperm species of five genera (*Abies*, *Larix*, *Picea*, *Pinus*

Table 2: Significant effects of chilling and photoperiod treatments on species' ability to reach budburst

Species	Family	Intercept	C2	C3	P2	P3	Number C1		Number C2		Number C3	
							tot	bb	tot	bb	tot	bb
<i>Cupressus funebris</i>	Cupressaceae	ns	ns	ns	ns	ns	18	12	18	12	18	13
<i>Ginkgo biloba</i>	Ginkgoaceae	ns	0.018	ns	ns	0.079	18	8	18	5	18	17
<i>Pinus massoniana</i>	Pinaceae	ns	0.008	0.036	ns	ns	18	3	18	11	18	9
<i>Pseudolarix amabilis</i>	Pinaceae	ns	ns	ns	ns	ns	18	11	18	18	18	18
<i>Podocarpus macrophyllus</i>	Podocarpaceae	ns	ns	ns	ns	ns	18	4	18	6	18	3
<i>Cryptomeria fortune</i>	Taxodiaceae	ns	ns	ns	ns	ns	18	13	18	17	18	17
<i>Cunninghamia lanceolata</i>	Taxodiaceae	ns	0.095	0.023	ns	ns	18	5	18	10	18	12
<i>Metasequoia glyptostroboides</i>	Taxodiaceae	ns	ns	0.018	ns	ns	18	9	18	12	18	16

Calculations were performed with binomial generalized linear models. Models without interaction terms showed lower Akaike's Information Criterion (AIC) values, hence *P* values refer to models without interaction terms. C: chilling, P: photoperiod, C2: intermediate chilling, C3: high chilling. Number: number of twigs with budburst per chilling treatment, tot: total number, bb: number with budburst within treatment period (8 weeks), ns: not significant (*P* > 0.10).

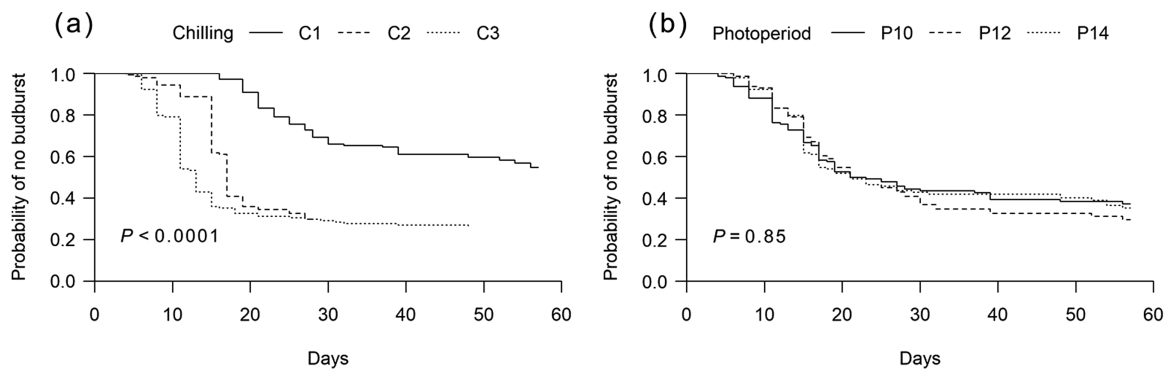


Figure 3: Budburst curves for all treatments, calculated for the number of days in climate; (a) chilling treatment only and (b) photoperiod treatment only. Treatments given as C1: low chilling, C2: intermediate chilling, C3: high chilling. Photoperiod P10: 10 h, P12: 12 h, P14: 14 h daily photoperiod.

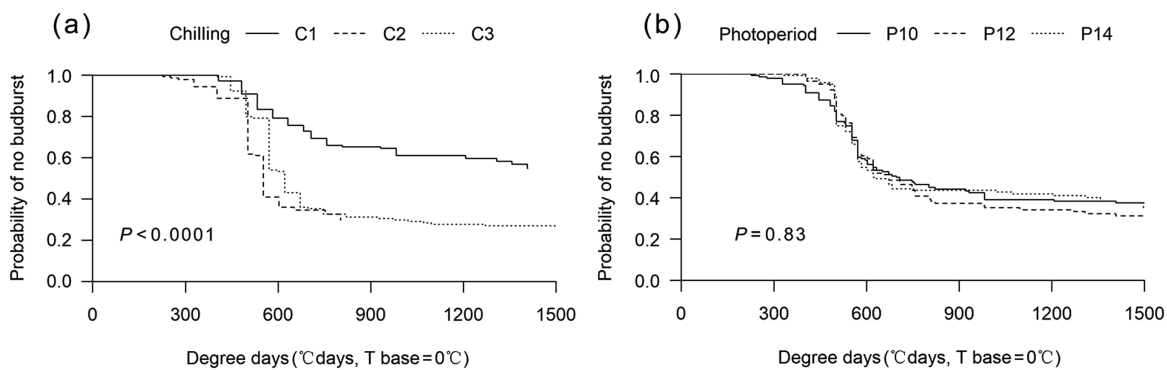


Figure 4: Budburst curves for all treatments, by absolute degree days of forcing (field and chamber) until budburst; (a) chilling treatment only and (b) photoperiod treatment only. Treatments given as C1: low chilling, C2: intermediate chilling, C3: high chilling. Photoperiod P10: 10 h, P12: 12 h, P14: 14 h daily photoperiod.

and *Pseudotsuga*) in Germany (Laube *et al.* 2014a). Laube *et al.* (2014a) found that increased chilling significantly enhanced budburst ability for eight out of nine gymnosperm species tested, and our study showed that the one genus common to these two studies, *Pinus*, is one of the most sensitive to chilling treatment, with both intermediate and high chilling treatments significantly enhancing budburst. In addition, our results showed that long chilling could also enhance spring development of other gymnosperm taxa like *Cunninghamia* spp., *Metasequoia* spp. and *Ginkgo* spp. Our findings are also consistent with previous studies conducted in Europe and North China for other temperate deciduous (e.g. Caffarra *et al.* 2011; Laube *et al.* 2014a; Wang *et al.* 2020) and subtropical evergreen angiosperm species (e.g. Du *et al.* 2019a; Song *et al.* 2020).

In our study, intermediate and strong chilling treatments clearly advanced budburst. We conclude that intermediate chilling (38 days) may be enough for gymnosperm species to advance spring development in the subtropics. As previous studies have shown,

120 chilling days are sufficient to complete winter dormancy for most angiosperm species in temperate regions (Dantec *et al.* 2014; Polgar *et al.* 2014). Our findings indicate that gymnosperms in subtropical evergreen forests need fewer chilling days. In our recent studies on subtropical angiosperm species in this forest, we found that intermediate chilling is also enough to trigger budburst and leaf-out (Du *et al.* 2019a; Song *et al.* 2020), which indicates that both angiosperm and gymnosperm species may need a moderate number of chilling days to advance spring phenology in subtropical regions. This is in line with one study along a latitudinal gradient in China, which showed that *Melia azedarach* experienced 38–81 chilling days in the subtropical and northern tropical zones but 143 days in the temperate zones (Chen *et al.* 2017). Another study on apricot flowering also indicated that in cold climates, spring timing of apricots is almost entirely determined by forcing conditions, but for apricots in warmer locations, chilling temperatures were the main driver of flowering date (Guo *et al.* 2015). This means that

even slight increases in winter temperature may disturb the dormancy of subtropical plants, and the insufficient chilling temperature accumulation for leaf-out and flowering may counteract or exceed the advancing effect of forcing temperature accumulation, thus resulting in delayed spring phenology in the subtropical zones (Chen *et al.* 2017; Guo *et al.* 2015). Understanding chilling requirements in subtropical species can contribute to more accurate predictions of how climate change will impact vegetation growth and carbon balance in this unique ecoregion.

Plants in intermediate and strong chilling treatments required less forcing than plants in low chilling treatments. Our study revealed the major importance of the chilling effect, which is consistent with studies in Switzerland on temperate angiosperms (Basler and Körner 2012), and also supports the ‘residual effect of dormancy’ hypothesis, which is defined as a reduction in forcing requirements as chilling increases (Campoy *et al.* 2011). Interestingly, the mean forcing requirement for the gymnosperm species in our study was greater than that for the angiosperms in the same forest in our previous study (1.2–1.4-fold; Du *et al.* 2019a) (C1: 1150 vs. 979 °C days; C2: 814 vs. 586 °C days; C3: 815 vs. 646 °C days). This indicates that subtropical gymnosperms may need more cumulative forcing to initiate budburst than subtropical angiosperms. Notably, the ecophysiological basis of these contrasting growth responses to temperature remain poorly discussed and resolved. A likely explanation is that differences in forcing requirements for leaf-out times might be related to the evolutionary history of these two phylogenetic groups. Unlike angiosperm, gymnosperms lack water transport vessels and nutrient transport sieve tubes, and instead only have less efficient tracheids and sieve cells (Hacke *et al.* 2001; Huang *et al.* 2020; Poorter *et al.* 2012). This key difference implies that gymnosperms need more time to grow in early spring and may explain why they start spring phenology later than angiosperms, as well as why they have a greater forcing requirement than angiosperms. The later spring phenology of gymnosperms compared with angiosperms has been observed and confirmed by previous studies also (e.g. Panchen *et al.* 2014). These differences suggest that these two groups may qualitatively differ in their relative responses of carbon dynamics and tree growth to increased spring temperatures (Carnicer *et al.* 2013). This could imply that gymnosperm phenology is less sensitive to global warming than that of

angiosperms (Carnicer *et al.* 2013). It remains to be tested, however, whether gymnosperms need more cumulative forcing than angiosperms in temperate and tropical regions as well.

We also found that the forcing requirement was much lower for temperate gymnosperms (Laube *et al.* 2014a; between 194 and 600 °C days) than for the species in our study, suggesting that subtropical gymnosperms may need more cumulative forcing than do temperate gymnosperms. This is consistent with previous findings that trees from the south usually require a greater heat sum accumulation before budburst and leaf-out than trees from the north (Olson *et al.* 2013; Sanz-Pérez *et al.* 2009). This would also explain the phenomenon where species adapted to more southern climates leaf-out later than native species in a more northerly common garden, as was shown in one common garden study in the Munich Botanical Garden (Zohner and Renner 2014). There, the southern species flushed on average 15 days later than the native species, likely because they had greater forcing needs than more northern species (Zohner and Renner 2014). Our results indicate that northern gymnosperms will flush bud earlier than southern gymnosperms under warmer spring temperatures in future and will be better able to take advantage of early season growing opportunities. Thus, northern gymnosperms may have a competitive advantage over southern ones in regard to early season growth (Olson *et al.* 2013).

Effects of photoperiod

In general, we found that photoperiod influenced the rate of budburst in gymnosperms, with a moderate photoperiod producing higher budburst rates. This may be because a day length of 12 h is the optimal photoperiod, and photoperiods that are too short or too long inhibit budburst rates. This finding is supported by the optimal day length hypothesis, which states that species may start leaf phenology when the day length is optimal, with leaf-out date determined by the trade-off between maximizing nutrient uptake to ensure competitive advantage by lengthening the growing season and reducing the risk of late frost damage (Fu *et al.* 2019). For *G. biloba*, however, longer photoperiod significantly reduced budburst rate. For temperate gymnosperms *Abies* and *Picea*, budburst was delayed by short photoperiods (Basler and Körner 2012). Short days seem to control annual transcriptome dynamics of gymnosperm species and to induce cessation of needle growth (Nose *et al.* 2020). For species controlled by

photoperiod, temperature seemed to trigger spring phenology only when the photoperiod exceeded a critical day length (Körner and Basler 2010). More studies are needed to test the effects of photoperiod on gymnosperm phenology across a wider range of species and ecosystems.

We found no effects of photoperiod on the number of days until budburst. Our results are consistent with one study on nine temperate gymnosperm species, which revealed no measurable photoperiod response (Laube *et al.* 2014a). However, another study on three gymnosperm species (*Larix* spp., *Abies* spp. and *Picea* spp.) in the Swiss Alps showed that short photoperiods delayed budburst for two of three species (Basler and Körner 2012). Our findings suggest a general, photoperiod-independent control of dormancy for the genera in our study.

For the total amount of forcing, longer photoperiods could lead to reduced forcing requirements in years with poor chilling (C1). This is consistent with prior results showing a reduction in the forcing requirement with longer photoperiods only in the low chilling treatment (Laube *et al.* 2014a) and where budburst was delayed by short photoperiods (Basler and Körner 2012). Our findings suggest that incomplete chilling may be a mandatory precondition for photosensitive responses (Laube *et al.* 2014a), which contradicts the previous idea that plants become receptive to photoperiod signals after chilling is satisfied (Körner and Basler 2010).

CONCLUSIONS

This experiment confirmed that twig cutting is a reliable method for assaying phenological responses to chilling and photoperiod in subtropical gymnosperms. We found that long chilling duration could advance budburst and increase percentage budburst for gymnosperms. In accordance with our recent studies on angiosperm species in this subtropical forest, gymnosperm species in subtropical regions may also need moderate chilling days to advance spring phenology. Plants in the intermediate and strong chilling treatments required less forcing than did plants in the low chilling treatment. Interestingly, the mean forcing requirement was greater for the gymnosperm species in this study than for the angiosperms in our previous study in the same forest (Du *et al.* 2019a). This suggests that gymnosperm species may need more cumulative forcing to initiate budburst than do angiosperms. Gymnosperm species may need more cumulative

forcing in subtropical than in temperate regions (e.g. Laube *et al.* 2014a). In contrast, photoperiod had less of an effect on advancing budburst, which suggests that chilling rather than photoperiod controls budburst for subtropical gymnosperm species. Future climate change may prevent most of these gymnosperm species from meeting their chilling requirement, thus delaying spring phenology.

Funding

This research was supported by the Innovative Research Team Program of Hainan Natural Science Fund (2018CXTD331), the Natural Science Foundation of Hainan Province (320RC504) and Hainan University (KYQD(ZR)1979).

Acknowledgements

We appreciate the Gutianshan Research Station for allowing us to carry out the experiment. We thank Dr. Haibao Ren and Dr. Xiangcheng Mi for sharing climate data. We would like to thank Elizabeth Tokarz of Yale University for her assistance with English language and grammatical editing.

Conflict of interest statement. The authors declare that they have no conflict of interest.

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