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The chilling requirement of subtropical trees is fulfilled by high temperatures: A generalized hypothesis for tree endodormancy release and a method for testing it

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ABSTRACT

Spring phenology is a key phenomenon mediating the effects of climate change on terrestrial plants and eco-systems, but in regard to subtropical trees, the dormancy mechanisms that regulate spring phenology are still poorly understood. It has been suggested recently that similarly to temperate and boreal trees, subtropical tree species also show endodormancy and a chilling requirement of endodormancy release. However, there are no previous experimental results on the chilling temperature range that is effective for endodormancy release in subtropical trees. We studied endodormancy and the chilling requirement in four subtropical tree species experimentally. In addition to chilling in natural conditions, we applied controlled chilling at several constant temperatures ranging from -2 to +15 °C. Our results show endodormancy and a chilling requirement in the tree species studied and reveal several differences among the four species in the manifestation and depth of endo-dormancy. Most importantly, our findings indicate that contrary to the prevailing mainline conception that chilling temperatures are generally restricted to those below +10 °C, higher temperatures of up to +15 °C are also effective for endodormancy release in the subtropical tree species examined. An exact upper threshold of +10.4 °C has been experimentally established for boreal *Betula pubescens*. We hypothesized that this difference would be explained by differences in the occurrence of low autumn temperatures between the two respective climates. We developed a method for testing the hypothesis by analysing long-term climatic records in relation to the experimental findings. Tentative results supported our hypothesis. On the basis of this novel result, we put forward the testable generalized hypothesis that in any climatic conditions where trees show endodormancy, the range of temperatures physiologically effective in endodormancy release represents the range of typical autumn air temperatures occurring in those particular climatic conditions.

1. Introduction

Bud dormancy is an essential phenomenon in the annual cycle of development of boreal and temperate trees (Perry, 1971; Sarvas 1972, 1974; Fuchigami et al., 1982). According to the classical conceptual model, the dormant period of buds in these trees consists of two major phases (Polgar and Primack, 2011; Hänninen, 2016; but see also Vegis, 1964; Cooke et al., 2012; Junttila and Hänninen, 2012; Lundell et al., 2020). After growth cessation and formation of terminal buds in the

autumn, buds enter a state of *endodormancy* (Lang et al., 1987; synon-ymous to *rest*, Fuchigami et al., 1982), where the onset of growth is prevented by physiological conditions in the buds. A prolonged exposure (days to months) to chilling temperatures is generally required for endodormancy release (synonymous to *rest break*, Fuchigami et al., 1982), i.e., removal of the growth-arresting physiological conditions (but see also Vitasse and Basler, 2013; Fu et al. 2019a,b). In this way, chilling acts as an environmental cue preventing a premature growth onset during intermittent warm periods in autumn and winter, which

would lead to serious damage during subsequent periods of frost (Sarvas, 1974; Cannell, 1985; Hänninen, 1991, 2016). When the chilling requirement is met, buds attain the state of *ecodormancy* (Lang et al., 1987; synonymous to *quiescence*, Fuchigami et al., 1982), where bud burst and growth onset are prevented by unfavourable environmental factors, such as low air temperatures occurring during winter. Bud burst will occur in ecodormant buds when they are exposed to high growth-promoting forcing temperatures for a prolonged period (days to weeks) (Fuchigami et al., 1982; Hänninen, 2016).

Endodormancy and the chilling requirement are crucial elements in process-based tree phenology models, which are currently used for assessing the effects of climate change on boreal and temperate trees (Chuine et al., 2013; Hänninen, 2016; Chuine and Régnière, 2017; Hänninen et al., 2019). In these models the progress of endodormancy release is simulated by accumulating specific chilling units, with the rate of accumulation depending on the ambient air temperature. Reduced chilling accumulation in a warming climate may reduce, or even reverse, the generally accelerating effect of warming on the phenological timing during spring (Murray et al., 1987; Ford et al., 2016). This phenomenon has recently been addressed in several studies where the effects of climatic warming on the spring phenology of trees that have already appeared were examined by analysing observational long-term climatic and phenological records (Fu et al., 2015a,b; Ma et al., 2018, Chen et al., 2018, 2019). However, in some regions the amount of chilling is projected to increase (Fraga et al., 2019), suggesting that in those regions climatic warming would cause especially rapid acceleration of the spring phenological development. This shows once again how the projecting of the ecological effects of climatic change requires proper mechanistic understanding of both meteorological and ecological phenomena.

Since its discovery by Coville (1920), the chilling requirement has been addressed in hundreds if not thousands of studies, with both naturally growing forest trees and horticultural crops (Perry, 1971; Fuchigami et al., 1982; Arora et al., 2003; Junttila, 2007; Rohde and Bhalerao, 2007; Hänninen and Tanino, 2011; Cooke et al., 2012; Luedeling, 2012). Given that background, it is surprising that the temperature response of the rate of endodormancy release has been determined experimentally in very few studies (Hänninen et al., 2019). Thus it is not known what chilling temperature range is effective in endodormancy release and what form the response will take within that range. This constitutes a major uncertainty in process-based tree phenology modelling (Hänninen, 2016). As an early exception, Sarvas (1974) determined the response experimentally for boreal *Betula pubescens*. His results established the lower and upper thresholds of chilling at -3.4 and +10.4 °C, with the maximum chilling effect at +3.5 °C (S1a). Due to a shortage of other similar studies, Sarvas's (1974) model has been applied to several other boreal tree species as well (Hänninen, 1990; Häkkinen et al., 1998; Partanen et al., 2016, 2020).

With several decades of shortage of explicit experimental ecophysiological data, various assumptions have been made on the chilling temperature range effective in endodormancy release (Table 1). Almost without exception, temperatures slightly above zero have been included in the effective range. Sometimes freezing temperatures are also regarded as equally effective in chilling accumulation, but mostly it is assumed to take place at above-zero temperatures only. Following the intuitive notion of the concept of chilling, most studies agree that no chilling accumulation is assumed at temperatures well above +10 °C (Table 1, but also see Section 4.4).

In addition to the boreal and temperate zones, the air temperature shows seasonal variation in the subtropical zone, too. In winter, many subtropical trees are exposed to episodic frosts (Larcher, 2003). In all, then, subtropical trees, too, need to synchronize their annual development with the annual cycle of air temperature. Given that background, it is surprising that so little experimental information is available on the environmental cues regulating the alternation of growth and dormancy in subtropical tree species. Recently, Du et al. (2019) and Song et al.

Table 1

Assumed temperature ranges of chilling accumulation in studies applying the chilling requirement concept in phenological examinations, analyses, and modelling. The letter 'F' in the 'lower threshold' column indicates that all freezing temperatures contribute to chilling accumulation equally to the higher temperatures indicated by the upper threshold. For the sake of brevity, the acronym T_{upp} is used in the text for the upper threshold. The studies listed in the table provide a small sample of the mainline concept, suggesting the T_{upp} for boreal and temperate trees is +10 °C or, depending on the species, lower. For contradictory studies suggesting higher values of T_{upp}, see Section 4.4.

Chilling accumulation range		Reference
Lower threshold (°C)	Upper threshold (°C)	
F	±0	Meng et al. (2019)
-3.4	+4.4	Lundell et al. (2020)
F	+5	Weinberger (1950)
		Cannell and Smith (1983)
		Murray et al. (1989)
		Du et al. (2019)
		Song et al. (2020)
		Zohner et al. (2020)
		Wang et al. (2020)
±0	+5	Fu et al. (2015a,b)
		Ma et al. (2018)
		Chen et al. (2018, 2019)
		Fu et al. (2019a,b)
±0	+7	Rezazadeh et al. (2018)
-3.4	+10.4	Hänninen (1990, 1996)[1]Hänninen (1991, 1995))
		Häkkinen et al. (1998)[1]
		Linkosalo et al. (2006)[1]
		Hänninen et al. (2007)[1](Hänninen et al., 2007)
		Partanen et al. (2016)[1]Partanen et al. (2020)

[1] Maximum chilling effect at +3.5 °C

(2020) were the first to provide experimental evidence for the endodormancy and the chilling requirement of subtropical woody species, addressing a total of 37 species. However, as they applied chilling in naturally fluctuating temperatures only, their results do not provide information on the temperature range effective in endodormancy release, nor on the temperature response of endodormancy release within that range, both of which are needed in process-based tree phenology models (Fig. S1a).

We carried out an experimental study on endodormancy in four subtropical tree species growing commonly in southeastern China. We first hypothesized that these tree species would show endodormancy and a chilling requirement. Second, we hypothesized that in these trees the upper threshold for the chilling temperature range releasing endodormancy, T_{upp}, would be different from the upper threshold of +10.4 °C found earlier for boreal *Betula pubescens* (Fig. S1a). To test the second hypothesis, we included controlled chilling treatments with temperatures ranging from -2 to +15 °C in one of our experiments. Third, we hypothesized that differences in the occurrence of low autumn temperatures between subtropical and boreal climates would explain the corresponding differences in T_{upp} between subtropical and boreal tree species. We tested the hypothesis tentatively with a method developed in the present study for analysing long-term climatic records in relation to the experimental findings concerning endodormancy release.

2. Materials and methods

2.1. Study site and plant materials

The experiments were conducted in the Zhejiang A&F University campus (30°14′N, 119°42′E) in Hangzhou, southeastern China in 2017 - 2019. The location has a subtropical, monsoonal climate (Peel et al., 2007; Fig. S2). The mean annual precipitation is 1614 mm. The mean

annual air temperature is +15.6°C and the mean monthly air temperature is +4.5°C in January and +28.9°C in July (Zhang, 2015). The experiments were carried out with four subtropical tree species growing commonly in subtropical southeastern China: *Castanopsis sclerophylla, Phoebe chekiangensis, Pseudolarix amabilis*, and *Torreya grandis* (Table 2; Editorial Committee for Flora of the Chinese Academy of Sciences, 1990). *Torreya* is coniferous and evergreen, *Pseudolarix* coniferous and deciduous, while *Castanopsis* and *Phoebe* are broadleaved and evergreen. In our subtropical study area, these four tree species generally show spring leafout from March to April, followed by active growth in May and June. From late July to early August, growth ceases in hot summers, but a second leafout is generally seen from late August to September. Bud set generally occurs from September to October. For all four species, local provenances were used (Fig. S2). First-year container seedlings and, with *Torreya*, also twigs detached from mature trees were used in the study (Table 2). The twigs were included in order to account for any differences between seedlings and adult trees (Ununger et al., 1988; Partanen et al., 2005, 2016; Hänninen, 2016). Below, the concept *material category* will be used for the combination of species and age (seedling/twig) in the experimental material.

The seedlings were obtained from a nearby nursery at the Tianmushan National Forest Station (30°24′N, 119°28′E, Hangzhou, China). All seedlings were produced and managed according to standard nursery practices. In brief, open-pollinated seeds were collected from native seminatural stands in the province of Zhejiang in autumn. The seeds were air-dried and stored at +5 °C. In the March of the following year, the seeds were sown to propagation beds in a greenhouse (+25±5 °C) to initiate germination. In May, when the developed seedlings had three to five leaves, they were transplanted into non-woven pots filled with a soil substrate containing 5 peat: 2 vermiculite: 1 perlite: 2 organic matter by volume (Universal potting soil, Hangzhou, China). Depending on the size of the seedlings, the volume of the pots varied between 0.5 and 1.2 L. These first-year container seedlings were then grown in the field and were irrigated with sprinklers to keep the growth medium well-watered. Weeds were removed by hand as needed. All seedlings were transferred to the university campus in early November. They were transplanted into larger white polyethylene pots (1.2L-2.2L, depending on the seedling size) filled with the same soil substrate as used earlier and were kept in the campus outdoor seedling collection until the beginning of the experiment.

Twigs of *Torreya* were collected from mature trees growing at an 18-year-old plantation located 20 km from the university campus. The stand had been established with a local provenance and had been managed by means of routine practices (Zhang et al., 2017). In each

sampling at the plantation, a single twig, approximately 20 cm long, with female flower buds, was randomly sampled and detached with scissors from the southward side of each tree, from the middle part of the crown. After detaching, the twigs were transferred in water-filled buckets to the campus, where they were put in water-filled test tubes, one twig per tube. In order to avoid embolism, a small part (less than one cm) was cut from the cut end of each twig under water both after the sampling and after placing the twig in the test tube.

2.2. Experimental design for testing the existence of endodormancy

When exposed to growth-promoting high-temperature forcing conditions, endodormant buds either do not burst at all or their bursting requires a longer time than is required after the chilling requirement of endodormancy release is met (Fuchigami et al., 1982; Hänninen, 1990; Cooke et al., 2012). On this basis, the existence of endodormancy and the chilling requirement in the different material categories was tested in three experiments, all sharing the same principle of a standard chilling-forcing design (Hänninen et al., 2019). The principle of the experimental design is explicated below; for all details of Experiments 1-3, see Table 2 and Sections 2.3 and 2.4.

The experimental seedlings and twigs were first exposed to chilling for periods of varying duration, either in natural conditions or in controlled conditions in growth chambers. Then they were transferred to high-temperature forcing conditions in growth chambers. Exceptionally, the first treatment groups were transferred at the beginning of the experiment directly from natural conditions to the forcing conditions (zero chilling). In each successive transfer, a new set of seedlings/twigs was sampled in the chilling conditions and transferred to the forcing conditions. In the forcing conditions a regrowth test was carried out by observing the occurrence and timing of bud burst in each seedling/twig. For each seedling/twig showing bud burst, the number of days to bud burst in the forcing conditions, DBB, was determined. Subsequently, the bud burst percentage, BB%, and the mean DBB were calculated for each transfer and plotted against the transfer time. The existence of endodormancy was examined by checking whether the BB% increased and/or mean DBB decreased with successive transfers from the chilling to the forcing conditions (i.e., with increasing duration of chilling), as implied by the concepts of endodormancy and chilling requirement (Hänninen, 2016; Hänninen et al., 2019, and the references therein). In order to test the robustness of the results, both fluctuating and constant temperatures were used in the experiments; and in order to address Hypothesis 2, several controlled chilling temperatures were applied in Experiment 2 (Table 2).

Table 2
The experimental material, temperatures, and schedules for the three experiments of the study. The experimental material is subdivided into five material categories: seedlings of three species and both seedlings and twigs of *Torreya grandis*. The starting day of each experiment marks the initiation of the controlled chilling and also the initiation of the forcing for the zero chilling treatment groups transferred directly to the forcing without any chilling treatment.

Tree species	Species characteristics	*Experiment 1* 2017-18	*Experiment 2* 2018-19	*Experiment 3* 2018-19
Castanopsis sclerophylla	Evergreen, broad-leaved	-	Seedling	-
Phoebe chekiangensis	Evergreen, broad-leaved	-	Seedling	-
Pseudolarix amabilis	Deciduous, coniferous	-	Seedling	-
Torreya grandis	Evergreen, coniferous	Seedling, twig	Seedling, twig	Seedling
Experimental temperatures				
Chilling		Natural, 6°C	Natural, -2, 5, 10, 15°C	Natural
Forcing		20°C	20°C	7~15, 12~20, 17~25, 22~30°C[a]
Experimental schedule				
Start of experiment		21 Nov 2017	23 Nov 2018	23 Nov 2018
Last transfer		12 Feb 2018	15 Feb 2019	15 Feb 2019
Number of transfers		7	9	9
Range of chilling duration		0-12 weeks	0-12 weeks	0-12 weeks
Cessation of experiment		23 April 2018	4 May 2019	20 May 2019
Duration of experiment		154 days	163 days	179 days

[a] Diurnally fluctuating temperature, the daily temperature range is indicated.

2.3. Sample size and growing conditions

The sample size was n = 10 seedlings/twigs for every transfer and material category in all experiments. In all, 2300 seedlings and 590 twigs were included in the experiment. With the seedlings, natural chilling took place in the campus outdoor seedling collection, and the same was attained with the twigs by leaving them intact in the trees at the plantation until the time of sampling for the forcing conditions. In the controlled chilling with twigs, two sampling phases were included: First, all of the twigs included in the controlled chilling were sampled at the beginning of the experiment and placed in test tubes as described above. Second, at the time of transfer into the forcing conditions, ten twigs in their respective test tubes were randomly sampled from the controlled chilling conditions.

Within the limits set by the experimental design, the conditions in the chilling and forcing chambers approximated those prevailing in natural conditions at the study site during the respective times of the year. Air temperature was recorded hourly with iButton Data Loggers (Model DS1912L, Embedded Data Systems Co., Ltd, KY, USA) in all growing conditions. Three loggers were used in each chamber, in the outdoor seedling collection in the campus, and in the *Torreya* plantation. The loggers were positioned at the approximate height of the terminal buds and shielded with white cylindrical PVC radiation shields. For documenting the temperature conditions, the mean of the temperature records of the three loggers was used.

Experiment 1 was carried out in 2017 – 2018 with *Torreya* seedlings and twigs representing mature trees, using both natural and controlled chilling. The controlled chilling was implemented at a constant temperature and forcing at another constant temperature (Table 2). Both forcing and controlled chilling took place in computer-controlled walk-in growth chambers (E-Lotus Technology Co., Beijing, China). The conditions in the controlled chilling were as follows: air temperature +6 °C, constant day length 10 hours 30 minutes, with the light period from 6.30 am to 5 pm, and a relatively low PPFD (150 µmol m^{-2} s^{-1}). Two chilling chambers (2.55 m × 2 m × 2.5 m) were used. Both had eight shelves (0.6 m × 1.1m), with LED lights located above each shelf. The forcing took place in the following conditions: air temperature +20 ± 0.09 °C, constant day length 12 hours, with the light period from 6.30 am to 6.30 pm, and a relatively high PPFD (400 µmol m^{-2} s^{-1}). Two forcing chambers (4.92 m × 2 m × 2.5 m) were used. Both had four tables (0.6 m × 2.3m), with LED lights located above them. In both chilling and forcing chambers, relative humidity was 70-80 %, concentration of CO_2 300-400 ppm, and air circulation was in operation four times a day, 20 minutes at a time. In the natural chilling conditions, the daily mean temperature dropped below +10 °C soon after the start of the experiment, then fluctuated between 0 and +10 °C. The daily maximum temperatures often rose above +20 °C and daily minimums often dropped below zero, sometimes even close to -10 °C (Fig. S3a and S3b).

Experiment 2 was carried out in 2018 – 2019 with all five material categories and using both natural and controlled chilling. The controlled chilling was implemented at several constant temperatures and forcing at one constant temperature (Table 2). The forcing and chilling treatments were implemented in the same walk-in-growth chambers as in Experiment 1 a year earlier. The conditions were similar to those in Experiment 1 except for the controlled chilling, where four constant air temperatures ranging from -2 to +15 °C were used (Table 2; the precision of the temperature control in the forcing conditions was 20 ± 0.098 °C). In the -2 °C chilling condition, the leaves of the seedlings/twigs started to wither in late December, indicating damage probably caused by impaired water uptake under the freezing temperatures. Therefore we stopped the transfers of the seedlings and twigs from the -2 °C chilling condition to the forcing condition after the transfer on 14 December 2018. The air temperature varied in the natural chilling conditions basically similarly to Experiment 1 a year earlier, but night frosts were less severe in Experiment 2 (Fig. S3).

Experiment 3 was carried out in 2018 – 2019 with *Torreya* seedlings, using natural chilling in the same natural conditions in the campus seedling collection as in Experiment 2 (Fig. S3c) and forcing in four daily fluctuating temperatures. The forcing took place in computer-controlled outdoor Whole Tree Chambers, WTCs (E-Lotus Technology Co., Beijing, China; Fig. S4). Four treatments with diurnally fluctuating air temperatures, each in one chamber, were applied (Table 2). Day length varied naturally in the WTCs. Incoming solar radiation also varied naturally, but the shading effect of the chambers made it slightly lower than the ambient level. Relative humidity and atmospheric concentration of CO_2 were maintained at their ambient levels in the WTCs.

There was no *a priori* information on the time when the species studied enter endodormancy. The starting time of the experiments was therefore decided on the basis of the visible phenological stage of development of the experimental material and the prevailing climatic conditions. At the start of the experiments the deciduous species had dropped their leaves and all species had well-formed buds. Only a limited amount of chilling had been accumulated in natural conditions before the start of the experiment (Fig. S3).

The seedlings were watered in the chilling and forcing chambers every three and two days, respectively. The water in the test tubes of the experimental twigs was changed twice a week, and each time this was done, a small part (less than one cm) was cut from the cut end of the twig under water once more. In chilling the twigs at -2°C, the cut ends of the twigs were sealed with paraffin to prevent water loss. Thereafter, the twigs were located in an open box in the -2°C chilling chamber. Weeds were removed by hand as needed in the chilling and forcing chambers.

2.4. Determination of 'bud burst': leafout and flowering

In the forcing conditions, the seedlings/twigs were inspected visually at intervals of two or three days to assess their phenological stage of development. In the seedlings of all four species, four developmental events of bud development were discerned: bud closed, bud swelling, leaf emergence, and leafout (Fig. S5). The timing of the last of the events, the leafout, was recorded. A similar procedure, involving four developmental events, was applied to *Torreya* twigs, but the last developmental stage whose timing was recorded was determined on the basis of the flower bud development by observing the time when three visible stigmas with drops appeared in the flower bud, indicating the receptiveness of the female flower bud to pollen grains (Fig. S5a). The occurrence of that developmental stage in the flower bud was preceded by three developmental events in the adjacent vegetative buds, similar to those observed in the seedlings of the four species: bud closed, bud swelling, and leaf emergence (Fig. S5). Despite these varying observations, the standard variable names referring to the generic concept of bud burst (BB% and DBB, see Section 2.2) were used in the present study in order to facilitate the communication of the results. In the calculation of the values of BB% and DBB, the twig/seedling was considered not to have had bud burst if the observed leafout/flowering was not followed by normal development of the shoot. This was done in order to exclude observations of abnormal bud burst, which typically occur as a result of insufficient chilling (Erez, 2000; Fig. S6).

2.5. Statistical analyses

In all the statistical analyses, the experimental unit was the seedling or the twig (n=10). In each experiment, differences in BB% among the treatments were analyzed separately for each of the five material categories by logistic regression with a binary response (bud burst/no bud burst; Partanen et al., 2016). The explanatory variables were the transfer time to the forcing conditions (*Transfer*, in all experiments), the chilling treatment (*Chilling*, natural or +6 °C in Experiment 1; natural, -2, +5, +10, or +15 °C in Experiment 2), and the forcing temperature in Experiment 3 (*Forcing*, four levels of daily fluctuating temperature; Table 2). The interactions among the explanatory variables were also

examined. Differences in DBB among treatments were analyzed with a two-way analysis of variance (ANOVA) separately for each of the five material categories (Partanen et al., 2016). A log transformation of DBB was used as the response variable, and the explanatory variables were the same as in the analyses of BB%. In addition, all interactions between the two explanatory variables were examined. All statistical analyses were conducted with the SPSS (Version 16.0, SPSS Inc., Chicago, USA).

2.6. Climatological comparison of occurrence of low temperatures in subtropical and boreal climates

When endodormancy release is regulated by chilling, then the necessary condition for trees getting a reliable environmental cue of the progress of autumn and winter (see Introduction) is that physiologically effective chilling temperatures, causing endodormancy release, occur at the growing site relatively regularly each year. We tested our third hypothesis on the basis of this necessary condition by comparing the upper threshold for the chilling temperature range releasing endodormancy, T_{upp} (Fig. S1a), in subtropical and boreal trees with the occurrences of low temperatures in the two respective native climates of the trees.

We examined the occurrence of low temperatures by means of climatic records over 61 years, measured in 1958 – 2018 in standard meteorological screens at two geographical locations: Hangzhou, southeastern China (30°14'N, 120°1'E), representing the climatic conditions of the subtropical tree species examined experimentally in the present study; and Jyväskylä, central Finland (62°14'N, 25°44'E), representing the typical climatic conditions of boreal *Betula pubescens* used for comparison in the present study (Fig. S1a). At both locations, the occurrence of low temperatures was examined by counting the Number of Chill Days (NCD), that is, the number of days with a daily mean temperature below a given threshold (Fig. S1b, $T_{mean} < T_{thr}$, see below) in a period of 120 days (henceforth, *chilling period*), representing autumn and early winter in Jyväskylä and autumn and early to mid-winter in Hangzhou. The chilling period examined was started annually on 1 September for Jyväskylä (Hänninen, 1990, 1991, 2016) and on 1 November for the warmer climate of Hangzhou. Due to missing temperature records, the NCD could not be determined for the chilling period of 1998 in Jyväskylä, and thus not in 1998-99 in Hangzhou, either. In all, the NCD was examined for 59 years at both locations. The threshold temperature T_{thr} corresponds to the upper threshold of the chilling temperature range releasing endodormancy (T_{upp}, Fig. S1a), but as no lower threshold is assumed in the theoretical model used in the climatological calculations (Fig. S1b), T_{thr} is referred to simply as the threshold temperature for the sake of brevity.

In order to examine the occurrence of various low temperatures at the two locations, six different values of the threshold temperature T_{thr}, ranging in steps of 2 °C from +6 °C to +16 °C, were used in the counting of the NCD. For both locations, the following steps were taken with each of the six values of T_{thr} (Fig. 1): for each of the 59 years examined, the daily value of NCD, (NCD(i) = the Number of Chill Days counted up to day i) was counted for each day of the chilling period (i= 1,120). Subsequently, for each day i of the chilling period, the minimum, mean, and maximum value of the NCD(i) over the 59 years were determined and plotted against time indicated by the day number i. The minimum value of NCD(120), denoted as NCD_{min}, was determined (Fig. 1). Subsequently, the curve for the maximum NCD was used to determine the earliest day, t_{early}, when NCD_{min} was attained. Finally, the Chilling Completion Time Range was calculated as CCTR = 120 – t_{early}. It indicates the year-to-year range of the time of endodormancy release completion for an idealized tree with the theoretical threshold temperature response of endodormancy release (Fig. S1b) and a chilling requirement equal to the maximum requirement allowed by value of NCD_{min}, that is, the minimum number of chill days occurring during the chilling period in the 59 years examined (Fig. 1). Thus, the CCTR was used as a measure of the year-to-year variation in the occurrence of low temperatures. With the Hangzhou climatic record, the following
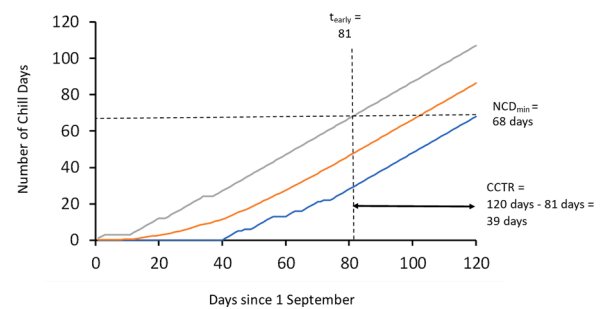

Fig. 1. The method used in assessing the year-to-year variation in the occurrence of low temperatures during autumn and early/midwinter by analysing long-term temperature records. The method is based on counting the Number of Chill Days, NCD, that is, the number of days with a daily mean temperature below the threshold temperature T_{thr}, for the 120-day chilling period of each year and subsequently examining the year-to-year variation in the NCD. The chilling period was started annually from 1 September (Jyväskylä) or 1 November (Hangzhou). The method is illustrated here with the case of the Jyväskylä temperature record and the threshold temperature $T_{thr} = +6$ °C. (1) The daily value of NCD (NCD(i) = the Number of Chill Days counted up to day i) was counted for each day of the chilling period (i= 1,120). This was done for each of the 59 years included in the temperature record. (2) For each day i of the chilling period, the minimum (blue), mean (orange, for descriptive purposes only) and maximum (grey) value of the NCD over the 59 years were determined and plotted against time. (3) The minimum of NCD(120) was determined and denoted as NCD_{min}. (4) The earliest day, t_{early}, of attaining NCD_{min} was determined. (5) The Chilling Completion Time Range was calculated as CCTR = 120 - t_{early} and was used as a measure of the year-to-year variation in the occurrence of low temperatures. For the rationale and the details, see the Material and methods section.

exceptional cases needed to be addressed: with the lowest three values of T_{thr}, NCD_{min} was attained one to four days before day 120 because no additional days with $T_{mean} < T_{thr}$ occurred during the last days of the chilling period. For this reason, day 120 was replaced in the calculations of the CCTR in those three cases as follows: for the threshold temperatures of +6 and +8 °C, with day 116 (February 24) and for +10 °C, with day 119 (February 27).

The CCTR was plotted against the value of T_{thr} used in the calculations, and the third hypothesis was tested by comparing the plots with the experimental results obtained in the present study for T_{upp} in subtropical trees and with the corresponding threshold of +10.4 °C found previously for boreal *Betula pubescens* (Fig. S1a). The hypothesis predicts that regardless of the climate, temperatures below the upper threshold imply a relatively low value of the CCTR, indicating regular occurrence of such temperatures during the chilling period in natural conditions.

3. Results

With successive transfers, either increasing bud burst percentage (BB %) or decreasing number of days to bud burst (DBB), or both, was found for all material categories (Fig. 2-4; Table 3). These findings are in accordance with the implications of the concepts of endodormancy and chilling requirement (see Material and methods), showing that all the four subtropical tree species examined have a chilling requirement of endodormancy release. From the main features of the BB% and DBB curves found for the different material categories, two dormancy patterns can be identified.

The first dormancy pattern is characterized by a BB% curve rising with successive transfers from 0 % until it reaches100 %, usually in the first few transfers. This pattern was found for *Torreya* twigs representing adult trees, hereafter '*Torreya* trees' (Fig. 2a, 3a), *Torreya* seedlings (Fig. 2c, 3c, 4a), and *Pseudolarix* seedlings (Fig. 3e). The BB% values of zero show that at the beginning of the experiments in late November, the buds were in deep endodormancy, as no leafout / flowering was seen during the subsequent prolonged forcing in growth-promoting
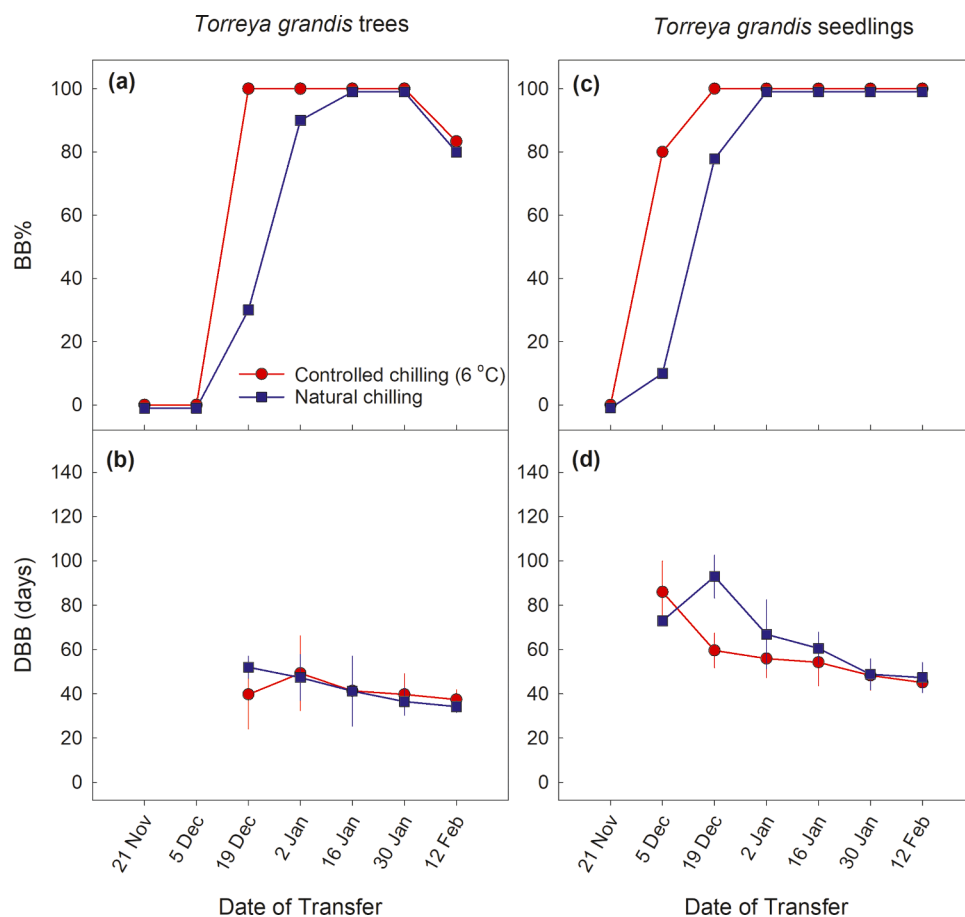
Fig. 2. Bud burst percentage (BB%, upper panels) and Days to Bud Burst (DBB, mean ± SE, lower panels) in Experiment 1 of *Torreya grandis* (a,b) twigs representing adult trees and (c,d) seedlings. The experimental material was first exposed to chilling in natural conditions outdoors (Fig. S3) or at constant +6 °C in walk-in growth chambers and then transferred to forcing conditions at constant +20 °C in walk-in growth chambers. Exceptionally, the first treatment groups were transferred at the beginning of the experiment on November 21 directly from natural conditions to the forcing conditions (zero chilling). In *Torreya* twigs representing adult trees, bud burst was determined on the basis of both vegetative buds and female flower buds. For details, see Material and methods and Fig. S5.

conditions. The increasing BB% values with successive transfers show that for an increasing part of the populations examined, there had been sufficient endodormancy release taking place in the chilling conditions during December to allow leafout / flowering to occur under subsequent high-temperature conditions. Further endodormancy release was generally seen as a descending DBB curve with successive transfers (Fig. 2d, 3d, 3f, 4b), but with *Torreya* trees (Figure 2b, 3b) the DBB did not vary as much with the transfer time as it did with the other material categories showing this dormancy pattern.

The second dormancy pattern is represented by *Castanopsis* (Fig. 3g, 3h) and *Phoebe* (Fig. 3i, 3j) seedlings, showing a BB% of 100 in all transfers. Endodormancy was manifested only as a significant decrease of DBB with successive transfers (Fig. 3h, 3j; Table 3). These results show that contrary to the species of the first dormancy pattern, these two species have a relatively shallow endodormancy, especially because the decrease in the DBB with successive transfers was relatively small in them (Fig. 3h, 3j).

From experiment 2, results for the lowest chilling temperature -2 °C are available for the first four transfers only. The limited results available for that temperature did not deviate drastically from the results for the other chilling temperatures (Fig. 3). Differences among the material categories were found in their responses to the different chilling temperatures between +5 and +15 °C (Fig. 3). Within the first dormancy pattern, differences were found in the responses to the highest two chilling temperatures, +10 and +15 °C (Fig. 3a-f). In *Torreya* trees, no bud burst was observed after chilling at +15 °C, and the BB% increased with successive transfers more slowly after chilling at +10 °C than after chilling at the lower temperatures (Fig. 3a). In *Torreya* seedlings (Fig. 3c) and *Pseudolarix* seedlings (Fig. 3e), the BB% results obtained after chilling at +10 °C did not deviate from those obtained after chilling at the lower temperatures; and with these seedlings, the chilling

temperature of +15 °C also caused endodormancy release, even though the BB% increased with successive transfers more slowly after chilling at +15°C than after chilling at the lower temperatures (Fig. 3c, 3e). Interestingly, the BB% curve found for +15 °C in *Torreya* seedlings (Fig. 3c) and *Pseudolarix* seedlings (Fig. 3e) is quite similar to the corresponding curve found for +10 °C in *Torreya* trees (Fig. 3a). Within the second dormancy pattern there were no major differences among the DBB values obtained for the different chilling temperatures (Fig. 3h, 3j), except that in the late transfers of *Castanopsis,* the DBB was lower for +15 °C than for the lower chilling temperatures (Fig. 3h).

In the climatological comparison, a major difference was found between the subtropical (Hangzhou) and the boreal (Jyväskylä) climate (Fig. 5). With low values of the threshold temperature, T_{thr}, the Number of Chill Days, NCD, during the chilling period showed more year-to-year variation in Hangzhou than in Jyväskylä (Fig. S7). This was caused by the relatively low frequency and large year-to-year variation in the occurrence of temperatures below +10 °C in Hangzhou during the chilling period addressed (November - February). For instance, with the lowest threshold temperature of +6 °C, the year-to-year minimum of NCD(120) was 15 days (Fig. S7b), indicating that in this particular year there were only 15 days with T_{mean} below +6 °C during the entire 120-day chilling period. The findings of this year determine the value of the parameter NCD_{min} (see Fig. 1): NCD_{min} = NCD(120) = 15 days. At the other extreme there was one year when the value of NCD(i) attained the value NCD_{min} = 15 days on day i = 43 (13 December) already (Fig. 5, S7b). This indicates that in this particular year, which determines the value of the parameter t_{early} (see Fig. 1), there were 15 days with T_{mean} below +6 °C within the first 43 days of the 120-day chilling period starting from 1 November. In all, the irregular occurrence of low temperatures resulted in Chilling Completion Time Range (CCTR) values of over 70 days with the lowest threshold temperatures +6 and +8 °C in

Table 3
Results of a logistic regression analysis with a binary response of factors affecting the bud burst percentage, BB%, and a two-way analysis of variance of factors affecting the days to bud burst, DBB, in forcing conditions for four subtropical tree species. *Transfer* represents the transfer time from chilling to forcing conditions, *Chilling* the details of the chilling treatment applied in Experiments 1 and 2, and *Forcing* the forcing temperature applied in Experiment 3 (for details, see Materials and methods). The logistic regression analysis was not applied on *Castanopsis sclerophylla* and *Phoebe chekiangensis*, since the BB% was 100% in all treatments.

		DF	BB% P	DBB F	P
Experiment 1	*Torreya grandis* trees				
	Transfer	4	<0.001	3.362	0.014
	Chilling	1	0.360	0.147	0.702
	Transfer x Chilling	4	<0.001	1.179	0.327
	Torreya grandis seedlings				
	Transfer	5	<0.001	25.025	<0.001
	Chilling	1	0.052	7.676	0.007
	Transfer x Chilling	5	<0.001	4.964	0.000
Experiment 2	*Castanopsis sclerophylla* seedlings				
	Transfer	8	-	266.018	<0.001
	Chilling	4	-	40.224	<0.001
	Transfer x Chilling	27	-	10.565	<0.001
	Phoebe chekiangensis seedlings				
	Transfer	8	-	49.107	<0.001
	Chilling	4	-	7.286	<0.001
	Transfer x Chilling	27	-	3.906	<0.001
	Pseudolarix amabilis seedlings				
	Transfer	7	0.012	178.143	<0.001
	Chilling	4	<0.001	67.441	<0.001
	Transfer x Chilling	20	<0.001	3.901	<0.001
	Torreya grandis trees				
	Transfer	6	<0.001	25.740	<0.001
	Chilling	2	0.254	5.127	0.007
	Transfer x Chilling	10	<0.001	5.802	<0.001
	Torreya grandis seedlings				
	Transfer	7	<0.001	22.577	<0.001
	Chilling	4	0.408	5.103	0.001
	Transfer x Chilling	19	<0.001	1.832	0.022
Experiment 3	*Torreya grandis* seedlings				
	Transfer	8	<0.001	101.042	<0.001
	Forcing	3	<0.001	226.624	<0.001
	Transfer x forcing	12	<0.001	1.993	0.028

Hangzhou (Fig. 5). In Jyväskylä, on the contrary, temperatures below +10 °C occurred frequently and regularly during the chilling period (September – December), so that with the lowest values of the threshold temperature, the CCTR in Jyväskylä was less than half of its value in Hangzhou (Fig. 5). In Jyväskylä the CCTR was lower than 40 days with all threshold temperatures, whereas in Hangzhou it was lower than 40 days only with the threshold temperatures of +12 °C or higher (Fig. 5).

4. Discussion

4.1. Chilling requirement in subtropical tree species

In accordance with our first hypothesis and the results of Du et al. (2019) and Song et al. (2020), our experimental findings provide evidence for the existence of a chilling requirement of endodormancy release in the four subtropical tree species examined. This showed up consistently in the increasing BB% and/or decreasing DBB with successive transfers from the chilling to the forcing conditions (Fig. 2-4; Hänninen et al., 2019). We applied chilling treatments in both natural and controlled constant temperature conditions. Most of our forcing treatments were carried out in constant temperature conditions, but in Experiment 3 with *Torreya* seedlings we applied diurnally fluctuating temperatures in the forcing. In order to examine whether there are differences in endodormancy release between seedlings and mature trees (Ununger et al., 1988; Partanen et al., 2005, 2016, Hänninen, 2016), we used both of these material categories in Experiments 1 and 2 with *Torreya*. In broad terms, the results of the different experimental treatments with different species and experimental materials representing different tree ages were similar, suggesting that our main conclusion in support of the existence of a chilling requirement in the four subtropical tree species examined is robust (for differences among the species, see below).

However, we found a major difference between *Torreya* seedlings and *Torreya* trees in the temperature range effective in endodormancy release, as +15 °C caused endodormancy release in the seedlings but not in the twigs (Fig. 3a, c). It should be noticed that even though the stage of development used to determine 'bud burst' in the *Torreya* twigs representing adult trees was based on a flowering event, the occurrence of that event was preceded by development in the adjacent vegetative buds (Fig. S5). Accordingly, the difference between *Torreya* seedlings and trees found in the present study is evidently a manifestation of differences between individuals of different ages, not of those between vegetative and flower buds. This finding suggests that results obtained with seedlings cannot necessarily be generalized to mature trees and the flower buds on them (Vitasse, 2013). The broader effective temperature range for endodormancy release in seedlings than in adult trees may
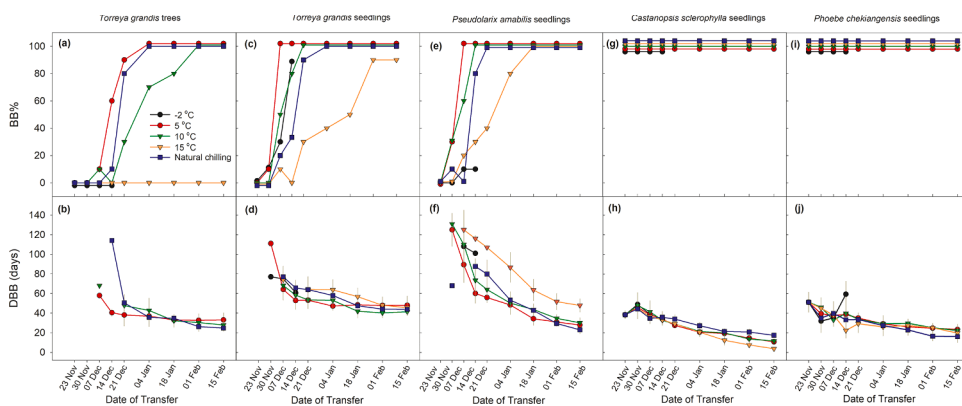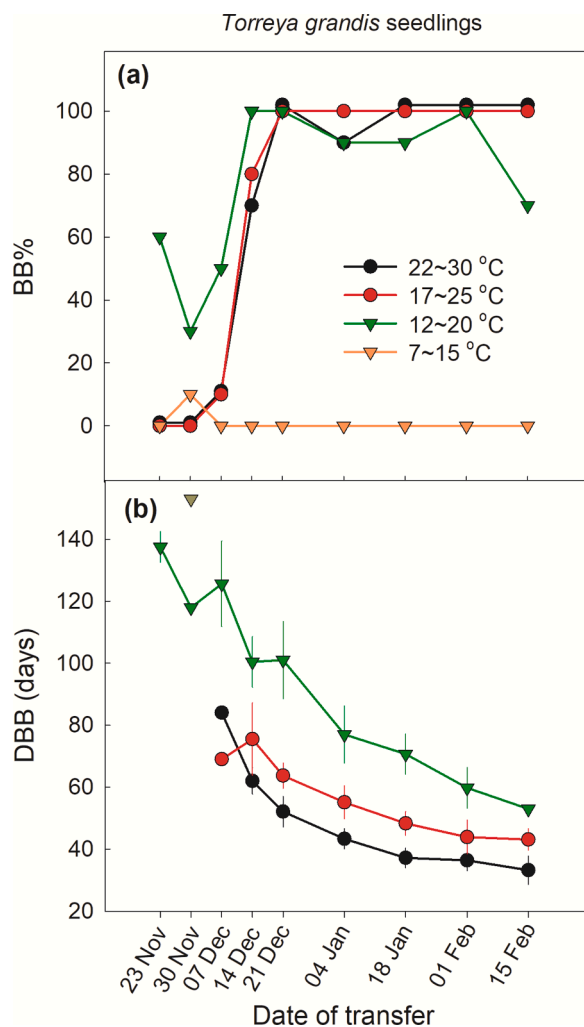

Fig. 3. Bud burst percentage (BB%, upper panels) and Days to Bud Burst (DBB, mean ± SE, lower panels) of (a,b) *Torreya grandis* twigs, representing adult trees, and seedlings of (c,d) *Torreya grandis*, (e,f) *Pseudolarix amabilis*, (g,h) *Castanopsis sclerophylla*, and (i,j) *Phoebe chekiangensis* in Experiment 2. The experimental material was first exposed to chilling in natural conditions outdoors (Fig. S3) or at constant -2, +5, +10, or +15 °C in walk-in growth chambers and then transferred to forcing conditions at constant +20 °C in walk-in growth chambers. Exceptionally, the first treatment groups were transferred at the beginning of the experiment on November 23 directly from natural conditions to the forcing conditions (zero chilling). In *Torreya* twigs representing adult trees, bud burst was determined on the basis of both vegetative buds and female flower buds. For details, see Material and methods and Fig. S5.

Fig. 4. Bud burst percentage (BB%, upper panel) and Days to Bud Burst (DBB, mean ± SE, lower panel) of *Torreya grandis* seedlings in Experiment 3. The experimental material was first exposed to chilling in natural conditions outdoors (Fig. S3) and then transferred to forcing conditions in Whole Tree Chambers (Fig. S4). Exceptionally, the first treatment groups were transferred at the beginning of the experiment on November 23 directly from natural conditions to the forcing conditions (zero chilling). In the forcing conditions, four treatments with diurnally fluctuating air temperature were applied. The daily minimum and maximum temperatures for the four treatments are shown in the upper panel. In each treatment the range of diurnal temperature variation was 8 °C. The treatments formed a temperature gradient such that the average temperature was raised by 5 °C from one treatment to the next.

reflect the more opportunistic growth strategy of the former (Vitasse and Basler, 2014). However, despite the wide use of detached twigs in endodormancy experiments, it is possible that any difference observed in the present study between the detached twigs and the seedlings of *Torreya* were due to physiological disturbances in the twigs caused by the detaching. However, when addressing this potential source of error, Partanen et al. (2016) did not observe any major differences between control seedlings and those where the root system was cut by detaching the shoot from the root system. Similarly, Vitasse and Basler (2014) concluded that detached twigs provide a good proxy of phenological responses of naturally growing adult trees.

In the manifestation and depth of endodormancy, however, there were major differences among the species. On the basis of these differences, the species were classified into two dormancy patterns. The species of the first pattern (*Torreya, Pseudolarix*) were found to have relatively deep endodormancy because no bud burst was found in them
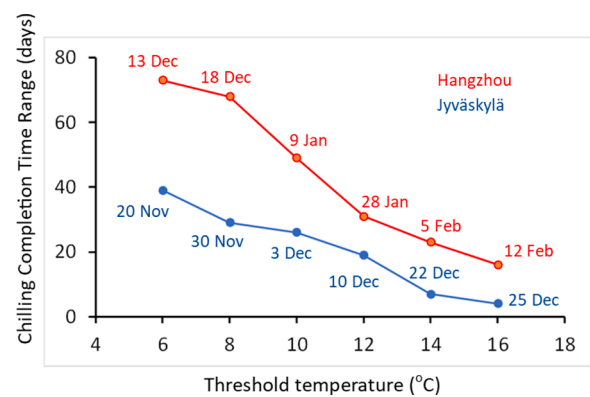

Fig. 5. Comparison of climatic conditions at two geographical locations: Hangzhou, southeastern China (30°14'N, 120°1'E), representing the growing sites of the subtropical trees studied in the experiments of the present study; and Jyväskylä, Central Finland (62°14'N, 25°44'E), representing typical climatic conditions of the boreal trees used for comparison in the present study (Fig. S1a). The variable Chilling Completion Time Range, CCTR, on the vertical axis indicates the year-to-year variation in the occurrence of air temperatures below the threshold indicated by the horizontal axis. For details, see Material and methods and Fig. 1.

after the first transfers from the chilling to the forcing conditions; whereas the species of the second pattern (*Castanopsis, Phoebe*) were found to have relatively shallow endodormancy, manifested only by a relatively small, yet significant, shortening of the time required for bud burst in the forcing conditions with successive transfers (Fig. 2-4; Table 3). One may even question whether *Castanopsis* and *Phoebe* show any endodormancy and chilling requirement at all, for regardless of the duration of the chilling exposure, all seedlings of these two species showed bud burst in the experiment (Fig. 3g, 3i). However, according to the established criterion, the decline of DBB with successive later transfers alone is sufficient to show the existence of endodormancy and a chilling requirement (Hänninen, 2016, Hänninen et al., 2019). Quite often, the BB% values are even not reported at all when the existence of endodormancy is inferred from the DBB-curve (Worrall and Mergen, 1967; Leinonen et al., 1995; Leinonen, 1996; Myking, 1997, 1998).

As none of the tree provenances examined in the present study was from the southern part of the subtropical zone (Fig. S2), one should not generalize the results to more southern subtropical tree species and provenances. Rather, one could even hypothesize that subtropical trees near the borderline of the tropical zone would not have endodormancy at all, or at least not a chilling requirement.

4.2. Upper threshold for the chilling temperature range releasing endodormancy, T_{upp}

Though calculations of chilling accumulation are widely used in studies addressing springtime tree phenology, there is no agreement on the chilling temperature range effective in endodormancy release (Table 1, Section 4.4), nor on the temperature response of the rate of endodormancy release within that range. This is a reflection of the shortage of experimental studies in which the effects of various temperatures on endodormancy release are determined explicitly (Hänninen, 2016). In the absence of such experimental studies, Du et al. (2019) and Song et al. (2020) followed a procedure commonly applied with temperate and boreal trees in their study of the chilling requirement of subtropical woody plants: they assumed that only temperatures below +5 °C are effective in endodormancy release. Contrary to that assumption and in accordance with our second hypothesis, we found that the air temperature response of the rate of endodormancy release in subtropical trees is different from the responses often assumed (Table 1) and, in a few studies, also found (Fig. S1a; Sarvas, 1974) for the more northern trees. Accordingly, we found that depending on the species,

temperatures of up to +15 °C can be effective for endodormancy release in subtropical tree species. Furthermore, with the exception of *Torreya* trees, the highest chilling temperature (+15 °C) caused endodormancy release in the present study (Fig. 3). This implies that the value of T_{upp} may be even higher than +15 °C for sub-tropical trees. The limited experimental results available for boreal and temperate trees, mainly for temperate horticultural crops, suggest that such high temperatures are usually non-effective in endodormancy release (Sarvas, 1974; Naor et al., 2003), and temperatures above +15 °C have also been found to cause negation of the endodormancy-releasing effect of the previous chilling (Overcash and Campbell, 1955; Erez and Lavee, 1971; Richardson et al., 1974; Erez et al., 1979a(Erez et al., 1979b; Hänninen and Pelkonen, 1989).

Process-based tree phenology models are currently used for assessing the effects of climatic change on boreal and temperate trees (Hänninen and Kramer, 2007; Kramer and Hänninen, 2009; Chuine et al., 2013; Hänninen, 2016; Chuine and Régnière, 2017; Hänninen et al., 2019). Our experimental results suggest that when the scope of process-based tree phenology modelling is broadened to cover subtropical trees (Chen et al., 2017), the concept of chilling has to be broadened to include higher temperatures than those usually assumed for boreal and temperate trees (Table 1). Otherwise, the model prediction for meeting the chilling requirement of endodormancy release will be too late in comparison with the true response of the trees.

In the absence of explicit experimental data, the air temperature response of endodormancy release is often approximated by various fixed rules for calculating the chilling accumulation. Accordingly, the response determined experimentally by Sarvas (1974) for boreal *Betula pubescens* (Fig. S1a) has subsequently been applied with several other boreal tree species as well (Hänninen 1990, 1991, 1995, Häkkinen et al. 1998, Linkosalo et al. 2006). Similarly, the threshold model (Fig. S1b) has been applied with the threshold temperature of +5 °C in several recent studies addressing temperate tree species (Fu et al., 2015a,b, 2019a,b, Chen et al. 2018, 2019, Ma et al. 2018). Our results, however, show that the real temperature response can vary even among tree species growing at the same geographical region. This shows that there is an urgent need to determine the real temperature response experimentally for as many tree species as possible (Harrington et al., 2010; Hänninen et al., 2019).

4.3. A generalized hypothesis for a temperature range physiologically effective in endodormancy release

In our climatological comparison, a decreasing Chilling Completion Time Range, CCTR, was found with increasing threshold temperature, T_{thr}, in both subtropical Hangzhou and boreal Jyväskylä (Fig. 5). This result is axiomatic: the CCTR is a measure of the year-to-year variation in the occurrence of chill days, that is, days with the daily mean temperature below T_{thr}. This implies that with an increasing value of T_{thr} an increasing part of the days will be classified as chill days, so that the year-to-year variation measured by the CCTR decreases. It is also axiomatic that with any given value of T_{thr}, the CCTR is higher in Hangzhou than in Jyväskylä (Fig. 5). This is because temperatures are higher in Hangzhou than in Jyväskylä, so that with any given value of T_{thr} a larger number of days will be classified as chill days in Jyväskylä than in Hangzhou.

However, with successively lower temperatures from just above +10 °C, the CCTR increased more rapidly in Hangzhou than in Jyväskylä (Fig. 5). This shows that temperatures below +10.4 °C, required for endodormancy release in boreal *Betula pubescens* (Fig. S1a), occur so rarely and irregularly in the autumn and winter of the subtropical climate that relying only on them as an environmental cue would result in a year-to-year range of over two months in the time of endodormancy release completion in the idealized tree assumed in the calculations (see Section 2.6). When higher temperatures of up to +16 °C were also assumed to be effective in endodormancy release, the range predicted

for subtropical trees narrowed to the level typically predicted for boreal trees (Fig. 5). This accords with our third hypothesis: differences in the occurrence of low autumn temperatures between subtropical and boreal climates (Fig. 5) explain the corresponding differences in the response to chilling temperatures between subtropical (Fig. 3) and boreal (Fig. S1a) tree species.

Due to the limited empirical data, our results are to be considered preliminary. Our climatological comparison is a simplifying one, being based on many assumptions about matters such as the period in which chilling is effective in endodormancy release. Furthermore, only two geographical locations were included in our first-time comparison. Accordingly, rather than presenting conclusive results, our study introduces the principle of, and the method for, the climatological comparison to be used in later studies. Similarly, our experimental results are restricted because due to the limited growth chamber facilities, only four subtropical tree species were examined and no replicates were included in our experiments. Finally, as discussed above, there is only a limited number of earlier experimental studies addressing the temperature response of the rate of endodormancy release in boreal and temperate trees, either.

Despite the limitations of our study, we found that our results, when examined together with Sarvas's (1974) findings (Fig. S1a), support our third hypothesis, concerning the reason for the differences in the temperature response of the rate of endodormancy release in trees originating from different climatic conditions. Encouraged by this, we put forward a testable generalized hypothesis: in any climatic conditions where trees show endodormancy, the range of temperatures physiologically effective in endodormancy release represents the range of typical autumn air temperatures occurring in those particular climatic conditions.

The geographical differences in endodormancy release that we hypothesize are analogous to other geographical differences among tree species and even provenances of a given species, which are well-documented for several tree traits related to the annual cycle of growth and dormancy (Hänninen, 2016). For instance, it has been known for long that the critical night length triggering growth cessation and bud set in late summer is shorter in northern species and provenances than in southern ones (Ekberg et al., 1979; Junttila, 1980; Howe et al., 1995). This is clearly an adaptation to the night length prevailing in the native growing conditions, which before autumn equinox is shorter in the north than in the south. Along this line of reasoning, we now hypothesize that the endodormancy release has been similarly adapted to the temperature conditions prevailing in the native growing site of the trees.

Our hypothesis can be readily tested by applying the methods of the present study on tree species and climatic records from different climatic zones. This calls for further research that combines climatological analyses with experimental studies addressing the temperature response of the rate of endodormancy release.

4.4. Earlier studies with $T_{upp} > 10 \, °C$

Contrary to the mainline research documented in Table 1, there are earlier studies where temperatures above +10 °C have been suggested to be effective in endodormancy release in boreal and temperate trees and other plants as well (Richardson et al., 1974; Landsberg, 1974; Tanino and Wang, 2008; Chavarria et al., 2009). Such results have sometimes been found in studies where process-based tree phenology models have been fitted to observational phenological records. Upper thresholds well above +10 °C have been estimated in these studies. However, due to the methodological problems already revealed by Hunter and Lechowicz (1992) and recently discussed by Hänninen et al. (2019), estimates based on observational phenological records have an exceptionally high degree of uncertainty. Hunter and Lechowics (1992) used the models to generate theoretical phenological records and then used the generated data for testing other models. They found that besides the 'realistic'

model used for generating the data, several other models fitted to the generated data predicted the phenological timing in the generated data set equally well. This shows that a modelling approach based on observational phenological records will not enable us to identify a realistic model that reflects true causal relationships. For another example discussed in Hänninen et al. (2019), Kramer (1994) used a modelling approach based on observational phenological records to estimate a T_{upp} value of +165.8 °C for *Fraxinus excelsior*, which cannot be a realistic value. These problems are a consequence of the information in the observational phenological records being insufficient for identifying and parameterizing process-based tree phenology models. This shows that process-based tree phenology modelling needs to be based on experimental approaches (Hänninen et al., 2019). In all, then, modelling studies based on observational phenological records do not provide strong evidence for the existence of T_{upp} values above +10 °C in boreal and temperate trees.

Values of T_{upp} well above +10 °C have been suggested for boreal and temperate trees in experimental studies, too. Garber (1983), for instance, concluded that temperatures from 0 to +12 °C are equally effective in endodormancy release of loblolly pine, and Harrington et al. (2010) found the value of $T_{upp} = +16$ °C for Douglas-fir. However, rather than being based on an experimental design explicitly set up for determining the value of T_{upp} with a set of various constant temperatures, i.e., the design used in the present study and in Sarvas's study (1974) (Fig. S1a), Garber's (1983) and Harrington et al.'s (2010) conclusions were based on indirect inferences from results obtained by chilling in various fluctuating temperatures, which renders some uncertainty to their conclusions. In contrast, by using an explicit experimental design, Gilreath and Buchanan (1981) and Erez and Couvillon (1987) found the value of approximately +14 °C for T_{upp} in peach, thus providing conclusive evidence against the mainline concept (Table 1) for the varieties of peach they studied. Using a similar explicit experimental approach, Heide and Prestrud, 2005 found +6 and +9 °C effective but +12 °C only marginally effective for endodormancy release in apple and pear. Observations of values of T_{upp} above +10 °C for horticultural crops grown in temperate zones may actually be well in line with our hypothesis presented in Section 4.3, as many of these tree species originate from more southern conditions.

In all, it is possible that T_{upp} values well above +10 °C occur in boreal and temperate tree species, too. It should be noticed, however, that the relatively infrequent findings supporting this notion are not inconsistent with our hypothesis. This is because our hypothesis implies that the value of T_{upp} should not be so low that temperatures below it occur infrequently and irregularly in the native climate of the trees. In other words, our hypothesis addresses the problem of insufficient chilling in cases where T_{upp} is so low that the air temperature does not drop below it sufficiently often. Conversely, if the value of T_{upp} for boreal trees is increased from +10.4 °C (Sarvas 1974, Fig. S1a) to +16 °C (Harrington et al. 2010), this will inevitably increase the amount of chilling available. In that case the shortage or irregular occurrence of chilling would not be a problem for the trees.

The trees may then face a different problem, however, not addressed in our hypothesis: in boreal climates in particular, temperatures between +10 and +16 °C often occur in early autumn already. If those temperatures cause endodormancy release in the trees, as suggested by Harrington et al. (2010), then the trees may run a risk of premature endodormancy release in the autumn. In other words, if temperatures well above +10 °C are effective in chilling accumulation, then chilling accumulation in boreal conditions may not provide the trees with a reliable environmental cue of the progress of autumn and winter any longer (Sarvas, 1974).

Together with the empirical findings discussed above, these theoretical considerations call for further experimental studies explicitly addressing the temperature response of endodormancy release, also in boreal and temperate trees (Harrington et al., 2010; Hänninen et al., 2019).

5. Conclusions

Our experimental results suggest that contrary to the established idea of chilling temperatures, higher temperatures of up to +15 °C are effective for endodormancy release in the subtropical tree species examined. These results will facilitate the development of realistic process-based models of tree phenology for use in assessing the effects of climatic warming on subtropical trees and ecosystems. We introduced a novel method for analysing long-term climatic records in relation to experimental findings in order to explain differences in the temperature response of endodormancy release in trees originating from different climatic conditions. Tentative results obtained with this method suggest that the difference in the temperature response of endodormancy release found between tree species originating from the boreal and the subtropical climate can be explained by the differences in the occurrence of low autumn temperatures between these two zones. On the basis of this novel result, we put forward the testable generalized hypothesis that in any climatic conditions where trees show endodormancy, the range of temperatures physiologically effective in endodormancy release represents the range of typical autumn air temperatures occurring in those particular climatic conditions. Due to the limitations of our study, we confine ourselves here to presenting the hypothesis and a method for testing it in forthcoming studies rather than providing conclusive results.

Declaration of Competing Interest

None.

Acknowledgements

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2020.108296.

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