

Convergence of leaf-out towards minimum risk of freezing damage in temperate trees

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Summary

1. Within the same forest stand, temperate deciduous trees generally exhibit a distinct pattern in leaf-out timing, with some species flushing earlier than other species. This study aimed to explain the timing of leaf-out of various temperate tree species in relation to the risk of freezing damage to leaves.

2. We combined long-term series of leaf-out date (14–32 years) of five temperate tree species located in both low and high elevations in Switzerland, daily minimum temperatures recorded at the same sites and species-specific freezing resistance (LT_{50}) of emerging leaves. We calculated temperature safety margins (the temperature difference between absolute minimum temperature during leaf-out and species-specific LT_{50} values), and date safety margins (time lag between the last day when temperature falls below species-specific LT_{50} values and the date of leaf-out).

3. Leaf-out occurred when the probability to encounter freezing damage approaches zero, irrespective of climatic conditions (low vs. high elevation) and species (early- and late-flushing species). In other words, trees leaf out precisely at the beginning of the probabilistically safe period. Interestingly, the temperature safety margins did not differ significantly between low and high elevation. Yet, the date safety margin was smaller at high elevation, presumably due to a faster increase in temperature during the leaf-out period at high elevation.

4. When species-specific freezing resistance is taken into account, the time of leaf-out converges among species towards a marginal risk of freezing damage. Thus, leaf-out time has likely evolved in a way that the risk of freezing damage is minimized over a large spectrum of climatic conditions. Species with a small safety margin against freezing temperature, like *Fagus sylvatica*, appear to employ photoperiod co-control of spring phenology, whereas species with a large safety margin depend largely on temperature for the right timing of leaf-out.

5. Our results offer a new avenue to explain the differences in leaf-out timing among co-occurring tree species. They further suggest that in a warming climate, tree species can expand their distribution range to the extent their phenology matches the stochasticity of freezing temperatures in spring.

Key-words: bud break, cold hardiness, evolution, frost, LT_{50} , phenology, safety margin, temperate deciduous forest

Introduction

Leaf-out of temperate deciduous trees is likely driven by a trade-off between minimizing the risk of freezing damage and maximizing the length of the growing season (Cannell 1997). Accurate timing of development is crucial for

temperate deciduous trees in spring. Thus, temperate and boreal trees employ sophisticated mechanisms to control the time of flushing, including a combination of temperature and photoperiod cues. Low winter temperatures (chilling) and increasing day-length from the winter solstice onwards (photoperiod) are involved in dormancy release, and warm temperatures in spring modulate the date of leaf-out (Polgar & Primack 2011; Basler & Körner 2012).

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Although it is still unclear how temperature and photoperiod contribute to metabolic activation of bud tissue in spring (Cooke, Eriksson & Junttila 2012), a simple accumulation of degree-days has been widely used in agronomy and agroforestry to assess the onset of vegetative growth since the 18th century (Réaumur 1735).

Leaf-out dates can differ among temperate tree species growing within the same forest by more than a month (Kramer 1995; Augspurger 2009; Vitasse 2013). Lechowicz (1984) suggested that such differences among species could arise from phylogenetic constraints, ecological disequilibrium caused by climate change, and trade-offs of leaf-out with other traits of a given tree species. The author further assumed that freezing temperatures alone could not satisfactorily explain the difference in leaf-out dates among species, since all species within a forest stand would then have to leaf out at approximately the same time. However, leaf-out in temperate tree species can only be explained by freezing temperature when the species-specific freezing resistance of emerging leaves is known, since it varies substantially among species (Vitasse *et al.* 2014).

Temperate deciduous trees escape freezing damage to leaves in winter by shedding them in autumn and producing winter-hardy buds. In spring, a new leaf canopy is built from the overwintering buds. These emerging leaves are very vulnerable to freezing damage. A major limitation assessing the risk of freezing damage in temperate plants is the absence of long-term data about freezing damage in spring, except for montane wildflowers in the United States where an increase in freezing damage was attributed to earlier snowmelt (Inouye 2008). No such long-term records exist for long-lived trees, although particularly widespread freeze events in North America as well as in central Europe were recently documented (Gu *et al.* 2008; Augspurger 2009; Kreyling, Stahlmann & Beierkuhnlein 2012). Hence, freezing damage in spring is generally attributed to a common threshold temperature across different tree species, ranging from -4°C to 0°C (e.g. Kramer 1994; Leinonen & Hänninen 2002; Bennie *et al.* 2010; Augspurger 2013). For instance, Augspurger (2013) used a temperature threshold of -1.7°C to assess the risk of freezing damage in 20 woody species after bud break in Illinois (USA) during the 20th century. Yet, young and mature leaves are more freezing resistant in spring than this arbitrary temperature threshold, and freezing resistance depends strongly on species (e.g. Till 1956; Taschler, Beikircher & Neuner 2004; Charra-Vaskou *et al.* 2012; Lenz *et al.* 2013). For instance, LT_{50} values (the temperature that is lethal for 50% of samples) of emerging leaves ranged from -9°C to -3°C in eight dominant tree species at their upper elevational range limit in the Swiss Alps (Lenz *et al.* 2013). Similar LT_{50} values were found in seven tree species in lowland conditions (Vitasse *et al.* 2014). Using accurate and species-specific LT_{50} values is crucial to assess the risk of freezing damage, since one or two degrees of difference correspond to about 400 m of elevation or a shift of several days of the safe time period.

In a recent study, we showed that temperate trees delay their flushing with increasing elevation so that the risk of freezing damage remains constant along the elevational gradient in the Swiss Alps (Lenz *et al.* 2013). In other words, deciduous broadleaved trees experience the same low-temperature extremes during leaf-out irrespective of elevation. However, in this previous study the exact frequency of freezing damage (the risk of a damage in a particular year) was predicted using a thermal time model and not actual observations. Temperature and phenology show a large variation along temporal and spatial scales, which is not well estimated by current phenological models (Basler 2016). To better approximate the frequency of freezing damage, actual phenological observations must be combined with temperatures recorded at the same site. Here, we combine for the first time long-term data of leaf-out phenology and onsite temperature with freezing resistance of emerging leaves. We address the following questions: (i) When do trees leaf out relative to the probability of freezing damage? (ii) Do trees experience the same risk of freezing damage at low and at high elevations? (iii) Do growing degree-days accumulated during spring correlate with freezing damage to emerging leaves across spatial and temporal gradients?

Materials and methods

STUDY SITE AND SPECIES

We selected five winter-deciduous tree species with contrasting leaf-out: *Sorbus aucuparia* L., *Prunus avium* L., *Tilia platyphyllos* Scop., *Acer pseudoplatanus* L. and *Fagus sylvatica* L. (from the earliest- to the latest-flushing species). For brevity, we refer to each species by its genus. We chose species for which long-term leaf-out data and LT_{50} values of emerging leaves were available. Absolute minimum temperatures are difficult to predict statistically from weather stations because of their high regional variability, especially in complex terrain such as in mountain areas (Kollas *et al.* 2014). Therefore, we selected eight stations with phenology and temperature records on the same site, or within 100 m of elevation (Table 1). Sites were distributed throughout Switzerland. We categorized sites into low-elevation (<500 m a.s.l.) or high-elevation sites (>900 m a.s.l.; Table 1). Sites are characterized by a temperate continental climate, with a mean annual precipitation ranging from 700 to 1700 mm (Table 1), and dominated by mature mixed forest stands, with calcareous or sandstone as bedrock.

PHENOLOGY, TEMPERATURE AND FREEZING RESISTANCE DATA

Leaf-out was observed weekly by one to two persons per site, following the protocol of MeteoSwiss. A tree was considered to have leafed out when 50% of its leaves were unfolded (leaf surface and leaf base visible; Brügger & Vasella 2003). Leaf-out dates were interpolated between weekly sampling events. Either one or a minimum of 10 individual trees were observed (Brügger & Vasella 2003); however, only the aggregate response of all the observed trees at a site was recorded. We extracted leaf-out dates from 1981 to 2012 for all species, except for *Prunus*, for which only flowering data were available. However, *Prunus* generally flowers very shortly before or simultaneous to leaf-out (Brügger & Vasella

Table 1. Location and mean annual precipitation of the study sites. The study sites are ordered from low to high elevation

Site	Elevation (m)			Latitude	Longitude	Precipitation (mm)
	Climate Station	Phenology recordings	Class			
Wädenswil	485	400	Low	47°13'N	8°41'E	1390
Altdorf	438	470	Low	46°52'N	8°40'E	1186
Hallau	419	430	Low	47°42'N	8°27'E	1072
Wynau	422	450	Low	47°15'N	7°49'E	1129
Elm	958	1000	High	46°55'N	9°10'E	1619
Chaumont	1073	1150	High	47°03'N	6°59'E	1289
Adelboden	1320	1350	High	46°30'N	7°34'E	1338
Scuol	1303	1240	High	46°48'N	10°18'E	706

Mean sum of annual precipitation for the period 1981–2010.

2003). Depending on species and station, 14–32 years of leaf-out data were available for the investigated period from 1981 to 2012 (Table 2). Leaf-out data were visually checked for consistency within species among stations, and among species within stations (see Figs S1 and S2, Supporting Information).

Air temperature was recorded by climate stations every 10 min in the vicinity of the observation sites for phenology from 1981 to 2012. Hereafter, we use °C for absolute temperature values and K for temperature differences, to avoid confusion, as suggested by McVicar & Körner (2013).

Freezing resistance of emerging leaves in spring primarily depends on the phenological stage, and only to a very small extent on temperature (Larcher & Mair 1968; Campbell & Sorensen 1973; Ibanez *et al.* 2010), and is not genetically differentiated among distinct populations (Flint 1972; Li *et al.* 2003). Since development largely overrules environmental or genetic components, we used freezing resistance data from different sites, but assessed at the same phenological stage (emerging leaves) with exactly the same method. LT₅₀ values were obtained from Lenz *et al.* (2013) for *Sorbus*, *Prunus*, *Acer* and *Fagus* at high elevation, and from Vitasse *et al.* (2014) for *Tilia* at low elevation (see Table S1).

CALCULATION OF LEAF-OUT RELATIVE TO THE PROBABILITY OF FREEZING DAMAGE

To estimate the risk of freezing damage in relation to the date of leaf-out, two independent probabilities need to be estimated, the

probability of trees having leafed out and the probability of the temperature falling below the species-specific LT₅₀ value for each spring date. These probabilities need to be estimated on a common time-scale, which accounts for phenological variation among stations. This allows us to test the hypothesis that, regardless of elevation or temporal variation in climate, leaf-out happens only after risk of freezing damage is minimal. Thus, we scaled the date of leaf-out at each station by the mean date of leaf-out, so that day 0 corresponds to the mean date of leaf-out across all years at each station for a species.

The data from MeteoSwiss represent a single leaf-out date for each station, species and year. For each scaled date, we converted the leaf-out dates to binomial count data, representing the number of years, across all available years, in which leaf-out had already occurred. These data represent a cumulative distribution function of leaf-out data at a given station (see Fig. S3), which can be estimated using logistic regression (Venables & Ripley 2002). The cumulative distribution function provides an estimate of the probability of a species having leafed out by a given scaled date, across all stations and years. Logistic regression was fit in *R* using *glm* with a log-link function across all stations, with the scaled date as the independent variable, and the leaf-out dates converted to binomial counts as the dependent variable.

Finally, we calculated the probability that the absolute minimum air temperature was below the species-specific LT₅₀ value for each day on the same relative date scale per station. This probability was calculated by summing up all years in which the absolute

Table 2. Start year of leaf-out observations, with the number of years of available leaf-out records for every species

	Start year of observation					Number of years					No. of observers
	<i>Sorbus</i>	<i>Prunus</i>	<i>Tilia</i>	<i>Acer</i>	<i>Fagus</i>	<i>Sorbus</i>	<i>Prunus</i>	<i>Tilia</i>	<i>Acer</i>	<i>Fagus</i>	
Low-elevation sites											
Wädenswil	1996	1981	1996	1996	1981	16	32	16	16	31	2
Altdorf	1999	1999	1999	1996	1981	14	14	14	14	14	2
Hallau	1996	1981	1996	1996	1981	17	32	17	17	30	1
Wynau	1996	1981	1996	1996	1981	17	32	17	17	32	2
High-elevation sites											
Elm	1996	1981	1996	1996	1994	17	31	17	17	32	1
Chaumont	1996	1994	1996	1996	1981	17	19	17	17	19	1
Adelboden	1996	1981	1996	1996	1981	16	30	12	16	31	2
Scuol	1996	1987	1996	1997	1981	17	26	17	16	32	1

Phenology was observed on either one or at least 10 individual trees up to the year 2012. In some stations, no records are available for 1 or 2 years, except for *Tilia* in Adelboden, where records are missing for 5 years. The study sites are ordered from low to high elevation.

minimum temperature was below the species-specific LT_{50} value of emerging leaves, divided by the total number of years. Freezing damage is only probable during the time period when the two probabilities overlap.

CALCULATION OF SAFETY MARGINS

To assess the risk of freezing damage at different elevations, we calculated the safety margin against freezing damage for low- and high-elevation sites similar to Lenz *et al.* (2013). We calculated two different safety margins for each species, site and year. The 'temperature' safety margin was defined as

$$\text{Temperature safety margin} = T_{\min} - LT_{50}$$

where T_{\min} corresponds to the absolute minimum air temperature from 5 days prior to 5 days after the date of leaf-out, and LT_{50} corresponds to the species-specific LT_{50} value. The 'date safety margin' was defined as

$$\text{Date safety margin} = \text{Doy}_{\text{Leaf-out}} - \text{Doy}_{\text{Last freeze}}$$

where $\text{Doy}_{\text{Leaf-out}}$ corresponds to the day of year of leaf-out in a specific year, and $\text{Doy}_{\text{Last freeze}}$ corresponds to the last day of year with an absolute minimum temperature below the species-specific LT_{50} value in late winter and spring. Positive values of both safety margins indicate that leaf-out occurred during the safe period when potential freezing damages were excluded, whereas a negative value indicates potential freezing damage.

RECURRENCE OF ABSOLUTE MINIMUM TEMPERATURES DURING LEAF-OUT

Due to the long lifespans of trees and the stochastic nature of low-temperature extremes, we calculated the probability of the recurrence of absolute daily minimum temperatures during leaf-out for a period of up to 100 years, using generalized extreme value distributions (GEV; Coles 2001), as described in detail in Kollas, Körner & Randin (2014). Briefly, we extracted all daily minimum temperatures at low- and high-elevation stations from 1981 to 2012. Then, we fitted a GEV distribution to all absolute daily minimum temperatures at low- or high-elevation stations for every week of the year, using the R-package 'ISMEV' version 1.39 (Heffernan & Stephenson 2012). We calculated the return rate of weekly absolute minimum temperatures by inverting the equation of the GEV distribution (see Eq 1 in Kollas, Körner & Randin 2014). For example, a return rate of 5 years for -4°C means that this temperature is expected to occur every fifth year. Absolute temperature minima were found to increase by 0.34 and 0.26 K per decade at low, respectively, high elevation since 1981, due to climate change. We thus calculated two different GEV models for every week of the year for low and high elevation, one GEV with constant location, scale and shape parameters, and one GEV with a linear trend in the location parameter to account for the warming trend. Accounting for climate warming, return rates were generally 0.6% less frequent at low elevation, and 1.3% less frequent at high-elevation sites from 1981 to 2012.

DATA ANALYSES

We fit linear mixed effects models to the date of leaf-out and both safety margins. Species, elevation and the interaction between the two were considered as fixed factors, the individual sites, and species nested within the site were considered as random effects. We visually tested for a normal distribution and

homoscedasticity of residuals of the chosen models. If results were significant, we calculated Tukey-HSD post hoc tests. All analyses were performed using R 2.15.3 (R Core Team 2013), using the R-package 'NLME' version 3.1-108 (Pinheiro *et al.* 2013) to calculate mixed effects models, and the R-package 'MULTCOMP' version 1.2-17 (Hothorn, Bretz & Westfall 2008) to calculate post hoc tests.

Results

SPATIAL AND TEMPORAL PHENOLOGICAL PATTERN

Leaf-out dates varied greatly among species and years, ranging from the earliest-flushing *Sorbus* on day 77 (in Wädenswil) to the latest-flushing *Fagus* on day 161 (in Adelboden; Fig. 1). Leaf-out dates differed also significantly between low and high elevations, but the interaction between species and elevation was nonsignificant (Table 3). For a given species, populations growing at high elevation flushed 15–21 days later than populations growing at low elevation, with a large year-to-year variability of 32–53 days within both low- and high-elevation sites (Fig. 1).

MINIMUM TEMPERATURES DURING SPRING

Mean minimum air temperatures ranged from ~ 2 to 9°C at low-elevation sites and were slightly cooler (1 K) at high-elevation sites during the leaf-out period from 1981 to 2012 (Fig. 2a,b). Absolute minimum temperature was around -7.5°C at low elevation and -14°C at high elevation during this period (Fig. 2a,b). Interestingly, the return rate of absolute minimum temperature was ~ 1.5 times larger at high elevation compared to low elevation during the respective leaf-out period (Fig. 2c,d). While temperatures of -1 and -2°C occurred every 21 or 52 years at high elevation in the middle of the leaf-out period (day 127, Fig. 2d), the corresponding return rates were every 30 and 76 years at low elevation (day 113,

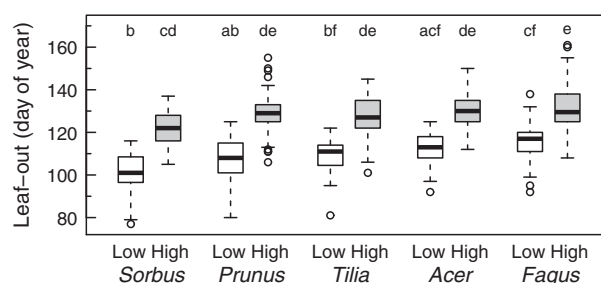


Fig. 1. The date of leaf-out for each species across low- and high-elevation sites. White boxes represent low-elevation sites, while grey boxes represent high-elevation sites. The boxplots show the median, the first and third quartile, and the extreme point. Any point extending more than 1.5 times the interquartile distance from the first to the third quartile is drawn as an outlier. Species are ranked from left to right from early to late leaf-out date. Different letters indicate significant ($P < 0.05$) differences in the mean leaf-out date tested by Tukey's HSD test.

Table 3. Summary of the analysis of variance using general linear models on the date of leaf-out, the temperature safety margin and the date safety margin

Source of variation	Degrees of freedom		Date of leaf-out		Temperature safety margin		Date safety margin	
	Num	Den	F	P	F	P	F	P
<i>Fixed effects</i>								
Species	4	24	11.0	< 0.0001	34.6	< 0.0001	15.2	< 0.0001
Elevation	1	6	49.3	< 0.001	0.6	0.46	16.4	< 0.01
Species × Elevation	4	24	1.0	0.44	2.9	< 0.05	2.8	0.05
<i>Random effects</i>								
Station			Sigma		Sigma		Sigma	
Species within station			3.44		0.43		3.2	
Residual variation			7.5		2.30		17.7	

Degrees of freedom are the same for all models.

Num, numerator degrees of freedom; Den, denominator degrees of freedom; F, F-value; P, P-value shown in bold when $P < 0.05$.

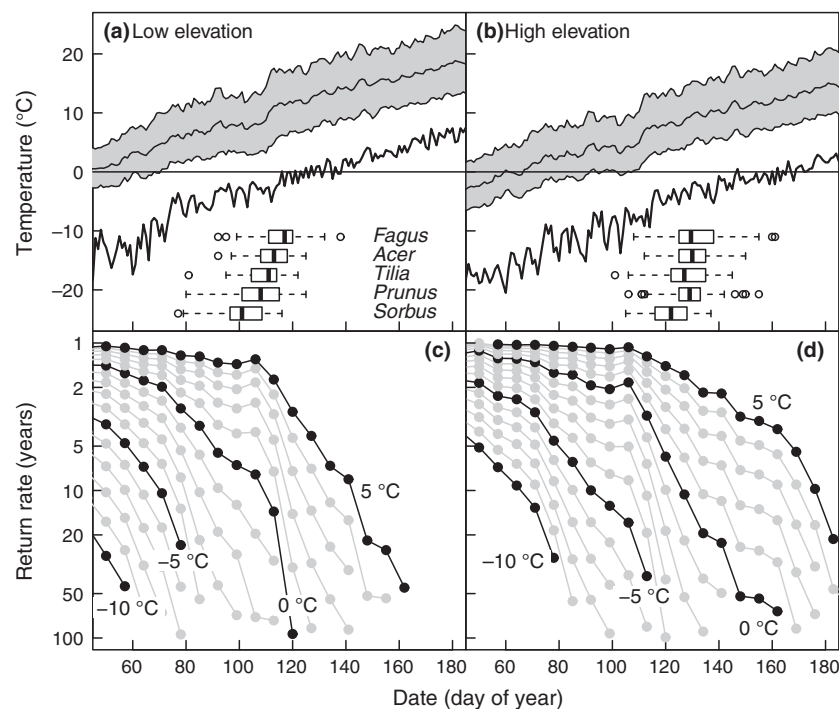


Fig. 2. Air temperature records for low- and high-elevation stations during the leaf-out period. Mean minimum, mean and mean maximum air temperature are shown as shaded area, with the absolute minimum temperature (thick line), and the leaf-out time of species for (a) low- and (b) high-elevation sites. The return rate (time interval of recurrence) of absolute minimum temperatures of -10 °C to $+5$ °C in steps of 1 K for (c) low- and (d) high-elevation sites. A return rate of 5 years means that a given temperature is expected to occur every fifth year. Note the logarithmic and reverse scale in the lower panels (c, d).

Fig. 2c). The occurrence probability of freezing temperatures declined rapidly during the leaf-out period, and towards the end of the leaf-out period, no more freezing temperatures occurred (Fig. 2). Interestingly, temperatures increased slower at low elevation than at high elevation during the leaf-out period. For instance, the return rate of 5 °C was below 1.5 years at low elevation until day 113, before it increased when most species had already leafed out (Fig. 2c). On the other hand, the return rate of 5 °C increased steadily at high elevation from 1 to 3 years during leaf-out (Fig. 2d).

ESCAPE OF FREEZING DAMAGE IN SPRING

Leaf-out converged relative to the probability of late freezing damage in spring in all investigated species irrespective of elevation (Fig. 3). Thus, all trees escaped freezing temperatures well during the investigated period. Leaf-out occurred among all species at high elevation on average 35–44 days after the time when the mean probability of species-specific freezing damage to emerging leaves reached 5%, except for *Fagus*, which had only a time lag of 25 days. Similarly, the time lag for leaf-out after potential

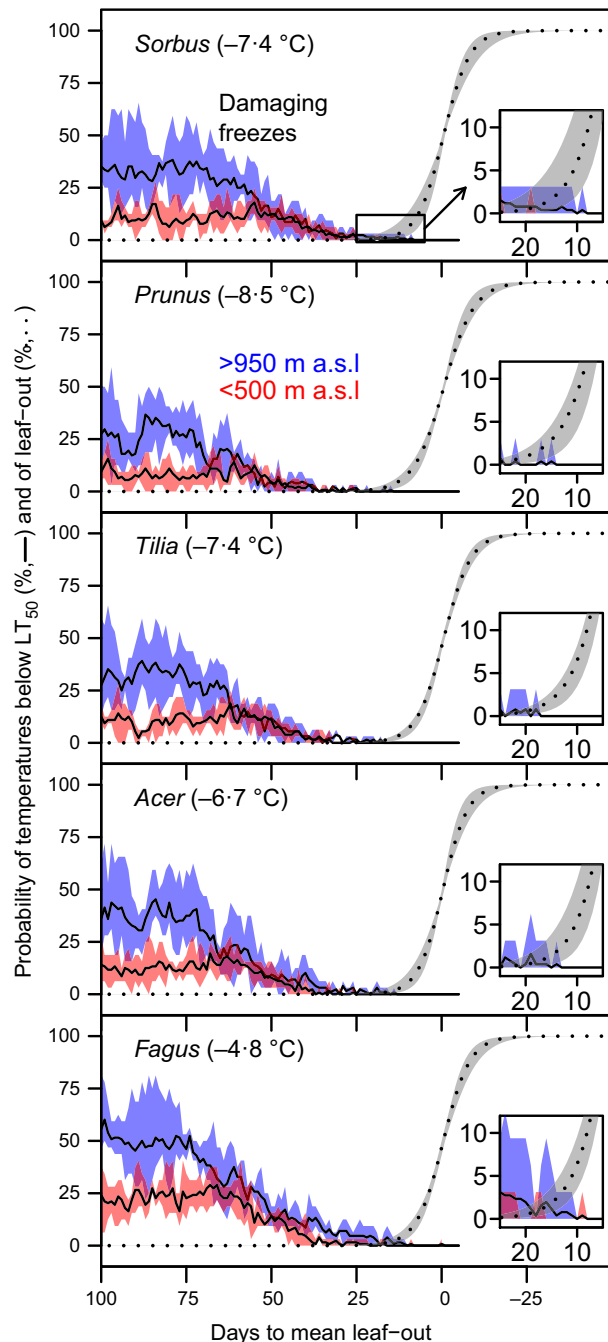


Fig. 3. The mean probability of daily absolute minimum temperatures below species-specific LT_{50} values for low- and high-elevation sites (solid lines with the range across stations for high- and low-elevation sites in blue and red) in relation to the leaf-out timing. Leaf-out data across years were fitted by a logistic regression (dashed line), with the range across stations in grey. Day 0 corresponds to the mean date of leaf-out (see Materials and methods). Species-specific LT_{50} values are given in brackets behind species names. The inset diagrams show the time period of 25 to 5 days prior to the mean date of leaf-out using the same axis labels, but note the different scale. Species are ranked from top to bottom from early to late leaf-out date.

damaging freezes was 38–50 days at low elevation. *Fagus* exhibited the shortest time interval between the probabilistically dangerous freezing period and leaf-out (Fig. 3).

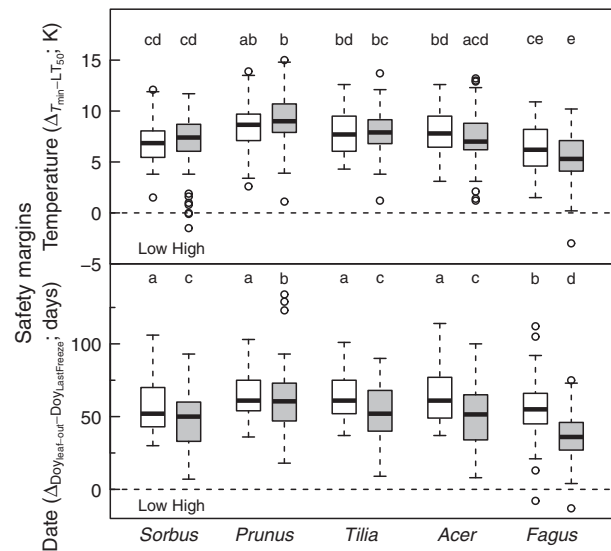


Fig. 4. The temperature and date safety margin against freezing damage at low (white) and high elevation (grey) for each species. The temperature safety margin was defined as the difference between absolute minimum temperature from 5 days prior to 5 days after leaf-out and the species-specific freezing resistance of foliage during leaf-out. The date safety margin was defined as the difference in days between the leaf-out date and last freeze event below the species-specific LT_{50} value. The boxplots show the median, the first and third quartile, and the extreme point. Any point extending more than 1.5 times the interquartile distance from the first to the third quartile is drawn as an outlier. The species are ranked by leaf-out date from left to right (early to late). Different letters indicate significant ($P < 0.05$) differences tested by Tukey's HSD test.

Interestingly, earlier-flushing species such as *Sorbus* were not at a greater risk of freezing damage than late-flushing species. For each of the five investigated species, the probability of freezing temperatures below the species-specific LT_{50} values was zero when 10% or 50% of trees had leafed out. Thus, the timing of leaf-out was such that the risk of freezing damage was virtually absent during the observation period (1981–2012).

SAFETY MARGINS AGAINST FREEZING DAMAGE

Irrespective of elevation, absolute minimum temperatures ranged from -5°C to $+5^{\circ}\text{C}$ during leaf-out, that is from 5 days prior to 5 days after leaf-out (Fig. 5). Thus, absolute minimum temperatures were generally well above species-specific LT_{50} values during leaf-out, and the temperature safety margin was generally positive (Fig. 4). Consequently, all investigated tree species exhibited an average temperature safety margin against freezing damage during early spring of 7.0 – 9.2°K at both high and low elevation over the period from 1981 to 2012 (16–32 years, depending on species), except for *Fagus*, which had a significantly lower temperature safety margin of (on average) 5.3°K at high elevation and 6.4°K at low elevation (Fig. 4). Elevation had no significant effect on the temperature safety margin; however, species and the interaction

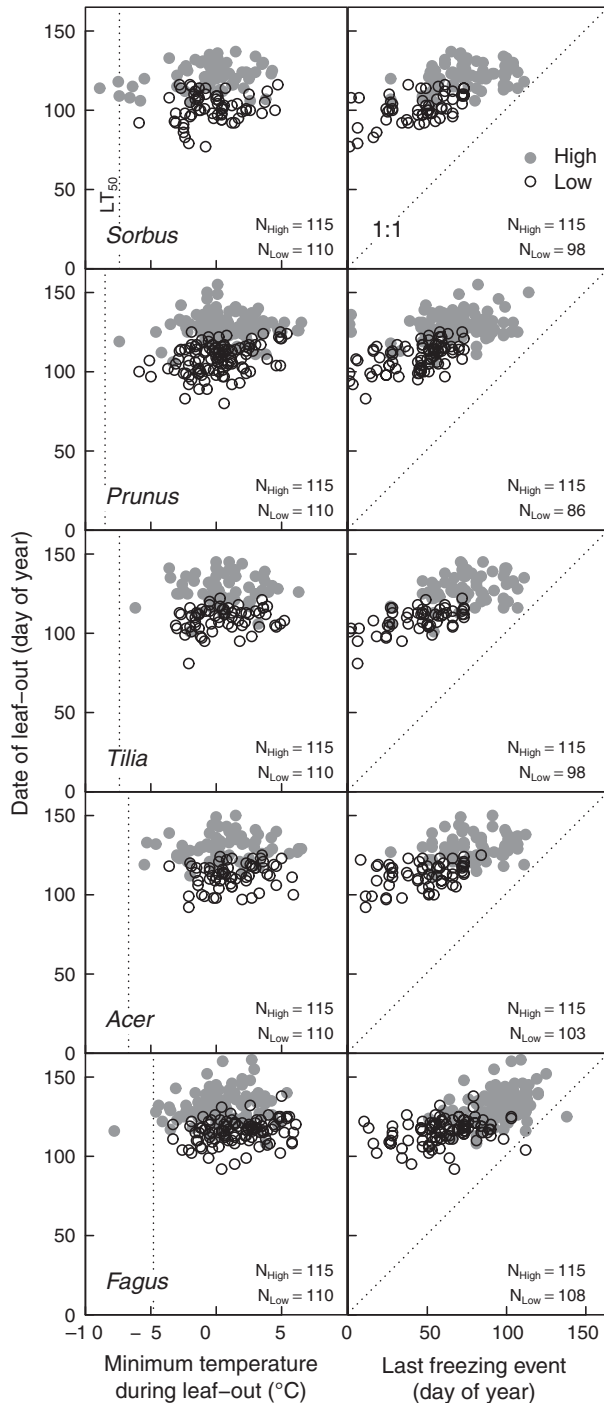


Fig. 5. The date of leaf-out for each species vs. absolute minimum temperature during leaf-out (left panel) and the date of the last freeze event below species-specific LT_{50} values (right panel) for low- (open circles) and high (grey circles)-elevation sites. The absolute minimum temperature during leaf-out corresponds to the absolute minimum air temperature that occurred between 5 days prior to 5 days after leaf-out. The last freeze event corresponds to the day of year when temperatures fell for the last time below the species-specific LT_{50} value. On the left panel, the species-specific LT_{50} values are drawn as a vertical dotted line, on the right panel, the 1:1 line is drawn as dotted line. Values to the left of the LT_{50} values or below the 1:1 line indicate potential freezing damage.

between species and elevation were both significant (Table 3). The interaction was significant because earlier-flushing species (*Sorbus* and *Prunus*) had a slightly nonsignificantly larger temperature safety margin at high elevation, whereas late-flushing species (*Acer* and *Fagus*) had a smaller safety margin at high elevation (Fig. 4). Overall, *Prunus*, *Acer* and *Tilia* were never at risk of freezing damage to emerging leaves in any of the study sites, whereas a negative temperature safety margin (potential freezing damage) was found for *Fagus* in the year 1997 (3 K below LT_{50}) and for *Sorbus* in the years 1991 and 1997 (0.1 and 1.5 K below LT_{50} , respectively). Interestingly, *Sorbus* was the earliest-flushing species and *Fagus* the latest.

While temperatures were very similar between low- and high-elevation sites during leaf-out (Fig. 5), the time lag between the last freeze event below species-specific LT_{50} values and leaf-out (the date safety margin) was shorter at high-elevation sites compared to low-elevation sites (Figs 4 and 5). Consistently, species, elevation and the interaction between the two had a significant effect on the date safety margin (Table 3). *Fagus* was the only species with incidences of a negative date safety margin, namely two times in the records (Figs 4 and 5). Interestingly, the first incidence was 8 days after leaf-out at low elevation in Hallau in 1991, and the second was thirteen days after leaf-out at high elevation in Elm in 2012. Absolute minimum temperature was -5.1°C at low elevation, and -5.3°C at high elevation during these incidences, that is only slightly below the LT_{50} value of -4.8°C of *Fagus*. Trees were most likely not damaged due to the advance in leaf development in the 8–13 days after leaf-out that preceded the damaging freezing event.

RELATIONSHIP BETWEEN LATE SPRING FREEZE AND DEGREE-DAYS

The accumulated growing degree-days above 5°C from February to April as a proxy for spring warmth among sites strongly correlate with the date of the last freeze event below species-specific LT_{50} values (Fig. 6), yet with quite substantial year-to-year variation. Thus, while mean growing degree-days from February to April are a good proxy for the average occurrence of critical minimum temperatures late in spring over a large climatic gradient, they cannot capture the stochastic nature of such extreme events in a particular year.

Discussion

The comparison of leaf-out data with species-specific freezing resistance and *in situ* minimum temperatures revealed that deciduous temperate tree species employ sufficiently large safety margins so that the risk of freezing damage to young leaves in spring is almost zero on long time-scales, irrespective of the climate of sites (low vs. high elevation).

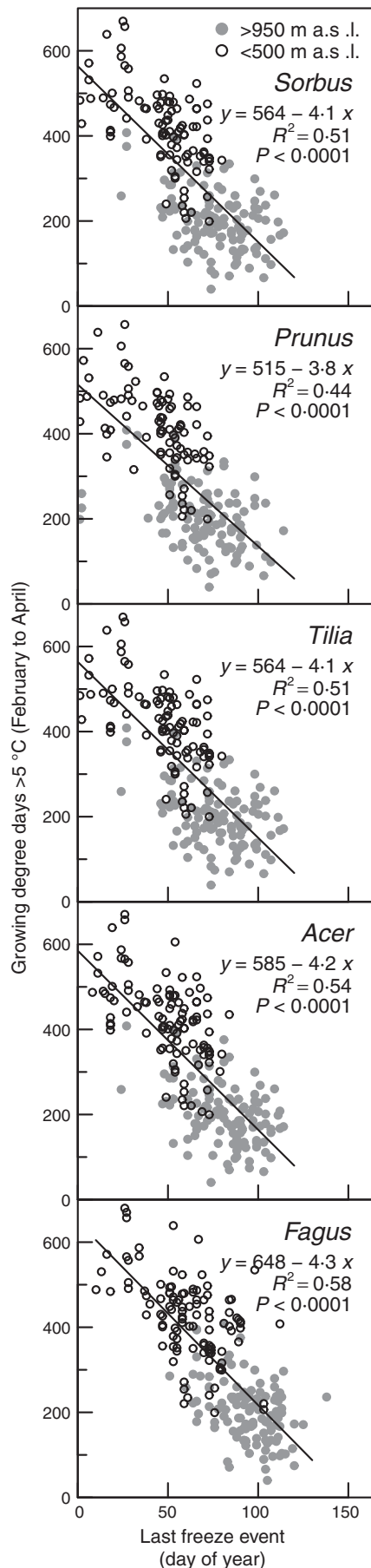


Fig. 6. Correlation between growing degree-days above 5 °C in spring (February to April) for low- (open circles) and high (grey circles)-elevation sites and the date of the last freeze event below the species-specific LT_{50} value for each species. Growing degree-days were used as a proxy for spring warmth. Correlations were calculated across all data.

The large safety margins enable trees to survive very stochastic freeze events on centennial time-scales, even though they would be safe from freezing damage when leafing out earlier in most years. Using modelled phenology data combined with freezing resistance data, it was found recently that the risk of freezing damage remains constant along elevation (Lenz *et al.* 2013). Using true phenology observations and temperature data, we confirm the result of this previous study and are now able to accurately define the size of the safety margin. While the temperature during leaf-out (temperature safety margin) is very similar between low- and high-elevation sites, leading to the same risk of freezing damage irrespective of elevation, the date safety margin is smaller at high-elevation sites. The convergence of flushing date to the same temperature conditions (whenever these occur) across elevation suggests that leaf-out phenology evolved in a way so that a critical safety margin against freezing temperatures is achieved. Consistently, temperatures increase faster during the leaf-out period at high elevation than at low elevation (see Fig. 2), explaining the difference between the date safety margins of low- and high-elevation sites. While the date safety margin suggests an increase in freezing risk with elevation, the risk of freezing damage remains actually constant along elevation when temperatures are considered. The risk of freezing damage should therefore be assessed by a temperature safety margin rather than a date safety margin, especially when trees along elevational gradients are compared. The large temperature safety margin found for all species, that is the extent to which trees escape freezing damage in spring, suggests selection against freezing damage. The large variation in the date safety margin (time of leaf-out vs. last LT_{50} event) suggests plasticity in timing of leaf-out in the light of the year-to-year variation in weather. Tree species with a higher freezing resistance can thus flush earlier than less freezing-resistant tree species in cold climates (Lenz *et al.* 2013; Vitasse *et al.* 2014; CaraDonna & Bain 2015), leading to the same risk of freezing damage among species (Lenz *et al.* 2013).

THE LINK BETWEEN PHENOLOGY AND FREEZING RESISTANCE

Freezing resistance in spring (during de-acclimation) is tightly linked to bud development (Kalberer, Wisniewski & Arora 2006; Pagter & Arora 2013), with the same circadian clock genes involved in the acquisition of freezing tolerance and in dormancy release (Ibanez *et al.* 2010). In other words, freezing resistance and leaf-out are part of the same photoperiod related trait syndrome, in which it is

possible to derive the direction/extent of one trait based on the other trait. Once bud development starts in spring, freezing resistance is largely and irreversibly lost, and trees cannot (or only to a very small extent) re-acclimate (i.e. become more freezing resistant) in response to cold temperatures (Larcher & Mair 1968; Campbell & Sorensen 1973; Pagter & Arora 2013; Vitasse, Lenz & Körner 2014). Thus, leaf-out is likely under selective pressure in relation to the inherent freezing tolerance of developing leaves in spring. Consequently, we found no difference in the temperature safety margin between low- and high-elevation sites. Besides the lack of re-acclimation within a single tree, freezing resistance is very similar among distinct populations of the same species in spring (Flint 1972; Alexander, Flint & Hammer 1984; Li *et al.* 2003). Due to this very low genetic variation, we suggest that freezing resistance is a rather fixed trait in tree species. Freezing resistance is a very complex trait, including many physiological, ultra-structural, and in some species even morphological adjustments that enable a species to survive cold temperature. Onset of growth in spring can have a direct impact on freezing resistance, by changing physiological properties or competing for resources within the plant, such that an increase in freezing resistance is not possible once leaf-out commences (Kalberer, Wisniewski & Arora 2006). It thus might be easier for a plant to adjust the timing of leaf-out, rather than to maintain a high level of freezing tolerance throughout leaf development. We hypothesize that there is no selective pressure for a high plasticity in freezing resistance in spring because temperate trees show a high plasticity in leaf-out over spatial and temporal scales. This plasticity in leaf-out timing enables trees to avoid freezing damage in the vast majority of years without the need of a short-term acclimation of freezing resistance to climate conditions.

ADJUSTING SPRING PHENOLOGY TO ESCAPE FREEZING DAMAGE

The biological drivers behind the timing of leaf-out in spring can be viewed from two different temporal scales, first on the evolutionary time-scale, integrating all potential freezing damages to leaves in spring over the lifetime of several generations (several centuries), and second on a year-to-year basis of an individual tree that responds to environmental cues with its given life-history traits (i.e. chilling and warmth requirements, photoperiod thresholds). If the pattern of leaf-out in spring is viewed from the perspective of a single year, no link between freezing resistance and the timing of leaf-out can be detected, but only hypothesized. The link between freezing resistance and leaf-out timing can only be observed when several years are combined and larger geographic areas are considered. Consequently, similar absolute temperature extremes were found at elevational limits of temperate tree species in the Swiss Alps and at latitudinal limits of the same species in northern Europe over the last 100 years (Kollas,

Körner & Randin 2014). Evolutionary selection operates via extremes over the entire lifetime of an organism. The safety margin has to be large to cover the stochastic nature of freezing events (i.e. cold spells can occur unexpectedly and independently of seasonal mean temperature). Theoretically, trees could flush earlier in many years and would still be safe from freezing temperature. However, over long time-scales, the risk (probability of damage) selects for large mean safety margins.

Over long time periods and on a large regional scale, the last day with minimum temperatures below species-specific LT_{50} values correlates well with growing degree-days accumulated during spring. Thus, relying on warmth (once chilling and photoperiodic requirements are fulfilled), offspring from a given tree population can avoid freezing damage when transferred to colder climatic conditions. Accordingly, leaf-out occurs between 2.5 and 6 days later for 1 K of temperature decrease (i.e. increase in elevation by ~200 m) in seedlings of temperate tree species grown in common gardens (Vitasse *et al.* 2013). Interestingly, the environment (temperature) has a much stronger influence on leaf-out than the genetic adaptation of tree populations from contrasting climates (Vitasse *et al.* 2013), and gene flow seems to be abundant along elevational gradients (Alberto *et al.* 2010). This high plasticity allows trees to leaf out early in the warmest locations and late in colder sites. Besides, leaf-out dates vary generally strongly within the same population (Alberto *et al.* 2011), potentially enhancing survival of a significant part of a population in critical years.

THE SEVERITY OF FREEZING EVENTS

Whatever the exact environmental controls of phenology of tree species (tracking chilling, photoperiod and concurrent temperature), it leads to the same pattern among species: they all flush at a time when the probability of freezing damage approaches zero. *Fagus sylvatica* is the species with the lowest temperature safety margin, especially at high elevation. *Fagus* has the earliest leaf-out timing in respect to the risk of freezing damage. Interestingly, *Fagus* also exerts strong sensitivity of leaf-out to photoperiod (Vitasse & Basler 2013). Presumably, the sharp photoperiod co-control of leaf-out permits this species to operate at a narrow safety margin with regard to the risk of freezing damage compared to species with a strong requirement to thermal forcing. Interestingly, the wood formation of *Fagus* is strongly dependent on the length of the growing season at its range limit, potentially increasing the pressure for an early leaf-out in cold conditions (Lenz *et al.* 2014), as found in common garden experiments (von Wuehlisch, Krusche & Muhs 1995; Vitasse *et al.* 2013).

Conclusion

The timing of leaf-out is the evolutionary outcome of the interaction of a species' inherent freezing tolerance and the

probability of the occurrence of critically low temperatures. This interaction of phenology with freezing tolerance offers a new avenue to explain the diversity in leaf-out among coexisting deciduous temperate tree species. Species like *F. sylvatica* that employ photoperiod co-control of leaf-out can leaf out closer to the period with a high probability of freezing damage than species relying more strongly on temperature control. As the climate keeps warming, species can expand their distribution range to the extent the stochasticity of freeze events matches their controls over phenology.

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Data accessibility

Freezing resistance data are published in this paper, and climate and phenology data are available in a data base from MeteoSwiss, the Swiss federal office of meteorology and climatology (<https://gate.meteoswiss.ch/idaweb/>).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. Freezing resistance during leaf-out given as the LT₅₀ value for each species.

Figure S1. Date of leaf-out of the five deciduous broadleaved tree species used for the study for each station during the time period from 1981 to 2012.

Figure S2. Date of leaf-out for each of the five species used among all eight stations during the period from 1981 to 2012.

Figure S3. The mean probability of daily absolute minimum temperatures below species-specific LT₅₀ values for low and high elevation sites (solid lines with the range across stations for high and low elevation sites in blue and red) in relation to the leaf-out timing.