

European deciduous trees exhibit similar safety margins against damage by spring freeze events along elevational gradients

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Received: 26 March 2013

Accepted: 3 July 2013

New Phytologist (2013)

doi: 10.1111/nph.12452

Key words: cold hardiness, flushing, frost, phenology, range limit, spring, summer, winter.

Summary

- Minimum temperature is assumed to be an important driver of tree species range limits. We investigated during which period of the year trees are most vulnerable to freezing damage and whether the pressure of freezing events increases with increasing elevation.
- We assessed the course of freezing resistance of buds and leaves from winter to summer at the upper elevational limits of eight deciduous tree species in the Swiss Alps. By reconstructing the spring phenology of these species over the last eight decades using a thermal time model, we linked freezing resistance with long-term minimum temperature data along elevational gradients.
- Counter-intuitively, the pressure of freeze events does not increase with elevation, but deciduous temperate tree species exhibit a constant safety margin (5–8.5 K) against damage by spring freeze events along elevational gradients, as a result of the later flushing at higher elevation. Absolute minimum temperatures in winter and summer are unlikely to critically injure trees.
- Our study shows that freezing temperatures in spring are the main selective pressure controlling the timing of flushing, leading to a shorter growing season at higher elevation and potentially driving species distribution limits. Such mechanistic knowledge is important to improve predictions of tree species range limits.

Introduction

Among the abiotic factors that control the global distribution of taxa, freezing temperatures are possibly the most decisive. For plants, this selective filter varies with life stage, tissue type and, most importantly, seasonal development (Sakai & Larcher, 1987). In temperate climates, deciduous trees have adopted a strategy to escape winter freezing damage to foliage by shedding their leaves in autumn. However, deciduous trees must exhibit freezing tolerance in all other overwintering organs, particularly in buds, because they contain pre-formed leaves and flowers for the following growing season. Freezing damage is ultimately linked to the rupture of biomembranes (Ziegler & Kandler, 1980; Sung *et al.*, 2003; see review by Larcher, 2005). Therefore, a major part of freezing resistance is to maintain membrane fluidity during the freezing process and to tolerate freezing-induced dehydration in the cell plasma. To do so, plants synthesize dehydrins and antifreeze proteins and reduce the amount of membrane-located carriers and receptors during the pre-hardening stage in late autumn. Next, plants change the ultrastructure of the cytoplasm and increase the proline and polyol concentration during the early stage of hardening. The final stage of hardening is reached by a repeated exposure to freezing temperatures during dormancy in winter, leading to the species-specific maximum freezing resistance. In early spring, before bud burst,

freezing resistance decreases progressively as temperature rises (dehardening period), reaching a minimum when the new leaves emerge (Till, 1956; Weiser, 1970). Once development starts in spring, freezing resistance is irreversibly lost and plants cannot re-acclimate to low temperatures (Sakai & Larcher, 1987; Repo, 1991; Rapacz, 2002). During the maturation of the new leaves, the freezing resistance of foliage increases slightly by 2–3 K; the maximum freezing resistance of active leaves is reached by mid-summer (Till, 1956). By the end of summer, after budset, freezing resistance begins to increase again (hardening period) in response to the shortening photoperiod and decreasing temperature (Weiser, 1970; Christersson, 1978; Larcher, 2005). The freezing resistance of deciduous trees is therefore tightly linked to their phenology, especially the state of bud dormancy (Larcher & Mair, 1968; Weiser, 1970; Campbell & Sorensen, 1973; Ibanez *et al.*, 2010).

Past attempts to explain species range limits have largely adopted a correlative approach, looking for correlations between species boundaries and some presumably important isotherms (e.g. Iversen, 1944; Woodward, 1987). Until now, it has remained unclear which facet of the temperature regime is critical and at which time of the year or developmental stage this critical temperature acts in a decisive way. In long-lived organisms, such as trees, freezing resistance controls species persistence over long time scales, with a single extreme event potentially eliminating a

species beyond a certain isotherm. The good relationship between the winter freezing resistance of tree species and the minimum annual temperatures at the distribution limits of tree species suggests that winter temperatures control cold distribution limits (e.g. Sakai & Weiser, 1973; Sakai, 1978). However, several studies have suggested that spring freezing events are most important for the distribution limit of deciduous temperate tree species (e.g. Rubner, 1921; see review by Parker, 1963). Trees are particularly vulnerable in spring, when they start to grow and lose their freezing resistance during a period in which freezing events are still likely. Spring freezing events can seriously affect the growth and reproduction of trees at the cold edge of their range through either the loss of new leaves or damage to flowers, subsequently affecting the tree's reproductive success (Inouye, 2000; Augspurger, 2009; Hufkens *et al.*, 2012). The loss of a first cohort of leaves and the need for a new cohort may delay seasonal xylogenesis and can lead to a significant reduction in annual ring width (Dittmar *et al.*, 2006).

Earlier spring phenology as a result of climate warming has been observed for >400 plant species in Europe (Menzel *et al.*, 2006), as well as for many tree species in Europe and America (reviewed in Bertin, 2008). Earlier spring phenology and subsequently earlier dehardening of tree tissues can possibly lead to a higher risk of freezing damage (Cannell & Smith, 1986; Gu *et al.*, 2008), particularly for early flushing species. However, warming affects phenology only after trees have received sufficient periods of chilling winter temperatures, and some tree species employ photoperiodic controls of phenology as a safeguard against warm spells at the 'wrong time' (Cannell, 1997; Körner & Basler, 2010; Basler & Körner, 2012). Thus, dormancy release is co-controlled by several factors, with temperature controlling the last step. In spite of this co-control of dormancy release, a freezing event in spring damaged both crops and tree species in the eastern part of the USA in 2007 as a result of an exceptionally warm early spring that caused a very early bud burst (Gu *et al.*, 2008; Augspurger, 2009). In most regions, freezing events in spring are generally more severe at higher elevations because of the decline in temperature as elevation increases. However, because the beginning of the growing season is also delayed at higher elevations, it remains unclear whether tree populations

growing close to their upper elevational limits are at greater risk of freezing damage than those inhabiting lower elevations.

In this study, we investigated the freezing resistance of buds and leaves before, during and after the period of leaf emergence in eight major European deciduous broadleaved tree species at their upper elevational limits in the Swiss Alps. The high sampling resolution allowed us to assess freezing resistance according to the development of buds in spring. Because freezing resistance in spring is physiologically linked to phenology (e.g. Larcher & Mair, 1968; Campbell & Sorensen, 1973; Ibanez *et al.*, 2010), we reconstructed the spring phenology of these species over the last eight decades via a thermal sum model. This allowed us to link freezing resistance with long-term minimum temperature data along elevational gradients. We addressed the following questions. What is the seasonal variation in the freezing resistance of deciduous broadleaved trees at their elevational limit? Does the difference between the minimum temperature experienced and the freezing resistance of a certain species, that is the temperature safety margin against freezing damage, approach a critical level at the tree species limit? The results of this study permit a better understanding of the decisive controls of low temperature on species range limits.

Materials and Methods

Study area and species

We investigated an elevational transect situated in the western Swiss Alps near Morcles (46°11'55"N–46°12'55"N; 07°02'00"E–07°02'58"E), where a number of tree species currently reach their upper elevational distribution limits (Vitasse *et al.*, 2012; Randin *et al.*, 2013). We selected eight deciduous tree species that have wide distribution ranges in Europe and reach their distribution limits at contrasting elevations in the study area (Vitasse *et al.*, 2012), namely *Sorbus aucuparia* L., *Sorbus aria* L., *Acer pseudoplatanus* L., *Laburnum alpinum* (Mill.) Brecht. & J. Presl., *Prunus avium* L., *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl. and *Fraxinus excelsior* L. We selected mostly 12 (6–13) adult trees for each species at 200–600 m below their respective elevational limits for repeated sampling (Table 1).

Table 1 Mean sampling elevation and mean, minimum and maximum day of the year of bud burst (phenological stage 2) reconstructed by the thermal time model for the eight examined deciduous tree species at 1500 m above sea level (asl) and at their species-specific elevational limit from 1931 to 2011

Species	Sampling elevation (m asl)	Elevational limit (m asl) ¹	Mean bud burst date (min, max)	
			1500 m asl	Species-specific elevational limit
<i>Sorbus aucuparia</i>	1675	2150	117 (97, 136)	141 (118, 167)
<i>Sorbus aria</i>	1550	2000	130 (102, 155)	148 (121, 171)
<i>Acer pseudoplatanus</i>	1550	1875	141 (122, 158)	155 (134, 174)
<i>Laburnum alpinum</i>	1550	1875	140 (119, 157)	154 (133, 173)
<i>Prunus avium</i>	1150	1665	117 (91, 137)	123 (99, 150)
<i>Fagus sylvatica</i>	1325	1600	142 (121, 165)	146 (127, 170)
<i>Quercus petraea</i>	950	1525	145 (121, 169)	147 (125, 171)
<i>Fraxinus excelsior</i>	1150	1500	140 (112, 168)	140 (112, 168)

¹Adult species limit recorded in the same area (extracted from Vitasse *et al.*, 2012).

Assessment of freezing resistance and spring phenology

Twigs with buds, and later with leaves, were collected weekly from six individuals from the selected population of 6–13 trees for each tree species from March to May 2011 and once during deep dormancy in winter 2012 (January 13 or March 6, depending on species). For each tree, a well-exposed branch in the upper crown part was collected and all buds/leaves of this branch were sampled. The phenological stage of each sampled bud was recorded on a categorical scale according to Vitasse *et al.* (2013), namely bud closed (stage 0), bud swelling (stage 1), bud burst (stage 2), leaf emergence (stage 3) and leaf unfolding (stage 4), except for *Sorbus aucuparia* and *Quercus petraea*, for which the assessment of stage 1 was unclear or missed and was therefore removed from further analysis. Immediately after cutting, the twigs were kept cool (0–4°C) for 7–12 h and then placed in freezers. For each sampled tree, several twigs (5–10 cm long) comprising 3–10 buds (depending on species) were equally distributed among seven target freezing temperatures and one control chamber at 4°C. We used commercial freezers (Liebherr GN 1056 Premium No Frost, with an integrated heating system; Liebherr, Ochsenhausen, Germany) modified to be computer controlled. The freezing system allowed for an independent freeze–thaw cycle for each freezing treatment. The temperature within the chambers was recorded using Pt-100 temperature sensors (Pt-100, DIN EN 60751; Pollin Electronic GmbH, Pförring, Germany). The samples in each freezer were frozen at a rate of 3 K h^{−1} until reaching the target temperature, and then kept for 4 h at the target freezing temperature before being thawed by 3 K h^{−1} until reaching 4°C (Fig. 1). The range of target freezing temperatures among the seven freezers was adjusted to the development of freezing resistance for each sampling occasion (Supporting Information Table S1). In winter 2012, one additional sample served as a negative control and was kept at −80°C throughout the freeze–thaw cycle. After freezing, the samples were cut and leaf primordia (later leaves) were visually observed for survival. Freezing damage is manifested by a discoloration caused by the oxidation of polyphenols or a characteristic

odour caused by the de-compartmentalization and autolysis of the protoplast (Sakai & Larcher, 1987). After visual observation, the samples were placed in distilled water in Falcon tubes and kept at 4°C for 24 h. Then, electric conductivity (i.e. electrolyte leakage after treatment) was measured using a conductivity meter before autoclaving the samples at 120°C for 20 min. The autoclaved samples were kept for 24 h at 4°C before measuring the electric conductivity a second time (i.e. maximum electrolyte leakage). The measurements of electric conductivity before and after autoclaving allowed for the calculation of injury values according to Flint *et al.* (1967).

Freezing resistance data are stress data and represent threshold values above which investigated tissue survives and below which death of the investigated tissue occurs. In this study, freezing resistance was expressed as the lethal temperature at which 50% of the samples died (LT₅₀). LT₅₀ was calculated using logistic regression for visual damage and nonlinear Gompertz models for electrolyte leakage data according to Lim *et al.* (1998). LT₅₀ values were calculated separately for each sampled tree of each species ($n=6$ per species). LT₅₀ values calculated from visual damage assessment and electrolyte leakage showed a high correlation ($LT_{50 \text{ Electrolyte}} = -1.7 + 0.8 \times LT_{50 \text{ Visual}}$, $R^2 = 0.65$, $P < 0.001$). We were interested in freezing damage to leaf primordia in buds of the broadleaved trees. Because, for technical reasons, electrolyte leakage could only be determined in the bulk sample of tissue including living scales, this method could not account for the tissue specificity of critical freezing damage. In addition, we considered the visual test for living vs dead tissue to be more precise than the proportional increase in electrolyte leakage in stressed tissue. Therefore, we used only the visually assessed damage data for further analyses, except for the winter freezing resistance of *Sorbus aucuparia*, *Acer pseudoplatanus* and *Laburnum alpinum*, for which LT₅₀ values were calculated from the electrolyte leakage data.

Reconstruction of minimum temperature and spring phenology

We calculated three seasonal temperature lapse rates for winter (December–February), spring/summer (March–July) and autumn (August–November) separately for daily mean and minimum air temperatures using temperatures recorded at a height of 2 m in full shade with seven data loggers (TidbiT v2; Onset Computer Corp, Cape Cod, MA, USA) distributed from 400 to 2160 m above sea level (asl) in the study area from June 2010 to July 2011. The specific lapse rates ranged from −0.35 to −0.51 K 100 m^{−1} for mean temperature and from −0.30 to −0.46 K 100 m^{−1} for minimum temperature. The daily mean and minimum air temperatures recorded along the studied elevational gradient were highly correlated with the temperatures recorded from the nearest climate station that offered long-term records (Château-d'Oex, 46°28'35"N, 7°08'31"E, 985 m asl; slope > 0.9, $R^2 > 0.9$). The seasonal temperature lapse rates were then used to extrapolate the long-term daily mean and minimum air temperatures from the reference climate station to the study area for the period 1931–2011.

Phenology is very difficult to model on a mechanistic basis because it involves influences from low temperatures in winter

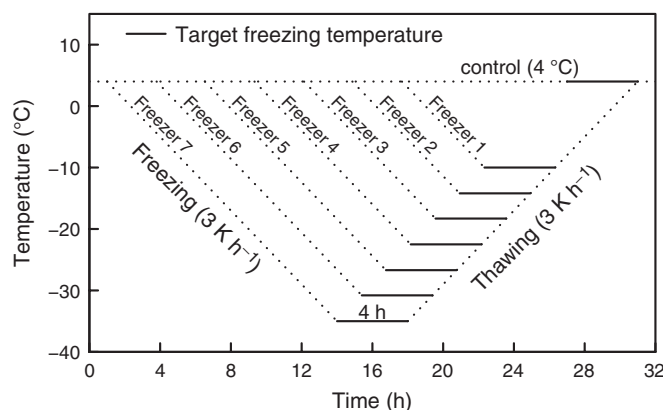


Fig. 1 Parallel freeze–thaw cycles in seven computer-controlled freezers used for the assessment of the freezing resistance of eight deciduous tree species before, during and after bud burst. Shown is an example of programmed temperature ramps for the assessment of freezing resistance in week 11, 2011.

(chilling), photoperiod and warm temperatures in spring (forcing; Polgar & Primack, 2011). Assuming that the species studied here all receive sufficient chilling in every year, which minimizes the interaction between photoperiod and forcing temperatures in spring (Caffarra & Donnelly, 2011; Vitasse & Basler, 2013), the remaining start term, year-to-year variation in the bud burst date, is largely temperature related. The assumption that trees in this study receive sufficient chilling seems reasonable, as we focused on an area with cold winters and temperatures were reconstructed for past decades with, on average, colder temperatures. Hence, we can approximate phenology using a thermal time model (Cannell & Smith, 1983), which we fitted using seedlings growing in common gardens at different elevations along the transect (Vitasse *et al.*, 2013). Although seedlings are known to exhibit earlier spring phenology than mature trees (Vitasse, 2013), we observed only a slightly earlier leaf unfolding date for the seedlings, 3.5 ± 4.7 d earlier (mean \pm SE) across species, compared with selected adult trees near the common gardens during the study year (Fig. S1). However, because of the potential seedling/adult discrepancy, we conducted an uncertainty analysis to determine the extent to which earlier or later spring phenology influences the results (shifting the phenology phase time window by -10 , -5 , $+5$, $+10$, $+15$ and $+20$ d).

The thermal time model was calibrated using phenological observations in spring 2011 of seedlings originating from the study area and belonging to the same species as studied here. These 2-yr-old seedlings were monitored for bud development in eight common gardens (except for *Quercus petraea*, which was grown in only four common gardens) along two elevational transects ranging from 437 to 1708 m asl in the Swiss Alps (Vitasse *et al.*, 2013). The thermal time model has three parameters: t_0 , the starting date for temperature accumulation; t_b , the temperature threshold required to accumulate temperature; and F , the critical sum of degree days required for flushing. The model was fitted using the whole dataset for each species at phenological stage 2 (the best-documented stage). Model parameters were derived using a simulated annealing algorithm employing PMP software (Chuine *et al.*, 2013). We then fixed the parameters t_0 and t_b and fitted the parameter F for each species and for phenological stages 1, 3 and 4 (Table S2). The model efficiency (ME; fraction of variance explained; see Vitasse *et al.*, 2011) ranged from 0.70 to 0.99 with a low root-mean-square error (RMSE, 1.1–4.8 d). A leave-one-out cross-validation yielded an ME ranging from 0.39 to 0.97 and an RMSE from 1.5 to 9.9 d, with most of the models having an RMSE of < 4 d (Table S3). With this model, we predicted the start date of each phenological stage per species along the studied elevational gradient for the period 1931–2011, using the daily mean temperature extrapolated from the reference climate station.

Calculation of the safety margin against freezing damage during the flushing period

We extracted minimum temperatures for the predicted periods of the different phenological stages (1–4) from 1931 to 2011 for

each year and species. We extracted the minimum temperature for the time window between the mean of the dates of the previous and corresponding stages and for the mean of the dates of the corresponding stage and the following stages. For stage 1, the pre-stage 1 window was set equal to the length of the post-stage 1 window between stages 1 and 2. In a similar way, the time window after stage 4 was set equal to the length of the pre-stage 4 window. The safety margin against freezing damage was defined as the temperature difference between the minimum air temperature calculated for the respective phenological stages and the LT_{50} value of freezing tolerance for that phenological stage. Values below zero indicate freezing damage, whereas values above zero indicate safety.

To assess the error related to the calculated safety margin, we combined the errors resulting from both phenology predictions and freezing resistance quantification as follows. The predicted phenology data have an associated RMSE obtained from the leave-one-out cross-validation. We shifted the time window for the minimum temperature calculation for each stage backwards and forwards by the extent of the corresponding RMSE, yielding a new temperature dataset at both ends of the time window. Then, an RMSE was calculated between the original minimum temperature dataset according to phenology and the new temperature datasets at both ends. The RMSEs for both ends were combined with the SE of the LT_{50} data and subtracted from or added to the safety margin.

Calculation of winter and summer temperature minima

To reconstruct 'winter' temperature minima, we calculated the lowest temperature value between November of the previous year and the mean date of stage 1 in the following year for each species at its species-specific elevational limit (i.e. day 132). For 'summer', we calculated the lowest temperature between the mean date of stage 4 of all species at their species-specific elevational limits (i.e. day 159) and July 30. All analyses were performed using R 2.12.2 (R Development Core Team, 2011).

Results

The development of the freezing resistance of buds and leaves from full dormancy to full activity

The lethal temperatures at which 50% of the samples died (LT_{50}) closely followed the phenological state of the trees from winter to spring in all investigated tree species (Fig. 2). The lowest LT_{50} values were found in winter with substantial differences among species, ranging from -42°C in *Sorbus aucuparia* to -21°C in *Fagus sylvatica*. In early spring, LT_{50} increased dramatically before and during bud burst and reached a maximum at the time of leaf unfolding (Fig. 2).

Early flushing species were more freeze resistant than late flushing species during early bud development. For instance, during bud burst (phenological stage 2), the three earliest flushing species, *Prunus avium*, *Sorbus aucuparia* and *Sorbus aria* (Table 1), had LT_{50} values below -9.6°C , whereas all of the other species

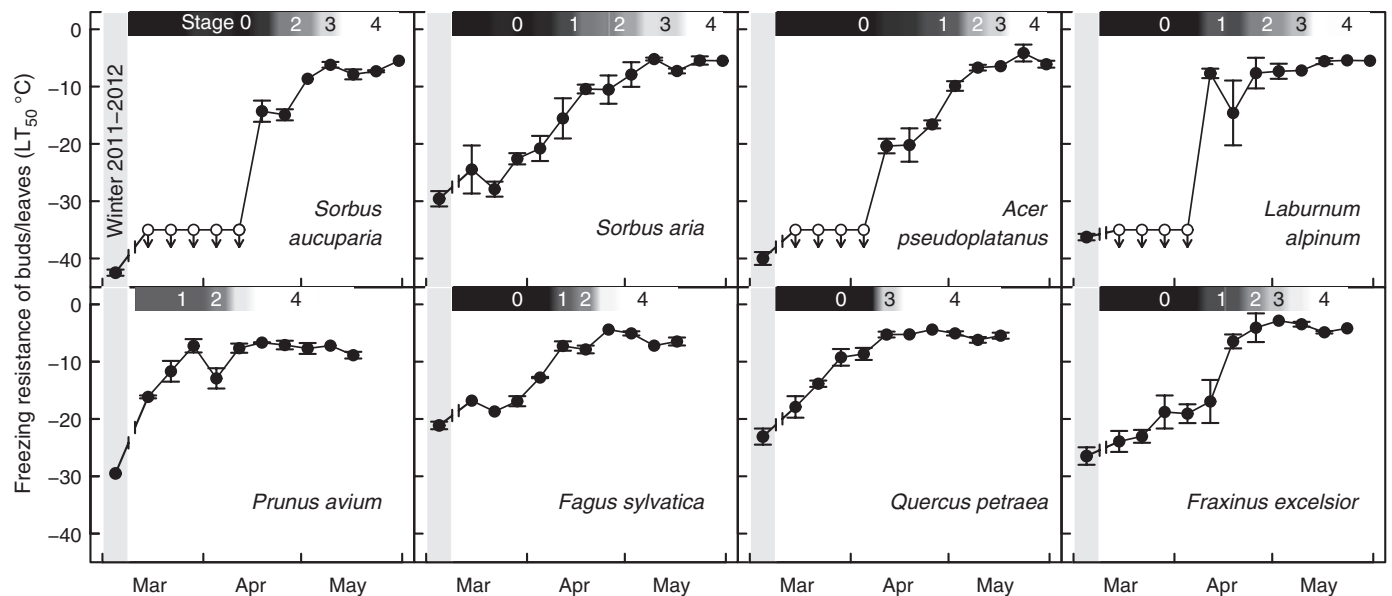


Fig. 2 Mean freezing resistance of the buds and leaves of eight deciduous tree species during winter and early spring expressed as the lethal temperature at which 50% of samples are dead (LT_{50}). Points within grey bars represent freezing resistance during winter 2011–2012. Open circles with arrows indicate freezing resistance below -35°C . The shaded bars on top indicate phenological stages: stage 0, buds closed; stage 1, buds swelling; stage 2, buds bursting; stage 3, leaves emerging; stage 4, leaves unfolding. Error bars, \pm SE ($n = 6$).

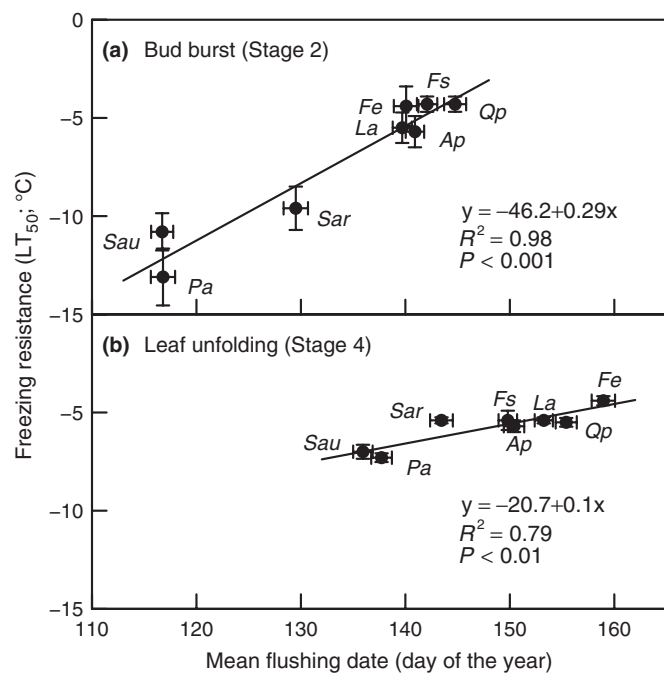


Fig. 3 Relationship between freezing resistance ($LT_{50} \pm$ SE) and the timing of spring phenology (\pm SE) reconstructed by the thermal time model at 1500 m above sea level (asl) in eight deciduous tree species. Correlations are shown for bud burst (a, stage 2) and leaf unfolding (b, stage 4). Ap, *Acer pseudoplatanus*; Fe, *Fraxinus excelsior*; Fs, *Fagus sylvatica*; La, *Laburnum alpinum*; Pa, *Prunus avium*; Qp, *Quercus petraea*; Sar, *Sorbus aria*; Sau, *Sorbus aucuparia*.

had LT_{50} values above -6°C (Fig. 3). Surprisingly, there were still substantial differences among the freezing resistances of the investigated tree species during leaf emergence (i.e. phenological stage 3), with LT_{50} values ranging from -8.5 to -3.2°C for

Prunus avium and *Fraxinus excelsior*, respectively. Nevertheless, the variation in freezing resistance among species decreased gradually during bud development. Thus, the difference in maximum freezing resistance among species was > 20 K when the buds were closed, c. 10 K when the buds were swelling and bursting (phenological stages 1 and 2), c. 5 K when the leaves were emerging (stage 3) and < 3 K after the leaves had unfolded (stage 4, Fig. 3). As the new leaves matured, the freezing resistance increased slightly, but the small differences in freezing resistance among species persisted into summer (Fig. 2). After leaf maturation, the freezing resistance of leaves was not correlated with the upper limits of the species, but was related to the timing of spring phenology; early flushing tree species, such as *Sorbus aucuparia* and *Prunus avium*, were always more resistant than late flushing tree species to freezing temperatures (Fig. 3).

The risk of freezing damage in spring during the period 1931–2011

Over the period 1931–2011, the risk of spring freezing damage remained the same along the entire elevational gradient, with all species exhibiting an average safety margin against freezing damage at their elevational limit of 6.7–8.2 K during early spring, except for *Fraxinus excelsior*, which had an average safety margin of 5.6 K (Fig. 4). The slopes of the safety margin along the elevational gradient were extremely flat, ranging from -0.5 to 0.3 K for 1000 m of elevation increase across species, except for *Fagus sylvatica*, which had a slope of -1.3 K per 1000 m of elevation (in comparison, the expected lapse rate along 1000 m of elevation is 4–5 K). This safety margin was associated with an error of 1.9–2.7 K, yielding a minimum safety margin of 2.9 K in *Fraxinus excelsior*. However, our results also revealed that most

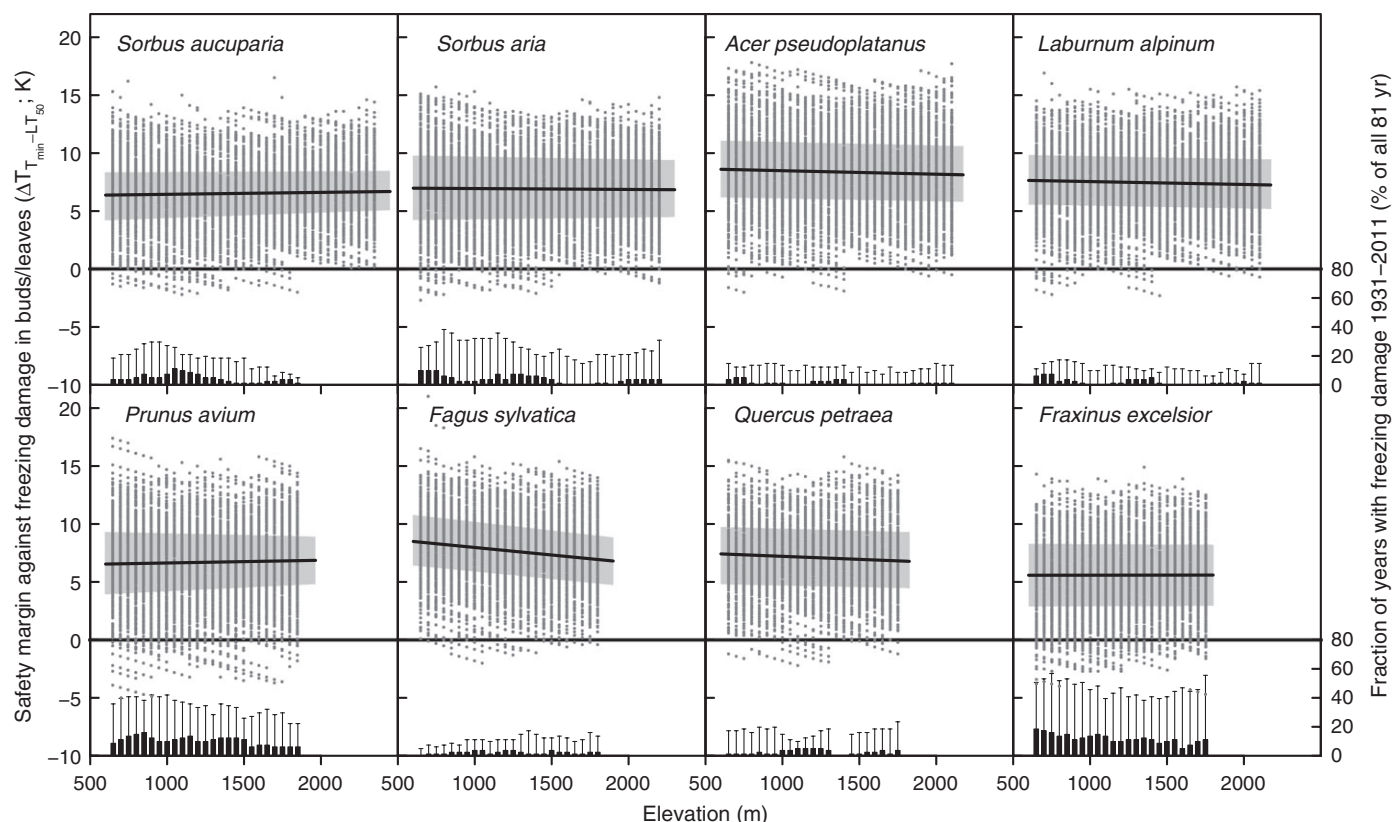


Fig. 4 Safety margin against freezing damage in eight deciduous tree species at different elevation during flushing (stages 1–4), expressed as the difference between the minimum air temperature and mean freezing resistance (LT_{50}) according to phenological stage. The points indicate safety margin values from 1931 to 2011 for each elevation. Negative values of the safety margin indicate potential freezing damage. The thick line indicates the mean safety margin across elevations with the associated error (grey shade, see the Materials and Methods section). Values are shown up to 200 m above species-specific elevational range limits, which were extracted from Vitasse *et al.* (2012). Bars (+ combined error) show the fraction of years with potential freezing damage (negative safety margin value) during the period 1931–2011.

tree species probably experienced freezing damage during the 1931–2011 period. As a result of the elevational shift in phenology, however, no increase in the risk of freezing damage with elevation was detected in any of the investigated species (Fig. 4). During the flushing period, we found that at least one potential instance of freezing damage occurred in < 5% of the examined years for all species (i.e. in < 4 of 81 yr i.e. less than every 16th year), except for *Prunus avium* and *Fraxinus excelsior*, for which damaging freezing events occurred twice as frequently (10–12% of all years; Fig. 4). When the error terms were taken into account, the years with potentially damaging events at least doubled (35–45% in *Prunus avium* and *Fraxinus excelsior*, 10–25% for the other species). Apparently, all investigated tree species delayed bud development in spring with increasing elevation in a manner that kept the risk of freezing damage at a constantly low rate along the elevational gradient. Because the reconstruction of phenological stages in spring relied on seedlings, which are known to exhibit earlier phenology, adult trees may be affected to a lesser extent (see the respective comments in Materials and Methods). However, the uncertainty analysis showed that, by shifting spring phenology up to 10 d earlier or up to 20 d later, the mean safety margins were not affected significantly. Hence, our results are quite robust against such ontogenetic shifts in phenology (Fig. S2).

The risk of freezing damage in winter and summer

Species-specific maximum freezing resistance was reached in winter. Interestingly, the LT_{50} values of deciduous broadleaved tree species were significantly related to the elevational limits of tree species during winter ($n = 8$, $r^2 = 0.68$, $P = 0.01$), although *Sorbus aria*, a high elevation species, had a rather high LT_{50} value during winter (Fig. 5). Counter-intuitively, tree species reaching higher elevations were generally safer from freezing damage in winter than species with a lower elevational limit (Fig. 5). For instance, the three species with the lowest elevational limits encountered potential freezing damage every 3–27 yr. In contrast with winter, the risk of being damaged by freezing events during summer was very low for all species and higher for species having higher elevational limits (Fig. 5). Minimum air temperature reconstructions showed that potential freezing damage occurred only once during the 81-yr period, namely in 2007.

Discussion

Our study demonstrated that deciduous broadleaved trees of temperate regions are prone to freezing damage during winter and spring, but are mostly safe during summer. However, late

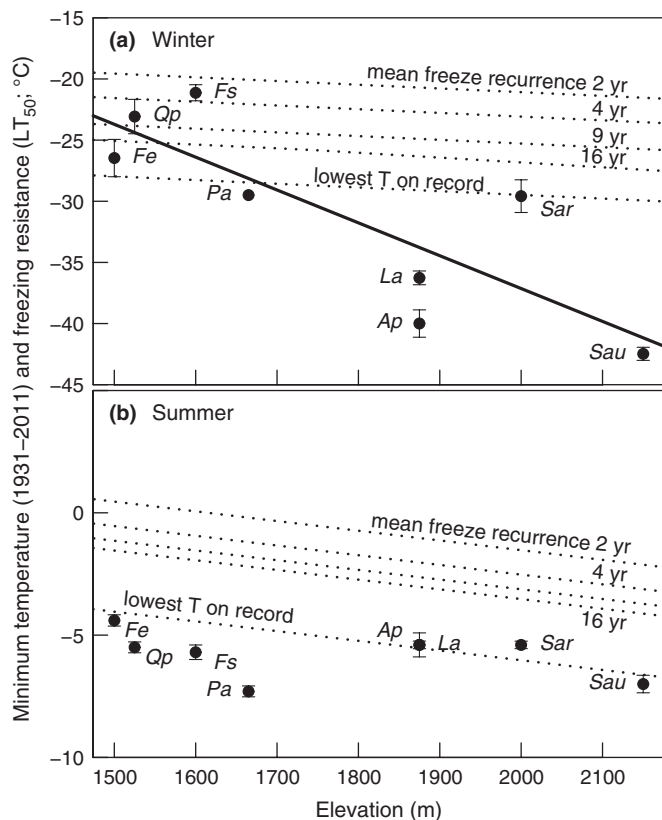


Fig. 5 Minimum temperatures from 1931 to 2011 scaled to the studied elevational gradient and the mean freezing resistance (\pm SE) of eight deciduous tree species at the elevational limit in winter (a) and summer (b). Dotted lines indicate the mean recurrence time of freezing events. Thick lines show the correlation between the freezing resistance of tree species at the elevational limit and elevation (in summer there is no significant correlation). Ap, *Acer pseudoplatanus*; Fe, *Fraxinus excelsior*; Fs, *Fagus sylvatica*; La, *Laburnum alpinum*; Pa, *Prunus avium*; Qp, *Quercus petraea*; Sar, *Sorbus aria*; Sau, *Sorbus aucuparia*. Error bars represent SE ($n = 6$). Note the different y-axis scales.

spring freezing events have a higher probability of damaging tree species than freezing events during winter. As a result of the high sampling resolution, we could measure directly the freezing resistance of the investigated tree species at defined phenological stages, which allowed us to compare freezing resistance with long-term temperature records during the flushing period in spring. Interestingly, as a result of the phenological shift in response to a decrease in temperature, we did not find an increase in potentially damaging freezing events with increasing elevation during the flushing period. By contrast, our results show, for the first time, that temperate deciduous tree species experience similar risks of freezing damage along an elevational gradient from 600 m up to the species-specific maximum elevational limits, and exhibit a mean safety margin against freezing damage of 5–8.5 K (Fig. 4). This similar mean safety margin against freezing damage across elevations suggests a probabilistic linkage between leaf-out phenology, the course of spring freezing resistance and the regional likelihood of occurrence of a critical freezing temperature (Leinonen, 1996; Cannell, 1997). The results underline that tree phenology has evolved in such a way that trees face similar

risks of freezing damage in spring under various climatic conditions. The analysis also reveals that damaging events mostly occur with a recurrence rate of 8–16 yr depending on species. However, this frequency of potentially damaging freezing events does not reveal the severity of damage *per se*. Freezing events only slightly below the LT₅₀ of leaf primordia or leaves are certainly less severe than strong frosts well below the LT₅₀. Strong freeze events might also damage meristematic tissue, wood parenchyma and phloem, which generally have LT₅₀ values several K below that of leaves, and consequently lead to severe damage of the entire tree (Sakai & Larcher, 1987; Augspurger, 2011).

Freezing resistance from full dormancy to full activity

Overall, observed maximum freezing resistance values during winter are in line with those of previous studies on other temperate deciduous tree species (Till, 1956; Sakai & Weiser, 1973; Sakai, 1978). For the dehardening period, only few freezing resistance data are available, and data of high temporal resolution are particularly scarce (Till, 1956; Tranquillini & Plank, 1989). Our assessment of freezing resistance on a weekly basis permitted the assessment of freezing resistance during defined developmental stages in spring. To our knowledge, this assessment has only been performed once previously, by Taschler *et al.* (2004). Those authors studied three conifer species, one dwarf shrub and *Sorbus aucuparia* at the treeline, but, unfortunately, the freezing resistances during distinct phenological stages were not compared with long-term temperature records to assess the long-term risk of freezing damage in spring in these species. Thus, the present study is the first to provide a long-term risk assessment along a large elevational gradient.

Once metabolic activity is resumed in spring and the development of buds begins, freezing resistance is irreversibly lost (Sakai & Larcher, 1987). Therefore, it is crucial that early flushing species are more freezing resistant than late flushing species in early phenological stages, as was found here. Hence, freezing resistance during the flushing period is not closely related to the elevational limit of tree species, but depends more strongly on the phenological stage of development and the phenological strategy (i.e. early or late flushing species). However, within an individual tree, the timing of flushing, which is known to be highly responsive to temperature, is adjusted to actual environmental conditions rather than the actual freezing resistance during flushing. This adjustment may explain why no difference was reported in freezing resistance in spring among different provenances of various tree species from contrasting latitudes, whereas, in autumn and winter, large differences were found (Flint, 1972; Alexander *et al.*, 1984; Li *et al.*, 2003). Our study therefore adds to the old knowledge that the timing of flushing secures an appropriate 'escape' from risk periods, and thus the long-term persistence of deciduous temperate trees at a given location.

Risk of freezing damage during the flushing period

In temperate climates, the beginning of the growing season differs between understorey and canopy trees, with the phenology of

the understorey generally earlier by several days or weeks (Vitasse, 2013). Our uncertainty analysis revealed, first, that all investigated uncertainties lie within the error of the model used to calculate the mean safety margin against freezing damage, and, second, that, overall, the pattern of a constant safety margin against freezing damage along elevation does not change substantially if the model is run with slightly earlier or later flushing dates. Obviously, the mean safety margin must increase when a later flushing date is assumed (Fig. S2). Any projection of future risks of freezing damage and species range limits will thus depend on accurate predictions of phenology.

It seems that the spring phenology of deciduous trees has evolved to optimize the timing of bud burst in relation to the probability of spring freezing events (Cannell, 1997). However, trees do not 'measure' directly the occurrence of extreme temperatures, but have developed complex mechanisms to adjust the onset of their bud development in spring to the complex interaction of photoperiod and temperature (Körner & Basler, 2010; Polgar & Primack, 2011; Basler & Körner, 2012). The probability of certain means or sums coinciding with certain extremes is a central issue in plant–climate interactions and in the global warming debate. Both an increase and/or decrease in freezing damage in a future climate have been suggested (e.g. Cannell & Smith, 1986; Inouye, 2000).

Trade-off between freezing damage and growing season length

Species range limits are assumed to be driven by a trade-off between growing season length and escape from damaging freezing events (Loehle, 1998; Koehler *et al.*, 2012). The constant temperature safety margin against freezing damage across elevations found here indicates that freezing events are such a strong selective pressure that tree species delay flushing until they are safe from damage caused by freezing temperatures. Vitasse *et al.* (2013) reported delays in the date of leaf unfolding for the studied tree species of between 2.6 d K^{-1} (c. 200 m increase in elevation) in *Fagus sylvatica* and 5.4 d K^{-1} in *Fraxinus excelsior* at the seedling life stage. Similar values were found for adults of the same species in the Pyrenees Mountains (Vitasse *et al.*, 2009b). This delay is such that the period available to recover from occasional spring freezing damage before the end of the growing season becomes dramatically shorter at high elevations. This shortened period may explain why common garden experiments generally showed that, in deciduous tree species, populations from high elevation are genetically differentiated from low elevation populations by exhibiting later spring phenology irrespective of actual weather (Vitasse *et al.*, 2009a; a review of the older literature in Körner, 2012; Vitasse *et al.*, 2013). A short growing season restricts fruit ripening and seed maturation in deciduous trees (particularly large-seeded species), potentially shaping northern distribution limits (Chaine & Beaubien, 2001; Morin *et al.*, 2008). This may explain why seed size often decreases with decreasing temperatures (Murray *et al.*, 2004; Moles *et al.*, 2007; Kollas *et al.*, 2012), with fewer seeds sometimes produced at higher latitudes (Moles *et al.*, 2009). In addition to seed

maturation, latitudinal tree species distribution has been suggested to be limited by minimum metabolic requirements to fulfil life history traits for different tree species (Morin & Chaine, 2006). Within a recent growth chamber study, we found that deciduous trees developed no late wood and immature leaf buds when treated with short and cold growing seasons typically found at temperate alpine treelines (A. Lenz & G. Hoch, unpublished data). We suggest that tree species differ in their minimum requirement of growing season length that enables them to complete their annual life cycle successfully with respect to species-specific life history traits, for instance, seed-related traits, wood anatomy, bud formation or leaf traits. Thus, species-specific minimum growing season length requirements may be the ultimate range-limiting factor, with thermal conditions during the growing season modulating that requirement in a non-linear fashion (the cooler the conditions, the longer the required minimum growing season).

Risk of freezing damage in winter and summer

The actual freezing resistance in winter depends on the depth of dormancy and shows a high plasticity to actual *in situ* temperatures (Pisek & Schiessl, 1947; Sakai & Larcher, 1987). We found an increase in maximum freezing resistance during full dormancy with an increase in the elevational limits of species (i.e. species having a higher elevational limit have a higher freezing resistance). Because genetic differentiation in freezing resistance among populations growing at contrasting elevations has been reported, especially in winter (Eiga & Sakai, 1984; see review by Körner, 2012), it is important that we sampled populations growing near their upper elevational limits. The increase in freezing resistance with elevational limit found here was much stronger than the minimum temperature lapse rate along the same elevational gradient. As a result, tree species having the highest elevational limits exhibit freezing resistances that largely exceed actual minimum temperatures in winter. As a result of the high plasticity of freezing resistance to temperature in winter, the freezing resistance values obtained here are most probably too low for species with a low elevational limit and damage is probably overestimated. Thus, winter freezing resistance most probably does not explain the upper elevational limits of temperate deciduous trees. By contrast, freezing resistance in summer shows no correlation with the elevational limits of species. Indeed, the leaves of the examined tree species showed similar freezing resistance between -7 and -4°C during summer, similar to the observations by Taschler & Neuner (2004). Our study demonstrated that, over the past 81 yr, deciduous trees have generally been safe from damaging freezing events during summer at their upper elevational limits.

In conclusion, the risk of freezing damage to the buds or leaves of deciduous tree species is close to zero in summer and rare or zero in winter. By contrast, freezing damage during spring occurs every 7–60 yr (mostly 8–16 yr) depending on species, with a similar mean safety margin against freezing damage in all species at all elevations controlled by species-specific and elevation-specific phenology. Freezing events during flushing appear to be the main

selective pressure controlling the timing of flushing in the studied temperate deciduous tree species. However, this tracking of climate by phenology inevitably leads to shorter growing seasons at higher elevations. We therefore suggest that trees have a species-specific minimum requirement for growing season length that is tied to their life history and freezing resistance during flushing, which, in turn, defines the required timing of spring phenology.

Acknowledgements

We would like to thank Walter Larcher for his initial advice on the sampling design, Gerhard Zotz for critical questions provoking us to further explore the phenology–freezing interaction along the elevational gradient, Lukas Zimmermann for development and support of the freezing laboratory, Isabelle Chuine for providing support in the calibration of the thermal sum model, Chris Kollas and Christophe Randin for initial site selection within the TREELIM project and on-site temperature data, the Swiss Federal Office of Meteorology and Climatology (Meteo-Swiss) for providing climate station temperature data, and the Lavey-Morcles municipality and local foresters for their support. We would like to thank Richard Norby, David Inouye and another anonymous reviewer for helpful comments on the manuscript. This work was funded by European Research Council (ERC) grant 233399 (project TREELIM).

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Comparison between seedling and adult phenology.

Fig. S2 Uncertainty analysis of the mean safety margin against freezing damage.

Table S1 Target freezing temperatures used for the assessment of freezing resistance

Table S2 Parameter estimates used for the thermal time models

Table S3 Performance of the thermal time models

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