

Physiological ecology of the low temperature range limit of European deciduous tree species

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Chapter 1

General Introduction

Armando Lenz

Tree species range limits

All species occupy a certain geographic space beyond which they cannot thrive, forming more or less clear boundaries at the range limit. Some species are generalists while other species have a very narrow range. One of the central questions in ecology is, why species occur where they do, and what the reasons behind the absence of a species and the formation of species-specific range limits are. In the 18th century, Alexander von Humboldt adopted a global view of plant distribution, the formation of vegetation belts and the consistent change in elevation of these vegetation belts along latitude due to temperature (Fig. 1.1, von Humboldt & Bonpland 1807). Since Humboldt's time, two different approaches were employed to explain the formation of species distribution. The first is the correlative approach. The principle is that the correlation of any factor with the presence and absence of a species reveals the most decisive (biotic or abiotic) factor for the formation of a species' range limit. For instance, northern or high elevation range limits of species do usually correlate with some isotherms (e.g. Iversen 1944; Woodward 1990). While this correlative approach allows to predict well the current realised niche of a given species, the transferability of such models is limited in space and time because these models lack of biological processes. So, many abiotic factors like for instance mean annual temperature have no biological meaning per se, but

may contribute in model calibration due to autocorrelation with other more ecologically relevant factors (e.g. absolute minimum temperatures during a particular time). Wilhelm Schimper was among the first to realise that the physiology of the plant needs to be studied when the biological reasons for the global formation of range limits should be understood (Schimper 1898). This physiological approach allows to arrive at a functional explanation of tree species limits. For instance, a direct limitation of tissue formation by low temperature is the most plausible cause for the formation of the alpine treeline (the life form limit of trees; Körner 2012). However, the effect of low temperature on species-specific elevational limits of deciduous tree species not reaching the treeline is still largely unknown. To understand the mechanisms for the formation of the species-specific upper elevational limit in deciduous temperate tree species, the fundamental niche of a species, rather than its realised niche, is of interest. Thus, abiotic factors having a direct influence on the fundamental niche are of key interest.



Fig. 1.1 The elevational distribution of plants follows well the annual mean temperature isotherm. From Bromme (1851)

Abiotic factors consistently changing with elevation and latitude

Regarding high elevation or high latitude range limits, factors that change consistently with elevation and latitude are most promising to test for a functional explanation of a species' range limit. On a global scale, there are only four ecologically important abiotic factors that change consis-

tently with increasing elevation, namely a decrease of the barometric pressure and thus the partial pressure of O₂ and CO₂, an increase of the clear sky radiation, an increase of the fraction of UV radiation in the solar radiation and an adiabatic reduction of atmospheric temperature (Körner 2003; Körner 2007). Clear sky radiation and UV radiation are increasing with elevation, but the radiation a plant experiences is strongly influenced by cloudiness and thus, is not elevation or latitude specific. Precipitation changes with elevation (and also latitude), however, trends vary greatly across the globe. The amount of precipitation decreases with increasing elevation in the equatorial and polar regions, increases at lower elevations and decreases at higher elevations in subtropical regions, and it commonly increases with elevation in the temperate zone (Lauscher 1976; Körner 2007). Another factor not specific to elevation is the available length of the season for growth and reproduction. In the humid tropics, the period for growth does not change significantly along elevation (i.e. a full year season). In the temperate zone, the length of the available freezing-free season gets shorter with increasing elevation.

Taking into consideration not only elevation but also latitude in the temperate climate of Europe, only the adiabatic decrease of temperature and the shortening of the length of the growing season remain as potential factors to explain the distribution limits of major European deciduous broadleaved tree species. Low temperature can affect trees in two ways. First, extreme temperature acts as a threshold beyond which trees will be damaged. Second, temperature can act gradually on the performance of trees either directly or via the length of its action. Thus, the length of the growing season is a gradual effect of temperature.

The TREELIM project – a functional explanation of deciduous tree species limits

This thesis was part of the larger ERC project ‘TREELIM – A functional explanation of low temperature tree species limits’. The TREELIM project assessed the three major potential causes for the formation of a distribution limit related to low temperature effects (Fig. 1.2). Its general structure is as such applicable to any range limit, be it a drought limit or a temperature

limit: (1) the biogeography and climatology at the range limit, (2) population processes and evolutionary adjustments at the range limit and (3) the growth and stress physiology of species at the range limit (Fig. 1.2). In the TREELIM project more than 20 European deciduous tree species were investigated. The analyses presented in this thesis were conducted on the following species: *Sorbus aucuparia* L., *Sorbus aria* L., *Acer pseudo-platanus* L., *Laburnum alpinum* (Mill.) Brecht. & J. Presl., *Prunus avium* L., *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl., *Fraxinus excelsior* L. and *Tilia platyphyllos* Scop.. These species cover a wide range of contrasting elevations, with the first species reaching its limit at very high elevation near the treeline and the last species with a range limit at rather low elevation.

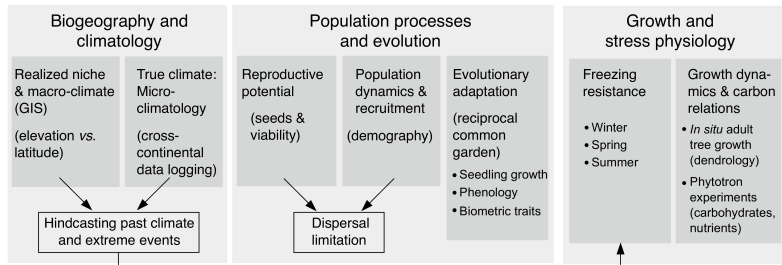


Fig. 1.2 The TREELIM project and its major structure to assess the effect of low temperature on the range limit of tree species. This thesis focuses mainly on the growth and stress physiology part, with some co-authorship in the population processes and evolution part (with courtesy from Ch. Körner)

The biogeography and climatology part included database and geographic information system work with the aim to find and compare the range limit of temperate deciduous tree species at high elevation in the Swiss Alps with the positions of the range limit at northern latitude in Scandinavia (Randin et al. 2013), as well as the assessment of the microclimatology (the ‘true’ temperature trees experience) at the range limit and the reconstruction of past temperature conditions throughout the year trees experienced (Kollas, Körner & Randin 2014a; Kollas et al. 2014b).

In the second part of the project, the viability of seed was compared between trees from the range limit and from low elevation (Kollas et al.

2012) and the change in population structure along elevation was assessed (Chapter 5, Vitasse et al. 2012). Further, large transplant experiments with tree populations from low and high elevation were established at four different elevations, fully replicated in two different regions in the Swiss Alps. These gardens were used to disentangle the environmental from the genetic effects on tree growth, leaf traits and spring and autumn phenology (Chapter 5, Vitasse et al. 2013; Vitasse et al. 2014).

The present thesis explores the physiologically limiting temperature effects. The focus of the thesis is first on threshold effects of temperature (freezing temperature) affecting the physiology of a tree (Chapters 2 and 3). Next, gradual temperature effects on wood formation along elevational gradients are investigated (Chapter 4). In Chapter 5, further related publications which I wrote or co-authored during the thesis are shortly summarised.

Threshold effects of temperature – stress physiology

Freezing temperatures below a certain threshold will directly affect survival of individual tissues as well as entire trees, and can potentially affect trees during any time of the year. Correlations of winter freezing resistance with the distribution limit of deciduous tree species led to the hypothesis, that maximum freezing resistance in winter is the main range limiting factor of deciduous temperate tree species (Charrier, Cochard & Améglio 2013; Sakai 1978; Sakai & Weiser 1973), although many species show a considerable potential to acclimate to lower temperatures than actually experienced in mid winter (Pisek & Schiessl 1947; Sakai 1966). In contrast, all species are very vulnerable to freezing temperatures in spring and recently several damaging freezing events in the US as well as in Europe have been reported (Augsburger 2009; Gu et al. 2008; Hufkens et al. 2012; Kreyling, Stahlmann & Beierkuhnlein 2012). Within my thesis, I experimentally assessed freezing resistance of deciduous temperate tree species in a freezing laboratory with seven computer-controlled freezers which allow for a simultaneous freezing of all samples at different temperatures. The aims of the freezing experiments were

- to determine during which period of the year trees are most vulnerable to freezing temperatures at their upper elevational range limit (Chapter 2),
- to quantify the change of the risk to encounter freezing damage with increasing elevation (Chapters 2 and 3),
- to explore how well trees escape freezing damage to developing leaves in spring (Chapters 2 and 3) and
- to assess the hardening potential of different beech provenances in mid winter (Chapter 5.2).

Gradual temperature effects — growth dynamics

Low temperatures during the growing season can generally slow down photosynthetic carbon uptake, nutrient uptake or meristematic activity and consequently tissue formation. Of these three processes, the meristems are the most susceptible to low temperature effects (Körner 2006). Meristematic activity (cell division) generally stops at temperatures between 2 – 5 °C, while net photosynthesis is still running at approximately 40-60% of full capacity at these temperatures (Körner 2006; Körner 2013). Similarly, the uptake of nutrients is less temperature limited than tissue formation (Ehrhardt 1961; Körner 2012), and there is no indication of increasing nutrient limitation with increasing elevation (Birmann & Körner 2009; Körner 2012). Meristematic activity (growth) seems to be the most likely point of action of low temperature and is thus the focus of this thesis. We tested for gradual temperature effects on tree growth in two ways.

First, we experimentally tested gradual low temperature effects on tissue formation in potted tree seedlings. These seedlings were grown in thermostated water-baths, inducing a vertical temperature gradient from warm temperatures (20 °C) to cold temperatures (2 °C) in the root space. Roots could grow in this temperature gradient to the species-specific limiting temperature for tissue formation. We asked following questions

- What are the low temperature limits for tissue formation of deciduous temperate tree species (Chapter 5.8),

- Do the low temperature limits for tissue formation differ among species and do they match the elevational distribution limit of species (Chapter 5.8)?

Second, we investigated gradual temperature effects on mature tree growth by collecting increment cores of mature trees along natural elevational gradients, to ask

- Is radial growth declining gradually or abruptly along elevational gradients (Chapter 4)?
- How do non-structural carbohydrate concentrations (as a proxy for carbon pools) change along elevation (Chapter 4)?
- Is the frequency of very narrow tree rings as an indicator of bad summers or early season freezing damage increasing with increasing elevation (Chapter 4), and finally
- Is in situ radial growth of temperate tree species more limited by low temperature or by the length of the growing season (Chapter 4)?

The use of in situ measurements and experimental approaches used here allowed us to disentangle both, threshold and gradual temperature effects on tree growth and survival, with the core of this thesis presented in Chapters 2 to 4. The results enabled us to formulate a functional concept for the formation of species-specific cold range limits.

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Chapter 2

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

Armando Lenz, Günter Hoch, Yann Vitasse and Christian Körner

Abstract

- 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 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 to 8.5 K) against damage by spring freeze events along elevational gradients, due to 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

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growing season at higher elevation and potentially driving species distribution limits. Such mechanistic knowledge is important to improve predictions of tree species range limits.

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

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, with 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 rupture of biomembranes (Ziegler & Kandler, 1980; Sung et al., 2003; see review by Larcher, 2005). So, a major part of freezing resistance is to maintain membrane fluidity during the freezing process and tolerate freezing induced dehydration in the cell plasma. To do so, plants synthesise dehydrins and antifreeze proteins and reduce the amount of membrane located carriers and receptors during the prehardening 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 increasing 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 at explaining 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 minimum annual temperatures at the distribution limits of tree species would suggest that winter temperatures control cold distribution limits (e.g., Sakai & Weiser, 1973; Sakai, 1978). On the other hand, 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 when 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 trees 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 due to climate warming has been observed for more than 400 plant species in Europe (Menzel et al., 2006), as well as for many tree species in Europe and in 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 United States in 2007 due to 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 due to 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 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 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, i.e. 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 to 600 m below their respective elevational limits for repeated sampling (Table 2.1).

Table 2.1 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)

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

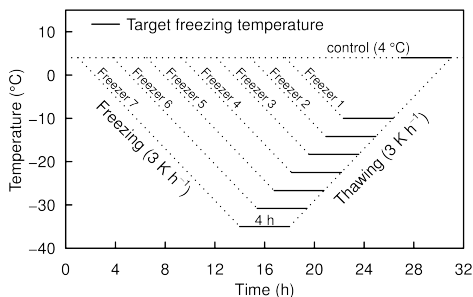
Assessment of freezing resistance and spring phenology

Twigs with buds, and later with leaves, were collected weekly from six individuals out of 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). Of 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 (1), bud burst (2), leaf emergence (3), and leaf unfolding (4), except for *Sorbus aucuparia* and *Quercus petraea*, for which the assessment of stage 1 was unclear or missed and therefore removed from further analysis. Immediately after cutting, the twigs were kept cool (0–4 °C) for 7–12 hours 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; 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, then kept for 4 h at the target freezing temperature before being thawed by 3 K h⁻¹ until reaching 4 °C (Fig. 2.1). 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 A1). 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 becomes manifested by a discoloration due to oxidation of polyphenols or a characteristic odour due to de-compartmentalisation 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 autoclav-

ing the samples at 120°C for 20 min. The autoclaved samples were kept for 24 h at 4°C before measuring 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 (LT_{50}). LT_{50} was calculated using logistic regression for visual damage and non-linear Gompertz models for electrolyte leakage data according to Lim et al. (1998). LT_{50} values were calculated separately for each sampled tree of each species ($n = 6$ per species). LT_{50} values calculated from visual damage assessment and electrolyte leakage showed a high correlation ($LT_{50}^{Electrolyte} = -1.7 + 0.8 * LT_{50}^{Visual}$, $R^2 = 0.65$, $P < 0.001$). We were interested in freezing damage to leaf primordia in buds of those broadleaved trees. Because for technical reasons, electrolyte leakage could be only determined in 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 of 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 LT_{50} values were calculated from the electrolyte leakage data.

Fig. 2.1 Parallel freeze-thaw cycles in 7 computer-controlled freezers used for the assessment of the freezing resistance of 8 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.



Reconstruction of minimum temperature and spring phenology

We calculated three seasonal temperature lapse rates for winter (Dec-Feb), spring/summer (March-July) and autumn (Aug-Nov) 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 m to 2160 m 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 (chilling), photoperiod and warm temperatures in spring (forcing; Polgar & Primack, 2011). Assuming that the species we are studying here do all receive sufficient chilling in every year, which minimises 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, since we focussed 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 fit 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 days earlier (mean \pm s.e.) across species compared to selected adult trees near the common gardens during the study year (Fig. A1). 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 days).

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 studied here. These 2-year-old seedlings were monitored for bud development in 8 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 by using a simulated annealing algorithm using 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 A2). 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 to 4.8 days). A leave-one-out cross-validation yielded a ME ranging from 0.39 to 0.97 and an RMSE from 1.5 to 9.9 days, with most of the models having an RMSE of less than 4 days (Table A3). 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 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 (i) the mean of the dates of the previous and corresponding stages and (ii) 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 0 indicate freezing damage, whereas values above 0 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: predicted phenology data have an associated RMSE obtained from the leave-one-out cross-validation. We shifted the time window for minimum temperature calculation for each stage backward and forward by the extent of the corresponding RMSE, yielding a new temperature data set at both ends of the time window. Then, an RMSE was calculated between the original minimum temperature data set according to phenology and the new temperature data sets 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 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. 2.2). 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. 2.2).

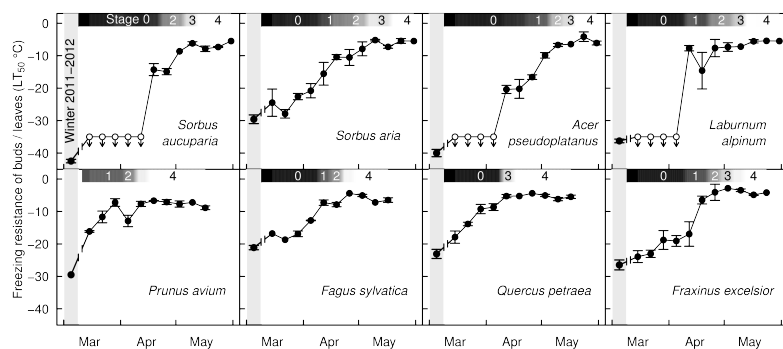
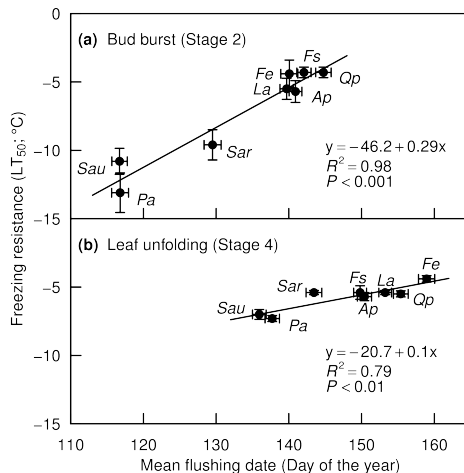


Fig. 2.2 Mean freezing resistance of the buds and leaves of 8 deciduous tree species during winter and early spring expressed as the lethal temperature at which 50% of samples are dead (LT_{50}). Points within grey bars represent freezing resistance during winter 2011–2012. Open circles with arrows indicate freezing resistance below -35°C . The shaded bars on top indicate phenological stages: Buds closed (Stage 0), buds swelling (1), buds bursting (2), leaves emerging (3) and leaves unfolding (4). Error bars correspond to the standard error ($n = 6$).

Early flushing species were more freeze-resistant during early bud development than late flushing species. For instance, during bud burst (phenological stage 2), the three earliest flushing species, *Prunus avium*, *Sorbus aucuparia* and *Sorbus aria* (Table 2.1), had LT_{50} values below

Fig. 2.3 Relationship between freezing resistance ($LT_{50} \pm$ s.e.) and the timing of spring phenology (\pm s.e.) reconstructed by the thermal time model at 1500 m asl in 8 deciduous tree species. Correlations are shown for bud burst (a, stage 2) and leaf unfolding (b, stage 4). *Sau* *Sorbus aucuparia*, *Sar* *Sorbus aria*, *Ap* *Acer pseudoplatanus*, *La* *Laburnum alpinum*, *Pa* *Prunus avium*, *Fs* *Fagus sylvatica*, *Qp* *Quercus petraea*, *Fe* *Fraxinus excelsior*.



-9.6°C , whereas all of the other species had LT_{50} values higher than -6°C (Fig. 2.3). 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°C to -3.2°C for *Prunus avium* and *Fraxinus excelsior*, respectively. Nevertheless, the variation in freezing resistance among species gradually decreased during bud development. Thus, the difference in maximum freezing resistance among species was more than 20 K when the buds were closed, approximately 10 K when the buds were swelling and bursting (phenological stages 1 and 2), approximately 5 K when the leaves were emerging (stage 3) and less than 3 K after the leaves had unfolded (stage 4, Fig. 2.3). As the new leaves became mature, freezing resistance increased slightly, but the small differences in freezing resistance among species persisted into summer (Fig. 2.2). After leaf maturation, the freezing resistance of leaves was not correlated with the species' upper limits but was related to the timing of spring phenology; early flushing tree species, such as *Sorbus aucuparia* and *Prunus avium*, were always more resistant to freezing temperatures than late flushing tree species (Fig. 2.3).

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. 2.4). 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 would be 4–5 K). This safety margin was associated with an error of 1.9 to 2.7 K, yielding a minimum safety margin of 2.9 K in *Fraxinus excelsior*. However, our results also revealed that most tree species likely experienced freezing damage during the 1931–2011 period. Due to 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. 2.4). During the flushing period, we found that at least one potential instance of freezing damage occurred in less than 5% of the examined years for all species (i.e., in less than 4 out of 81 years, that is, 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. 2.4). 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 way 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 above). However, the uncertainty analysis showed that by shifting spring phenology up to 10 days earlier or up to 20 days later, the mean safety margins were not significantly affected. Hence, our results are quite robust against such ontogenetic shifts in phenology (Fig. A2).

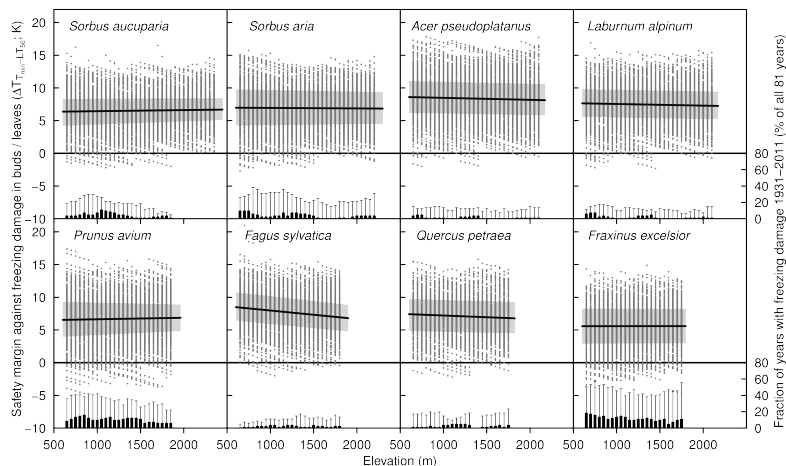


Fig. 2.4 Safety margin against freezing damage in 8 deciduous tree species at different elevation during flushing (stages 1 to 4), expressed as the difference between the minimum air temperature and mean freezing resistance (LT_{50}) according to phenological stage. The points indicate safety margin 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 M&M). 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 to 2011.

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. 2.5). Counter-intuitively, tree species reaching higher elevations were generally safer from freezing damage in winter than species with a lower elevation limit (Fig. 2.5). For instance, the three species with the lowest elevational limits encountered potential freezing damage every 3–27 years. In contrast to winter, the risk of being damaged by freezing events during summer was

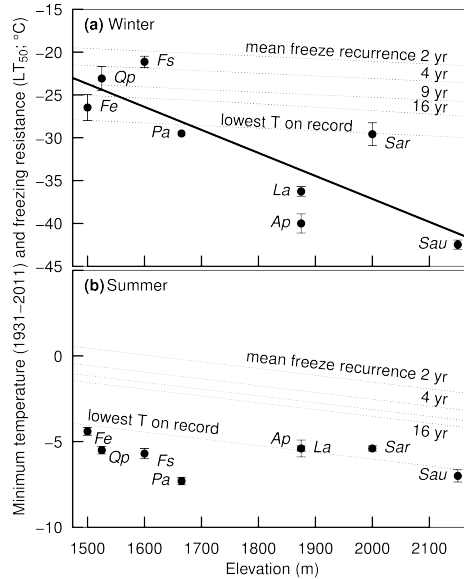


Fig. 2.5 Minimum temperatures from 1931 to 2011 scaled to the studied elevational gradient and the mean freezing resistance (\pm s.e.) of 8 deciduous tree species at the elevational limit in winter (a) and summer (b). Dotted lines indicate the mean recurrence time of freezing events. Thin lines indicate the lowest temperature reconstructed from 1931 to 2011. 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). Sau *Sorbus aucuparia*, Sar *Sorbus aria*, Ap *Acer pseudoplatanus*, La *Laburnum alpinum*, Pa *Prunus avium*, Fs *Fagus sylvatica*, Qp *Quercus petraea*, Fe *Fraxinus excelsior*. Error bars represent standard errors ($n = 6$). Note the different y-axis scales.

very low for all species and higher for species having higher elevational limits (Fig. 2.5). Minimum air temperature reconstructions showed that potential freezing damage occurred only once during the 81 year 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 spring freezing events have a higher probability of damaging tree species than freezing events during winter. Due to the high sampling resolution, we could directly measure 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, due to the phenological shift in response to temperature decrease, we did not find an increase in potentially damaging freezing events with increasing elevation during the flushing period. In 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 elevation limits and exhibit a mean safety margin against freezing damage of 5–8.5 K (Fig. 2.4). 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 to 16 years 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 LT_{50} of leaf primordia or leaves are certainly less severe than strong frosts well below the LT_{50} . Strong freeze events might also damage meristematic tissue, wood parenchyma and phloem, which generally have LT_{50} 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 us to assess freezing resistance during defined developmental stages in spring. To our knowledge, this assessment has only previously been performed once, by Taschler et al. (2004). Those authors studied three conifer species, one dwarf shrub and *Sorbus aucuparia* at the tree-line, 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 providing 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 in early phenological stages than late flushing species, 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 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 understory and canopy trees, with the phenology of the understory 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. A2). 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 optimise 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 a 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 days K^{-1} (approx. 200 m increase in elevation) in *Fagus sylvatica* and 5.4 days 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 (Chuine & 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). Besides 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 & Chuine, 2006). Within a recent growth chamber study, we found deciduous trees to develop no late wood and immature leaf buds when treated with short and cold growing seasons typically found at temperate alpine treelines (Lenz & 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 max-

imum freezing resistance during full dormancy with species' elevational limits (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. Due to 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 likely overestimated. Thus, winter freezing resistance most likely does not explain the upper elevational limits of temperate deciduous trees. In contrast, freezing resistance in summer shows no correlation with the elevational limits of species. In fact, the leaves of the examined tree species showed similar freezing resistance between -7 and -4°C during summer, similar to what Taschler & Neuner (2004) reported. Our study demonstrated that over the past 81 years, 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. In contrast, freezing damage during spring occurs every 7 to 60 years (mostly 8–16 years) 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.

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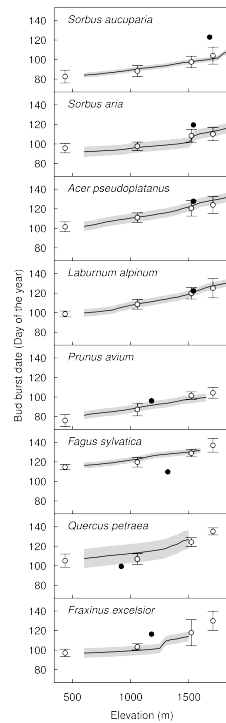
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Supporting Information

Comparison between seedling and adult phenology

Fig. A1 The mean bud burst date observed in seedlings growing in common gardens (open circles \pm SD), predicted by a thermal time model (line \pm RMSE of the leave-one-out cross validation) and observed in adults used for assessment of freezing resistance (closed circles, no SD reported due to changing individuals) according to elevation in 2011.



Uncertainty analysis of the mean safety margin against freezing damage

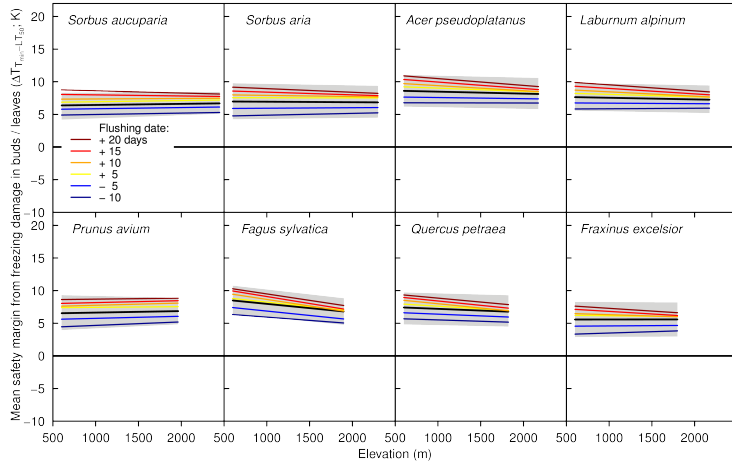


Fig. A2 Mean safety margin against freezing damage of 8 deciduous tree species along an elevational gradient (black line \pm Error; grey shade; see material and methods) along with mean safety margins obtained from the uncertainty analysis (coloured lines). Uncertainties were calculated by shifting the time window of minimum temperature calculation from 10 days earlier towards 20 days later flushing. Note: the figure has the same axis scale as the Fig. 2.2 in the Manuscript.

Table A1 The seven target freezing temperatures (FT¹, in °C) used for the assessment of freezing resistance of broadleaved trees before, during and after flushing in the week of the year 2011 and in winter 2011/2012.

Week	FT1	FT2	FT3	FT4	FT5	FT6	FT7
11	-10.0	-14.2	-18.3	-22.5	-26.7	-30.8	-35.0
12	-10.0	-14.2	-18.3	-22.5	-26.7	-30.8	-35.0
13	- ²	-5.0	-11.0	-17.0	-23.0	-29.0	-35.0
14	-5.0	-10.0	-15.0	-20.0	-25.0	-30.0	-35.0
15	-5.0	-10.0	-15.0	-20.0	-25.0	-30.0	-35.0
16	-5.0	-10.0	-15.0	-20.0	-25.0	-30.0	-35.0
17	-5.0	-9.2	-13.3	-17.5	-21.7	-25.8	-30.0
18	-3.0	-6.7	-10.3	-14.0	-17.7	-21.3	-25.0
19	-3.0	-6.2	-9.3	-12.5	-15.7	-18.8	-22.0
20	-3.0	-5.5	-8.0	-10.5	-13.0	-15.5	-18.0
21	-3.0	-5.5	-8.0	-10.5	-13.0	-15.5	-18.0
22	-3.0	-5.5	-8.0	-10.5	-13.0	-15.5	-18.0
Winter ³	-10.0	-18.0	-21.5	-25.0	-28.5	-32.0	-40.0

¹ Target freezing temperatures were randomly distributed among the 7 freezers.

² One freezer did not work in week 13 2011.

³ For the assessment of winter freezing resistance an additional freezing treatment without controlled ramps at -80°C was included as a '100% dead' reference, using a storage deep freezer.

Table A2 Parameter estimates of the thermal time models used for hindcasting of flushing dates in the study area.

Species	t_0	t_b	F_{Stage1}	F_{Stage2}	F_{Stage3}	F_{Stage4}
<i>Sorbus aucuparia</i>	66	0.2	70	115	169	231
<i>Sorbus aria</i>	82	4.1	14	53	78	108
<i>Acer pseudoplatanus</i>	75	0.0	200	275	316	355
<i>Laburnum alpinum</i>	72	0.1	203	264	326	383
<i>Prunus avium</i>	58	1.8	23	74	121	170
<i>Fagus sylvatica</i>	104	4.1	65	91	107	133
<i>Quercus petraea</i> ¹	92	4.1	69	111	130	168
<i>Fraxinus excelsior</i>	89	6.5	17	38	62	95

t_0 and t_b were fitted for Stage 2 and fixed to those values for all other stages; t_0 day of the year when accumulation of degree days starts; t_b critical temperature above which degree days are accumulated; F critical sum of forcing units required for flushing of the respective stage.

¹ For *Q. petraea* $n = 4$.

Table A3 Performance of the thermal time models used for hindcasting of flushing dates in the study area for the calibration based on the whole dataset of phenological observation, and for the ‘leave-one-out’ cross-validation.

Species	Whole dataset (<i>n</i> =8)			Cross-validation (<i>n</i> -1)		
	RMSE	ME	Bias	RMSE	ME	Bias
<i>Sorbus aucuparia</i>						
Stage 1	3.6	0.70	0.3	4.2	0.61	0.0
Stage 2	1.9	0.91	0.0	2.6	0.84	-0.2
Stage 3	1.6	0.96	-0.2	1.9	0.94	-0.2
Stage 4	1.9	0.96	-0.2	2.1	0.94	-0.2
<i>Sorbus aria</i>						
Stage 1	2.3	0.86	0.0	2.9	0.76	-0.2
Stage 2	3.0	0.81	0.9	5.3	0.41	-0.3
Stage 3	4.2	0.79	-0.7	4.8	0.72	-0.6
Stage 4	4.4	0.81	-0.8	4.5	0.80	-0.8
<i>Acer pseudoplatanus</i>						
Stage 1	1.8	0.93	-0.2	2.1	0.91	-0.2
Stage 2	3.7	0.83	0.2	4.9	0.70	0.7
Stage 3	3.1	0.89	0.7	3.7	0.84	0.5
Stage 4	2.7	0.91	-0.3	3.2	0.88	-0.2
<i>Laburnum alpinum</i>						
Stage 1	2.0	0.94	0.0	2.3	0.91	0.0
Stage 2	3.1	0.88	-0.3	4.0	0.81	-0.2
Stage 3	2.4	0.94	0.5	2.8	0.92	0.3
Stage 4	3.1	0.91	-0.5	3.7	0.87	-0.2
<i>Prunus avium</i>						
Stage 1	3.3	0.87	0.3	3.9	0.82	0.6
Stage 2	2.7	0.90	0.1	4.2	0.77	-0.5
Stage 3	2.2	0.95	0.0	2.6	0.92	0.2
Stage 4	3.0	0.92	-0.3	3.8	0.88	-0.4
<i>Fagus sylvatica</i>						
Stage 1	2.5	0.79	-0.1	2.7	0.75	0.1
Stage 2	1.9	0.91	0.0	3.1	0.78	-0.3
Stage 3	1.9	0.92	-0.1	2.3	0.88	-0.1
Stage 4	1.7	0.93	0.1	2.0	0.91	0.1
<i>Quercus petraea</i> ¹						
Stage 1	1.1	0.99	0.5	1.5	0.98	0.0
Stage 2	4.3	0.88	1.2	9.9	0.39	-2.8
Stage 3	1.9	0.97	0.4	2.0	0.97	0.0
Stage 4	2.3	0.97	-0.7	3.6	0.92	-0.3
<i>Fraxinus excelsior</i>						
Stage 1	2.1	0.89	1.0	4.3	0.53	-0.2
Stage 2	2.4	0.95	-1.0	5.0	0.78	-1.7
Stage 3	3.9	0.91	-0.8	6.1	0.79	-1.7
Stage 4	4.8	0.88	0.9	5.5	0.85	0.8

RMSE root mean square error; ME model efficiency; Bias mean difference between predicted and observed dates.

¹ For *Q. petraea* *n* = 4.

Chapter 3

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

Armando Lenz, Günter Hoch, Christian Körner and Yann Vitasse

Abstract

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 on 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. The timing of 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 prob-

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abilistically 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 of 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 minimising the risk of freezing damage and maximising 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 photoperiodic 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). 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 couldn't 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 US where an increase in freezing damage was attributed to earlier snowmelt (Inouye 2008). No such long-term records exist for long-lived trees, though particularly wide-spread 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 by 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 on-site temperature with freezing resistance of emerging leaves. We address the following questions: (1) When do trees leaf-out relative to the probability of freezing damage? (2) Do trees experience the same risk of freezing damage at low and at high elevations? (3) 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 3.1). 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 3.1). Sites are characterised by a temperate continental climate, with a mean annual precipitation ranging from 700 to 1700 mm (Table 3.1), and dominated by mature mixed forest stands, with calcareous or sandstone as bedrock.

Table 3.1 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.

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

was 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 1981–2012 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 2003). Depending on species and station, 14 to 32 years of leaf-out data were available for the investigated period from 1981–2012 (Table 3.2). Leaf-out data were visually checked for consistency within species among stations, and among species within stations (see Figures S1 and S2 in Supporting Information).

Air temperature was recorded by climate stations every 10 minutes in the vicinity of the observation sites for phenology from 1981–2012. 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. LT_{50} 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 LT_{50} 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 corre-

sponds 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 Figure 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 LT_{50} 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 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

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

Table 3.2 Start year of leaf-out observations, with the number of years of available leaf-out records for every species. 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

	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>
<i>Low elevation sites</i>											
Wädenswil	1996	1981	1996	1996	1981	16	32	16	16	31	2
Altdorf	1999	1999	1999	1999	1981	14	14	14	14	14	2
Hallau	1996	1981	1996	1996	1981	17	32	17	17	32	1
Wynau	1996	1981	1996	1996	1981	17	32	17	17	32	2
<i>High elevation sites</i>											
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
Scul	1996	1987	1996	1997	1981	17	26	17	16	32	1

Table 3.3 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	<0.0001	34.6	<0.0001	15.2	<0.0001
Elevation	1	6	49.3	<0.001	0.6	0.46	16.4	<0.01
Species x Elevation	4	24	1	0.44	2.9	<0.05	2.8	0.05
<i>Random effects</i>								
Station			<i>Sigma</i>		<i>Sigma</i>		<i>Sigma</i>	
Species within station			3.44		0.43		3.2	
Residual variation			3.34		0.28		3	
			7.5		2.3		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 $P < 0.05$

where $Doy_{Leaf-out}$ corresponds to the day of year of leaf-out in a specific year, and $Doy_{Last\ freeze}$ 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 generalised extreme value distributions (GEV; Coles 2001), as described in detail in Kollas, Körner and Randin (2014). Briefly, we extracted all daily minimum temperatures at low and high elevation stations from 1981–2012. 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 K 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 analysis

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 con-

sidered 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. 3.1). Leaf-out dates differed also significantly between low and high elevations, but the interaction between species and elevation was non-significant (Table 3.3). 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. 3.1).

Minimum temperatures during spring

Mean minimum air temperatures ranged from ca. 2 to 9°C at low elevation sites, and were slightly cooler (1K) at high elevation sites during the leaf-out period from 1981 to 2012 (Fig. 3.2a,b). Absolute minimum temperature was around -7.5°C at low elevation and -14°C at high elevation during this period (Fig. 3.2a,b). Interestingly, the return rate of absolute minimum temperature was approximately 1.5 times larger at high elevation compared to low elevation during the respective leaf-out period (Fig. 3.2c,d). While temperatures of -1°C and -2°C occurred every 21 or 52 years at high elevation at in the middle of the leaf-out period (day 127, Fig. 3.2d), the corresponding return rates were every 30 and 76 years

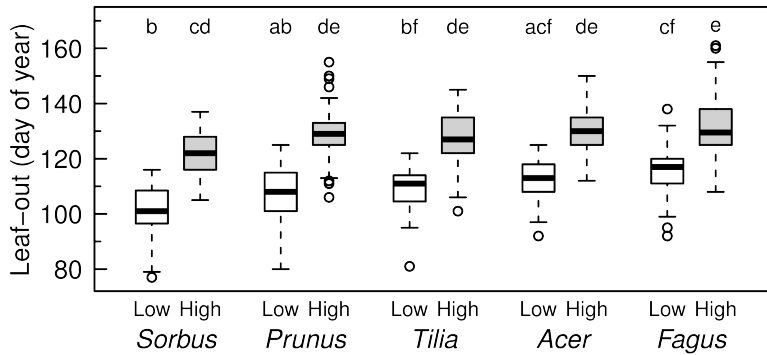


Fig. 3.1 The date of leaf-out for each species across low- and high-elevation sites. White boxes represent low-elevation sites, while grey boxes represent high-elevation sites. The boxplots show the median, the first and third quartile, and the extreme point. Any point extending more than 1.5 times the 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.

at low elevation (day 113, Fig. 3.2c). 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. 3.2). Interestingly, temperatures increased rather 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. 3.2c). On the other hand, the return rate of 5°C increased steadily at high elevation from 1 year to 3 years during leaf-out (Fig. 3.2d).

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. 3.3). Thus, all trees escaped freezing temperatures well during the investigated period. Leaf-out occurred among all species at high elevation on average 35

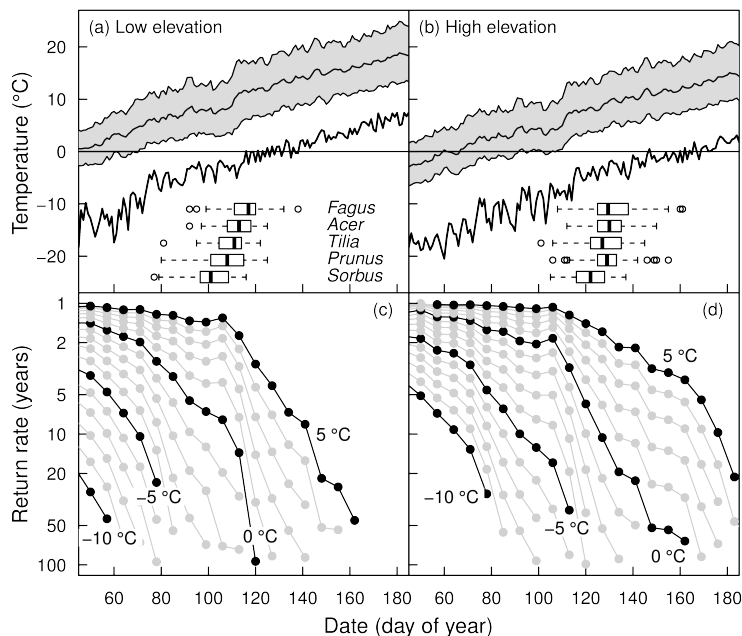


Fig. 3.2 Air temperature records for low and high elevation stations during the leaf-out period. Mean minimum, mean, and mean maximum air temperature is shown as shaded area, with the absolute minimum temperature (thick line), and the leaf-out time of species for (a) low, and (b) high elevation sites. The return rate (time interval of recurrence) of absolute minimum temperatures of -10°C to $+5^{\circ}\text{C}$ 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).

to 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 damaging freezes was 38 to 50 days at low elevation. *Fagus* exhibited the shortest time interval between the probabilistically dangerous freezing period and leaf-out (Fig. 3.3). 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 proba-

bility 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, i.e. from 5 days prior to 5 days after leaf-out (Fig. 3.5). 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. 3.4). 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 1981–2012 (16 to 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. 3.4). Elevation had no significant effect on the temperature safety margin, however species and the interaction between species and elevation were both significant (Table 3.3). The interaction was significant because earlier flushing species (*Sorbus* and *Prunus*) had a slightly non-significantly larger temperature safety margin at high elevation, whereas late flushing species (*Acer* and *Fagus*) had a smaller safety margin at high elevation (Fig. 3.4). 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. 3.5), 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 (Fig. 3.4, 3.5). Consistently, species, elevation and the interaction between the two had a significant effect on the date safety margin (Table 3.3). *Fagus* was the only species with incidences of a negative date safety margin,

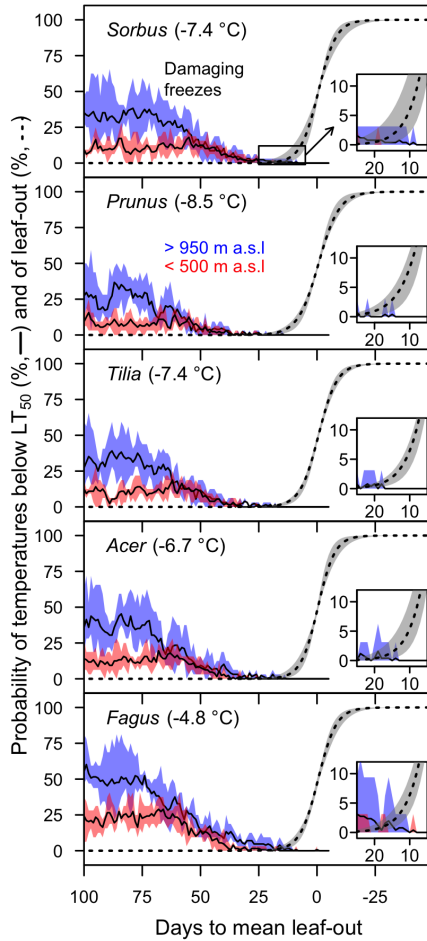


Fig. 3.3 The mean probability of daily absolute minimum temperatures below species-specific LT_{50} values for low and high elevation sites (solid lines with the range across stations for high and low elevation sites in blue and red) in relation to the leaf-out timing. Leaf-out data across years were 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 days 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.

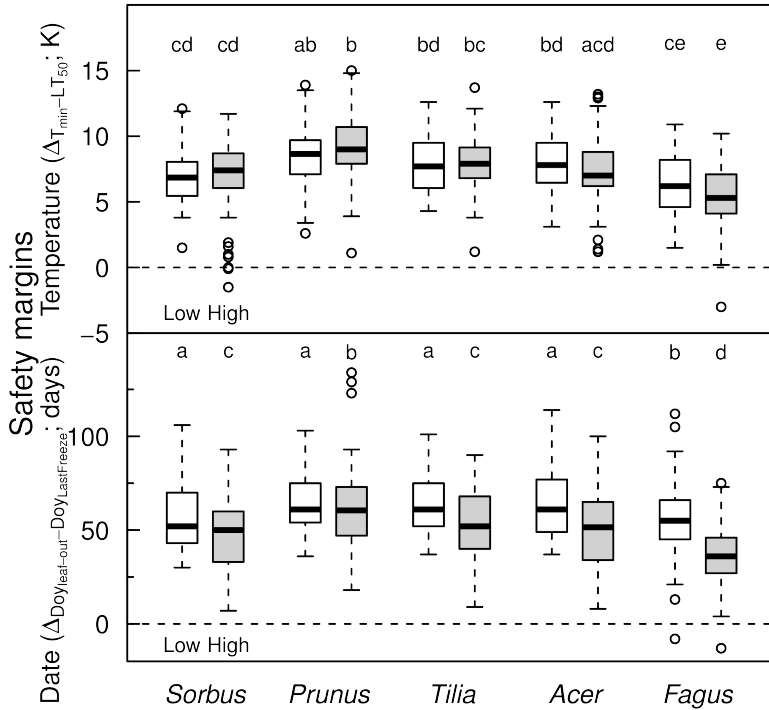


Fig. 3.4 The temperature and date safety margin against freezing damage at low (white) and high elevation (grey) for each species. (a) The temperature safety margin was defined as the difference between absolute minimum temperature from 5 days prior to 5 days after leaf-out and the species-specific freezing resistance of foliage during leaf-out. (b) 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.

namely 2 times in the records (Fig. 3.4, 3.5). Interestingly, the first incidence was eight 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, i.e. 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. 3.6), 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). 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

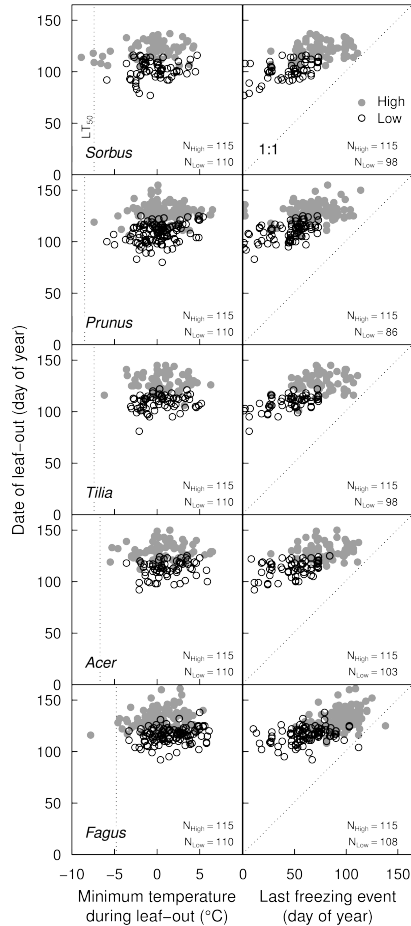


Fig. 3.5 The date of leaf-out for each species versus absolute minimum temperature during leaf-out (left panel) and the date of the last freeze event below species-specific LT_{50} values (right panel) for low (open circles) and high (grey circles) elevation sites. The absolute minimum temperature during 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.

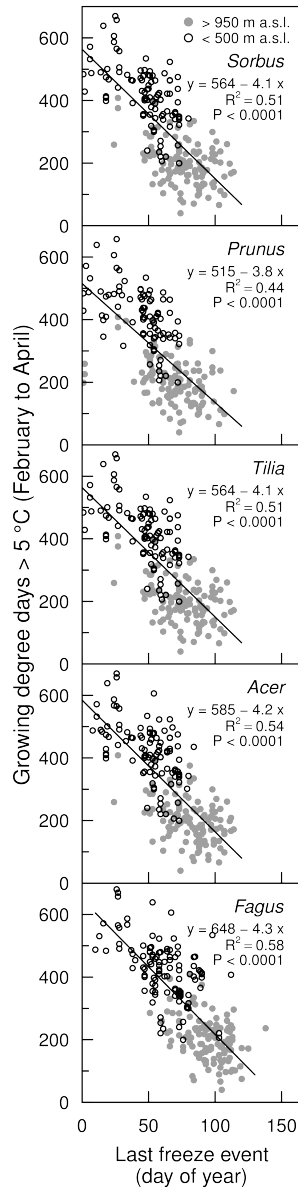


Fig. 3.6 Correlation between growing degree days above 5 °C in spring (February to April) for low- (open circles) and high- (grey circles) elevation sites with to the date of the last freeze event below the species-specific LT50 value for each species. Growing degree days were used as a proxy for spring warmth. Correlations were calculated across all data.

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 the 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. 3.2), 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, i.e. 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 deacclimation) is tightly linked to bud development (Kalberer, Wisniewski & Arora 2006; Pagter & Arora 2012), 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 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 2012; 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 hypothesise 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 hypothesised. 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 in elevational limits of temperate tree species in the Swiss Alps and 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 life-time 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 to 6 days later for 1K of temperature decrease (i.e. increase of elevation by ca. 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. In-

terestingly, *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 Wuehlich, 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 co-existing deciduous temperate tree species. Species like —*Fagus 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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Supporting Information

Table S1 Freezing resistance during leaf-out given as the LT_{50} value for each species. For each species, 5 individuals were sampled. Species differ significantly in freezing resistance (ANOVA, d.f = 4, F = 18.37, P < 0.0001).

Species	LT_{50} (°C)	Tukey HSD post-hoc groups
<i>Sorbus aucuparia</i>	-7.4 ± 0.6	bc
<i>Prunus avium</i>	-8.5 ± 0.1	c
<i>Tilia platyphyllos</i>	-7.4 ± 0.3	bc
<i>Acer pseudoplatanus</i>	-6.7 ± 0.2	b
<i>Fagus sylvatica</i>	-4.8 ± 0.0	a

LT_{50} values were extracted from Lenz et al. (2013), except for *Tilia*, where the LT_{50} value was extracted from Vitasse et al. (2014).

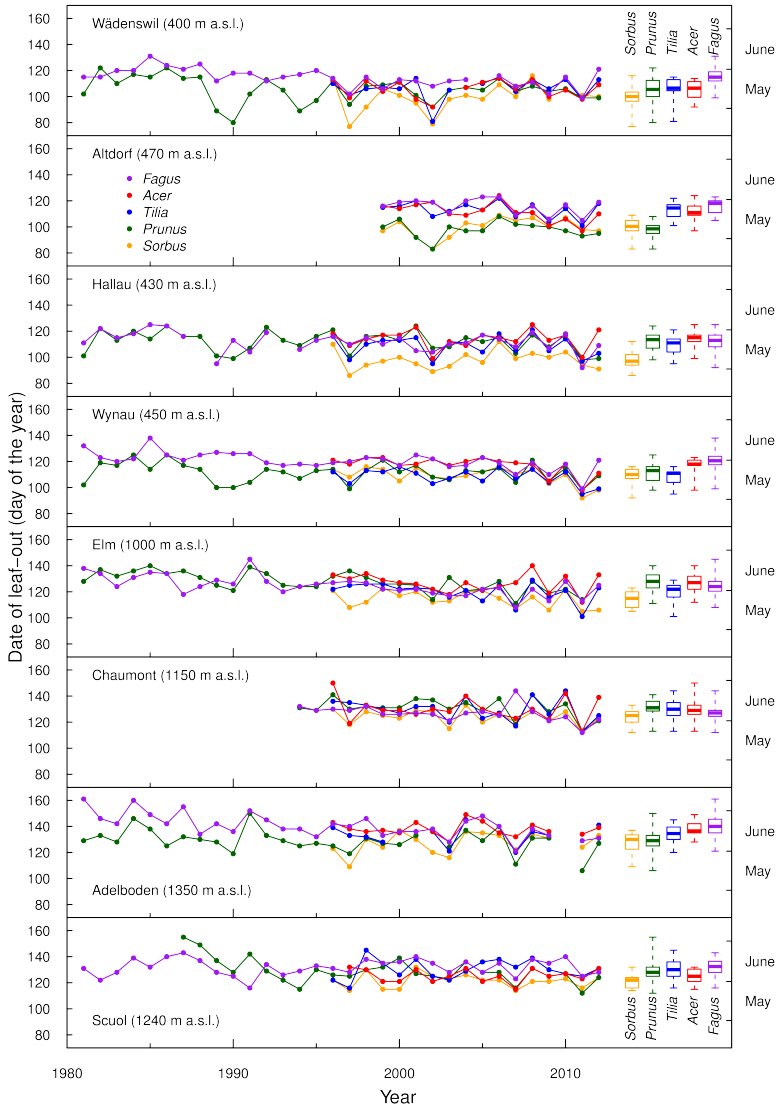


Fig. S1 Date of leaf-out of the five deciduous broadleaved tree species used for the study for each station during the time period from 1981 to 2012.

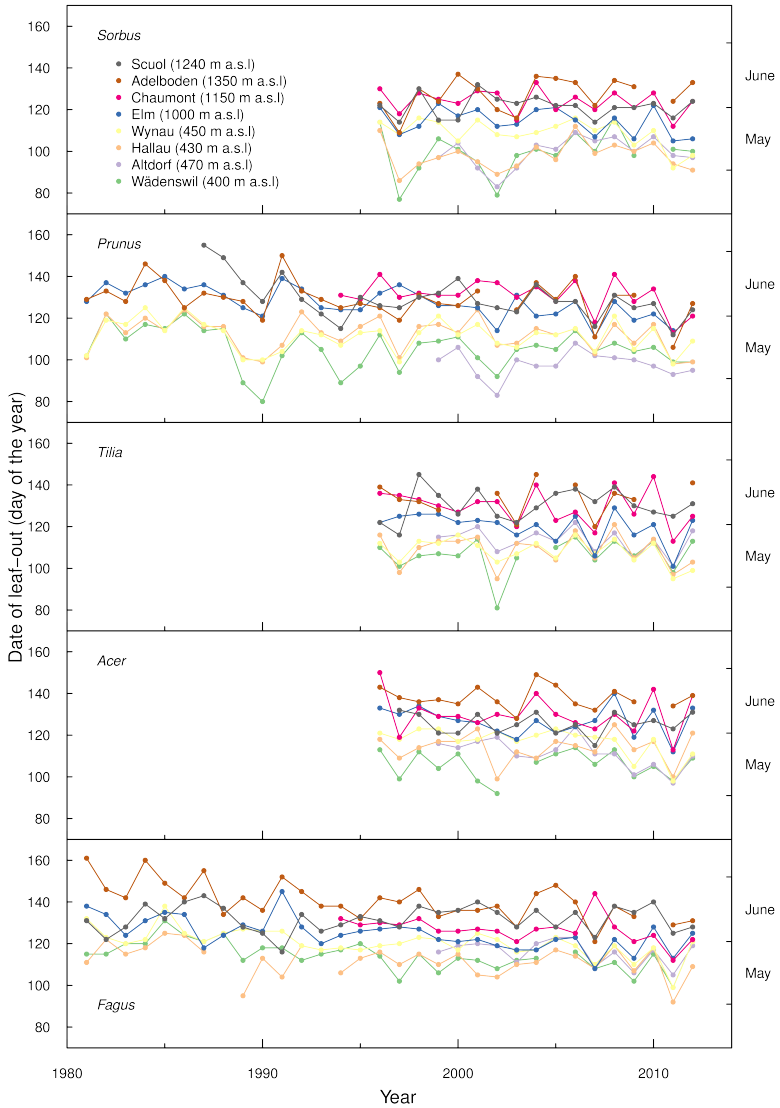


Fig. S2 Date of leaf-out for each of the five species used among all eight stations during the period from 1981 to 2012.

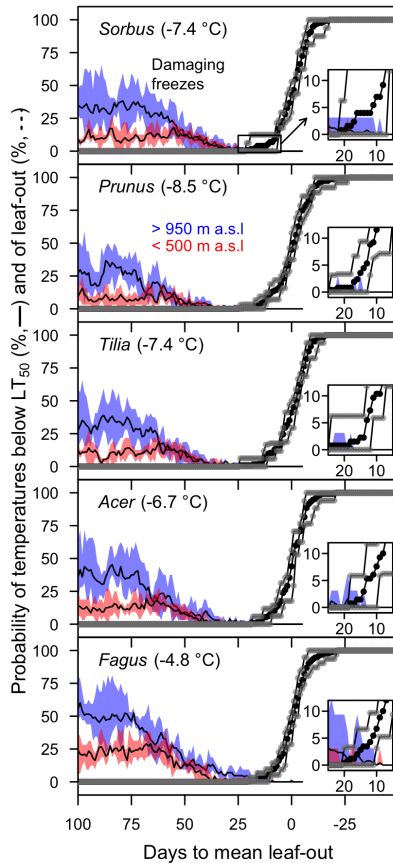


Fig. S3 The mean probability of daily absolute minimum temperatures below species-specific LT_{50} values for low and high elevation sites (solid lines with the range across stations for high and low elevation sites in blue and red) in relation to the leaf-out timing. The cumulative probability of leaf-out is shown, and calculated as the sum of years with leaf-out divided by the total number of years in which leaf-out was observed. The earliest and latest station is represented by grey dots, with the mean across stations as black dots. 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 days 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.

Chapter 4

Growth and carbon relations of temperate deciduous tree species at their upper elevation range limit

Armando Lenz, Yann Vitasse, Günter Hoch and Christian Körner

Abstract

1. Temperature is one of the most important drivers of range limits in plants. Here, we aimed at disentangling the direct effect of low temperature and the indirect temperature effect via the length of the growing season on radial growth and carbon resources of deciduous temperate tree species at their high-elevation limit in the Swiss Alps.
2. Trees of eight species were cored along three elevational gradients of ca. 1000 m up to the specific high-elevation range limit. We correlated basal area increment (BAI) with mean temperature during the growing season and length of the growing season, derived from a thermal time model. Stem sapwood of cored trees was analysed for non-structural carbohydrate concentrations.
3. The frequency of negative event years (exceptionally narrow tree rings) did not significantly increase with elevation except for *Fagus sylvatica*. Late season non-structural carbohydrate concentrations remained at a high level across elevations in all species, suggesting that trees are not carbon-limited at their high-elevation limit.
4. Annual BAI showed no significant change over a wide range of elevations, before it gradually decreased over the last few hundred (300–

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500) metres below the range limit, with an abrupt decrease in few species at the range limit. Annual BAI correlated with the mean temperature during the growing season in the uppermost 400 m of elevation. The length of the growing season had only a significant effect on BAI at warm mean temperatures during the growing season (i.e. at lower elevation or during warmest summers).

5. *Synthesis*: While temperature has a strong effect on wood formation, the length of the growing season is negligible for stem growth at high elevation due to the low rate of wood formation at low temperature and the over-proportional increase of growth with warmer temperatures. We ruled out a direct growth limitation by low temperature as the limiting factor of the upper distribution limits and rather suggest that the formation of range limits (not necessary the rate of growth) is set by a minimum requirement of warmth and season length to fully mature key tissues such as seeds, shoots or winter hardy buds.

Key words: Basal area, carbohydrates, niche, phenology, plant-climate interactions, season length, species distribution, spring frost, temperature, tree ring

Introduction

Temperature and precipitation are regarded as the major drivers of plant distribution at the global scale. Northern and high-elevation range limits of plant species usually correlate well with mean or extreme temperature (e.g. Iversen 1944; Woodward 1987; Körner 2012). A low-temperature-related range limit of temperate tree species is supported by similar elevational and latitudinal limits, when the thermal distance to the climatic treeline is used as a reference (Randin et al. 2013). Yet, temperature could control tree distribution through either gradual or threshold phenomena (Körner 2006).

Among the gradual influences induced by low temperature, a slowing of photosynthetic or meristematic activity or insufficient soil nutrient uptake could restrict tree growth. Based on the actual temperature dependencies, meristems are the most likely tissues that are directly af-

ected by low temperature (Körner 2006, 2013). Meristematic activity in woody species, such as above-ground secondary growth (xylogenesis), root growth, as well as leaf growth of wild grasses or winter crops approaches zero below mean temperatures of ca. 5°C (Alvarez-Uria & Körner 2007; Körner 2008; Rossi et al. 2008), temperatures which exert comparatively minor effects on photosynthesis (Körner 2006). Hence, the generally higher tissue concentration of non-structural carbohydrate reserves in trees at the alpine treeline have been interpreted to result from the relative overabundance of carbon assimilates in these trees (Hoch & Körner 2012). In temperate areas, temperatures vary strongly during the seasonal growth period and such critical temperatures thresholds may occur at any time of the year. It can be assumed that winter deciduous trees need a minimum sum of warm hours during the growing season to successfully complete their annual growth cycle. For treeline trees, a mean seasonal air temperature of 6.4°C was found to represent the best global proxy isotherm (Körner 2012). Most deciduous tree species find their distributional limit substantially below treeline in the northern hemisphere. Thus, such taxa operate at significantly higher mean growing season temperatures, although the physiological thresholds for meristematic activity may be similar (Alvarez-Uria & Körner 2007). These species are likely to require more hours with temperatures above 5°C per year to complete their seasonal growth cycle.

Similar to carbon assimilation, the acquisition of nutrients is usually less temperature-limited than tissue formation (Ehrhardt 1961; Tranquillini 1979; Körner 2012). There is no indication that treeline trees face a higher nutrient limitation than trees at lower elevation (Birmann & Körner 2009; Körner 2012). Would the nutrient cycle play an important role in defining species range limits, cold edge limits would change their position following soil fertility (e.g. soil depth) and would not follow consistently elevational or latitudinal isotherms. It was shown experimentally that the effect of a low mean temperature (6°C) on the growth of treeline seedling cannot be mitigated by nutrient addition (Hoch 2013).

In contrast to gradual effects, threshold effects of temperature include absolute minimum temperatures (for instance the lowest temperature ever recorded in a century) or critically low-temperature extremes during sensitive developmental stages such as during flushing. Basically, freezing temperatures could impact tree survival at high elevation at any time of

the year. A close relationship between winter freezing resistance and the absolute minimum temperature at range limits of some temperate tree species suggests a role of temperature extremes in setting such limits (Sakai & Weiser 1973; Sakai 1978; Charrier, Cochard & Améglio 2013). Although the freezing resistance of deciduous tree species does correlate with the position of their elevational limits, they commonly experience far warmer minimum temperatures than what would be causing tissue damage (Lenz et al. 2013). Substantial acclimation potential during prolonged exposure to low temperatures contributes to this large safety margin (Pisek & Schiessl 1947; Sakai 1966; Sakai & Larcher 1987). However, late spring freezing events can severely damage trees (Rubner 1921; Augspurger 2009, 2011; Hufkens et al. 2012). Indeed, the loss of leaves from a spring freeze event may have considerable negative consequences for deciduous trees in terms of nutrient storage, growth, reproduction or canopy development (Augspurger 2009, 2011). Depending on the degree of damage, the formation of new leaves demands a high amount of storage compounds and assimilates and strongly affects the current seasonal growth. Thus, similar to summer drought effects, years with a late spring freeze event occurring after leaf emergence can lead to negative event years, that is narrow tree rings, or even ‘frost rings’ that are visible by a distortion of xylem cells (Schweingruber 1996; Dittmar, Fricke & Elling 2006), especially at high elevation due to the shorter remaining growth period to recover from such damages.

In spring, freezing resistance of deciduous trees is tightly linked to their phenological stage (Larcher & Mair 1969; Ibanez et al. 2010). Since the flushing stage is the most sensitive stage, early flushing trees require a higher tolerance against freezing than late flushing trees (Lenz et al. 2013). Interestingly, deciduous trees are able to delay the timing of flushing at higher elevations to such an extent that the risk encountered by freezing remains the same across a range of elevations (Lenz et al. 2013). Evolutionary controls over phenology are thus tracking the risk of spring freezing damage. Yet, a delay in development (flushing) inevitably leads to a shorter growing season at higher elevation. In European temperate tree species, the shift in phenology (e.g. date of flushing) with decreasing spring temperature varies among species, ranging from approximately -2 days K^{-1} (approximately 200 m increase in elevation) in *Fagus sylvatica* up to -7 days K^{-1} in *Fraxinus excelsior* both along elevational (Vitasse

et al. 2009b; Cufar et al. 2012; Pellerin et al. 2012) and latitudinal gradients (Kramer 1995; Phillimore et al. 2013). A shorter growing season restricts all aspects of tissue maturation, including fruit ripening and seed maturation, bud and bark maturation and completion of tree rings, potentially shaping low-temperature distribution limits of deciduous taxa. Thus, a trade-off between the avoidance of late spring freezing damage and the length of the growing season might be the main driver of deciduous tree species range limits (Lenz et al. 2013; Kollas, Körner & Randin 2014).

Here, we explore the effect of declining temperature on growth as one approaches the species low-temperature range limit. We investigated the annual radial stem increment of eight deciduous tree species from the centre of their elevational distribution to the upper elevation range limit of adult individuals. The aim was to disentangle the influence of minimum temperature, mean temperature and the length of the growing season on tree growth and carbon balance towards the upper elevation range limits. We also correlated annual basal area increment (BAI) with the date of the onset of the growing season, derived from a thermal time model, and the mean temperatures of the growing season, in order to examine the influence of these two factors in shaping deciduous tree species range limits. Specifically, we asked the following questions: (i) Does the BAI of temperate tree species decrease, and if so, does it decrease gradually or abruptly towards their upper elevational distribution limit? (ii) Does the frequency of negative event years (exceptionally narrow annual tree ring increments) increase with increasing elevation? (iii) Is there any evidence that tree species fall short in carbon reserves at their distribution limit? (iv) To what extent is annual growth of deciduous temperate tree species restricted by either the mean temperature of the growing season, or the effective length of the growing season?

Materials and methods

Study area and species

A preliminary exploration of dominant deciduous tree species distribution in Switzerland revealed one region in the Western Swiss Alps where a

number of tree species currently reach their upper elevational distribution limits (Vitasse et al. 2012; Randin et al. 2013). For this study, we focused on three elevational transects in this area, situated near St. Maurice (Table 4.1). The region is characterised by a temperate continental climate, with a mean annual precipitation of 1472 mm at 1500 m a.s.l (Vitasse et al. 2012). The climatic treeline in this region is at ca. 2200 m a.s.l. We focused on eight deciduous tree species belonging to five plant families. These species currently reach their distribution limit at contrasting elevations in the study area (Table 4.1 and Vitasse et al. 2012).

Sampling and measuring of tree cores

Increment cores of all species were collected along three elevational gradients from the uppermost tree found downwards, in approximately 50 m steps of elevation for 1000 m of elevation (Table 4.1). In total, 419 trees were cored. Because trees show a strong age-dependent growth when they are young, trees being younger than 30 years at coring height were removed from further analysis. Thus, in total 298 trees along the elevational gradient were used, ranging from 27 to 46 individuals per species (Table 4.1). The precise elevation was recorded with an atmospheric pressure altimeter (Suunto Core, Vantaa, Finland), which was calibrated several times a day with topographical maps (1:25 000, 20 m contour interval). Areas of obvious human influence and forest management, as well as abrupt changes in slope aspect or soil properties were avoided. Only mature dominant trees were considered. Increment cores were extracted with a 5 mm increment borer (Suunto, Vantaa, Finland) at breast height on the lower tree side to avoid reaction wood. Two cores per tree were extracted. One core was used to measure ring width, while the other core was used to quantify non-structural carbohydrates in the last 3 cm of sapwood. Ring width was measured on an electronic analysis bench (± 0.01 mm, LINTAB 6, Rinntech, Heidelberg, Germany) using a stereomicroscope.

Table 4.1 The range of sampling elevations (m), the number of individuals used for analysis and the location of the tree study transects

Species	Transect 1		Transect 2		Transect 3		Total	
	Elevation (m)	<i>n</i>	Elevation (m)	<i>n</i>	Elevation (m)	<i>n</i>	Elevation (m)	<i>n</i>
<i>Sorbus aucuparia</i>	1004–1842	13	1034–2084	12	1304–2031	15	1004–2084	40
<i>Sorbus aria</i>	912–1907	14	1039–2055	9	1193–1943	18	912–2055	41
<i>Acer pseudoplatanus</i>	843–1708	18	1031–1775	9	1215–1910	19	843–1910	46
<i>Laburnum alpinum</i>	987–1778	7	1035–1806	11	1165–1882	17	987–1882	35
<i>Prunus avium</i>	650–1640	12	1032–1318	4	948–1722	11	650–1722	27
<i>Fagus sylvatica</i>	745–1714	18	1040–1406	9	–	–	745–1714	27
<i>Quercus petraea</i>	602–1507	15	1038–1170	3	955–1641	24	602–1641	42
<i>Fraxinus excelsior</i>	498–1403	17	1028–1314	4	806–1578	19	498–1578	40
<i>Site characteristics</i>								
Latitude	46° 11' N		46° 09' N		46° 08' N			
Longitude	07° 02' E		07° 03' E		07° 04' E			
Slope aspect	SW		SW		S			

Calculation of basal area increment and negative event years

For the analysis of growth trends along elevation, we calculated the BAI of each tree based on the radius measurements and the onsite diameter

measurements at coring height. BAIs were calculated from the phloem towards the pith, assuming a constant annulus. We used BAI since no age-dependent growth trend was apparent in BAI data on the remaining 298 trees (i.e. excluding trees younger than 30-year-olds).

We defined negative event years *sensu* Schweingruber et al. (1990), by a ring width that was reduced by 60% compared to the mean ring width of the 4 previous years. Negative event years were calculated for the period of 1980–2011 for trees which were older than 30 years.

Analysis of non-structural carbohydrates

Non-structural carbohydrates (NSC) are defined here as free, low molecular sugars (glucose, fructose and sucrose) and starch. NSC concentration was analysed in an aqueous extraction using enzyme digestion, following the method of Wong (1990) as described in detail in Hoch et al. (2002). Total sugar, starch and total NSC concentration are expressed as a percentage of dry matter.

Phenology, growing season length and temperature data

We calculated elevational lapse rates of daily mean temperature for winter (Dec–Feb), spring/summer (March–July) and autumn (Aug–Nov) using temperatures recorded inside the forest 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 m to 2160 m a.s.l. in the study area from June 2010 to July 2011 (see Lenz et al. 2013). Because temperature is highly dependent on slope aspect and vegetation cover and differs from inside the forest to standard weather stations (Kollas et al. 2014), we used data loggers distributed inside the forest rather than standard climate stations to get an as accurate as possible lapse rate for the study region. The season-specific lapse rates for mean air temperature ranged from $-0.35 \text{ K } 100 \text{ m}^{-1}$ in winter, to $-0.51 \text{ K } 100 \text{ m}^{-1}$ in spring/summer and $-0.46 \text{ K } 100 \text{ m}^{-1}$ in autumn (see Fig. S1 in Supporting Information). The variation among monthly lapse rates within each considered season was lower than $0.1 \text{ K } 100 \text{ m}^{-1}$ (see Fig. S1). We therefore used the

seasonal lapse rate through all analyses. The daily mean air temperatures recorded along the studied elevational gradient were highly correlated with the temperatures recorded at the nearest climate station that offered long-term records (Château-d'Oex, 46°28'35"N, 7°08'31"E, 985 m a.s.l.; slope > 0.96, $R^2 = 0.96$). The season-specific temperature lapse rates were then used to extrapolate the long-term daily mean air temperatures from the reference climate station to the study area for the period 1980–2011.

We reconstructed the timing of bud burst for the last 31 years using a thermal time model, calibrated by phenological observations obtained in spring 2011 along two elevational gradients from seedlings originating from the study area and belonging to the same species studied here (see details in Lenz et al. 2013). Although seedlings are known to flush earlier than conspecific adult trees (Vitasse 2013), the phenological observations of adult trees corresponded to the ones of seedlings in the year of observation, leading to a valid model for adults (Lenz et al. 2013). With this model, we predicted the bud burst date of each species for the elevation of occurrence of each individual along the studied gradient for the period 1980–2011, using daily mean temperature extrapolated from the reference climate station. We defined the growing season length as the period between the date of the predicted bud burst for each year and 1 September. We assumed a fixed date for the end of the growing season across all elevations, since the end of the growing season is mainly determined by photoperiod (Rossi et al. 2006; Tanino et al. 2010). This is a conservative approach; since the photoperiod-controlled induction of dormancy related phenology in autumn may be ecotypically differentiated as well but in the direction of an earlier bud maturation and leaf abscission layer formation at high elevation, potentially leading to further shortening of the growing season.

Statistical analysis

To estimate the combined effects of temperature and growing season length on growth along elevation for all trees of a given species, the boundary line of maximum growth is of interest, rather than examining the mean growth of all the trees, including those that grew less for reasons not directly related to temperature (e.g. poor edaphic conditions, pathogens and

light conditions). We fitted a generalized extreme value distribution (GEV) on all BAIs of the years 1980–2011 along the elevational gradient for each species separately. A line was fit through the 95% tails of the GEV using the R-package VGAM (Yee & Wild 1996; Yee 2012). The effect of elevation on the number of negative event years during the period 1980–2011 was calculated using a generalized linear model with a quasi-Poisson distribution for each species separately. The effect of elevation on the non-structural carbohydrates concentration was tested using linear regressions.

Mean temperature of the growing season and the length of the growing season autocorrelate along elevation. Thus, to estimate the effect of the mean temperature of the growing season and the length of the growing season on radial growth, we used all BAI data irrespective of elevation for calculations. We calculated a linear mixed effects model on log-transformed BAI for each species with the following sources of variation: the mean temperature of the growing season treated as a continuous variable, the length of the growing season treated as a continuous variable and the interaction between the two as fixed effects. Since we were interested in the effect of the mean temperature of the growing season or the length of the growing season on BAI among individual trees, and not within individual trees, we used the year of the tree ring and the tree nested within the year as random effects. Most species showed a significant interaction between the mean temperature of the growing season and the growing season length (Table 4.3). To visualize the effect of the interaction on BAI, we used bivariate linear interpolation according to Akima (1978) using the R-package *akima* (Gebhardt et al. 2013) on log-transformed BAI. With this interpolation, it is possible to derive the BAI at any given mean temperature of the growing season or length of the growing season. To further analyse the individual effect of temperature or the length of the growing season on BAI, we binned BAI data into five evenly distributed classes of the length of the growing season or the mean temperature during the growing season for each species. We then calculated ANCOVAs of the effect of the mean temperature of the growing season on BAI for the different classes of the length of the growing season and vice versa for each species. The classes of the longest growing season and of the warmest mean temperature of the growing season were removed from ANCOVA analyses, since we could already observe a decreasing BAI in these classes, most probably due to water limitation. In this study, we are only interested in

the declining growth towards the cold range edge, and not the warm range edge. However, all data were used for linear interpolation, and the decline in BAI at the warmest temperature class and the longest growing season can be observed. Further, the number of classes used had no effect on the analysis of the interaction. All analyses were carried out using R 2.15.3 (R Core Team 2013).

Results

Tree age and diameter at breast height along elevation

Mean tree diameter at breast height ranged from 16 to 55 cm at low elevation. In the uppermost third of the investigated species' range, mean diameter above breast height ranged from 9 to 32 cm (Table 4.2), corresponding to a 20–50% reduction across all species from the lowest third of the investigated gradient to the highest third of the gradient, with the strongest reduction observed in both *Sorbus* species, *Laburnum* and *Fagus* and the weakest reduction observed in *Quercus* and *Fraxinus* (Table 4.2). In contrast to tree diameter, tree age estimated at coring height after discarding trees younger than 30 years did not significantly change with elevation, except for *Quercus*, where older trees were found at higher elevations (Table 4.2). Across all elevations, the species with the youngest individuals were *Laburnum*, *Prunus* and *Sorbus aucuparia* (known for their limited age) with a mean age of less than 55 years. *Acer* and *Fraxinus* showed intermediate mean ages of approximately 90 years, whereas *Quercus* and *Fagus* had a mean age of more than 100 years. The oldest trees cored belonged to the species of *Acer*, *Quercus* and *Fagus* with more than 240 years and were always found at the highest elevation (Table 4.2), with actual age 10–20 years greater than at 1.3 m coring height.

Basal area increment along elevation

Overall, the generalised extreme value distribution showed no substantial change of basal area increment (BAI) over the lower half of the gradient,

followed by a decrease of BAI over the last few hundred meters of elevation (Fig. 4.1). In most species, BAI decreased gradually with elevation, whereas in some species (e.g. *Prunus* or *Fagus*) a sharp decrease of BAI towards the species' range limit was observed (Fig. 4.1). For instance, 50% of the maximum BAI was reached at 200–350 m below species-specific elevational limits in all species, except for *Prunus* and *Fraxinus*, where 50% of the maximum BAI was reached only 60–100 m below species' elevational limits. Finally, 30% of the maximum BAI was reached at 150–250 m below the species limit, except for *Quercus* and *Prunus*, which reached 30% of the maximum BAI at less than 50 m below the species limit, and *Fraxinus* exhibited hardly any reduction until the limit is reached.

Within the uppermost 50 m below species-specific elevational limits, the mean BAI was below $1 \text{ cm}^2 \text{ year}^{-1}$ in both *Sorbus* species and *Fagus*, between 2 and $5 \text{ cm}^2 \text{ year}^{-1}$ in *Laburnum*, *Prunus*, *Quercus* and *Acer* and more than $10 \text{ cm}^2 \text{ year}^{-1}$ in *Fraxinus*. These BAIs correspond to a reduction of 75 to 98% compared to the species-specific maximum BAI, except for *Fraxinus*, which still exhibited 60% of its maximum BAI at its current elevational limit.

Number of negative event years along elevation

For most of the species, the number of negative event years (i.e. the number of years with a ring width of less than 40% of the mean ring width of the 4 preceding years) did not significantly change with elevation during the period from 1980–2011 (Fig. 4.1), except for *Fagus*, where the frequency increased significantly (*Quercus* showed a similar, marginally significant trend; Fig. 4.1). The increase of negative event years with elevation remained consistent in *Fagus*, when a longer time period of 60 years (1951–2011) was used (data not shown). For all other species the data indicate that their timing of development is such that tree rings remain largely unaffected by exceptionally bad years, despite the decline in mean tree ring width as one approaches the species limit.

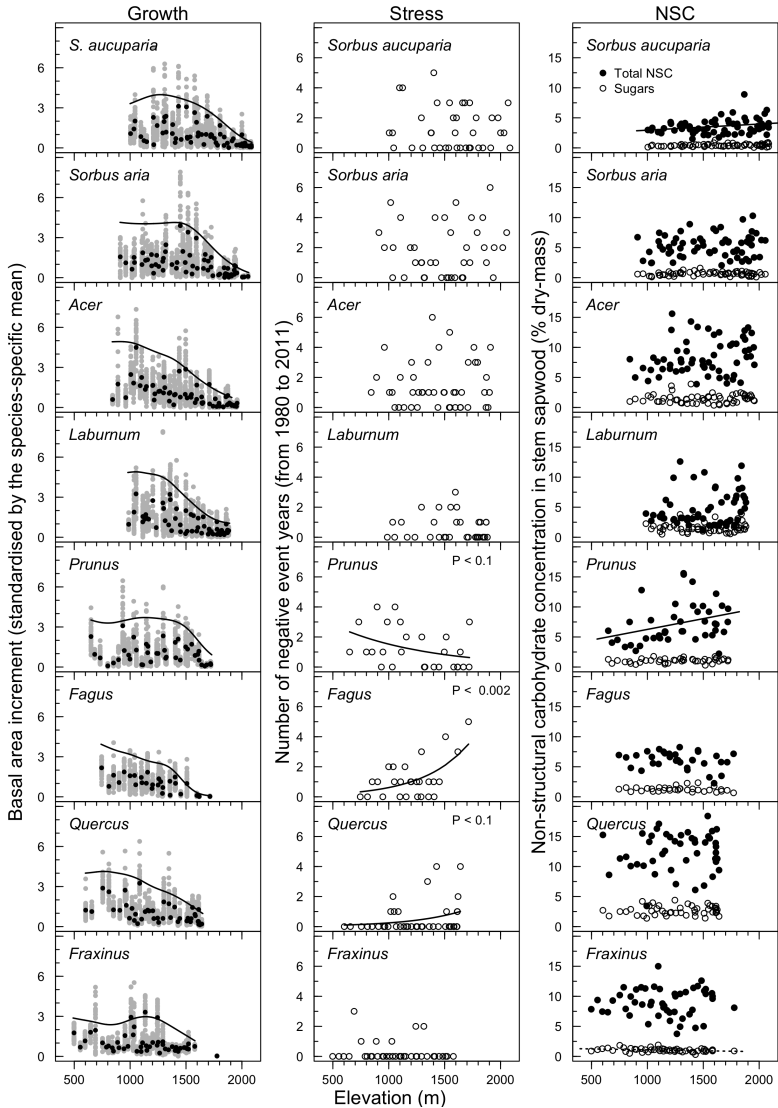


Fig. 4.1 Basal area increment (BAI; growth), the number of negative event years (stress) and the concentration of non-structural carbohydrates (NSC) along elevation for eight different deciduous temperate tree species. BAI is shown for the years from 1980 to 2011 for each tree along the elevational gradients. Grey dots represent individual years, black dots represent mean values per tree and lines represent the 95th percentile of fitted general extreme value distributions. The number of negative event years was fitted with a quasi-Poisson-regression. Total non-structural carbohydrate concentrations as well as concentration of sugars were fitted with linear regressions. Only significant ($P < 0.05$) regressions are drawn.

Table 4.2 Mean diameter at breast height (\pm se) and mean tree age (minimum, maximum in brackets) across all individuals per species separated in three classes of elevation (Low, Mid and High)

Species	Diameter at breast height (cm)			Age (years*)		
	Low	Mid	High	Low	Mid	High
<i>Sorbus aucuparia</i>	19.3 \pm 1.1 ^a	17.5 \pm 1.5 ^a	11.2 \pm 1.8 ^b	48 (33, 69)	49 (32, 100)	53 (31, 78)
<i>Sorbus aria</i>	16.7 \pm 1.4 ^a	16.8 \pm 1.5 ^a	9.4 \pm 1.4 ^b	62 (35, 126)	71 (34, 144)	54 (32, 84)
<i>Acer pseudoplatanus</i>	38.7 \pm 2.7 ^a	34.7 \pm 2.4 ^{ab}	24.2 \pm 4.4 ^b	72 (37, 175)	94 (37, 193)	93 (32, 250)
<i>Laburnum alpinum</i>	22.6 \pm 2.8 ^a	14.2 \pm 1.5 ^b	12.0 \pm 1.1 ^b	55 (42, 86)	52 (32, 81)	52 (31, 79)
<i>Prunus avium</i>	26.4 \pm 2.9	25.9 \pm 2.4	19.4 \pm 3.4	55 (38, 64)	47 (34, 78)	52 (39, 77)
<i>Fagus sylvatica</i>	55.7 \pm 2.9 ^a	40.0 \pm 4.6 ^b	29.5 \pm 8.4 ^a	106 (60, 162)	96 (39, 173)	150 (36, 241)
<i>Quercus petraea</i>	35.5 \pm 4.0	29.5 \pm 4.0	27.5 \pm 2.8	84 (34, 161) ^a	80 (37, 120) ^a	138 (41, 261) ^b
<i>Fraxinus excelsior</i>	42.3 \pm 2.8 ^a	43.3 \pm 4.7 ^a	31.1 \pm 2.2 ^b	84 (32, 130)	99 (35, 153)	92 (31, 143)

Elevation classes were evenly distributed across the total elevational range per species given in Table 4.1. Different letters within species among the three classes of elevation indicate significant differences tested by Tukeys honestly significant difference (HSD) tests.

* Age was determined at coring height, only accounting for actual tree rings observed in the sample, assuming that we randomly missed the same amount of tree rings to the pith in all samples and does not represent true tree age. All individuals younger than 30 years at coring height were removed from the analysis and are not reported in this table.

Table 4.3 ANCOVA table of basal area increment tested in dependence of growing season mean temperature among different growing season length classes and in dependence of growing season length among different growing season mean temperature classes (see Fig. 4.2 for classes)

Source of variation	<i>Sorbus aucuparia</i>		<i>Sorbus aria</i>		<i>Acer pseudoplatanus</i>		<i>Laburnum alpinum</i>		<i>Prunus avium</i>		<i>Fagus sylvatica</i>		<i>Quercus petraea</i>		<i>Fraxinus excelsior</i>	
	df	F	df	F	df	F	df	F	df	F	df	F	df	F	df	F
<i>Temperature effects</i>																
Temperature (T)	1	20.**	1	16.**	1	116.***	1	51.***	1	13.**	1	139.***	1	42.***	1	3.3
GSL class	3	1.4	3	1.5	3	3.4(*)	3	2.6	3	0.4	3	7.2*	3	1.3	3	0.4
T × GSL class	3	1.0	3	0.3	3	2.0	3	8.1*	3	1.0	3	1.5	3	1.1	3	1.8
Residuals	6	—	7	—	6	—	6	—	7	—	7	—	7	—	7	—
<i>Growing season length effects</i>																
Growing season length	1	170.***	1	52.***	1	53.***	1	67.***	1	2.8	1	54.***	1	12.*	1	1.6
Temperature class	3	71.***	3	29.***	3	28.***	3	31.***	3	3.6(**)	3	47.***	3	11.**	3	1.8
GSL × T class	3	17.**	3	5.7*	3	2.6	3	29.***	3	0.8	3	3.8(*)	3	0.8	3	3.0
Residuals	6	—	7	—	6	—	6	—	7	—	7	—	7	—	7	—

Elevation classes were evenly distributed across the total elevational range per species given in Table 4.1. Different letters within species among the three classes of elevation indicate significant differences tested by Tukey's honestly significant difference (HSD) tests.

* Age was determined at coring height, only accounting for actual tree rings observed in the sample, assuming that we randomly missed the same amount of tree rings to the pith in all samples and does not represent true tree age. All individuals younger than 30 years at coring height were removed from the analysis and are not reported in this table.

Non-structural carbohydrate concentration along elevation

The total concentration of non-structural carbohydrates (NSC) in stem sapwood ranged between 1 and 19% of dry-mass, with the highest concentrations found in *Quercus* and the lowest concentrations in both *Sorbus* species. Along the elevational gradient, no consistent change in NSC concentrations was apparent across species. In *Sorbus aucuparia* and *Prunus*, NSC concentrations increased significantly with elevation (mainly due to starch), whereas concentration of sugars (only) decreased significantly in *Fraxinus* (Fig. 4.1). Trends were very weak in all cases, with R^2 values below 0.1.

Growth responses to temperature and length of growing season

As expected from the elevation trends, BAI strongly declined with the mean temperature of the growing season and with decreasing length of the growing season across all species (Fig. 4.2a, Table 4.3). Consequently, the smallest BAI (below 0.8 cm^2) was observed at low growing season temperature (mostly below 11°C) and at a short growing season length (from flushing to 1 September) between approximately 80 to 100 days (Fig. 4.2a). Interestingly, the maximum BAI found across species (greater than $33 \text{ cm}^2 \text{ year}^{-1}$) was neither associated with maximum growing season temperature nor maximum growing season length, but rather with a moderate mean growing season temperature of $15 - 16^\circ\text{C}$ and an intermediate growing season length (from flushing to 1 September) of 110 to 120 days. Generally, high mean temperatures during the growing season can compensate for a shorter length of the growing season, whereas a longer growing season did not necessarily compensate for a lower mean growing season temperature (Fig. 4.2b,c). Accordingly, temperature had always a stronger effect on BAI (irrespective of the growing season length class) and, most importantly, no interaction of temperature with growing season length class could be observed (except for *Laburnum*; Table 4.3). In contrast to temperature, the length of the growing season had inconsistent effects on BAI. BAI did not correlate with season length in the coldest temperature class, but BAI correlated significantly with season length in warmer temperature classes, resulting in significant interactions between

the length of the growing season and temperature class in most species (Fig. 4.2b,c, Table 4.3). *Fagus* and *Acer* had the strongest growth decline with decreasing temperature and length of the growing season, whereas *Fraxinus* was insensitive to temperature or duration of the growing season within its elevational range and showed a rather sudden decline in growth near its range limit (Fig. 4.2).

Discussion

The elevational decrease of temperature does not lead to a proportional reduction in stem growth in any of the eight examined broadleaved deciduous tree species. Basal area increment (BAI) hardly changed over a large part of the gradient, but only declined over the last few hundred metres of elevation. In some species, growth declined rather abruptly over a relatively short vertical distance as one approaches the range limit. This suggests a species-specific climatic threshold beyond which trees are not able to produce and mature sufficient tissue. Since the mean temperature and the length of the growing season correlate with each other, their combined influence is difficult to disentangle. At low elevation, the season is not only warmer, but also longer. At high elevation, the season is shorter and colder. Since metres of elevation as such exert no influence, we analysed the BAI data for temperature and season length effects irrespective of elevation. It turned out that the mean temperature of the growing season exerts a dominant effect on BAI irrespective of the length of the growing season across the entire studied range of elevation. On the other hand, the length of the growing season leads to greater BAI only under warmer temperature, an effect that becomes most pronounced at the range limit during warm seasons. In other words, the length of the growing season has a negligible effect on growth in cold years, which are actually almost all years at the range limits. The most likely explanation for the negligible influence of season length on growth at cold mean temperatures is the non-linear temperature dependency of cell division and growth. The time required for producing new cells increases exponentially with colder temperatures. So, the cell doubling time (the rate at which new cells are formed) is approximately 24 h at 20°C, but more than 60 h at 10°C and already several

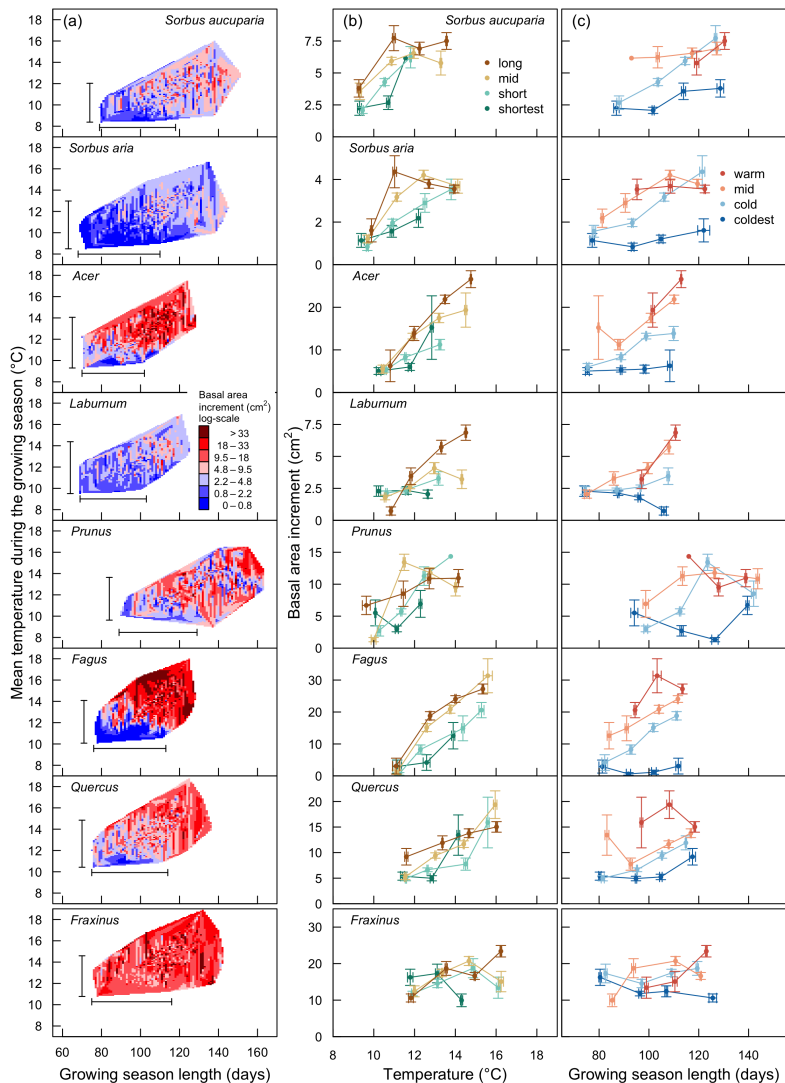


Fig. 4.2 (a) The basal area increment (BAI) of each individual tree ring (log scale, cm^2) in dependence of the mean temperature during the growing season and the respective length of the growing season from flushing to 1 September for eight deciduous temperate species, linearly interpolated among temperature and season length values. Low values of the BAI are in blue and high values are in red. Black lines indicate the range of mean temperature and season length for all trees occurring within the uppermost 100 m of the range. (b) BAI in dependence of mean temperature during the growing season for different classes of the season length, and (c) in dependence of the length of the growing season for different classes of mean temperature. The points at the coldest temperature in figure b correspond to the points in the coldest temperature class in figure c, and vice versa for the length of the growing season. Note that the warmest temperature class and the longest season length class were removed from figures b and c for clarity, since the focus of the study lies on the cold temperature range limit of species.

days at 5°C (Körner 2003). An increase of the season length by several days will only lead to few more cells at cold temperature, whereas at warm temperature, many more cells can be formed. Interestingly, at their upper elevational range limit, the examined tree taxa experience mean temperatures of the growing season significantly higher than what is known to be the physiological low-temperature limit for tissue formation in temperate trees (Alvarez-Uria & Körner 2007; Schenker et al. 2014). However, fine root growth of seedlings was found to be limited at colder mean temperature in soils, the higher the elevation limit of a species was (Schenker et al. 2014), meaning that species from higher elevation have a larger potential for tissue formation at cold temperature. A similar trend for a growth potential was observed in 24 species of the Salicaceae family from across North America, with species from more northern latitudes having a larger potential for growth at cold mean temperatures (Savage & Cavender-Bares 2013). Although the potential for growth differed among the latitudes species originated from, these authors concluded that phenology-related cues associated with freezing resistance rather than the ability to grow at low temperatures are setting the northern range limit of species.

Negative event years and non-structural carbohydrates along elevation

Given that temperature and season length clearly influence BAI, particularly at high elevation, we expected particularly unfavourable years to leave obvious signals in tree ring chronologies. Both cold temperatures per se and freezing events in spring can lead to the formation of such ‘pointer’ years (Dittmar, Fricke & Elling 2006; Neuwirth, Schweingruber & Winiger 2007). Since we investigated BAI of trees from mid montane to high elevations, we expected the incidence of such ‘pointer’ years to become more frequent with increasing elevation. However, we did not find an increase of negative event years with elevation, except for *Fagus* and to a lower extent for *Quercus*. Such event years with exceptionally small ring width could either be related to early season freezing events (frost rings) or to a cool or dry summer. Freezing events are, however, unlikely to cause event years, because European deciduous tree species delay their flushing at high elevation in such a way that the risk of being affected by freeze events early in the season remains very low and constant along the

entire elevational gradient (Lenz et al. 2013). Nevertheless, *Fagus* is again the only species examined that is facing quite regular freezing damage at higher elevation, but it seems to be able to cope with this (Dittmar, Fricke & Elling 2006). Although our data showed an increase of negative event years in this species, we did not notice so-called frost rings associated to negative event years. In fact, these negative event years may have other causes. For instance, mast years could also contribute to negative event years in large-seeded species such as *Fagus* and *Quercus* (Schweingruber 1996 and references therein), particularly close to the range limit (Holmsgaard 1956; Han et al. 2008; Drobyshev et al. 2010). At low elevation, such an impact of mast years on tree ring width was not observed under current atmospheric conditions (Mund et al. 2010). In addition, beech is not carbon-limited during mast years and is in fact using mostly new carbon assimilated during the mast year for fruit production (Hoch 2005; Hoch & Keel 2006; Hoch et al. 2013). At higher elevation, with shorter and colder growing seasons, a high fruit production could still be a competing sink for nutrients and carbon, leading to more frequent negative event years with increasing elevation in species that perform masting behaviour. Yet, we neither see such a trend in ring width nor did we detect any reduction in non-structural carbohydrate stores with elevation in the sampling year, which, however, was not a mast year (see below).

No reduction in non-structural carbohydrate concentrations was detected when approaching the species' range limit in any species. There is solid evidence that low mean temperature constrains meristematic activity long before significantly limiting photosynthetic carbon gain, causing a source – (structural) sink imbalance that increases non-structural pools (Körner 2013). There is an ongoing debate about the minimum levels of NSC concentrations in trees under stress conditions like low temperature or drought stress and their indicative value for the C-source-sink balance of plants (e.g. McDowell 2011; Sala, Woodruff & Meinzer 2012; Wiley & Helliker 2012; Palacio et al. 2014). However, empirical evidence suggests that tissue formation is affected long before photosynthetic carbon gain under water or low-temperature stress (Muller et al. 2011; Körner 2013; Palacio et al. 2014). The absence of reductions of NSC concentrations along the elevational gradients of this study across species, at least allows us to exclude an acute carbon limitation in deciduous tree species at their upper range limit. Overall, our data thus may support the assumption that

deciduous temperate tree species, like the trees at the alpine treeline, are not limited by an insufficient carbon supply towards the species-specific range limit.

Temperature and growing season length effects on tree growth

The trade-off between maximizing the length of the growing season while escaping freezing damage in spring is apparent in the growth of most species observed here. The two most extreme cases are *Fagus sylvatica* and *Fraxinus excelsior*. *Fagus* is the species in which BAI is most strongly limited by low temperature during the growing season and might therefore be under evolutionary pressure to maximize the length of the growing season. Consistently, positive correlations between spring or summer temperatures and ring width of high-elevation *Fagus sylvatica* could be observed in Spain as well as in the Alps (Di Filippo et al. 2007; Jump, Hunt & Penuelas 2007). Accordingly, but contrasting the response seen in other deciduous broad-leaved taxa, common garden experiments have revealed that high-elevation provenances of *Fagus* exhibited earlier (rather than later) leaf out than low-elevation provenances (Vitasse et al. 2009a, 2013), thus leading to a longer growing season at the expense of an increasing risk of freezing damage (Dittmar, Fricke & Elling 2006; Gömöry & Paule 2011; Lenz et al. 2013). The other extreme species – *Fraxinus excelsior* – showed no growth response to mean temperature of the growing season or length of the growing season across the entire gradient. Accordingly, *Fraxinus* is (i) one of the species having the strongest response of phenology to temperature when transplanted along elevational gradients (Vitasse et al. 2013), (ii) exhibits stringent genetic differentiation with later leaf out of high-elevation provenances (Vitasse et al. 2009a), and (iii) is one of the most sensitive temperate tree species to freezing temperatures during leaf out (Lenz et al. 2013). Due to the late flushing of *Fraxinus* in colder areas, its active shoots exploit the warmest part of the season only. By the restriction to increasingly shorter fractions of the season, growth and development are confined to the warmest part of the year, thus removing the elevation effect of temperature. Tree ring formation is a process that happens early in the season. The full size of a ring is commonly reached by midsummer, with the remaining length of the season used for late wood

thickening (Cufar et al. 2011; Michelot et al. 2012; Lenz, Hoch & Körner 2013). In contrast, the maturation of new shoots, roots, but also fruits is a late season process and thus may fail if the season is terminated too early. This would explain the dominant temperature signal for BAI, with the other traits most likely experiencing a stronger season length effect.

Conclusion

The use of elevational transects allowed us to examine the effect of the length of the growing season and the mean temperature during the growing season as factors influencing radial stem growth of deciduous temperate tree species. Our data suggest a strong effect of temperature on growth only over the last few hundred metres of elevation. Interestingly, the length of the season becomes effective for growth only in warmer years. The negligible effect of season length on growth at cold temperature may be related to the non-linearity of the temperature response of tissue formation, with slow growth at cold temperatures and an over-proportionally faster growth at warm temperatures. While sap wood formation of deciduous temperate tree species at the cold range limit is strongly dependent on temperature, the range limit as such is most likely set by an interaction between species-specific freezing tolerance during spring, the dependent spring phenology (to avoid freeze damage) and the species' growth potential at cold temperatures, allowing a species to meet crucial life-history requirements to grow and survive the next winter (Lenz et al. 2013).

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Supporting Information

Comparison of seasonal and monthly lapse rates

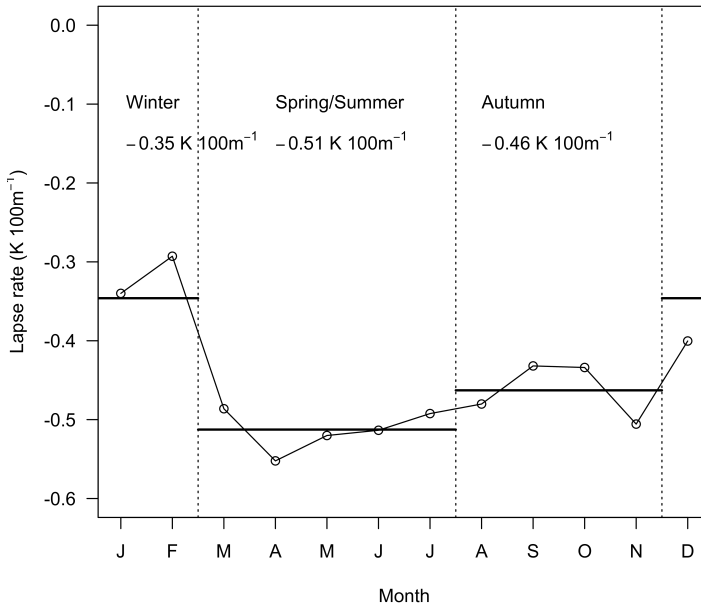


Fig. S1 The three seasonal lapse rates used in the study for winter, spring/summer, and autumn (bold solid line) and the corresponding monthly lapse rates (circles). Lapse rates were calculated on 7 temperature loggers distributed from 400 to 2160 m a.s.l. in the study area from June 2010 to July 2011. Note the small variation in monthly lapse rates within a defined season.

Chapter 5

Further publications

In this chapter I present further publications I wrote or co-authored during my thesis. Only the abstract and the main figure of each publication (or manuscript in preparation) are shown. The first paper in this chapter deals with the population dynamics of temperate tree species at the range limit. In the next three papers we investigated threshold effects of temperature. Specifically, we discuss the acclimation potential of beech in winter, potential changes of freezing resistance during a tree's ontogeny, and the de-hardening period of the native evergreen liana *Hedera helix* in spring. The following articles deal with gradual temperature effects. The first two of these articles present results from the large TREELIM common garden experiments, reporting adaptation and plasticity of phenological patterns and of growth. The last two articles focus on the dynamics and the thermal limits of tissue formation.

5.1 Tree recruitment of European tree species at their current upper elevational limits in the Swiss Alps

Yann Vitasse, Günter Hoch, Christophe F. Randin, Armando Lenz, Chris Kollas and Christian Körner

Abstract

Aim The physical and physiological mechanisms that determine tree line position are reasonably well understood, but explanations for tree species-specific upper elevational limits below the tree line are still lacking. In addition, once these uppermost positions have been identified, questions arise over whether they reflect past expansion events or active ongoing recruitment or even upslope migration. The aims of this study were: (1) to assess current tree recruitment near the cold-temperature limit of 10 major European tree species in the Swiss Alps, and (2) to rank species by the extent that their seedlings and saplings exceed the elevational limit of adult trees, possibly reflecting effects of the recent climate warming.

Location Western and eastern Alps of Switzerland.

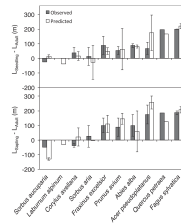
Methods For each species, occurrences were recorded along six elevational transects according to three size classes from seedlings to adult trees in 25-m-elevation steps above and below their regional upper elevational limit. Two methods were used to compare upper elevational limits between seedlings, saplings and adults within species. First, we focused on the uppermost occurrence observed in each life stage for a given species within each studied region; and second, we predicted their upper distribution range using polynomial models fitted to presence/absence data.

Results Species exhibited a clear ranking in their elevational limit. Regional differences in species limits (western versus eastern Swiss Alps) could largely be attributed to regional differences in temperature. Observed and predicted limits of each life stage showed that all species were represented by young individuals in the vicinity of the limit of adult trees. Moreover, tree recruitment of both seedlings and

saplings was detected and predicted significantly beyond adult tree limits in most of the species. Across regions, seedlings and saplings were on average found at elevations 73 m higher than adult trees.

Main conclusions Under current conditions, neither seed dispersal nor seedling establishment constitutes a serious limitation of recruitment at the upper elevational limits of major European trees. The recruits found beyond the adult limits demonstrate the potential for an upward migration of trees in the Alps in response to ongoing climate warming.

Fig. 5.1 Difference in elevation (m) between observed or predicted seedling and sapling limits and the adult tree limits. The observed limits correspond to the highest individual observed for each life stage within all three transects of each region. The predicted limits were derived for each life stage from generalized linear models with a probability of occurrence of 10%. Error bars show the standard deviation between the eastern and western regions of Switzerland.



5.2 Fast acclimation of freezing resistance suggests no influence of winter minimum temperature on the range limit of European beech

Armando Lenz, Günter Hoch and Yann Vitasse

Abstract Low temperature extremes drive species distribution at a global scale. Here, we assessed the acclimation potential of freezing resistance in European beech (*Fagus sylvatica* L.) during winter. We specifically asked (1) how do beech populations growing in contrasting climates differ in their maximum freezing resistance, (2) do differences result from genetic differentiation or phenotypic plasticity to preceding temperatures, and (3) is beech at risk of freezing damage in winter across its distribution range. We investigated the genetic and environmental components of freezing resistance in buds of adult beech trees from three different populations along a natural large temperature gradient in north-western Switzerland, including the site holding the cold-temperature record in Switzerland. Freezing resistance of leaf primordia in buds varied significantly among populations, with LT_{50} values (lethal temperature for 50% of samples) ranging from -25°C to -40°C correlating with mid-winter temperatures of the site of origin. Cambial meristems and the pith of shoots showed high freezing resistance in all three populations, with only a trend to lower freezing resistance at the warmer site. After hardening samples at -6°C for 5 days, freezing resistance of leaf primordia increased in all provenances by up to 4.5 K. After additional hardening at -15°C for 3 days, all leaf primordia were freezing resistant to -40°C . We demonstrate that freezing resistance of *F. sylvatica* has a high ability to acclimate to temperature changes in winter, whereas the genetic differentiation of freezing resistance among populations seems negligible over this small geographic scale but large climatic gradient. In contrast to the assumption made in most of the species distribution models, we suggest that absolute minimum temperature in winter is unlikely to shape the cold range limit of beech. We conclude that the rapid acclimation of freezing resistance to winter temperatures allows

beech to track changing climatic conditions, especially during unusually warm winters interrupted by very cold weather.

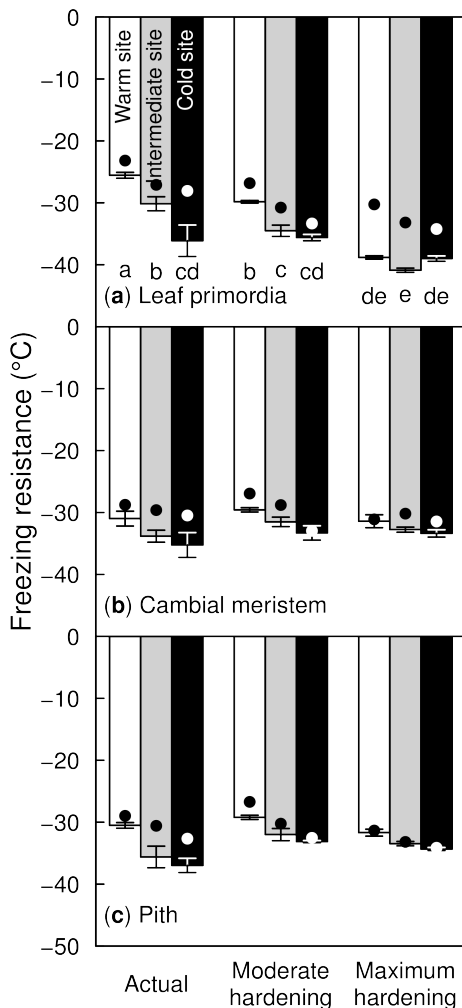


Fig. 5.2 The actual freezing resistance, freezing resistance after the moderate hardening treatment, and freezing resistance after the maximum hardening expressed as LT_{50} values of (a) leaf primordia in buds, (b) cambial meristems, and (c) pith tissue in twigs of beech among the three sites. LT_{10} values are shown as points. Letters indicate significant differences calculated by a Tukey-HSD post hoc test, if the interaction between site of origin and treatment was significant.

5.3 Earlier leaf-out rather than difference in freezing resistance puts juvenile trees at greater risk of freeze damage than adult trees

Yann Vitasse, Armando Lenz, Günter Hoch and Christian Körner

Abstract

1. In temperate climates, seedlings and saplings have often been assumed to be more sensitive to late-spring freezes than conspecific adult trees. Yet, no data is available to compare the freezing resistance of juvenile and adult trees at their phenologically most sensitive stage, i.e. during leaf-out.
2. Emerging leaves of seedlings, saplings and adult trees were collected in spring 2013 in seven temperate tree species in a mature mixed forest in the foothills of the Swiss Jura Mountains. Freezing resistance of these emerging leaves was assessed using different target temperatures (-13°C to $+4^{\circ}\text{C}$) in 7 computer-controlled freezers. Additionally, we assessed the risk that species encounter freeze damages based on temperature data recorded since 1898.
3. The different study species showed contrasting freezing resistance, with the LT_{50} (median lethal freezing temperature) of emerging leaves ranging from $-3.5 \pm 0.2^{\circ}\text{C}$ (*Fraxinus excelsior*) to $-8.3 \pm 0.2^{\circ}\text{C}$ (*Prunus avium*). Within species, juvenile trees (seedlings or saplings) were found to be as sensitive to freezing temperatures as mature trees when the same developmental stage of foliage was compared. Based on phenological observations made during spring 2012, long time-series of temperatures indicate a very low risk of freeze damage at the study site, especially for adult trees.
4. *Synthesis*: We conclude that seedlings and saplings are more prone to freeze damage than adult trees because of their earlier flushing rather than due to a higher sensitivity to freezing as such. Our study highlights that the timing of spring phenology has evolved in such a way

that it minimizes the risk of freeze damage according to the species-specific LT_{50} . Early flushing species are among the most freezing resistant species during flushing, whereas late flushing species are among the least resistant. We suggest that process-based species distribution models should account for the ontogenetic effects acting on phenology. We conclude that for the examined species the species-specific freezing resistance during leaf emergence could be extracted from either adult or juvenile trees, as long as it is estimated at a same phenological stage.

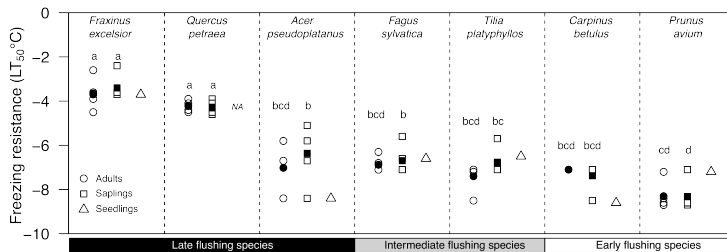


Fig. 5.3 Freezing resistance (LT_{50} , °C) of emerging leaves in adult trees, saplings and seedlings of the seven study species. Different letters indicate significant differences in the LT_{50} across species and ontogenetic stages (Tukey's honestly significant difference tests). Note that seedlings values were not included in the analysis of variance because no replicate was available (pooled data from $n = 20 - 30$, see methods). Closed symbols correspond to the mean LT_{50} values of the five replicates per species and life-stage for sapling and adult trees or the one mean value of the all-seedlings pool, whereas open symbols correspond to LT_{50} values of each individual for sapling and adult trees.

5.4 Spring patterns of freezing resistance and photosynthesis of two leaf phenotypes of *Hedera helix*

Evan Rehm, Armando Lenz, Günter Hoch and Christian Körner

Abstract Subdominant evergreen broad-leaved plants occurring in deciduous forests throughout temperate zones have only a short window of optimum photoassimilation in spring before canopy closure. Yet increasing photosynthetic and metabolic activity occurs concurrently with reductions in freezing resistance, resulting in vulnerability of plant tissues to late spring freezing events. Our goal was to document the temporal patterns of photosynthesis versus freezing resistance during spring in adult and juvenile leaf phenotypes of *Hedera helix* in Switzerland. Freezing resistances in all leaves were well below longterm minimum temperatures experienced at the study site, with adult leaf phenotypes in the forest canopy being more freezing resistant than juvenile leaves occurring closer to the ground. Reductions in freezing resistance were followed by increases in leaf photosynthetic capacities, which appeared synchronized among leaf phenotypes. Adult canopy leaves maintained a higher freezing resistance but lower photosynthetic capacity than juvenile leaves through the end of winter and into early spring. However, shortly after the cessation of freezing temperatures, adult leaves greatly increased their photosynthetic capacity relative to juvenile leaves, yet maintained freezing resistances sufficient to resist late spring freezing events. These patterns highlight the importance of the tradeoff in *H. helix* between exposure to potentially damaging cold temperatures in late spring and the need for high photosynthetic carbon gains before full canopy closure.

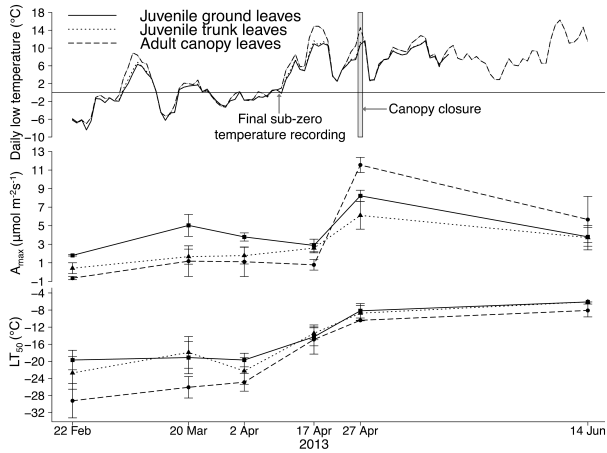


Fig. 5.4 Seasonal variation of daily low temperature ($^{\circ}\text{C}$), maximum photosynthetic capacity (A_{max}) and freezing resistance (LT_{50} ; $^{\circ}\text{C}$) as measured by the visual method for two phenotypic leaf types of *H. helix* at three different heights in the forest canopy (during each sampling interval $n = 3$ and 6 for each height/phenotype combination for photosynthetic capacity and freezing resistance respectively). Shaded gray area represents the date of full canopy closure.

5.5 Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species

Yann Vitasse, Günter Hoch Christophe F. Randin, Armando Lenz, Chris Kollas, J. F. Scheepens and Christian Körner

Abstract Phenological events, such as the initiation and the end of seasonal growth, are thought to be under strong evolutionary control because of their influence on tree fitness. Although numerous studies highlighted genetic differentiation in phenology among populations from contrasting climates, it remains unclear whether local adaptation could restrict phenological plasticity in response to current warming. Seedling populations of seven deciduous tree species from high and low elevations in the Swiss Alps were investigated in eight common gardens located along two elevational gradients from 400 to 1700 m. We addressed the following questions: are there genetic differentiations in phenology between populations from low and high elevations, and are populations from the upper elevational limit of a species' distribution able to respond to increasing temperature to the same extent as low-elevation populations? Genetic variation of leaf unfolding date between seedlings from low and high populations was detected in six out of seven tree species. Except for beech, populations from high elevations tended to flush later than populations from low elevations, emphasizing that phenology is likely to be under evolutionary pressure. Furthermore, seedlings from high elevation exhibited lower phenological plasticity to temperature than low-elevation provenances. This difference in phenological plasticity may reflect the opposing selective forces involved (i.e. a trade-off between maximizing growing season length and avoiding frost damages). Nevertheless, environmental effects were much stronger than genetic effects, suggesting a high phenological plasticity to enable tree populations to track ongoing climate change, which includes the risk of tracking unusually warm springs followed by frost.

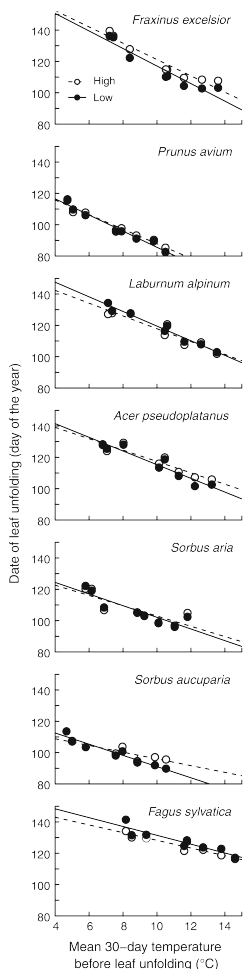


Fig. 5.5 Relationship between mean 30-day air temperature recorded in the common gardens prior to the flushing period and leaf unfolding dates for low and high elevation provenances of the seven studied tree species. The leaf unfolding date corresponds to the mean of all individuals per common garden for low and high elevation provenances (both regions of origin pooled) with standard errors and regression lines. For each species, the temperature range corresponds to the mean air temperature during 30 days before the leaf unfolding date of the earliest provenance until the unfolding date of the latest provenance. Black circles and solid lines correspond to populations from low elevation

5.6 Genetic vs. non-genetic responses of leaf morphology and growth to elevation in temperate tree species

Yann Vitasse, Armando Lenz, Chris Kollas, Christophe F. Randin, Günter Hoch and Christian Körner

Abstract

1. At high elevation, temperate trees generally exhibit adaptive genetic differentiation in their morphological and physiological traits. On account of this directional selection, we hypothesized that tree populations growing near their upper cold elevational limits exhibit lower phenotypic plasticity of growth and leaf morphological traits in response to temperature changes than populations growing at lower elevations.
2. Seedlings of six common deciduous tree species originating from low and high elevations were transplanted into eight common gardens along two elevational gradients in the Swiss Alps. The aim of the experiment was to evaluate the genetic differentiation in growth and leaf morphology between populations from low and high elevations and to quantify the phenotypic plasticity of these traits to temperature changes.
3. In contrast to growth that decreased with increasing elevation, leaf mass per area (LMA) showed no significant change with elevation of common garden, except for a decrease in *Laburnum alpinum* for both low- and high-elevation provenances. Interestingly, leaf density was found to decrease with elevation of the gardens for all species. Genetic differentiation between low- and high-elevation populations was found in both leaf morphology and growth: high-elevation populations tended to have slower growth rate than low-elevation populations, while no consistent trend was found for LMA across species. Interestingly, for *Acer pseudoplatanus* and *Fraxinus excelsior* high-elevation populations exhibited a lower phenotypic plasticity of growth in response to temperature compared to lower populations, whereas no interactions between the elevation of a provenance and the elevation of the garden was detected for the four other species.
4. Hence, during young life stages, the expected increase in tree growth in future warmer climates might be lower in populations living in the coldest part of the species distribution range in temperate species such as *Acer pseudoplatanus* and *Fraxinus excelsior*, but similar in other tree species, disregarding other environmental changes.

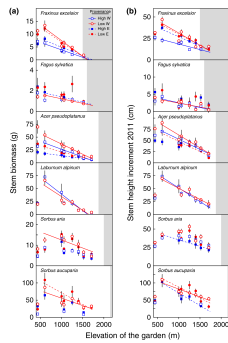


Fig. 5.6 Linear regressions between elevation of the gardens and (a) stem biomass, and (b) height increment in 2011 for each studied provenances and species. Open symbols and solid regression lines indicate provenances from the Western Swiss Alps (High W and Low W). Filled symbols and dotted lines indicate provenances from the Eastern Swiss Alps (High E and Low E). Low-elevation provenances are represented with red circles (means \pm SE) and red lines, whereas high-elevation provenances are represented with blue squares (means \pm SE) and blue lines. Only regression lines are shown with slopes significantly different from 0 ($P \leq 0.05$). The grey shaded areas represent elevations beyond the upper elevational limit of adult trees for each species within the two studied regions and were extracted from Vitasse et al. (2012).

5.7 Early season temperature controls cambial activity and total tree ring width at the alpine treeline

Armando Lenz, Günter Hoch and Christian Körner

Abstract

Background Temperature directly affects xylogenesis at high-elevation treelines. The low-temperature limitation of meristematic processes is thus key to understand treeline formation.

Aims We aimed to experimentally test in situ the direct low-temperature effect on wood tissue formation at the alpine treeline.

Methods We applied controlled Peltier-mediated cooling and warming (± 3 K) to branch segments in *Pinus uncinata* at the treeline in the Swiss Alps. In addition, we studied xylogenesis in untreated trees during the growing season by sequential micro-coring.

Results Micro-cores indicated that the cambial zone was fully developed by the time the cooling and warming treatment started, shortly after snowmelt. Presumably, because of this, experimental cooling of branches did not significantly reduce the number of cells produced per season. Warming extended the formation of early wood into the late season, and thus reduced the fraction of late wood.

Conclusions We conclude that temperatures very early in the season determine the width of the cambial zone which, in turn, strongly controls the number of tracheids produced during the remaining growing season. Temperatures later in the season mainly determine the early wood to late wood ratio. These data provide an empirical basis for the mechanistic understanding of tree growth at the treeline in response to temperature.

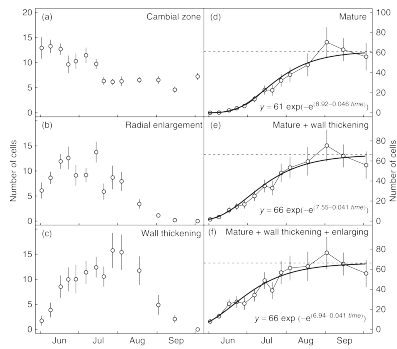


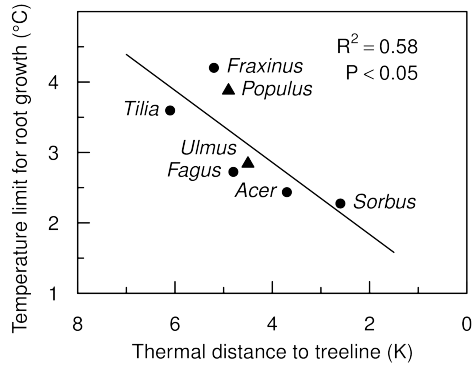
Fig. 5.7 Natural xylogenesis of *Pinus uncinata* studied by micro-cores in 2009. Mean number of cells (\pm s.e.) in the cambial zone (a), in the radial enlargement stage (b) and in the secondary wall-thickening stage (c). The calculated Gompertz growth models on the mean number (\pm s.e.) of mature cells (d), the sum of mature and enlarging cells (e) and the sum of mature, enlarging and wall-thickening cells (f) with the respective model equations. Note the difference in the axis scale between the left and right panel. All data were corrected by a circumference correction following Rossi et al. (2003).

5.8 Physiological minimum temperatures for root growth in seven common European broad-leaved tree species

Gabriela Schenker, Armando Lenz, Christian Körner and Günter Hoch

Abstract Temperature is the most important factor driving the cold edge distribution limit of temperate trees. Here, we identified the minimum temperatures for root growth in seven broad-leaved tree species, compared them with the species' natural elevational limits and identified morphological changes in roots produced near their physiological cold limit. Seedlings were exposed to a vertical soil-temperature gradient from 20 to 2°C along the rooting zone for 18 weeks. In all species, the bulk of roots was produced at temperatures above 5°C. However, the absolute minimum temperatures for root growth differed among species between 2.3 and 4.2°C, with those species that reach their natural distribution limits at higher elevations also tending to have lower thermal limits for root tissue formation. In all investigated species, the roots produced at temperatures close to the thermal limit were pale, thick, unbranched and of reduced mechanical strength. Across species, the specific root length (m g^{-1} root) was reduced by, on average, 60% at temperatures below 7°C. A significant correlation of minimum temperatures for root growth with the natural high elevation limits of the investigated species indicates species-specific thermal requirements for basic physiological processes. Although these limits are not necessarily directly causative for the upper distribution limit of a species, they seem to belong to a syndrome of adaptive processes for life at low temperatures. The anatomical changes at the cold limit likely hint at the mechanisms impeding meristematic activity at low temperatures.

Fig. 5.8 Linear correlation between the minimum temperature for root growth and the natural upper limit of the seven investigated tree species (circles, 2-year-old seedlings; triangles, current-year seedlings). The upper limit is given as a thermal distance relative to treeline (ΔT TL, in K) as reported by Randin et al. (2013), except for *Ulmus*, where the upper elevation limits were estimated from literature sources and personal observations (see Material and methods for further details on the definition of the thermal distance to tree-line). Note that for *Tilia*, only the lower temperature threshold from 2-year-old seedlings was used for correlation.



Chapter 6

Summary & Conclusion

Armando Lenz

The overarching symmetry between elevational and latitudinal range limits of major European deciduous tree species calls for a common low temperature influence, the nature of which is still unknown. Low temperature can either act as a threshold, directly affecting survival of trees, or gradually affecting growth and reproduction. Gradual temperature influences include the direct effect of low temperature on tissue formation and tissue maturation as well as an indirect effect via the length of the growing season. The aim of this thesis was to arrive at a functional explanation of temperate tree species cold range limits by disentangling (1) the influence of absolute minimum temperature during critical periods, (2) the effects of mean temperatures during the growing season and (3) the effects of the length of the growing season on survival and growth of trees. The results reveal that the interaction of freezing temperature, spring phenology and growing season length is key for survival and growth of trees near the species-specific low temperature range limit.

Freezing temperatures pose a small risk to trees

European deciduous trees are rarely damaged by freezing temperature during winter or summer. Although freezing resistance correlates with

the current distribution limit of tree species in winter and summer, long-term (80 years) minimum temperatures are significantly higher than what would be damaging to the examined taxa (Chapter 2). In addition, trees do exhibit a considerable potential to acclimate to cold temperatures in winter, resulting in a substantial safety margin against freezing damage (Chap. 5.2). The most critical period for trees in terms of freezing temperature is spring (Chapter 2). However, trees delay their flushing at higher elevation to such an extent, that the probability of a freezing damage remains the same as at lower elevation, i.e. close to zero (Chapters 2 and 3). To achieve this high safety margin against freezing temperatures in spring, trees need to have sophisticated control mechanisms that cause them to flush sufficiently late so that unexpected cold waves in spring do not affect them. Leaf-out data in transplants of trees originating from low and high elevation to different elevations showed a much stronger influence of environmental conditions than of genetic adaptation (provenance; Chapter 5.5). Thus, because leaf phenology is only to a small extent genetically fixed, but mostly controlled by current spring temperature (once chilling and photoperiod requirements are met), offspring from a given tree population can avoid freezing damage even if they are dispersed a long distance from a mother tree's climatic environment. While spring phenology is a very plastic trait, freezing resistance of leaves at flushing appears to be a rather fixed trait under strong phylogenetic control. Indeed, there is no evolutionary need for a high plasticity in freezing resistance since trees experience a high plasticity in phenology, enabling them to escape freezing damage in spring by delayed flushing (Chapter 3). Further, tree species with a higher freezing resistance during flushing can (and do) flush earlier than less freezing resistant tree species (Chapter 2). In summary, freezing temperatures are not directly related to the range limit of the tree species examined, but freezing resistance acts indirectly via selection for a certain spring phenology, with the resulting length of the growing season presumably setting the crucial maturation constrains at the cold limit (Chapters 2 and 3).

Tree growth at the limit

Secondary growth of deciduous temperate tree taxa shows no linear reduction with elevation as one approaches the species-specific upper elevational range limit. Tree stem growth does not substantially change over a wide range of elevations, before it starts to gradually decline along the last few hundred meters below the high elevation range limit, with an abrupt growth decline in some species near their range limit (Chapter 4). Non-structural carbohydrate concentrations reveal that trees at the range limit have similar mobile carbon stocks than trees at lower elevation, suggesting that carbon acquisition is not limiting trees at their high elevation range limit (Chapter 4). The frequency of negative event years as an indicator of particularly bad summers or late spring freeze events did not increase with increasing elevation, except for *Fagus sylvatica*. Summarising, neither a negative effect of mean temperature on carbon acquisition nor a negative effect of extreme temperature on tree growth could be observed. Our results rather revealed that basal area increment correlates with mean temperature of the growing season irrespective of the length of the season across the last few hundred meters of elevation. Interestingly, the length of the season becomes more effective for growth in warmer years. This paradox may be related to the non-linearity of the temperature response of growth and the accelerating rate (in a sigmoidal shape) of tissue formation with warmer temperatures. A longer time for tissue formation enables an over-proportionally higher number of cell divisions (and associated differentiation) at warm temperatures, whereas at cold temperature very little cell divisions are possible. Since the majority of years are generally cold at the species' range limit, the length of the season has a negligible influence on the formation of stem tissue at the upper species limit.

A direct growth limitation by low temperature during the growing season can be rejected as the causative explanation for the formation of tree species limits. The low temperature limits for the bulk of fine root formation were found to be around 5°C – a temperature value that is far below the mean temperature during the growing season for any temperate tree species at its range limit (Chapter 5.8). However, root growth at low temperature was found to be different among species. The absolute low temperature limits for root tissue formation ranged between 2.3 and 4.2°C depending on species (Chapter 5.8). Interestingly, this species-

specific thermal limit for tissue formation did correlate with the high elevation distribution limit of species, reflecting the potential for growth at low temperature. Species having their distribution limit at higher elevation experience colder temperatures during the growing season. The ability to produce tissues at colder temperatures might thus be crucial at high elevation in order to mature all tissues at the given short season and cold temperature. A species with higher growth rates at lower temperatures can mature newly formed tissues faster at colder temperatures, potentially enabling the species to have its limit at higher elevation. Therefore, the capability to produce tissue at cold temperature might be a potential trait explaining — at least partly — the position of the high elevation range limit of species.

A functional concept for the formation of species-specific range limits

Freezing temperature in spring is a very strong selective force that drives deciduous temperate tree species to delay their flushing to a safe time period. With increasing elevation, potentially lethal freezing temperatures to emerging leaves occur later in the season. Thus, the delay of flushing to a safe time period inevitably leads to a shorter remaining growing season at higher elevation (Fig. 6.1a). While at these high elevations wood formation is still possible (largely occurring during the first two months of the season), we suggest that the formation of the range limit as such is dependent on species-specific life history traits that require a minimum length of the growing season for the completion of maturation (Fig. 6.1b). Species-specific elevational range limits may have different causes, depending on species. Potentially important life history traits might be the size of seed and other seed related traits (e.g. seed viability), or the required time for tissue maturation in new shoots and winter bud formation. Further, the potential to produce tissue at low temperature interacts with tissue maturation. A species with more growth at a lower temperature can simply mature its tissues faster at colder temperatures, potentially enabling the species to have its limit at higher elevation. If the available season is too short or too cold for maturation of tissues, trees (or seeds) will not survive

the subsequent winter or fail to reproduce. While trees can sufficiently delay their flushing in spring to a safe time period, the resulting length of the remaining growing season becomes too short at the range limit to sufficiently mature and harden tissues before the next winter. These data permit for the first time to design a functional concept for the formation of species-specific cold range limits on the basis of observational and experimental results, and allow to define the cold range edge of the fundamental niche of deciduous temperate tree species. Our data open a new avenue to test for shifts in the fundamental niche due to climatic change, and to investigate deviations of the realised niche from the fundamental niche of a species.

Fig. 6.1 The shift in the occurrence of lethal freezing temperatures to developing new leaves in spring with elevation, the resulting length of the growing season and the position of the elevational limit of a species (a), as well as the interaction of environmental drivers with life history traits (b). Maturation related traits refer to traits important for maturation and hardening of tissues, for instance seed related traits, time required for tissue maturation and winter bud formation or the ability of a species to produce tissue at cold temperatures.

