

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

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Summary

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 are available to compare the freezing resistance of juvenile and adult trees at their phenologically most sensitive stage, that is, 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 to +4 °C) in seven 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 LT₅₀ (median lethal freezing temperature) of emerging leaves ranging from -3.5 ± 0.2 °C (*Fraxinus excelsior*) to -8.3 ± 0.2 °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 LT₅₀. Early flushing species are among the most freezing-resistant species during flushing, whereas late flushing species are among the least resistant. 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.

Key-words: bud burst, cold hardiness, flushing, frost, low temperature, ontogeny, phenology, plant–climate interactions, resistance, temperate deciduous forest

Introduction

In temperate deciduous tree species, the annual course of freezing resistance of buds is tightly associated with the state of bud dormancy and passes through the following three phases: (i) a hardening period in autumn in response to the shorter photoperiod and decreasing temperature, (ii) a period of maximum freezing resistance in winter during bud endodormancy, (iii) and a dehardening period in spring associated with a longer photoperiod and a rise in temperature, with the minimum freezing resistance reached when the new leaves

emerge (Weiser 1970; Sakai & Larcher 1987; Neuner 2007). Then, shortly after leaf emergence, freezing resistance of leaves increases slightly as leaves expand and mature (Taschler, Beikircher & Neuner 2004; Lenz *et al.* 2013). Because emerging leaves are very sensitive to freezing temperatures, temperate deciduous trees have developed sophisticated responses to environmental cues in order to optimize their seasonal activity while encountering a low risk of freeze damage (Polgar & Primack 2011). Thus, the timing of leaf-out of most of temperate trees is driven by a combination of temperature and photoperiodic cues, with longer duration of chilling decreasing the thermal time (i.e. the heat requirement) required for budburst (Wareing 1953; Sarvas 1972; Murray, Cannell & Smith 1989; Laube *et al.* 2014). Photoperiod (the only

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weather independent signal) possibly interacts with both the degree of chilling experienced during the phase of endodormancy and actual temperatures during the ecodormancy phase (Caffarra & Donnelly 2011; Basler & Körner 2012; Vitasse & Basler 2013). The phenological response to environmental factors must have been selected for to minimize freeze damage in a given environment or region while maximizing the growing season length, leading to genetic differentiation among populations inhabiting contrasted climatic conditions (reviewed in Langlet 1971; Morgenstern 1996 with references therein). Late freeze events are particularly critical in spring because freezing resistance of buds during the dehardening period in spring depends on the state of bud/leaf development and can hardly be enhanced in response to cold temperatures once an advanced stage of flushing has been reached (Taschler, Beikircher & Neuner 2004; Lenz *et al.* 2013).

Saplings and seedlings in the understory of temperate forests generally start their growing season earlier than conspecific adult trees (Seiwa 1999; Richardson & O'Keefe 2009; Vitasse 2013), allowing them to benefit from high light availability before canopy closure (Augsburger 2008). This phenological discrepancy between adult and juvenile trees has been recently assigned to ontogenic rather than microenvironmental effects (Vitasse 2013). Presumably, the seasonal development of young understory trees (seedlings and saplings) is set to lower thresholds of chilling requirement or shorter photoperiod to break dormancy than adult trees and is subsequently responsive to warm temperatures earlier in spring. To date, there is no evidence that emerging leaves of juvenile trees differ in freezing resistance from adult trees, though it is often assumed or expected (Larcher & Bauer 1981; Ningre & Colin 2007; Kreyling *et al.* 2012), perhaps because freeze damages in spring are frequently observed on juvenile trees (Y. Vitasse, unpubl. data). However, juvenile trees might be more prone to freeze damage than adult trees because of their earlier leaf-out rather than due to a higher sensitivity to freezing as such. Although we lack data for winter deciduous tree species, contrasting patterns of freezing resistance have been found between juvenile and adult trees in conifers and broad-leaved evergreen woody species. For instance, seedlings were found to be less freeze-resistant than conspecific adult trees in *Quercus ilex* (Larcher 1969) and *Nothofagus menziesii* (Neuner, Bannister & Larcher 1997). Similar freezing resistances between saplings and adults were found in *Pinus radiata* (Greer, Wardle & Buxton 1989), whereas seedlings were more freeze-resistant than adults in *Embothrium coccineum* (Alberdi & Rios 1983), *Pittosporum eugenioides* (Bannister, Colhoun & Jameson 1995) or in *Nothofagus dombeyi* (Rios *et al.* 1988). Yet, all these previous studies focused on conifers or broad-leaved evergreen tree species and compared the freezing resistance between juvenile and conspecific adult trees during the hardening period or in winter, when temperatures between the canopy and the understory strongly differ (Kollas *et al.* 2014). Thus, different freezing resistance between adults, seedlings and saplings in winter may be the consequence of different levels of hardening caused by different preceding temperatures.

To our knowledge, no study has so far investigated if there is a difference in freezing resistance between young and adult trees in winter deciduous temperate species during the most critical stage of the year, that is, during leaf emergence and shortly after. During this period, freezing resistance strongly depends on the phenological development of individuals and is not (or only to a very small extent) responsive to temperature, that is, trees cannot re-acclimate in response to colder temperatures once the irreversible process of bud break has been initiated (Larcher & Mair 1968; Pagter & Arora 2013). The comparison should also be conducted on the same phenological stages during flushing rather than at the same calendar date because freezing resistance decreases dramatically from bud swelling to leaf emergence (Taschler, Beikircher & Neuner 2004; Neuner 2007; Lenz *et al.* 2013). Because freezing resistance in spring is known to be tightly linked with bud development, we hypothesized that, at the same phenological stage, seedlings have the same freezing resistance as adult trees, but are more prone to freeze damage due to earlier leaf-out. We aimed at testing this hypothesis in seven common temperate tree species growing in a mature mixed forest by assessing their freezing resistance during the most sensitive phenological stage, that is, during leaf emergence.

Materials and methods

STUDY SITE AND STUDY SPECIES

The samples were collected in a mature mixed forest stand (c. 110 years old) near the village of Hofstetten (47°28'N, 7°30'E, 570–580 m asl), located 12 km south-west of Basel, Switzerland. Soils are of the Rendzina type on calcareous bedrock. The dominant tree species are *Fagus sylvatica* L. and *Picea abies* L., while *Acer campestre* L., *Acer pseudoplatanus* L., *Carpinus betulus* L., *Fraxinus excelsior* L., *Prunus avium* L. and *Tilia platyphyllos* Scop. occur as companion species. The site is situated on a north-facing slope with no access to the ground water table and has essentially rocky subsoil at 40–90 cm below the surface. The mean annual air temperature recorded at the nearest climate station (Binningen, 316 m asl, c. 10 km distant from the study site) is 10.3 °C, and the mean annual precipitation is 810 mm (1970–2011). At the study site, there are usually only a few weeks of slight snow cover during mid-winter.

We selected seven tree species that exhibit a large difference in their date of budburst in such a climate: two early flushing species, *C. betulus* L. and *P. avium* L.; two intermediate flushing species, *T. platyphyllos* Scop and *F. sylvatica* L.; and three late flushing species, *A. pseudoplatanus* L., *Quercus petraea* (Matt.) Liebl., and *F. excelsior* L. (ranking based on Vitasse 2013; Vitasse & Basler 2014). For clarity and brevity, hereafter, we refer to each species by its genus.

ASSESSMENT OF FREEZING RESISTANCE AND SPRING PHENOLOGY

We sampled three life stage categories: (1) adult trees (25–30 m, reaching the canopy height), (2) saplings (2–4 m), and (3) seedlings (<0.3 m). For adult trees and saplings, twigs with emerging leaves were collected from five individuals on 22 April 2013 for early and intermediate flushing species *Carpinus*, *Prunus*, *Tilia* and *Fagus* and on 29 April 2013 for later flushing species *Acer*, *Quercus* and

Fraxinus (Table 1). For adult trees, five species (*Carpinus*, *Fagus*, *Prunus*, *Quercus*, *Tilia*) were sampled from a 45-m-high construction crane (at the Swiss Canopy Crane facility, Pepin & Körner 2002) and two species (*Fraxinus*, *Acer*) were sampled by using a 7 m pole pruner at c. 5–9 m height. Whole seedlings (< 30 cm; 20–30 per species) were sampled on the same dates as adults and saplings. No samples of *Quercus* were collected at seedling stage, because this species was present with too few seedlings at the study site. Because, for the majority of seedlings, the number of leaves was too low to supply at least one leaf for each of the eight temperature treatment and the number of leaves per treatment was too low to get an accurate estimation of the LT₅₀ by randomly splitting up the data into different groups, we pooled leaves across the individual seedlings within a species for the freezing tests. Therefore, freezing resistances for the seedling stage are reported as unreplicated averages for randomly sampled individuals from the entire seedlings pool for each species. For sapling and adult trees, each individual sampled was assigned to a phenological stage according to Vitasse (2013), namely: bud closed (Stage 0), bud swelling (1), bud burst (2), leaf emergence (3), leaf unfolding (4). Then, for each individual tree we took care to select sample of buds that were exclusively at the corresponding stage previously assigned to the individual. For seedlings, we selected only individuals that were assigned to the phenological stage 4 and only used buds that were at this phenological stage for assessing freezing resistance (Table 1). Thus, even though samples of all life stages were collected on the same date within species, we achieved to compare the same phenological stages among life stages using advantage of (i) the phenological heterogeneity occurring within each tree, (ii) of the large phenological variation among individuals at young life stages, and (iii) of the weak phenological discrepancy between young and old trees during this specific spring (cool early spring delaying the commonly earlier leaf development in seedlings).

Immediately after cutting, twigs were kept in a cooling box (0–4 °C) during transport back to the laboratory (c. 30 min). On the same day, for each sampled tree, twigs were equally distributed to seven target freezing temperatures and one control at 4 °C. We used commercial freezers (Liebherr GN 1056 Premium No Frost, gross capacity = 102 L, with an integrated heating system; Liebherr, Ochsenhausen, Germany) modified to be computer controlled, allowing for an independent freeze-thaw cycle for each freezing treatment (see details in Lenz *et al.* 2013). Temperature within chambers was recorded using Pt-100 temperature sensors (Pt-100, DIN EN 60751; Pollin Electronic GmbH, Pförring, Germany) placed among samples.

The samples in each freezer were frozen at a rate of 3 K h⁻¹ until reaching the target temperature, and then, they were kept for 4 h at the target freezing temperatures before thawing at a rate of 3 K h⁻¹ until reaching +4 °C. The actual temperature of the treatments used for the analyses was calculated as the mean temperature recorded by the temperature sensors during the last two hours spent at the target temperature to ensure that the temperature was stabilized as close as possible to the target temperature. Among the seven freezers, the target temperature was setup with a step of 1.5 K between -2 and -8 °C and two other freezers were setup to a target temperature of -10 and -13 °C (Table 2). After freezing, samples were kept at constantly +4 °C for half a day before they were visually observed for survival. Freeze damage is obvious by a discoloration due to oxidation of polyphenols or a characteristic odour due to de-compartmentalization and autolysis of the protoplast (Sakai & Larcher 1987). Since a high correlation was found between visual observation and electrolyte leakage methods in a previous study conducted by the same authors on most of the species investigated here (Lenz *et al.* 2013), the visual assessment method was preferred for its simplicity and rapidity (Fig. 1).

DATA ANALYSIS

Lethal temperatures (LT) that caused damage to 10%, 50% and 90% of the samples (LT₁₀, LT₅₀ and LT₉₀) were calculated by logistic regression using the software R 2.12.2 (R Development Core Team

Table 2. Target and actual temperatures reached in each freezer for the two sampling dates (sampling 1: 22 April 2013; sampling 2: 29 April 2013)

Freezers	Temperature (°C)		
	Target	Sampling 1	Sampling 2
A	-2	-1.9	-1.8
B	-3.5	-2.3	-2.1
C	-5	-3.5	-2.8
D	-6.5	-5.6	-4.9
E	-8	-4.3	-4.5
F	-10	-8.7	-6.7
G	-13	-12.3	-10.2

Table 1. Date of harvesting and mean phenological score of each life stage for the samples considered for assessing the freezing resistance

Species	Phenology class	Sampling date (2013)	Mean phenological score		
			Seedlings	Saplings	Adults
<i>Fraxinus excelsior</i>	Late	04/29	4	4	3.2
<i>Quercus petraea</i>	Late	04/29	NA	4	3
<i>Acer pseudoplatanus</i>	Late	04/29	4*	4	4
<i>Fagus sylvatica</i>	Intermediate	04/22	4	3.8	3.4
<i>Tilia platyphyllos</i>	Intermediate	04/22	4	3.8	3.2
<i>Carpinus betulus</i>	Early	04/22	4	4	3.8
<i>Prunus avium</i>	Early	04/22	4	4	3

The mean phenological score was calculated on five individuals for saplings and adults. For seedlings, only individuals assigned to the phenological score 4 were harvested. The phenological classes were defined according to observations made on the previous and current year on the same species in the study site (ranking based on Vitasse 2013; Vitasse & Basler 2014).

*Leaves of *Acer* at seedling stage were beyond the phenological score 4 as a second cohort of leaves was out. We then discarded older leaves from the first cohort in the assessment of freezing resistance.

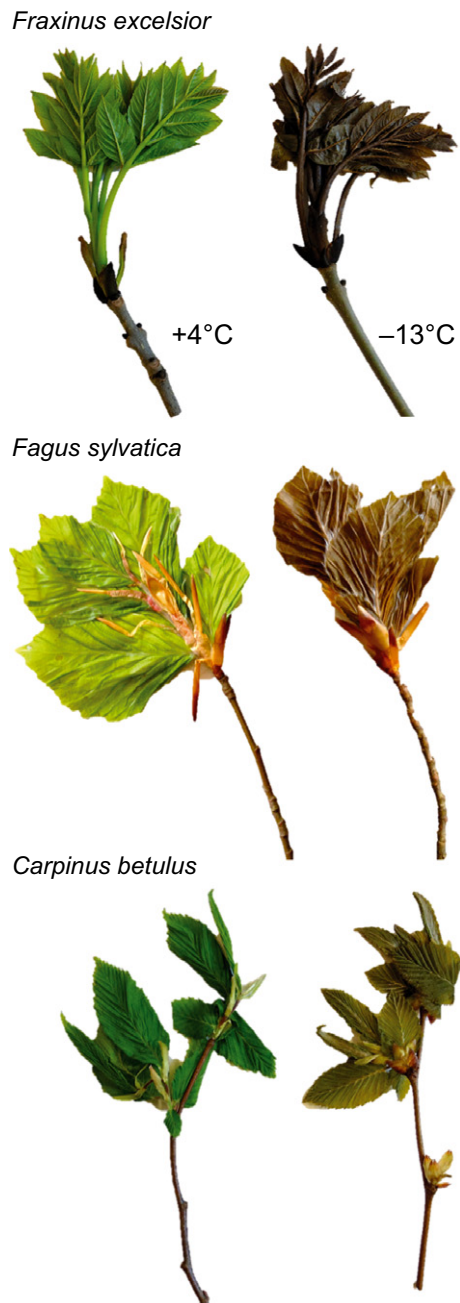


Fig. 1. Emerging leaves after the control treatment at +4 °C and the freezing treatment set-up at –13 °C of adult trees of *Fraxinus excelsior*, *Fagus sylvatica* and *Carpinus betulus* sampled in a mature mixed forest in the foothills of the Swiss Jura Mountains on 22 April 2013 for *C. betulus* and *F. sylvatica* and on 29 April 2013 for *F. excelsior*.

2011). LT₁₀ gives the initial damage, and LT₉₀ is the temperature below which damage is almost complete. The temperature range between LT₁₀ and LT₉₀ indicates the variability around LT₅₀ for each species. It also provides an estimation for how abruptly the damage occurs based on the temperature steps employed and the susceptibility of the species. For adult trees and saplings, LT values were calculated for each sampled tree of each species separately. Five trees per species and life stage were sampled, with c. 4–8 leaves per temperature treatment and replicate in species with a large terminal bud (*Acer* and

Fraxinus) and approximately 6–16 leaves for all other species. For seedlings, a similar number of leaves were used per treatment from the 20–30 seedling pool, that is, 3–4 seedlings were used per treatment. A two-way analysis of variance was performed to compare the LT₅₀ among species and life stage (i.e. sapling vs. adult trees). All factors were treated as fixed factors. Species was considered as a fixed effect because the species were specifically chosen for their differences in the timing of flushing (early-, intermediate- and late-flushing species). Within and among species, the mean LT values were compared among each life stage using Tukey's honestly significant difference (HSD) test performed using the software JMP 5.0.1.2 (SAS Institute, Cary, NC, USA). LT₅₀ values of seedlings were discarded from the statistical analyses because no replicate at the individual level was available (the seedlings had too few emerging leaves to distribute them in all seven freezers; see previous paragraph). However, given the pooled replicate of many individuals ($n = 20\text{--}30$), we consider LT₅₀ values for seedlings accurate and meaningful. Nevertheless, all data for seedlings were analysed and discussed with appropriate caution.

We compared the daily minimum temperature recorded directly at the study site at 2 m using shaded data loggers that recorded temperature every 30 min during the whole year 2011 (TIDBIT v2 UTBI-001; Onset Computer Corporation, Bourne, MA, USA) to the air temperature recorded in the nearest weather station (Binningen, 316 m asl, c. 10 km distant from the study site). Since we found an extremely good correlation between the two sites ($r^2 > 0.95$, $y = 1.02x - 0.42$), we directly used long-term series of temperature records from the weather station in order to assess the risk of freeze damage of the studied species in the past. We combined this temperature data set with phenological observations of seedlings and adult trees conducted at the same study site in spring 2012 (Vitasse 2013) to assess the risk of freeze damage. This phenological data set was also used to test if there is a correlation between the timing of leaf-out and the species-specific freezing resistance during leaf emergence. Because the timing of leaf-out in 2012 was similar or earlier than the average leaf-out dates recorded over the last 10 years on four of the studied species (2004–2013, leaf-out dates of *Acer*, *Fagus*, *Prunus* and *Tilia* observed in Therwil, 5 km distant from the study site, available from MeteoSwiss), we considered that using this specific year for assessing the risk of freeze damage in the past century lead to a realistic indication of the risk for the vast majority of years.

Results

FREEZING RESISTANCE AMONG SPECIES

The LT₅₀ values (LT that caused damage to 50% of the samples) of emerging leaves were significantly different among the seven tested species ($F = 60.9$, $P < 0.001$) and ranged from -3.5 ± 0.2 °C in *Fraxinus* to -8.3 ± 0.2 °C in *Prunus* (Table 3). Irrespective of life stage, the post hoc Tukey–Kramer test detected three distinct groups of species having significantly different LT₅₀ values: *Fraxinus* and *Quercus* were the least freezing-resistant species during flushing with an LT₅₀ higher than -4.3 °C; *Acer*, *Fagus*, *Tilia* and *Carpinus* exhibited intermediate LT₅₀ values ranged between -6.7 and -7.2 °C; the third group is constituted by a single species, *Prunus*, showing substantially higher freezing resistance with an LT₅₀ value of -8.3 °C (Table 3). Similar ranking among species was found with LT₁₀ and LT₉₀ values, but the

Table 3. LT₁₀, LT₅₀ and LT₉₀ values calculated per species across life stages

Species	Freezing resistance (°C)		
	LT ₁₀ ± SE	LT ₅₀ ± SE	LT ₉₀ ± SE
<i>Fraxinus excelsior</i>	-3.1 ^a ± 0.4	-3.5 ^a ± 0.2	-3.9 ^a ± 0.1
<i>Quercus petraea</i>	-3.5 ^a ± 0.1	-4.3 ^a ± 0.1	-5.0 ^b ± 0.1
<i>Acer pseudoplatanus</i>	-6.5 ^b ± 0.5	-6.7 ^b ± 0.4	-7.0 ^c ± 0.4
<i>Fagus sylvatica</i>	-6.2 ^b ± 0.3	-6.8 ^b ± 0.2	-7.4 ^c ± 0.2
<i>Tilia platyphyllos</i>	-6.9 ^{bc} ± 0.2	-7.1 ^b ± 0.2	-7.4 ^c ± 0.2
<i>Carpinus betulus</i>	-7.1 ^{bc} ± 0.4	-7.2 ^b ± 0.1	-7.5 ^c ± 0.2
<i>Prunus avium</i>	-8.1 ^c ± 0.2	-8.3 ^c ± 0.2	-8.6 ^d ± 0.2

Note that seedlings values were not included in the analyses because no replicate was available (see Materials and Methods).

Means with the same letter are not significantly different from each other within each threshold of Lethal Temperature (LT) tested by Tukey–Kramer test, $P < 0.05$.

temperature range comprised between these two LT values depended on species: between 0.3 °C for *Carpinus* to 1.5 °C for *Quercus* (Table 3).

FREEZING RESISTANCE AND LIFE STAGE

Neither life stage (i.e. sapling vs. adult trees, $F = 1.4$, $P = 0.24$) nor the interaction between species and life stage ($F = 0.6$, $P = 0.69$) showed an effect on LT₅₀ values of emerging leaves. The average absolute difference between LT₅₀ of saplings and adults was only 0.3 K (mean across species; Fig. 2) and never exceed 0.7 K for a given species. Although the LT₅₀ of seedlings could not be included in the same analyses (see Materials and Methods section), the LT₅₀ values of seedlings are comprised within the range of adults or saplings (Fig. 2).

RELATIONSHIP BETWEEN LEAF-OUT DATES, FREEZING RESISTANCE AND LONG-TERM MINIMUM TEMPERATURES

Based on phenological data observed in 2012 within the study site, the latest flushing species were found to be the least

freezing resistant during leaf emergence and the earliest flushing species were the more resistant ones (Fig. 3). Thus, a positive correlation was found across species between the date of leaf unfolding in 2012 and the species-specific freezing resistance during leaf emergence (Pearson's product moment correlation coefficient $r = 0.74$, $P = 0.058$ for adults and $r = 0.87$, $P = 0.052$ for saplings, Fig. 3).

Long-term air temperature records (1898–2012) from the nearest weather station showed that temperature below the species-specific freezing resistance of the studied tree species can occur at least once over this period until the c. 80th day of the year (21 March) for *Prunus* (the most freezing-resistant species studied here), and the 110th day of the year (20 April) for the least resistant species, *Fraxinus* (Fig. 4). If we assume the same leaf-out dates as the one observed on the same tree

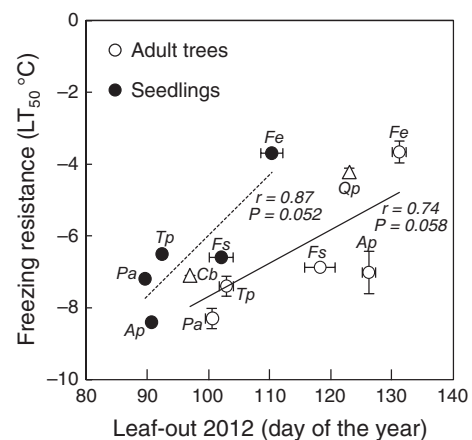


Fig. 3. Correlation between LT₅₀ values assessed in 2013 during leaf emergence calculated over life stages (reported in Table 3) and the timing of leaf-out recorded in 2012 in both seedlings and adult trees in the study site. The leaf-out dates in 2012 (phenological stage 4) were extracted from Vitasse (2013), except for *Carpinus* and *Quercus* for which they were deduced for adult trees according to pictures taken every 3 days in spring 2012 in the study site at a spot where two *Carpinus* and four *Quercus* individuals were present. These two latter species are represented with triangle symbols. r is the Pearson's product moment correlation coefficient. Ap, *Acer pseudoplatanus*; Cb, *Carpinus betulus*; Fe, *Fraxinus excelsior*; Fs, *Fagus sylvatica*; Pa, *Prunus avium*; Qp, *Quercus petraea*; Tp, *Tilia platyphyllos*.

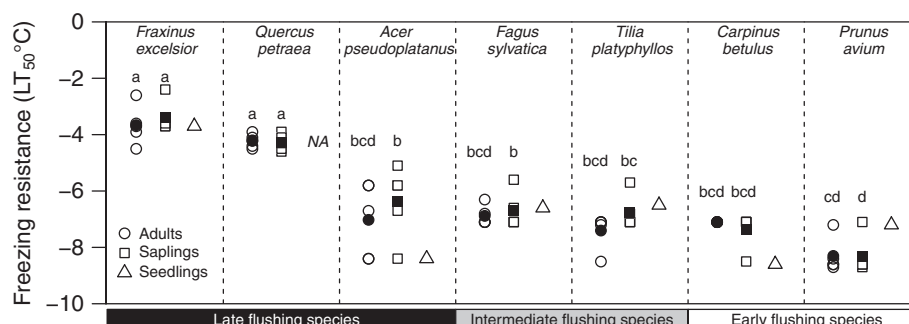


Fig. 2. Freezing resistance (LT₅₀, °C) of emerging leaves in adult trees, saplings and seedlings of the seven study species. Different letters indicate significant differences in the LT₅₀ across species and ontogenetic stages (Tukey's honestly significant difference tests). Note that seedlings values were not included in the analysis of variance because no replicate was available (pooled data from $n = 20$ – 30 , see Materials and Methods). Closed symbols correspond to the mean LT₅₀ 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 LT₅₀ values of each individual for sapling and adult trees.

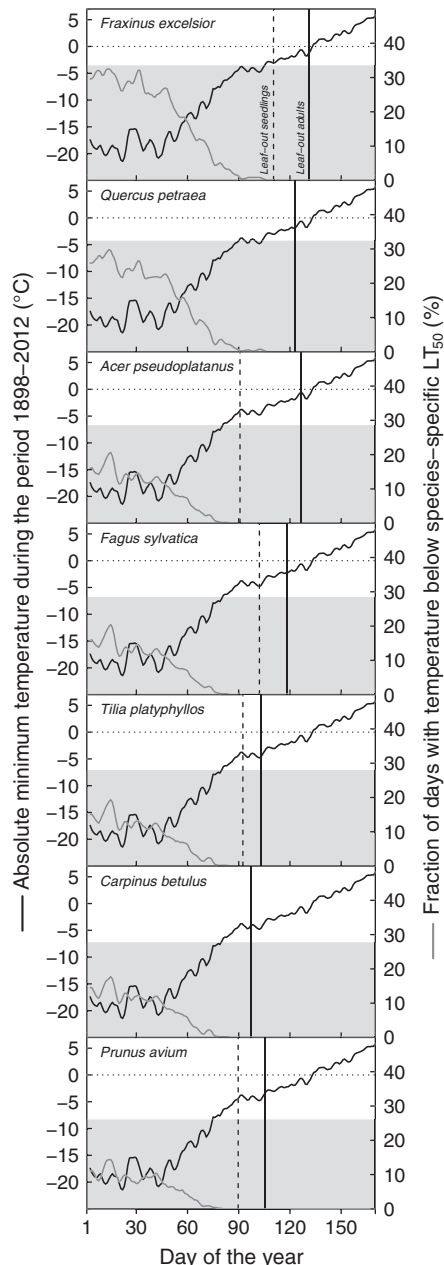


Fig. 4. Long-term daily minimum air temperatures recorded at 2 m in the nearest weather station (1898–2012, Binningen, 316 m asl, c. 10 km distant from the study site) in relation to the freezing resistance found during leaf emergence. Vertical lines represent the leaf-out dates (phenological stage 4) observed in 2012 in both seedlings (dotted lines) and adult trees (solid lines) of each species extracted from Vitasse (2013), except for *Carpinus* and *Quercus* for which they were deduced for adult trees according to pictures taken every 3 days in spring 2012 in the study site at a spot where two *Carpinus* and four *Quercus* individuals were present. The grey lines represent the fraction of days of temperature below species-specific LT_{50} values calculated across life stage (provided in Table 3) over the 1898–2012 period. The black lines represent the absolute minimum temperature during the period 1898–2012. The grey area corresponds to temperatures lower than the species-specific LT_{50} . Two-sided symmetric moving average of 5 days was plotted for both absolute minimum temperatures and the fraction of days of temperature below species-specific LT_{50} (weight coefficients: 0.125, 0.25, 0.25, 0.25, 0.125). Species were sorted by their species-specific LT_{50} .

population in 2012 for the whole period of the long-term temperature records, none of the species would flush during the unsafe period calculated for over more than a century. The leaf-out timing of deciduous temperate trees is known to greatly vary from year to year in relation to inter annual climate variability. Nevertheless, because the leaf-out dates in 2012 were average or, for some species, earlier than average leaf-out dates recorded nearby during the last decade (see Materials and Methods), this suggests that, in the study site, all the investigated species are very likely safe from late freezes during bud break in the vast majority of years (Fig. 4). In addition, based on these data, seedlings are more exposed to freeze damage than adult trees due to their earlier leaf development (Fig. 4).

Discussion

Our results demonstrated substantial differences in freezing resistance of emerging leaves among the tested tree species, but overall no ontogenetic effects within species. Seedlings and saplings did exhibit similar freezing resistance as adult trees, provided the same developmental stage of foliage is compared. Therefore, our results do not support the hypothesis that juvenile trees are less freeze resistant than conspecific mature trees but rather underline that juvenile trees are more prone to freeze damages as a result of their earlier leaf emergence.

TRADE-OFF BETWEEN FREEZING RESISTANCE AND TIME OF FLUSHING

Interestingly, the most freeze-sensitive species *Fraxinus* and *Quercus* are also the ones that exhibit the latest flushing, while the most freeze-resistant species, *Prunus*, *Carpinus* and *Tilia*, are among the earliest flushing species in this specific location (Vitasse 2013). Hence, the present study confirms the relationship between phenology and freezing resistance, as recently evidenced in the Swiss Alps in some of the same tree species as studied here (Lenz *et al.* 2013). This suggests that, in relation to species-specific freezing resistance of emerging leaves, late-spring freezes exert a strong selective pressure leading to different phenological sensitivity among the different species to environmental cues that are assumed to trigger budburst. Although the actual mechanism of dormancy release is very complex, including chilling requirement, photoperiod and heat requirement (Körner 2006; Polgar, Gallinat & Primack 2014), we assume that the dominant cues must be such that they account for the long-term probability of late-spring freezes. This could be achieved either by relying on photoperiod, that is, the only weather-independent environmental cue, or on weather-dependent cues given that long-term mean and minimum temperatures often correlate with low extreme temperatures. A crucial unresolved issue is to determine to which extent the long-term mean temperatures correlate with the likelihood of late spring freezes in a given year. For instance, freeze events are likely to occur later in the season toward higher elevations or higher latitudes. Thus,

under cooler climate conditions species having a low-freezing resistance during bud break (like *Quercus* and *Fraxinus*) must be able to sufficiently delay bud break towards warmer periods. Accordingly, *Fraxinus* and *Quercus* were found to be the most plastic tree species in terms of their phenological response to spring weather, exhibiting a delay of more than 6 days per 1 K temperature decrease in both adult and seedling stages (Vitasse *et al.* 2009b, 2010, 2013). This high plasticity allows them to leaf-out early in the warmest locations, whereas they exhibit rather late spring phenology in cooler sites (Vitasse *et al.* 2009c), leading to the same low risk of freeze damage along elevational gradients (Lenz *et al.* 2013).

The differential timing of flushing among populations from different climate has a genetic basis. Towards colder climate conditions, later flushing individuals might be selected for in species with a low tolerance to freezing temperatures and a low requirement in growing season length to complete the annual cycle of growth and development (including seed and winter bud maturation). In contrast, earlier flushing individuals would be selected for in species where a short season restricts fitness more than the risk of freeze damage does. In line with this hypothesis, *F. excelsior* may belong to the former group as it shows a strong delay of flushing in populations originating from high elevation when transplanted in common gardens at various elevations (Vitasse *et al.* 2009a, 2013), exhibits no decline in growth increment along elevational gradients in natural conditions (A. Lenz, Y. Vitasse, G. Hoch and C. Körner, unpubl. data) and is one of the most sensitive species to freezing temperatures, as demonstrated here. In contrast, *F. sylvatica* may belong to the second group for which short season restricts fitness more than the risk of freeze damage does. Common garden experiments showed earlier flushing for *F. sylvatica* populations originating from high elevations (vonWuehlisch, Krusche & Muhs 1995; Chmura & Rozkowski 2002; Vitasse *et al.* 2009a; Gomory & Paule 2011) despite the higher risk of freeze injuries at higher elevation for this specific species (Lenz *et al.* 2013). In the adult stage, beech populations exhibit a greater growth decline than other temperate tree species as one approaches the species' elevational limits (Lenz *et al.* unpubl. data).

FREEZING RESISTANCE IS NOT AFFECTED BY TREE AGE

When comparing leaves at the most sensitive phenological stage, that is, during leaf-out, no ontogenetic effect on freezing resistance was found in any of the studied species. Only *Acer* and *Carpinus* seedlings showed a LT₅₀ value ranging in the lowest part (higher freezing resistance), but still within the variance of conspecific saplings and adults (Fig. 2). Because we sampled leaves in adults and juveniles on the same day, this discrepancy might have resulted from slightly more expanded leaves at the seedling stage, given that freezing resistance increases with leaf expansion (Neuner 2007). Although our sampling protocol largely avoided phenological differences in LT₅₀, small developmental changes within our defined bud stages might have occurred, especially once

leaves are unfolded, as we did not have further phenological scores beyond the stage 4. *Acer* shows a particularly large phenological discrepancy between seedlings and adults (Fig. 4) and may be particularly prone to such difference in foliage expansion during our assessment.

To our knowledge, this study is the first to compare freezing resistance of emerging leaves between young and mature conspecific trees at the same developmental stage in winter deciduous tree species. In winter deciduous temperate trees that exhibit a range limit substantially below the treeline, species-specific freezing resistance of emerging leaves is probably to play a crucial role in setting their cold range limits (Lenz *et al.* 2013). The results highlighted here, especially the ones that have a crucial implication for process-based models that aim at predicting temperate tree species distributions, especially the ones that include phenology and species-specific freezing resistance of buds as parameters (e.g. PHE-NOFIT, Chuine & Beaubien 2001). The impact of stochastic late freeze events is likely to be more critical at the recruitment stage, because seedlings and saplings on average flush earlier. The good news is that the species-specific freezing resistance during leaf emergence could be extracted from either adult or juvenile trees, as long as leaves are at the very same developmental stage.

Conclusion

This study showed that freezing resistance of emerging leaves strongly depends on species, with early flushing species being more resistant than late flushing species in such cool climates. No ontogenetic effects were detected on freezing resistance of emerging leaves. We argue that juvenile trees are more prone to freeze damage than adult trees because of their earlier leaf-out rather than due to a higher sensitivity to freezing as such. Thus, competition for light leads juvenile trees to flush earlier than adult trees at the cost of a higher risk of freezing damage.

Acknowledgements

We thank Erwin Amstutz for his help in collecting leaves from the crane tower and Lukas Zimmermann for construction of and support with the freezing lab. We are grateful to MeteoSwiss, the Swiss federal office of meteorology and climatology for providing climate and phenology data. We thank three anonymous reviewers, the handling editor and the assistant editor for their helpful comments on an earlier version of the manuscript. This research was funded by the European Research Council (ERC) grant 233399 (project TREELIM).

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Received 8 October 2013; accepted 20 March 2014

Handling Editor: Frida Piper