Frost hardiness of tree species is independent of phenology and macroclimatic niche

M Hofmann[1,*] and H Bruelheide[1,2]

[1]Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle Wittenberg, Am Kirchtor 1, 06108, Halle (Saale), Germany

[2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103, Leipzig, Germany

*Corresponding author (Email, maria.hofmann@botanik.uni-halle.de)

The differences in timing in bud burst between species have been interpreted as an adaptation to late frost events in spring. Thus, it has been suggested that the degree of frost susceptibility of leaves is species-specific and depends on the species' phenology and geographic distribution range. To test for relationships between frost tolerance and phenology as well as between frost tolerance and distribution range across Central European tree species, we studied the frost hardiness of closed buds before bud burst and of freshly opened buds at the time of bud burst. We hypothesized that species with early bud burst and species distributed in eastern and northern areas were more frost tolerant than species with late bud burst and species distributed in western and southern areas. Frost hardiness was estimated by exposing twigs to 11 frost temperatures between −4°C and −80°C and by assessing tissue damage by the electrolyte leakage method. In contrast to our hypotheses, neither frost hardiness of closed buds nor frost hardiness of freshly opened buds were related to any variable describing species' macroclimatic niche. Furthermore, frost hardiness of freshly opened buds did not differ among species. Thus, the investigated species with early bud burst take higher risks of frost damage than the species with late bud bursts. These findings indicate that frost hardiness might not play the key role in limiting the geographic distribution ranges previously anticipated.

1. Introduction

In particular for trees, low temperatures are supposed to determine northern and eastern range boundaries in the boreal and temperate zone of the northern hemisphere (Sakai and Larcher 1987; Pither 2003; Kreyling 2010). Thus, clear relationships have been described between plant distribution ranges and macroclimatic variables such as minimum temperature (Sakai and Larcher 1987; Huntley 1990; Woodward 1997). For example, *Fagus sylvatica* is supposed to be excluded from regions with a mean temperature in January below −3°C (Bolte *et al.* 2007). However, mechanistic evidence for these claims is virtually lacking (Hofmann *et al.* 2013).

Stress from freezing temperatures results in damages at different levels, from the cell to the whole organism (Weiser 1970; Pearce 2001), either directly by ice formation or indirectly by freeze dehydration (Pearce 2001; Beck *et al.* 2004). In general, the degree of frost damage depends on (i) the duration and (ii) the intensity of frost stress, as well as on (iii) the rates of cooling (and rewarming) and (iv) the locality of ice formation (Beck *et al.* 2004). While intracellular ice crystals cause the disintegration of cell membranes and result in plasma efflux, with inevitably lethal effects for the cell,

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extracellular ice formation might dehydrate cells but not necessarily result in lethal injuries (Beck *et al.* 2004).

In temperate regions, frost is an annually recurring stressor; thus, species develop hardening mechanisms in autumn to impede intracellular ice formation and to tolerate extracellular ice formation (Beck *et al.* 2007). Because of the high energetic costs imposed by frost hardiness, the hardening level is only sustained when environmentally required (Huner *et al.* 1998). Therefore, plants modulate their frost resistance mechanisms in relation to environmental conditions (Weiser 1970). In addition, frost tolerance varies between organs, tissues, phenological stage and age of the species (Sakai and Larcher 1987; Calmé *et al.* 1994; Lennartsson and Ögren 2003; Bigras *et al.* 2004; Taschler *et al.* 2004; Augspurger 2009). In the case of deciduous trees, buds are the most important hibernation tissue, and bud hardening occurs gradually by accumulation of carbohydrates and progressive dehydration of the buds (Siminovitch *et al.* 1953; Sakai and Larcher 1987; Améglio *et al.* 2004; Morin *et al.* 2007; Callister *et al.* 2008).

Bud burst is a crucial phenological stage for trees (Pop *et al.* 2000) because increasing temperatures in early spring initiate bud dehardening (Leinonen *et al.* 1997; Beck *et al.* 2007; Hänninen *et al.* 2007) and freshly opened buds with young unfolded leaves are highly sensitive to frost damage (Cannell and Smith 1984; Dittmar *et al.* 2006; Augspurger 2009). Thus, earlier bud burst increases the risk of late frost damage on freshly opened buds and young leaves (Maxime and Hendrik 2011). As a result, trees face a trade-off between maximizing the duration of the photosynthetically active period and minimizing the risk of frost damage (Lockhart 1983; Saxe *et al.* 2001; Leinonen and Hänninen 2002; Kramer *et al.* 2010). For example, comparing juvenile trees of different deciduous species in the US, Augspurger and Bartlett (2003) found a significant correlation between time of bud burst and carbon gain. In contrast, early dehardening in spring bears the high risk of injuries by late spring frost events (e.g. Cannell and Smith 1986; Prozherina *et al.* 2003). This provides an explanation why northern populations of *Quercus petraea* displayed latest bud burst and highest late spring frost tolerance (Ducousso *et al.* 1996). Even if the frost damage at this stage was not lethal, it would ensue a reduced assimilation and reduced growth in that year (Dittmar and Elling 1999; Schweingruber and Nogler 2003; Clark 2010).

When comparing among-species differences in responses to environmental factors, within-species variation might be taken into account, as within-species can be higher than among-species variation at a particular site (Clark 2010). With respect to frost, testing this assumption would require taking samples from different climatic zones for different species, preferably also including the range boundaries of the different species. However, simultaneous sampling from different regions would involve the risk of including plants with a different degree of acclimatization and, thus, of comparing hardened with dehardened plants (Kathke and Bruelheide 2011). An alternative would be provenance trials of different tree species grown under the same common garden conditions, which, to our knowledge, do not exist. In any case, we would not expect that intra-specific adaptation overrules inter-specific differences in frost tolerance, as geographic range limits have been successfully interpreted by the species ecophysiological properties (Sakai and Larcher 1987; Jäger 1990; Milnes *et al.* 1998). This view is also supported by the findings that the potential geographic range of a species can be predicted from ecophysiological functions by mechanistic models that apply to the species as a whole (Pearson and Dawson 2003; Hijmans and Graham 2006).

The present study analysed frost damage of winter buds and freshly developed leaves of different deciduous tree species from Central Europe. We hypothesized that early-budding species show a higher frost resistance of freshly developed leaves after bud burst than species with a bud burst later in spring. Thus, late-budding species would escape the risk of frost damage by late frosts. Secondly, we hypothesized that the degree of frost hardiness of winter buds as well as freshly opened buds of a species is reflected in the species' geographic range, with species reaching farther into cold regions in winter and spring displaying higher frost tolerances. Hence, differences in frost hardiness among plant species should reflect the species' physiological potential to tolerate the minimum temperatures experienced over the whole geographical range where the species occurs.

2. Methods

2.1 *Study species and sampling design*

We investigated frost hardiness of closed buds and freshly opened buds with developed leaves of eight Central European deciduous tree species (table 1). The buds were taken from adult trees growing outside, thus acclimation followed outdoor conditions (figure 1). Frost hardiness was determined twice in 2010: first in March before bud burst and, depending on the species, in March or April exactly two days after bud burst (for the exact dates, see table 1). The study species all sampled in near vicinity to each other across the city of Halle (Saale), thus avoiding differences in climatic conditions that might affect hardening and dehardening. From the available trees at a site, individuals of a species were selected randomly. The measurements before bud burst represent frost hardiness close to the maximum, as the species had been exposed to frost temperatures throughout the winter and dehardening did not started yet. The

Table 1. Locality and sampling date before and after bud burst of the eight investigated tree species

Species	Locality (decimal degrees)		Sampling date	
	Latitude (N)	Longitude (E)	Before bud burst	After bud burst
Betula pendula Roth	51.492903	11.935010	08.03.2010	06.04.2010
Carpinus betulus L.	51.489379	11.962099	02.03.2010	31.03.2010
Fagus sylvatica L.	51.507332	11.925347	01.03.2010	25.04.2010
Fraxinus excelsior L.	51.488725	11.944768	01.03.2010	28.04.2010
	51.510041	11.954827		
Juglans regia L.	51.489339	11.958316	02.03.2010	19.04.2010
Salix x *rubens* Schrank	51.494918	11.953218	16.03.2010	28.03.2010
	51.493494	11.955545		
	51.493848	11.948703		
Tilia cordata Mill.	51.493047	11.939792	09.03.2010	15.04.2010
Ulmus laevis Pall.	51.502245	11.947365	16.03.2010	11.04.2010

Note that *Fraxinus excelsior* and *Salix* x *rubens* have been collected at different, but proximate locations.

measurements after bud burst represent the same phenological phase for all species, irrespective of the actual date of bud burst. The sampling directly after bud burst made sure that all species had the same degree of dehardening. In the following, we refer to the developing new leaves including the bud scales as freshly opened buds (figure 2).

2.2 Frost measurements

We measured frost hardiness in a climate test chamber (SANYO Atmos Chamber MTH−4400). Freshly harvested buds and young not yet unfolded leaves were exposed
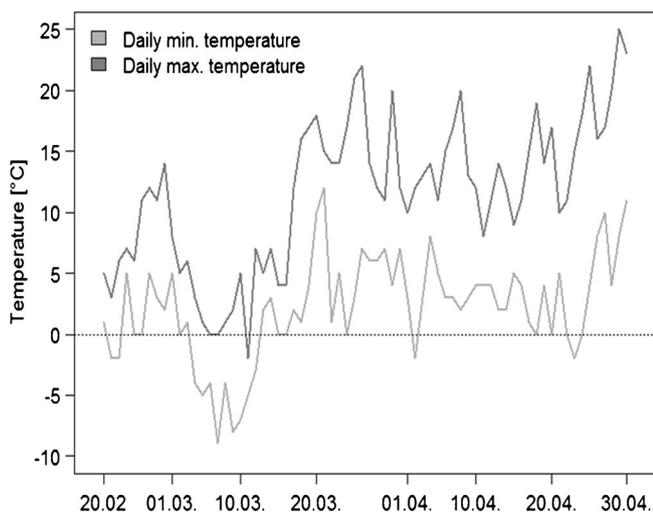

Figure 1. Climatic conditions during the study period in winter and spring 2010 of the sample according to *www.wetter-online.de* (weather station: Halle (Saale)).

stepwise to 11 temperature levels (+4°C, −4°C, −8°C, −12°C, −16°C, −20°C, −24°C, −28°C, −32°C, −40°C, −80°C) with eight replicates per level. Each temperature level took 45 min (resulting in 8.15 hours in total) and samples were freeze with a cooling rate of 0.13°C / min. At the end of one temperature level, one sample batch with buds was removed and stored at +4°C to the next day (see Hofmann *et al.* 2013). On the next day after frost exposure, the buds were transferred into test tubes with isopropanol solution and tested for electrolyte leakage according to Murray *et al.* (1989). The electric conductivity in the solution was measured six times: first immediately after preparing the whole buds (C_0), followed by four measurements after 4 h, 24 h, 48 h and 72 h after the transfer into test tubes (C_t), and a final measurement after boiling the samples for 20 minutes (C_b), which results in a complete destruction of the tissues and maximum electric conductivity. The first measurement was needed to define the baseline for electric conductivity, the final measurement served to scale the response to the maximum potential electric conductivity of that particular plant organ. We calculated the Relative Conductivity (*RC*) using formula 1 (Murray *et al.* 1989):

$$RC_t = \frac{C_t - C_0}{C_b - C_0} = 1 - e^{-k*t} \tag{1}$$

The rate of electrolyte leakage (*k* values) of every species was used to calculate a 4-parametric sigmoid regression according to formula 2:

$$k = f(T) = c + \frac{a}{1 + e^{-\left(\frac{T - LT_{50}}{b}\right)}} \tag{2}$$

The regression parameter LT_{50} describes the point of inflection of the resulting curve and is the temperature at which 50% of the maximum electrolyte leakage was reached.
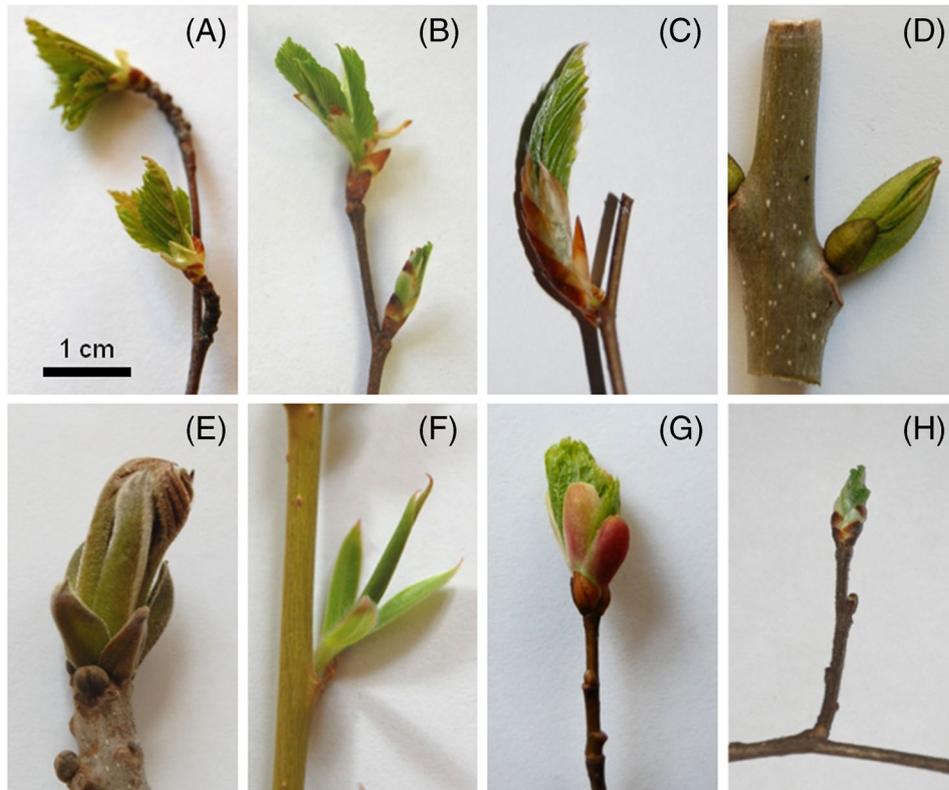
Figure 2. State of bud development defined as freshly opened buds of *Betula pendula* (**A**), *Carpinus betulus* (**B**), *Fagus sylvatica* (**C**), *Fraxinus excelsior* (**D**), *Juglans regia* (**E**), *Salix* x *rubens* (**F**), *Tilia cordata* (**G**) and *Ulmus laevis* (**H**).

2.3   Statistics

Frost hardiness as described by LT_{50} was related to the species' macroclimatic niches. This was achieved by compiling the global distribution for every species. The distribution range of the study species was derived from floristic atlases and online published databases. As the distribution data of *Salix* x *rubens* Schrank were not reliable, we combined the range data from the two parent species *Salix fragilis* L. and *Salix alba* L.. All distribution data were georeferenced and digitized using ArcMap (ESRI) and then used for extracting climatic data for all occurrence points from the Worldclim dataset (Hijmans *et al.* 2005).

To determine species macroclimatic niche we extracted the lower 1 %-percentile of annual mean temperature, annual precipitation, temperature annual range, minimum temperature of the coldest month and minimum temperature per month for November until April as well as the upper 1%-percentile of annual precipitation, temperature annual range, precipitation of the coldest quarter and precipitation per month for November until April. A total of 22 macroclimatic variables was compiled. The relationships of both LT_{50}

values of closed buds and freshly opened buds to all macroclimatic variables were tested by linear regression models. With multiple testing and assuming random distribution of predictor variables, we would expect 5% of the 22 models (i.e. 1.1 models) to be significant by chance. Thus, for assuming a significant relationship between frost hardiness and macroclimatic niche, more than one significant regression of LT_{50} values to macroclimatic variables would be required. Incremental model improvement by forward selection was employed to identify the variables with the highest absolute correlation coefficients.

The sigmoid regressions were calculated with Sigmaplot 11.0 (Systat Software 2008), whereas all the other statistical analyses were conducted using R 2.12.0 (R Development Core Team 2010).

3.   Results

All species showed a higher LT_{50} value of closed buds than of freshly opened buds. While frost hardiness of closed buds

differed between −17.1°C and −40.5°C, the freshly opened buds displayed a lower range from −8.5°C to −14.8°C (table 2). In addition, frost hardiness of closed buds was not significantly related to frost hardiness of freshly opened buds (p=0.943, figure 3).

The date of bud burst was neither related to the frost hardiness of the closed buds (p=0.950) nor the freshly opened buds (p=0.390, figure 4). While the species with earliest bud burst showed only moderate frost resistance (*Salix* x *rubens*, −10.2°C), the most frost tolerant species (*Ulmus laevis*, 14.8°C) had an intermediate bud burst phenology.

Among all macroclimatic variables tested, there was no significant correlation to the LT_{50} value of the closed buds (table 3). The species with the highest and the lowest levels of frost hardiness before bud burst (−40.5°C and −17.1°C for *Tilia cordata* and *Juglans regia*, respectively) had distribution ranges that extended into regions with similarly extreme frosts; thus both species displayed about −24°C as minimum temperatures of the coldest month (p=0.695, figure 5). Conversely, the species with lowest and highest minimum temperature in the coldest month (−50.8°C and −10.1°C for *Betula pendula* and *Fagus sylvatica*, respectively; for distribution maps see Appendix) did not differ in their frost hardiness of the closed buds (about −30°C).

There were only two significant correlations between the LT_{50} values of the freshly opened buds and the macroclimatic variables (table 3). The LT_{50} values of the freshly opened buds were significantly correlated with precipitation of the coldest quarter and precipitation in December in the species' distribution range (p=0.024, figure 6). The species with the lowest precipitation in the coldest quarter as well as lowest precipitation in December (*Ulmus laevis*) showed the highest frost hardiness, whereas the species with the highest precipitation values (*Fraxinus excelsior*) displayed the lowest frost hardiness.
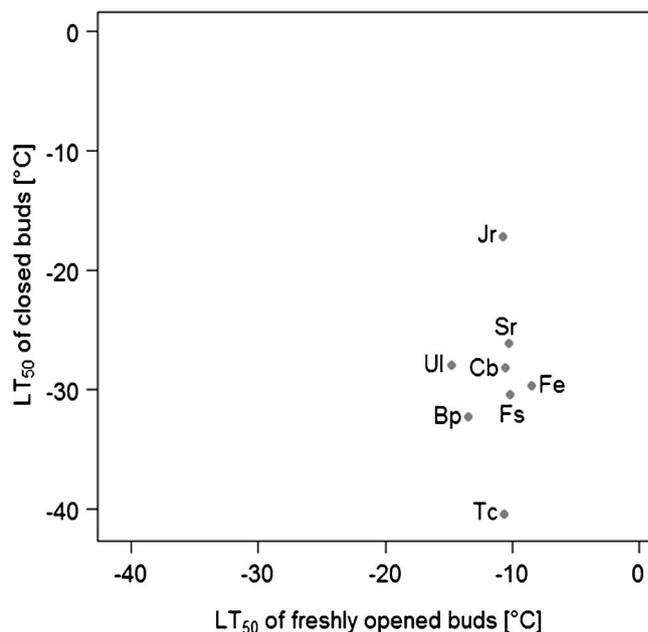

Figure 3. Frost hardiness of the closed buds expressed as LT_{50} values and frost hardiness of the freshly opened buds (p=0.943). Bp=*Betula pendula*, Cb=*Carpinus betulus*, Fe=*Fraxinus excelsior*, Fs=*Fagus sylvatica*, Jr=*Juglans regia*, Sr=*Salix* x *rubens*, Tc=*Tilia cordata*, Ul=*Ulmus laevis*.

4. Discussion

4.1 *Frost hardiness and date of bud burst*

Freshly opened buds, i.e. the young, yet unfolded leaves, of all species tested were more or less frost-resistant to the same degree. In addition, frost hardiness of the different species showed no correlation with the date of bud burst. Thus, our first hypothesis has to be rejected.

Table 2. Minimum temperature of the coldest month (lower 1%-percentile) as derived from the climate envelope of the study species and LT_{50} values from the 4-parametric sigmoid regression of the buds (± standard error) before and after bud burst

|	Min. temp. of coldest month	LT_{50} of closed buds	LT_{50} of freshly opened buds
Betula pendula	−50.8°C	−32.3°C ± 2.46°C	−13.5°C ± 2.15°C
Carpinus betulus	−10.3°C	−28.2°C ± 0.63°C	−10.5°C ± 1.12°C
Fagus sylvatica	−10.1°C	−30.4°C ± 0.88°C	−10.2°C ± 2.35°C
Fraxinus excelsior	−15.4°C	−29.7°C ± 1.32°C	−8.5°C ± 1.17°C
Juglans regia	−23.7°C	−17.1°C ± 0.67°C	−10.7°C ± 1.41°C
Salix x *rubens*	−16.1°C	−26.1°C ± 1.52°C	−10.2°C ± 1.02°C
Tilia cordata	−23.5°C	−40.5°C ± 1.28°C	−10.6°C ± 0.95°C
Ulmus laevis	−21°C	−27.9°C ± 1.60°C	−14.8°C ± 1.15°C
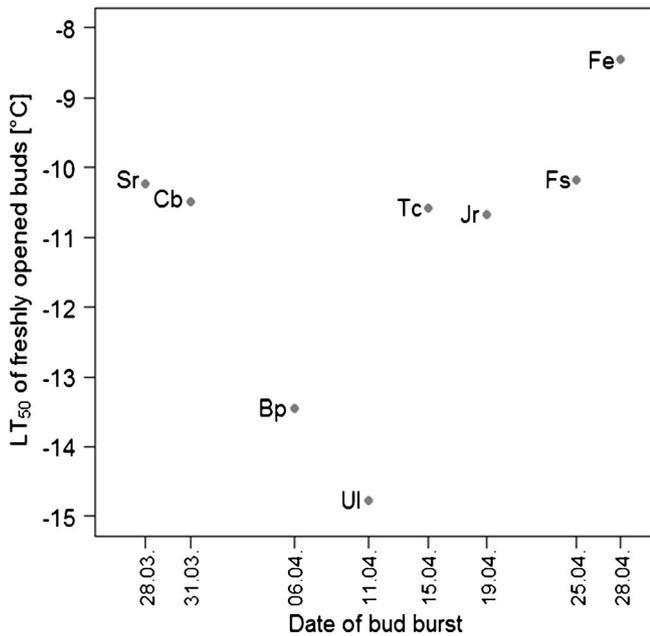
Figure 4. Frost hardiness of the freshly opened buds expressed as LT_{50} values as a function of the date of bud burst ($p=0.390$). Bp=*Betula pendula*, Cb=*Carpinus betulus*, Fe=*Fraxinus excelsior*, Fs=*Fagus sylvatica*, Jr=*Juglans regia*, Sr=*Salix* x *rubens*, Tc=*Tilia cordata*, Ul=*Ulmus laevis*.

The absence of a relationship between frost hardiness before and after bud burst can be explained by the limited number of ways growing tissues can cope with frost stress. The main strategy is the decrease in osmotic potential, either by accumulating sugar, organic acids or compatible solutes (e.g. Améglio *et al.* 2004; Morin *et al.* 2007). Nevertheless, there are osmotic constraints to lowering freezing temperatures, obviously confining this mechanism to frosts of −8°C to −14°C (Sakai and Larcher 1987). The significant relationship between precipitation in winter (precipitation of the coldest quarter as well as precipitation in December) and frost hardiness of the freshly opened buds has probably been caused by the tissue's water contents. Species usually experiencing dry winters might produce new developing shoots and leaves with lower water content, a hypothesis that has not been tested yet. Then, lower water contents of the buds would have indirectly resulted in a higher frost resistance (e.g. Améglio *et al.* 2004; Morin *et al.* 2007). Whatever the mechanism, there is probably no ecological relevance because the range of frost hardiness encountered was not large, and no relationship was encountered to the minimum temperatures to which the plants in the species range are exposed at the time of bud burst.

However, the degree of frost hardiness displayed by the different species was sufficient to withstand frost temperatures at the study site that occurred in the whole span of bud burst. Thus, the investigated species with early bud burst are not better protected but have the strategy of taking higher risks of frost damage than the late budding species. Frost damage should occur more frequently in early budding species and it can be assumed that early budding species are able to compensate the loss of injured tissues. Although late frost damages have been found to reduce net primary productivity (Awaya *et al.* 2009) and to reduce tree-ring growth (Dittmar *et al.* 2006), many species are able to flush a second time. Although this involves high costs (Augspurger 2009), the second flush leaves can reach even higher rates of photosynthesis (St. Clair *et al.* 2009).

4.2   *Frost hardiness and species' distribution*

Frost hardiness of the closed buds was not related to any variable that described the species' macroclimatic niches, although at least one significant correlation would have to be expected. Similarly, frost hardiness of the freshly opened buds was only related to the precipitation in the coldest month and to that of December. However, we have to consider that these correlations were false positives, and simply the result of multiple hypothesis testing. This interpretation is supported by the fact that there were no significant relationships to winter temperatures. Thus, we have to reject our second hypothesis, because the degree of frost tolerance of a species was not reflected by the temperatures in the species' geographic range.

There are several possible explanations for this outcome. Relationships between frost hardiness and biogeography might exist but we might have focused on the wrong life stage (e.g. Cannell and Smith 1984; Sakai and Larcher 1987; Repo *et al.* 2001) or measuring the wrong plant organ. It is well-known that frost hardiness varies between the ages of plants (e.g. Bigras *et al.* 2004, Taschler *et al.* 2004), with seedlings exhibiting a lower frost hardiness than adults. For example, Morin *et al.* (2007) showed for different European *Quercus* species that frost hardiness of adults was significantly higher than that of seedlings. Furthermore, it might be that frost affects adult trees more through xylem embolism caused by freeze–thaw events. In this case, frost hardiness of adult trees would be not determined by bud frost resistance but by wood properties. This would explain the relationship tracheid lumen diameter and altitudinal distribution of *Pinus sylvestris* provenances in Spain (Martín *et al.* 2010). Alternatively, relationships between frost hardiness and the temperatures tolerated in the species' geographical distribution range (i.e. the species' macroclimatic niches) do not exist across different species, which would have far-reaching implications for climate niche models and future climate predictions (see below). The frost experienced at the experimental site for all species was certainly well above the

Table 3. Summary of correlation coefficients (R) and *p*-values (*p*) of Pearson's rank correlation testing for relationships of LT_{50} values as obtained from the 4-parametric sigmoid regression against macroclimatic variables (from the Worldclim dataset)

	LT_{50} value of buds			
	Before bud burst		After bud burst	
	R	*p*	R	*p*
LT_{50} values of buds after bud burst [°C]	0.031	0.943	-	-
Date of bud burst	−0.027	0.950	0.354	0.390
Sampling date	−0.141	0.740	0.335	0.388
Annual mean temp.[°C]	0.063	0.883	0.471	0.239
Annual precipitation [mm] LP	−0.450	0.263	0.444	0.271
Annual precipitation [mm] UP	−0.396	0.331	0.705	0.051
Temp. annual range [°C] LP	0.581	0.130	−0.503	0.204
Temp. annual range [°C] UP	−0.210	0.618	−0.601	0.115
Min. temp. of coldest month [°C] LP	0.166	0.695	0.562	0.147
Precipitation of coldest quarter [mm]	−0.002	0.996	**0.734**	**0.038**
Min. temp. November [°C]	0.181	0.668	0.540	0.168
Min. temp. December [°C]	0.198	0.639	0.559	0.149
Min. temp. January [°C]	0.166	0.695	0.562	0.147
Min. temp. February [°C]	0.180	0.670	0.570	0.140
Min. temp. March [°C]	0.174	0.680	0.557	0.152
Min. temp. April [°C]	0.113	0.790	0.427	0.291
Precipitation November [mm]	−0.668	0.070	0.673	0.067
Precipitation December [mm]	−0.295	0.478	**0.776**	**0.024**
Precipitation January [mm]	0.032	0.941	0.698	0.054
Precipitation February [mm]	0.264	0.527	0.620	0.101
Precipitation March [mm]	0.342	0.407	0.562	0.148
Precipitation April [mm]	0.568	0.142	0.409	0.314

In all regressions, degrees of freedom=6. LP=lower 1%-percentile, UP=upper 1%-percentile.
Bold fonts indicate statistically significant differences.

minimum temperatures that the species are able to tolerate in other parts of the distribution range. Thus, the comparably mild winter period in the study area might have allowed the species to stay above their physiological limits. Differences in frost hardiness might only become apparent when plants are exposed to more extreme conditions (Nielsen and Rasmussen 2009). More extreme frost temperatures might also result in a higher degree of hardening. Support for this idea comes from the observation that frost hardiness increases in the course of the cold season (Sutinen *et al.* 1992; Martz *et al.* 2006; Morin *et al.* 2007; Nielsen and Rasmussen 2009; Poirier *et al.* 2010; Kathke and Bruelheide 2011). In deciduous tree species, an increase in frost hardiness is brought about by increasing dehydration of the buds (e.g. Sakai and Larcher 1987; Beck *et al.* 2007). Furthermore, adult trees might suffer much less from frost damage compared to seedlings and juveniles (e.g. Cannell and Smith 1984; Sakai and Larcher 1987; Woodward 1987; Repo *et al.* 2001; Bigras *et al.* 2004). Frost damage to

seedlings has been often described as potential cause of distribution range boundaries. For example, the northern distribution limit of *Pinus sylvestris* has been attributed to the seedlings' resistance to withstand low temperatures (Repo *et al.* 2001).

The alternative explanation would be that the investigated species actually do not differ in frost hardiness. In fact, although the correspondence of physiological performance and macroclimatic niche of different species is commonly assumed in biogeography (Jäger 1992), there are not many experimental studies supporting this idea. Such rare examples for a relationship of frost tolerance to the macroclimatic niche across different species are provided by different ericoid species in the British Iles (Bannister and Polwart 2001) and by eight temperate and subtropical tree species in Australia (Cunningham and Read 2006). At first sight, the absence of a relationship between macroclimate niche and frost hardiness across different species might be counterintuitive but might be explained by a strong intra-specific
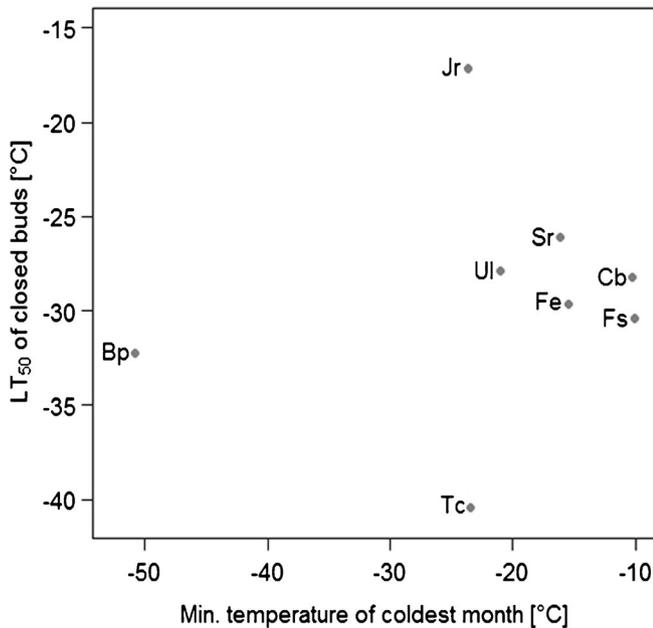
Figure 5. Frost hardiness of the closed buds expressed as LT_{50} values as a function of minimum temperature of the coldest month ($p=0.695$). Bp=*Betula pendula*, Cb=*Carpinus betulus*, Fe=*Fraxinus excelsior*, Fs=*Fagus sylvatica*, Jr=*Juglans regia*, Sr=*Salix* x *rubens*, Tc=*Tilia cordata*, Ul=*Ulmus laevis*.

Figure 6. Frost hardiness of the freshly opened buds expressed as LT_{50} values as a function of the precipitation in December (upper 1%-percentile) ($p=0.024$, $R^2=0.602$). Bp=*Betula pendula*, Cb=*Carpinus betulus*, Fe=*Fraxinus excelsior*, Fs=*Fagus sylvatica*, Jr=*Juglans regia*, Sr=*Salix* x *rubens*, Tc=*Tilia cordata*, Ul=*Ulmus laevis*.
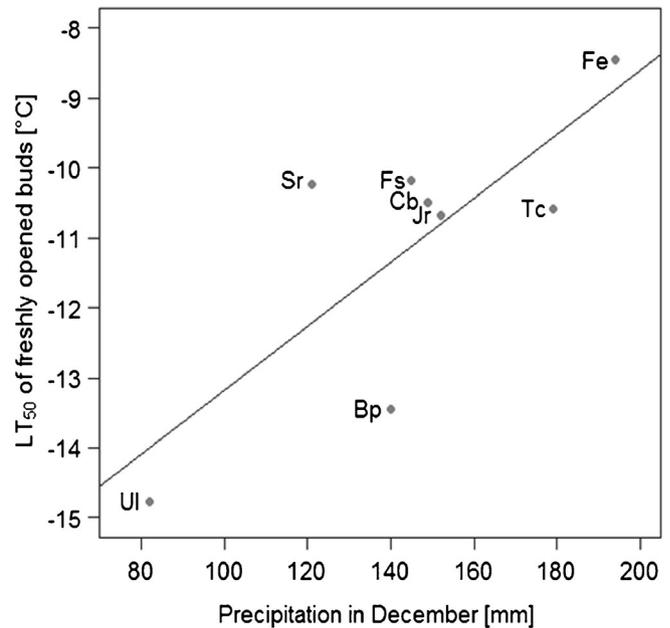
differentiation. Differences in frost hardiness between different provenances have been described for many tree species (Sakai and Weiser 1973; Lawes *et al.* 1995; Beuker *et al.* 1998; Leinonen and Hänninen 2002; Kathke and Bruelheide 2011). These differences in frost hardiness among provenances have often resulted in significant relationships to the climatic conditions at the origin localities. For the example of *Fagus sylvatica,* Visnjic and Dohrenbusch (2004) found a tight relationship between winter frost hardiness and the mean annual minimum temperature at the origin sites. Similarly, Kathke and Bruelheide (2011) detected a much lower LT_{50} in montane compared to lowland morphotypes of *Picea abies*. Repo *et al.* (2001) were able to relate frost hardiness of *Pinus sylvestris* provenances to latitude of origin. Morin *et al.* (2007) as well as Jensen and Deans (2004) described differences in frost hardiness of different provenances of *Quercus* species in Europe. However, there are also studies that failed to detect differences in winter frost hardiness among provenances (Deans and Harvey 1996; Beuker *et al.* 1998). Irrespective of the existence of a correlation between frost hardiness and temperatures at the populations' origins, it might well be that the variation in frost hardiness of different species growing at the same locality is lower than that of different provenances of the same species.

5. Conclusion

In summary, we have to conclude that the frost hardiness of the study species assessed at our site both in winter and at the time of bud burst did not correspond to the species' macroclimatic niche, as derived from the species' distribution ranges. This might have important implications for the current use of climate envelopes, which are much *en vogue* for predicting the climate change impacts on species distribution ranges (e.g. Iverson and Prasad 1998; Thomas *et al.* 2004; Thuiller *et al.* 2005). In almost all models, winter minimum temperatures are important predictors as they coincide, for example, with northern and eastern distribution boundaries in Europe (Huntley *et al.* 1995; Pompe *et al.* 2008; 2010). However, if these distribution boundaries are not mechanistically linked to frost hardiness, changing winter temperatures might not result in the foreseen range shifts of species in a changing climate. Given the high intraspecific differentiation in frost hardiness observed in many species (e.g. Lawes *et al.* 1995; Deans and Harvey 1996; Beuker *et al.* 1998; Leinonen and Hänninen 2002), species might rather respond with rapid evolution rather than with migration. Such evolutionary responses have been described for phenological shifts in response to periods of drought

(Franks *et al.* 2007), and might also apply to adaptive responses to frost.

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Corresponding editor: R Geeta