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Differences in frost hardiness of two Norway spruce morphotypes growing at Mt. Brocken, Germany

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ABSTRACT

Norway spruce (*Picea abies* (L.) Karst.) exhibits strong ecotypic variation along altitudinal gradients in morphological traits, e.g. slenderness of crowns or arrangement of second-order branches. We were interested whether montane and lowland morphotypes differ in a key trait for the survival in cold environments, i.e. frost hardiness, and asked: (i) are montane morphotypes more resistant to frost damage and (ii) do they have a lower risk of frost damage by late frosts in spring than lowland morphotypes?

We used the electrolyte leakage-method to measure frost hardiness on a monthly basis from October 2006 to May 2007 in stands of the montane and lowland morphotypes at Mt. Brocken in the Harz Mountains, Germany.

LT_{50} (i.e. the temperature that results in 50% of maximum electrolyte leakage) was assessed by freezing treatments in a frost chamber and was significantly influenced by morphotype, month and minimum ambient temperatures. LT_{50} was significantly lower in the montane than in the lowland morphotype, with −10.7 °C and −4.9 °C, respectively. However, the interactions between morphotype with minimum ambient temperature or month were not significant. Thus, as frost hardiness of the two morphotypes responded to temperature in the same way, both morphotypes can be supposed to be exposed to the same risk of frost damage during hardening in autumn and dehardening in spring.

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Introduction

A key trait for the survival of coniferous tree species in the boreal zone is frost hardiness. Frost hardiness of plants is associated to a multitude of physiological processes (Sutinen et al., 2001). Water loss by extracellular ice formation results in concentrating cellular solutes, decreasing water potentials and changes in membrane potentials (Beck et al., 2004, 2007), which the protoplast can tolerate by preceding synthesis of specific proteins and membrane lipids (Kozlowski and Pallardy, 2002). A widely distributed mechanism to prevent intracellular freezing and thus frost damage is deep supercooling (Sutinen et al., 2001). In some plants such as conifers, the concentrated cell solution can even turn into a state of glass (vitrification; Strimbeck et al., 2007). Plant tissues in this stage survive even temperatures of −196 °C (Larcher, 1994; Strimbeck et al., 2007).

In principle, frost hardiness consists of three determining components: first, genetically fixed traits, second, epigenetic effects and third, the physiological adaptation of individuals to environmental conditions. The genetically fixed component determines absolute thresholds of frost tolerance among species and is controlled by several genes (Beck et al., 2004). A considerable amount of genetic variation in frost hardiness has been found among and within conifer populations (Aitken and Hannerz, 2001; Savolainen et al., 2004), which is the basis of both present and future adaptations to the environment. The review by Aitken and Hannerz (2001) revealed that the degree of genetic control differs for different aspects of frost hardiness. The heritability for spring frost hardiness is generally high, while heritability for frost hardiness in autumn is lower and more variable. In addition, frost hardiness underlies epigenetic effects (Johnsen et al., 2005), i.e. changes in gene function which are mitotically and/or meiotically heritable but are not associated with changes in the DNA sequence (Wu and Moris, 2001). For example, in a study on *Picea abies* Johnsen et al. (1996) demonstrated that the environmental conditions experienced by the parent trees, especially in the period of female flowering, had a major impact on the frost hardiness of progenies. Flowering in an early and warm spring created less hardy progenies than flowering in a late and cold spring. Finally, frost hardiness is determined by the ability of physiological adjustment of individuals to the ambient environmental conditions, i.e. the ability of hardening and dehardening (Beck et al., 2004). Hardening and dehardening are strongly affected by ambient temperature (Jönsson et al., 2004; Søgaard et

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al., 2009), day length/photoperiod (Beck et al., 2004; Beuker et al., 1998), nutrients (Jönsson et al., 2001) and drought stress (Beck et al., 2007). While the initiation of hardening in autumn is mainly driven by photoperiod, the dehardening in spring is mainly regulated by temperature (Beck et al., 2004). For *P. abies* it has been demonstrated that dehardening starts already after five days with temperatures over +5 °C (Jönsson et al., 2004). This type of environmental control can be particularly crucial in the context of ongoing climate change, because higher temperatures will result in earlier dehardening (Hänninen et al., 2001). With an expected increase of magnitude and frequency of weather extremes (Hundecha and Bárdossy, 2005), the IPCC report predicts a rise in minimum and maximum temperatures as well as of mean temperatures in winter for the northern hemisphere (Solomon et al., 2007). The result will be an earlier dehardening, which might increase the risk of late frosts in spring, and thus, the risk of frost damage.

To assess this frost damage various methods have been developed, such as *in situ* determination (Buchner and Neuner, 2009; Taschler and Neuner, 2004) or visual assessment (Jensen and Deans, 2004) and, most commonly, electrolyte leakage measured as relative conductivity (Bruelheide and Heinemeyer, 2002; Bruelheide and Lieberum, 2001; Murray et al., 1989; Thomas and Sporns, 2009). Jensen and Deans (2004) compared the visual assessment and measurement of relative conductivity and demonstrated that the latter provided the most statistically consistent results.

Picea abies (L.) H. Karst. is one of the major coniferous tree species of the boreal zone in Europe with a high ability to tolerate low temperatures (Hannerz and Westin, 2005; Sakai and Okada, 1971) and a strong ecotypic variation along altitudinal and latitudinal gradients in frost hardiness (Johnsen and Skrøppa, 2000). Ecotypic variation is not only encountered at the continental scale, but also regionally. For example, Skrøppa (1991) found differences in frost hardiness between *P. abies* populations that were separated by only 60 km at the same altitude and longitude. When seedlings from origins of high and low altitudes were grown together at sea level, the latter developed their frost hardiness later (Skrøppa et al., 2007). Recently, regional, native ecotypes of *P. abies* have become the focus of nature conservation efforts because they represent valuable genetic resources from a time before industrial forestry started in the 18th century throughout Central Europe, and before lowland genotypes were planted at high altitudes. This mixing of provenances bears the risk of genetic erosion through introgression (Akimoto et al., 1999), and in consequence, the loss of certain traits such as a decrease in frost hardiness.

One well known example of such a risk is the native, i.e. montane morphotype of *P. abies* in the core zone of the Harz National Park at Mt. Brocken, Germany, which is today surrounded by planted, lowland morphotypes. This montane population includes individuals that are older than the initiation of forest management in this area. So far, the main evidence for its native status is its morphology, visible in a slender, cylindrical and tall growth habit (Greger, 1991) as well as in a higher number and reduced length of second-order branches, which are less horizontal compared to the lowland morphotype (Geburek et al., 2008; Greger, 1991). Consequently, the native, montane morphotype is known to show an increased resistance to snow and ice break (Greger, 1991) and is considered to be better adapted to the harsh environment at high altitudes, probably at the expense of lower growth rates (Oleksyn et al., 1998). That said, there is so far no empirical evidence for higher frost resistance of the native genotype. Likewise, no attempt has been made to relate frost hardiness to morphological traits that differ between montane and lowland morphotypes. Such morphological variation with regard to climatic variables has been ascribed for example to *Betula pendula* (Li et al., 2002), where an increase of frost hardiness was accompanied by an increase in specific leaf mass. Similarly, ecotypic variation in *Pinus sylvestris* was found for leaf dry matter content, which is in turn correlated to frost hardiness (Bresinsky et al., 2008).

We addressed the question of differences in absolute frost tolerance and timing in frost hardiness between different provenances of lowland and montane ecotypes of *P. abies* in the Harz Mountains, Central Germany. In particular, we first hypothesised that montane morphotypes are more resistant to frost damage than lowland morphotypes, and second, that montane morphotypes have a lower risk of frost damage by late frosts in spring than lowland morphotypes.

Materials and methods

Study area and sampling design

The study was conducted in the Harz Mountains in Saxony-Anhalt, Germany. Two different sampling sites were selected separated by a linear distance of about 6 km. Lowland morphotypes were taken from a planted Norway spruce (*Picea abies* (L.) Karst.) stand adjacent to the core zone in the Harz National Park at Mt. Brocken (51°44′08″N, 10°40′39″E, 550 m a.s.l.) and native (montane) morphotypes were sampled from spruce stands inside the core zone (51°47′21″N, 10°38′27″E, 930 m a.s.l.).

We selected 10 adult *P. abies* individuals per site at the edge of the stand to ensure that the sampled individuals did not differ in frost exposure. Only individuals with a minimum height of 15 m, a minimum circumference at breast height (cbh) of 1.0 m and without any crown break or indications of fungal infections were chosen. One first-order branch was selected per individual and marked. Second-order branches from all marked branches were sampled in monthly intervals and subsequently subjected to measurements of frost hardiness in the laboratory between October 2006 and April 2007 (sampling dates: 22/10/2006, 26/11/2006, 17/12/2006, 21/01/2007, 18/02/2007, 25/03/2007, 22/04/2007).

One of the lowland individuals broke down in January 2007 as a result of the storm “Kyrill”. Accordingly, from January onwards, only nine individuals of the lowland morphotype were sampled.

Ambient temperatures at both sampling sites were monitored with data loggers (Type Tinytag, Gemini), one installed per site on the marked branch of one of the target individuals. Temperature was logged every 30 min from October 2006 until May 2007.

Measurements of frost hardiness

Frost hardiness was assessed using the method of electrolyte leakage according to Murray et al. (1989). This method is based on the release of electrolyte leakage through membranes which have been damaged by intracellular ice formation. The increase of electrolytes in a solution is measured as electrical conductivity. Branches were exposed to the following temperature levels in a climate test chamber (Sanyo, MTH-4400): +4 °C (control), 0 °C, −8 °C, −16 °C, −24 °C, −32 °C, −40 °C, −80 °C and to liquid nitrogen (−196 °C). The temperature level of −80 °C was starting in December. All temperature levels lasted for 30 min in the climate test chamber, with the first four temperature levels having a cooling rate of $-4\,^{\circ}C\,h^{-1}$, and the last four temperature levels in the climate test chamber having a cooling rate of $-6\,^{\circ}C\,h^{-1}$. These cooling rates are in the range of those commonly applied in frost experiments (e.g. Hannerz and Westin, 2005; Thomas and Ahlers, 1999), but might represent a more extreme situation than normally encountered in field. At the end of each temperature level, twigs as parts of the branches from each individual were removed from the climate test chamber and stored at +4 °C in a refrigerator. One twig from each individual was placed into liquid nitrogen for 10 min after receiving all eight temperature levels in the climate chamber.

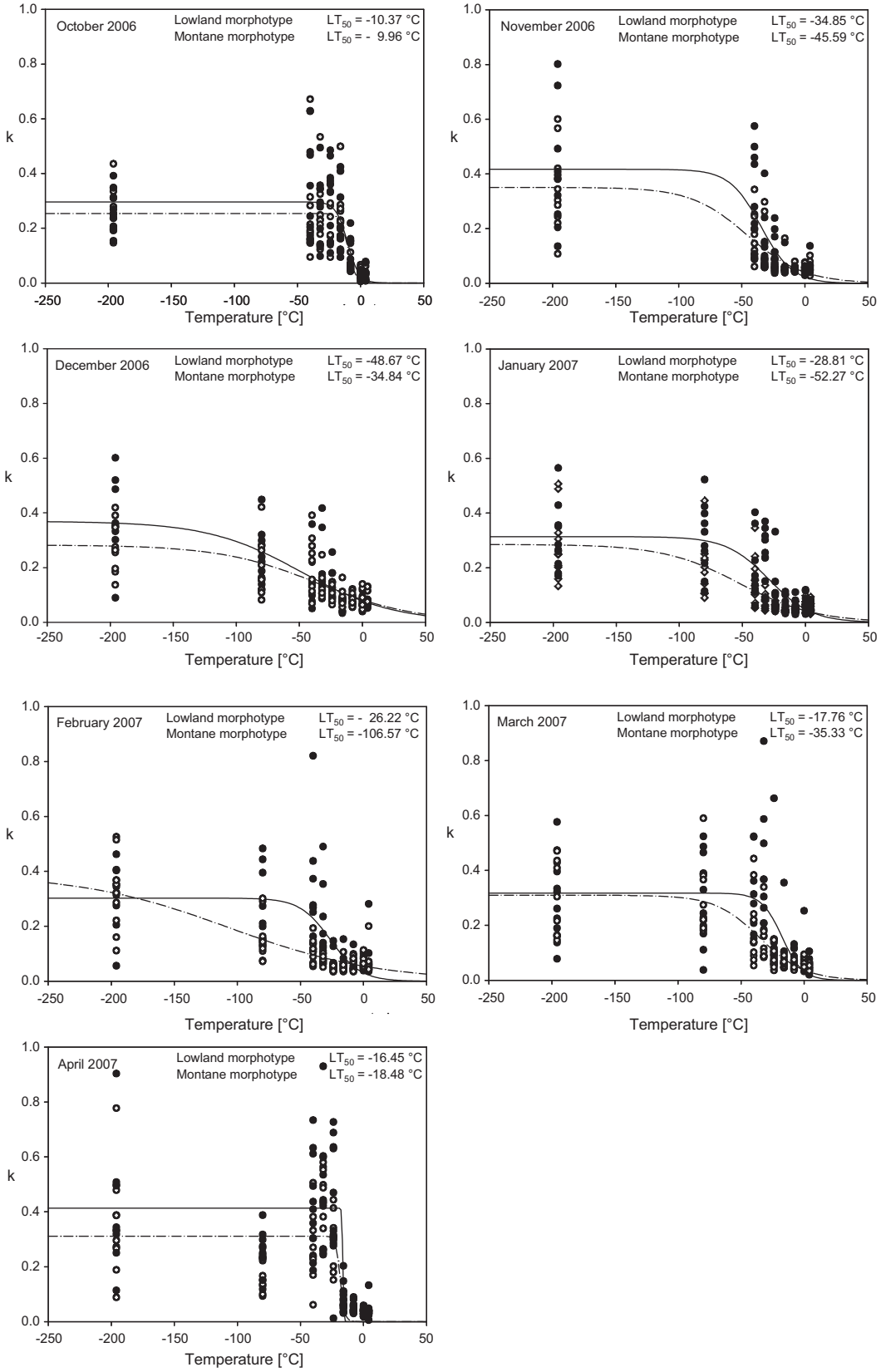

Fig. 1. Assessment of the LT_{50} value per month (October 2006 to April 2007) of the lowland (planted ●) and montane (native ○) morphotype by using k values and the corresponding temperature levels of the freezing treatment for a three-parameter sigmoid regression $f = a/(1 + \exp(-(x - x_0)/b))$, where x_0 is the LT_{50} value. All regressions were significant ($P < 0.0001$). Continuous lines indicate the regression curve of lowland morphotype, whereas dashed lines the montane morphotype.

Table 1
Results of the two-factorial ANOVA with temperature level of the freezing treatment and morphotype as fixed factors and *k* value for the different sampling times (month) as response variable. Shown are the results of the Type III test (proc glm, SAS, 2002).

Month	Effect	*DF*	Type III SS	MS	*F*	*P*
October	Temperature level	7	1.746	0.249	24.20	**<0.0001**
	Morphotype	1	0.187	0.019	1.81	0.1805
	Temperature level × morphotype	7	0.075	0.011	1.04	0.4072
November	Temperature level	7	1.792	0.275	31.56	**<0.0001**
	Morphotype	1	0.038	0.038	4.35	**0.0387**
	Temperature level × morphotype	7	0.064	0.009	1.06	0.3950
December	Temperature level	8	1.126	0.141	27.88	**<0.0001**
	Morphotype	1	0.012	0.012	2.28	0.1327
	Temperature level × morphotype	8	0.077	0.010	1.91	0.0620
January	Temperature level	8	1.382	0.173	30.75	**<0.0001**
	Morphotype	1	0.107	0.107	18.98	**<0.0001**
	Temperature level × morphotype	8	0.125	0.016	2.79	**0.0065**
February	Temperature level	8	1.430	0.179	26.58	**<0.0001**
	Morphotype	1	0.102	0.102	15.15	**0.0001**
	Temperature level × morphotype	8	0.200	0.025	3.72	**0.0005**
March	Temperature level	8	2.000	0.250	26.36	**<0.0001**
	Morphotype	1	0.117	0.117	11.86	**0.0007**
	Temperature level × morphotype	8	0.292	0.037	3.70	**0.0006**
April	Temperature level	8	5.247	0.656	44.16	**<0.0001**
	Morphotype	1	0.254	0.254	17.13	**<0.0001**
	Temperature level × morphotype	8	0.274	0.034	2.31	**0.0230**

Significant variables ($P < 0.05$) are in bold.

On the subsequent day, needles were clipped from each twig with a cutter above their stalk and then cut crosswise into two halves to allow better access of cell contents to the surrounding medium, which otherwise is impeded by the needle's thick wax cover. Ten needles per individual and temperature level were placed in test tubes with 10 ml 3% isopropanol, resulting in a total of 160 samples for October and November 2006 (2 morphotypes × 10 replicates × 8 temperature levels) and 180 samples from December 2006 onwards (2 morphotypes × 10 replicates × 9 temperature levels).

Frost damage was assessed by measuring the temporal increase of electrical conductivity (C) in the isopropanol solution in the test tubes at +4 °C after $t = 0$ h (C_0), 4 h, 24 h, 48 h, 72 h (C_t), using an automated sample processor equipped with a conductivity meter (Metrohm, 712 Conductometer). On the third day, after the last measurement, the samples were boiled in their tubes at +100 °C for 30 min to bring about complete electrolyte leakage. After cooling the solution to +4 °C, electrical conductivity was measured once again (C_{boiled}). Relative conductivity (RC) was calculated according to Murray et al. (1989) by using the following formula:

$$\text{RC} = \frac{C_t - C_0}{C_{\text{boiled}} - C_0} = 1 - e^{-kt} \quad (1)$$

Relative conductivity (RC) was fitted to a saturation curve using a one-parameter nonlinear regression (see formula (1), proc nlin, SAS 8.0, SAS Institute Inc., 2000). The estimate of parameter k can be taken as a measure for frost injury as k increases with the rate of electrolyte leakage from the damaged cells.

Statistical analysis

Differences in k were tested with a two-factorial ANOVA (proc glm, SAS 9.1, SAS Institute Inc., 2002), including the temperatures of the freezing treatment and morphotypes as fixed factors. In addition, all variables were also compared using contrasts of the control temperature of +4 °C versus all other temperatures of the freezing treatment (contrast and estimate statements, proc glm, SAS 9.1, SAS Institute Inc., 2002).

We used k to calculate the LT_{50} value, defined as the temperature at which the slope of electrolyte leakage reached 50% of the maximum k value. A three-parameter nonlinear regression was fitted to the k values according to the following formula:

$$f = \frac{a}{1 + \exp(-(x - x_0)/b)} \quad (2)$$

where x_0 is the LT_{50} value (SigmaPlot 9.0, Systat Software, 2004). LT_{50} was calculated for each month and each morphotype, using samples from all individuals.

To assess differences in ambient temperature (daily mean temperature and absolute monthly minimum temperature) between both sampling sites, we conducted a two-way ANOVA, including month as random factor (proc glm, SAS 9.1, SAS Institute Inc., 2002). Finally, the relationship of LT_{50} to observation month and morphotype were analysed in an ANCOVA, using ambient minimum temperature (absolute monthly minimum temperature) as covariate (proc glm, SAS 9.1, SAS Institute Inc., 2002). To obtain the best estimates of LT_{50} values, the ANCOVA model was optimised by removing non-significant interactions. Only minimum ambient temperature and morphotype were retained in the final model.

Results

The temperature level of the freezing treatment in the frost chamber had a significant effect on electrolyte leakage, visible in significantly different k values (in all months $P < 0.0001$; Table 1). As shown in Fig. 1, k decreases with increasing temperatures, following a sigmoid curve.

Morphotypes differed significantly in their k values for most of the months with native, montane morphotypes displaying generally lower k values, and thus, a lower degree of frost injury (Tables 1 and 2; Fig. 1). A different response of morphotypes to minimum temperatures is also evident in an interaction between freezing treatment and morphotype, which was significant from January 2007 onwards (Table 1). The different response of morphotypes to temperatures is also visible in different slopes of the regression curves in Fig. 1. In general, the montane morphotype

Table 2
Thresholds for frost damage of the different morphotypes in the different sampling months. The values show the lowest temperature levels of the freezing treatments that were not significantly different ($P > 0.05$) compared to the control (+4 °C) due to the contrast statement in the ANOVA (proc glm, SAS 9.1, SAS Institute Inc., 2002).

	Temperature level [°C]	
	Lowland morphotype	Montane morphotype
October	−8	−8
November	−32	−32
December	−24	−32
January	−24	−24
February	−24	−40
March	−24	−24
April	−16	−16

was characterised by more shallow slopes, especially in February 2007 (Fig. 1).

Fig. 1 also shows LT_{50} values per morphotype and month. Except for October and December 2006, the native provenances showed a higher degree of frost hardiness as displayed by lower LT_{50} values than the lowland morphotype. The largest difference in LT_{50} was encountered in January and February 2007 with observed LT_{50} values in the frost chamber between the two morphotypes of −29 °C versus −52 °C and −26 °C versus −107 °C, respectively. In contrast, the difference in LT_{50} values between both morphotypes was only 0.4 °C and 2 °C in October 2006 and April 2007, respectively.

Because of the different elevation of the two growth locations, the ambient daily mean temperature differed significantly between the lowland and montane sampling sites ($P < 0.0001$, Fig. 2). However, no significant differences between the two sampling sites were detected for absolute monthly minimum temperatures ($P = 0.3532$, Fig. 2). The seasonal variation in minimum temperatures was obvious. January 2007 was the coldest month at both sampling sites with −14.2 °C and −11.7 °C at the lowland and montane sampling site, respectively.

The analysis of covariance revealed a significant effect of minimum ambient temperature as covariate and of both class variables, morphotype and month, on LT_{50} (Table 3). The lower the minimum ambient temperature, the lower the LT_{50} value ($P = 0.0353$, Type I SS). The slope of this relationship did not differ between morphotypes, as the interaction between minimum ambient temperature and morphotype was not significant ($P = 0.4809$, Type I SS). There was also no significant interaction between minimum ambient temperature and month ($P = 0.4860$, Type I SS). In consequence, both interaction terms were removed from the model. To optimise our model, we removed all interactions except for the one between morphotype and month, which however also did not turn out to be significant ($P = 0.8544$, Type I SS; Table 3), and indicated the absence of differences in the seasonal course of LT_{50} between morphotypes.

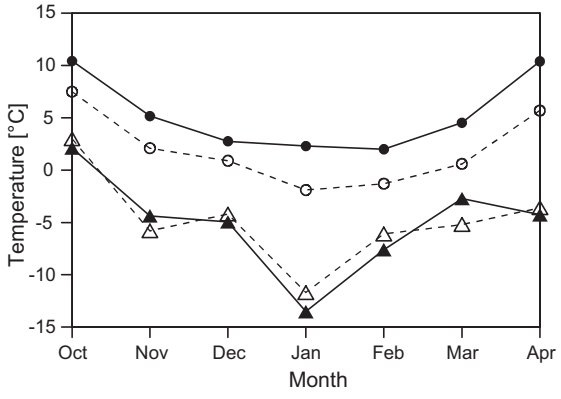

Fig. 2. Seasonal variation of ambient temperature at the lowland (planted) and montane (native) sampling sites. Daily mean temperature is significantly different between lowland (●) and montane (○) sampling sites (two-way ANOVA, $DF = 1$, $F = 266.69$, $P < 0.0001$), while absolute monthly minimum temperature was not significant between lowland (▲) and montane (△) sampling sites (two-way ANOVA, $DF = 1$, $F = 1.01$, $P = 0.3532$).

Table 3
Results for the ANCOVA of LT_{50} as response variable including minimum ambient temperature as covariate and morphotype and month as fixed factor. Shown are the results of the Type I SS (proc glm, SAS, 2002). To optimise the model, interactions of minimum ambient temperature with morphotype and between minimum ambient temperature and month were excluded as these interactions were not significant.

Effect	*DF*	Type I SS	MS	*F*	*P*
Minimum temperature	1	49750.406	49750	4.53	**0.0353**
Morphotype	1	50938.468	50938	4.64	**0.0332**
Month	6	251012.705	41835	3.81	**0.0016**
Morphotype × month	6	28631.760	4772	0.43	0.8544

Significant variables ($P < 0.05$) are in bold.

In a final model, we retained only minimum ambient temperatures and morphotype without including interaction terms. The result is shown in Fig. 3, where LT_{50} is plotted against minimum ambient temperatures. The two morphotypes show the same slope of decreasing LT_{50} with minimum temperatures ($P = 0.4369$), but differed in intercepts ($P = 0.042$), with lower LT_{50} values for native individuals.

Discussion

The most significant result of this study with regard to our first hypothesis is that lowland (planted) and montane (native) morphotypes exhibited significant differences in frost hardiness. This is consistent for both the *k* value as a measure for frost injury (for five of the seven observed months) and for the LT_{50} value with minimum temperature as covariate. The LT_{50} value as a measure of frost hardiness of the needles exhibited for the montane morphotype a minimum value of −106.6 °C, while LT_{50} of the lowland type attained a value of only −48.7 °C. Even though our frost treatment has been carried out under artificial conditions in the frost

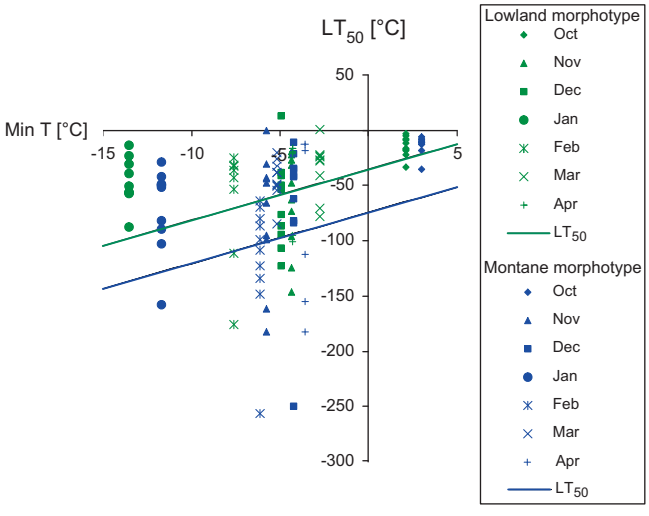

Fig. 3. LT_{50} values of the 10 sampled individuals per morphotype with the corresponding minimum ambient temperature for all seven investigated months. Additionally, the estimated LT_{50} values across all individuals and months of lowland (planted) and montane (native) morphotypes are assessed. Estimates for the regression lines were obtained from the final model that included only morphotype and minimum ambient temperatures, but no interaction terms. Both factors, morphotype and minimum ambient temperature were significant with $P = 0.042$ ($DF = 1$, $F = 4.21$) and $P = 0.044$ ($DF = 1$, $F = 4.11$), respectively.

chamber and cooling rates in nature might often be lower, the outcome for the lowland morphotype is in the range of results reported in the literature (−50 °C, Sakai and Okada, 1971; −45 °C, Hannerz and Westin, 2005). Although the frost hardiness of −106.6 °C of native individuals in February 2007 can be considered exceptionally high, values of similar magnitude were described by Stushnoff and Juntilla (1986) with −80 °C for Norway spruce.

To ensure survival, the frost hardiness should attain values substantially lower than the encountered ambient temperatures (DeHayes et al., 2001). At both sampling sites the minimum ambient temperatures did not fall under −15 °C during the mild winter of 2006/2007. Thus, both morphotypes would theoretically be able to grow on top of Mt. Brocken, albeit with a different safety buffer with respect to low temperature extremes.

An inherent problem of a study like ours with adult trees is that the response of individuals might be both dependent on the environment as well as on their genotype and on epigenetic effects. Therefore, genotype × environment interactions are generally tested with common garden experiments. These are however often not feasible with long-lived species such as trees. For this reason, the majority of studies on frost hardiness of different provenances have been carried out using seedlings and saplings (e.g. Hannerz and Westin, 2000; Jensen and Deans, 2004; Ögren et al., 1997; Savolainen et al., 2004). We can assume that the significance of frost hardiness is much more important for adult than for juvenile individuals, as the latter is able to escape damaging freezing temperatures under snow cover. Thus, even experimentally impeccable studies on saplings may have less relevance for adult trees. Although our field study did not involve completely equal site conditions as provided in a common garden experiment, the lowland and montane locations were largely comparable with respect to the most relevant site factor, i.e. minimum ambient temperatures. This might be surprising as the difference in elevation should also be reflected in minimum temperatures. The explanation is likely the complex topography of the Harz Mountains with narrow ridges and deep valleys, which very often display temperature inversions, as a result of downward movement of cold air streams (Glässer, 1994; Pflume and Bruelheide, 1994).

Under comparable minimum ambient temperatures at both sites, we found a statistically different frost hardiness between the two morphotypes, even at a time when dehardening occurs. Differences among ecotypes were also found for eleven north European provenances of *Quercus robur* cultivated side-by-side in a nursery in Denmark, with provenances from oceanic origins being less frost resistant than continental ones (Jensen and Deans, 2004).

With regard to our second hypothesis, we found lower frost hardiness of the lowland morphotype but no interaction between morphotype and month. Thus, there was no indication that timing and rate of hardening and dehardening during autumn and spring differed between the two morphotypes. In both morphotypes, frost hardiness fluctuated in the same way and, pointing to an equal response to dehardening temperatures in spring.

This result is not in line with results from glasshouse experiments conducted by Taulavuori et al. (2004) with five different Norwegian ecotypes of *Betula pubescens*. In their study, higher winter temperatures accelerated dehardening, with the highest rate of dehardening encountered in the alpine ecotype, whereas the oceanic type showed the slowest rate. The lack of differences between our *P. abies* provenances would also imply that both morphotypes would perform similarly under different global change scenarios with increased winter temperatures. Even if dehardening occurs earlier under these conditions, frost tolerance of needles in spring would be high enough in both morphotypes to prevent damage from late frost events. The frost hardiness of the lowland morphotype with LT_{50} values of about −17 °C in March and April would be sufficient to withstand frost events in this period, as long-term climate observations between 1948 and 2008 revealed that a minimum temperature of −16.8 °C, and −11.1 °C has never been transgressed in March and April, respectively (German Weather Service). Furthermore, taking into account that the results were obtained under artificial conditions in the frost chamber and not *in situ*, the safety margin might be even higher. In consequence, our study does not confirm theoretical predictions of greater frost damage under global warming scenarios that have been put forth by some authors (e.g. Hänninen, 1991). This conclusion conforms to modelling results. Evaluating two different models for temperature increase Kramer (1994) concluded that the probability of spring frost damage to trees will decrease in Germany. So far, this conclusion might hold only for needles but not for other organs that are essential to secure growth and survival such as buds. Although buds in most evergreen species have been found to be more frost resistant than needles (Bannister and Neuner, 2001), there are also studies showing a lower frost resistance of buds of *P. abies* (e.g. Beuker et al., 1998). However, Beuker et al. (1998) found the same seasonal course in frost hardiness of *P. abies* buds as observed in needles, with a frost hardiness being consistently lower than the ambient temperature.

In summary, our study demonstrated that the adaptation of the montane spruce morphotype is not only evident in crown-architecture but also in physiology. Nevertheless, with ongoing climate change, both morphotypes probably run the same risk of frost injuries during the period of hardening in autumn and dehardening in spring, as both morphotypes equally respond to seasonal course in temperature.

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