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Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species

XAVIER MORIN,[1,2] THIERRY AMÉGLIO,[3] REIN AHAS,[4] CATHY KURZ-BESSON,[5] VOJTĚCH LANTA,[6] FRANÇOIS LEBOURGEOIS,[7] FRANCO MIGLIETTA[8] and ISABELLE CHUINE[1]

[1] *Centre d'Écologie Fonctionnelle et Évolutive, Équipe BIOFLUX, CNRS, 1919 route de Mende, 34293 Montpellier cedex 5, France*

[2] *Corresponding author (xavier.morin@cefe.cnrs.fr)*

[3] *UMR PIAF (INRA - Université Blaise Pascal), Site INRA de Crouelle, 234 avenue du Brézet, 63100 Clermont-Ferrand cedex 2, France*

[4] *Institute of Geography, University of Tartu, Vanemuise 46, Tartu 51014, Estonia*

[5] *Instituto Superior de Agronomia, Dept. Botânica e Engenharia Biológica, Tapada da Ajuda, 1394-017 Lisboa, Portugal*

[6] *Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic*

[7] *UMR LERFOB (INRA-ENGREF), Équipe Écologie Forestière, ENGREF – Centre de Nancy, 14 rue Girardet, CS 14216, 54042 Nancy Cedex, France*

[8] *IBIMET (Institute of Biometeorology - CNR), Via Caproni, 8, 50145 Firenze, Italy*

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Summary Although cold hardiness is known to be a major determinant of tree species distribution, its dynamics and the factors that regulate it remain poorly understood. Variation in cold hardiness and carbohydrate concentration, from dormancy induction until bud burst, were investigated in populations of two deciduous (*Quercus robur* L. and *Quercus pubescens* Willd.) and one evergreen (*Quercus ilex* L.) European oak. Mean cold hardiness values in January were –56, –45 and –27 °C for *Q. robur*, *Q. pubescens* and *Q. ilex*, respectively. Soluble carbohydrate concentrations were closely related to instantaneous cold hardiness, estimated by the electrolyte leakage method, whereas total carbohydrate concentration was related to maximum cold hardiness. Both cold hardiness and carbohydrate concentration showed a close linear relationship with temperatures at the location of the sampled population. Our results show that temporal variation in both the inter- and intraspecific cold hardiness in European oaks can be related to variations in the concentrations of soluble carbohydrates and that these relationships appear to be driven by temperature.

Keywords: adult individuals, climate, distribution, frost damage, natural populations, phenology, Quercus robur, Quercus pubescens, Quercus ilex.

Introduction

Low temperatures play a key role in determining tree species distribution (Shreve 1914, Sakai and Weisser 1973, George et al. 1974, Sakai and Larcher 1987, Pither 2003), especially at the altitudinal and northern range limits (Gusta et al. 1983, Arris and Eagleson 1989, Cavieres et al. 2000). Global vegetation patterns are closely correlated with temperature zones (Woodward 1987). However, many temperate tree species exhibit a high resistance to cold, usually to –70 °C during winter, and their distributions seem unrelated to temperature extremes (Sakai and Weisser 1973). Determining the role of low temperature on the distribution of plant species is a major issue in biogeography (Pither 2003, Wiens and Donoghue 2004) and may become especially important in the context of climate change.

It has been hypothesized that the increase in temperature during this century could lead to an increase in frost damage to plant tissues (Cannell 1985, Hänninen 1991). Higher temperatures advance phenological development (Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003) and hasten spring dehardening. General Circulation Models (IPCC 2001) predict that the frequency of random frost events will remain quite stable as mean surface temperature increases. Therefore, advanced leaf development could be vulnerable to late frosts.

Freezing events may cause stress or injury to plants (Weiser 1970, Pearce 2001) from the cell to the whole-organism level, e.g., membrane injuries through ice formation in intracellular spaces, cell dehydration (by water loss due to extracellular ice formation), xylem embolism, sunscald due to frost and pathogen attacks (facilitated by lesions caused by frost). Tree species subjected to low temperatures, as can occur at high elevations in the tropics (Cavieres et al. 2000), have developed mechanisms to cope with such stresses (Weiser 1970).

The mechanisms enabling plants to withstand subfreezing conditions have been classified into two main categories: avoidance of intracellular ice formation and tolerance of extracellular ice formation and consequent cellular dehydration (Levitt 1978). Avoidance of intracellular ice formation involves supercooling and depends on the presence of anti-nucleation agents such as proteins (Welling et al. 1997, Sung et al. 2003) and polysaccharides (Almeida et al. 1994). To resist water loss, the cell can stabilize its membranes with specialized proteins (Pearce 2001) and with high concentrations of solutes, notably, low molecular weight carbohydrates (Lennartsson 2003, Cavender-Bares et al. 2005). Some extremely low-temperature-resistant boreal species have such high intracellular osmotic concentrations that aqueous glass formation occurs before the formation of intracellular ice (Franks 1985).

The energetic cost of freezing tolerance, as well as the impact of low temperature on mitotic activity (Calmé et al. 1994) does not allow organisms to be equally resistant throughout the year. Species have thus developed strategies to adapt their level of hardiness to environmental conditions (Weiser 1970). It has been shown that cold acclimation is mainly driven by temperature and photoperiod (Huner et al. 1998, Li et al. 2003) and is under strong genetic control (Xin and Browse 2000). Thus, variation in environmental conditions across a species' range can create differential selective pressures leading to the emergence of locally adapted ecotypes (Liepe 1993, Deans and Harvey 1996, Konig and Liepe 1996, Aitken et al. 1996, Boorse et al. 1998*b*, Li et al. 2003, Lu et al. 2003).

Our objective was to compare cold hardiness and carbohydrate composition and concentration throughout the hardening cycle in three European oak species growing in their native environments: the deciduous common oak (*Quercus robur*) and pubescent oak (*Quercus pubescens*), and the evergreen holm oak (*Quercus ilex*). *Quercus robur* and *Q. ilex* are dominant species in the European temperate and Mediterranean ecosystems, respectively. The northern range limit of *Q. robur* is in Scandinavia, whereas *Q. ilex* extends southward to northern Africa, and *Q. pubescens* has an intermediate distribution (see Figure 1). Cold hardiness and the concentration and composition of carbohydrates of stems were measured in adult trees from three populations of each species during early hardening, full hardening and before leaf unfolding. We sought to answer the following question: is cold hardiness related to the climate in the native habitat of the populations studied and to tissue carbohydrate composition or concentration?

Material and methods

Plant material

The studied populations were from the northern, central and southern portions of the European ranges of *Quercus robur*, *Q. pubescens* and *Q. ilex* (Figure 1 and Table 1). We collected 50-cm-long, 1-year-old twigs from 10 adult trees of each population on October 11, 2003 and January 15, 2004. Another sample collection was made just before bud burst on March 1, 2004 for *Q. ilex* and *Q. pubescens*, and on April 5, 2004 for the later flushing *Q. robur*. Successive samplings were made on different trees. The Oléron Island population was not sampled on the first collection date (October 2003). An additional sampling was made in January 2005 of three populations of *Q. robur*, the Montpellier population of *Q. pubescens* and the Montpellier and Mitra populations of *Q. ilex*. All samples were transported to the laboratory in Montpellier within 2 days, and freeze tests started on the third day after sampling. Samples were wrapped in moist paper and sealed in a plastic bag and stored at 5 °C until tested.

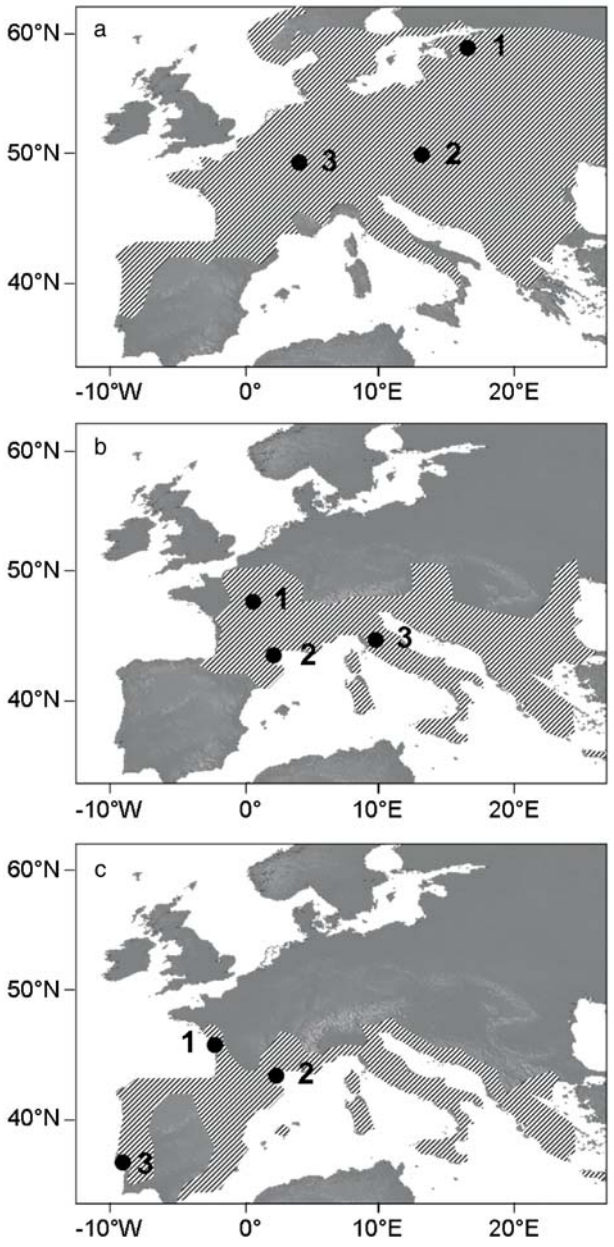

Figure 1. Geographical distribution of (a) *Quercus robur*, (b) *Q. pubescens* and (c) *Q. ilex* (hatched area), and location of the populations sampled (●). For names of population locations, see Table 1. Populations of *Q. ilex* also exist in North Africa.

Table 1. The location, map coordinates, elevation and temperature regime in the native habitat for each of the sample populations of three European oaks. Temperatures are monthly means of the sampling month (Mo.), means for the 14 days before sampling (14 d) and sampling-day means (1 d). Some daily values required to compute the temperature means were unavailable.

Species	Population	Code	Location	Elev. (m)	Temperature (°C)								
					October 2003			January 2004			March–April 2004		
					Mo.	14 d	1 d	Mo.	14 d	1 d	Mo.	14 d	1 d
Q. robur	Tartu, Estonia	1	58° N, 26° E	78	8.8	9.4	4.0	–7.3	–	–	2.0	–	–
	České Budějovice, Czech Republic	2	48° N, 14° E	400	10.6	10.7	11.9	–1.3	–1.6	2.5	4.7	4.7	6.3
	Nancy, France	3	48° N, 6° E	400	9.9	11.8	12.1	0.9	–	–	8.4	–	–
Q. *pubescens*	Orléans, France	1	47° N, 1° E	80	13.2	13.3	12.9	3.4	6.8	4.4	6.9	–	–0.4
	Montpellier, France	2	43° N, 3° E	200	14.7	11.7	13.2	5.8	5.8	4.4	9.5	–	–
	Florence, Italy	3	43° N, 11° E	350	13.5	–	–	7.4	7.8	9.9	7.7	–	4.1
Q. *ilex*	Oléron Island, France	1	46° N, 1° W	20	–	–	–	7.2	10.0	10.2	10.3	–	–
	Montpellier, France	2	43° N, 3° E	60	17.0	–	–	7.0	8.5	8.3	7.3	–	8.9
	Mitra, Portugal	3	8° N, 38° E	200	19.0	19.4	18.1	10.8	10.7	10.7	10.4	10.1	6.9

Freeze tests

Cold hardiness of each individual was estimated by the electrolyte leakage test (Zhang and Willison 1986), which determines frost damage to the plasma membrane by measuring electrolyte leakage from the symplast to the apoplast.

For each freeze test, shoot segments were cooled to one of four sub-zero temperatures. In addition, there was an unfrozen control. Five 5-cm-long segments were cut from each sample shoot, each being placed in a vacuum flask. To promote ice propagation after nucleation, 5 ml of deionized water was added to each sample. The control flask was kept at 5 °C in a cold room while the remaining flasks were placed in a –80 °C freezer until the temperature within the flasks, which was monitored with thermocouples, reached the target temperature. The rate of cooling averaged –5 °C h^{-1}. When the target temperature was reached, the flasks were transferred to a 5 °C cold room where they were held for 24 h to allow temperature equilibration. The tested temperatures were: –5, –10, –15 and –20 °C in October 2003; –5, –15, –30 and –45 °C in January 2004; –5, –10, –20 and –30 °C in March–April 2004; and –15, –30, –45 and –60 °C in January 2005.

After the freezing treatment, the segments were cut into 5-mm-long sections, which were placed in glass vials with 15 ml of deionized water. The vials were shaken for 24 h at 5 °C on a horizontal gravity shaker (Unimax 2010, Heidolph Instruments, Schwabach, Germany). The electrolytic conductivity of the water in which the stem segments were immersed (C_1) was measured at room temperature with a Cyberscan PC 300c meter (Oakton Instruments, Vernon Hills, USA). Samples were then autoclaved at 120 °C for 30 min, cooled to room temperature for 2 h and again shaken for 24 h at 5 °C before a second conductivity measurement at room temperature (C_2) was made. Relative electrolytic leakage (REL) was calculated as (C_1/C_2)100 (Zhang and Willison 1986). We assumed the following relationship between REL and percentage of cellular lysis (L) for each sample:

$$L = \frac{\mathrm{REL} - \overline{\mathrm{REL}_c}}{100 - \overline{\mathrm{REL}_c}} 100$$

where $\overline{\mathrm{REL}_c}$ is the mean relative electrolytic leakage of the control samples.

We then estimated the temperature causing 50% cell lysis (LT_{50}) by linear regression between L and freezing treatment temperature (Timmis 1976, Larcher 1995, Bigras 1997). Mean LT_{50} was calculated for each population from the individual LT_{50} values.

Carbohydrates

A 1-cm-long segment from each sample shoot was analyzed for carbohydrate concentration. Segments were immediately frozen in liquid nitrogen and subsequently lyophilized. Soluble carbohydrates (i.e., glucose, fructose and sucrose, denoted GFS) were extracted with 80:20 (v/v) hot ethanol:water and centrifuged. The pellet was used for starch determination. The superanatant was passed through an ion-exchange column (Bio-rad AG 1-X8 in the carbonate form, Dowex 50W in the H+ form), as described by Moing and Gaudillere (1992). Sucrose, glucose and fructose concentrations were determined spectrophotometrically at 340 nm after enzymatic assays (Boehringer 1984). Starch concentration was determined with a hexokinase-linked assay (Kunst et al. 1984) after hydrolysis of the pellet with amyloglucosidase (Boehringer 1984).

Statistical analyses

Effects of species, population and sample date on frost hardiness and carbohydrate composition were evaluated by analyses of variance (ANOVA) at the individual-tree level with the fixed factor population nested within the fixed factor species and the date of sampling also declared a fixed factor.

The relationship between LT_{50} and carbohydrate concentration was investigated by multiple regression analysis. We assessed the relationship between cold hardiness and climate by

multiple regression of LT_{50} against the mean temperature of the sampling month at the origin and carbohydrate concentrations at the population level. Linear regressions of: (1) mean LT_{50} values; (2) soluble carbohydrate concentration; and (3) total carbohydrate concentration against the mean monthly temperature at the population source were performed for each species separately and altogether. All statistical analyses were performed with R 2.1.1 software (Ihaka and Gentleman 1996).

Climate data

Temperature data were obtained from the meteorological station nearest each sample tree site (Table 1).

Results

Cold hardiness and carbohydrate concentration

The LT_{50}, as estimated by the electrolyte leakage test, varied significantly among species, dates and populations within species (Tables 2 and 3). Based on LT_{50} values, *Q. robur* was the hardiest species, and *Q. ilex* was the least hardy species (Table 2). Mean cold hardiness values in January were –56, –45 and –27 °C for *Q. robur*, *Q. pubescens* and *Q. ilex*, respectively. Student *t*-tests showed that cold hardiness in October varied significantly between the northern (Pop. 1) and the two other (Pop. 2 and 3) populations of *Q. robur* ($t = –2.91$, $P < 0.05$ and $t = –3.61$, $P < 0.01$, respectively) and of *Q. pubescens* ($t = –4.85$, $P < 0.001$ and $t = –5.18$, $P < 0.001$, respectively) (Figure 2). Cold hardiness in March–April varied significantly between the northern and the central populations of *Q. pubescens* ($t = –2.412$, $P < 0.05$). No differences in cold hardiness were detected among populations of *Q. ilex* at any time.

Total and soluble carbohydrate concentrations varied significantly among species (Table 3), especially in January 2004 (*t*-tests, $P < 0.001$ for every pair of species), and among populations within species (Table 3).

There were consistent differences in either cold hardiness or carbohydrate concentrations among populations and species over time (Table 2). Cold hardiness was minimal in October and maximal in January for all populations and species (Table 2, Figures 2a–c). Total carbohydrate concentration decreased from October to March in the deciduous species (*Q. robur* and *Q. pubescens*), whereas it increased in the evergreen species (*Q. ilex*) (Table 2, Figures 2d–f). The ratio between GFS and starch concentration increased in all populations between October and January (hardening period) and decreased from January to March–April (dehardening period) (Figures 2d–f).

Frost hardiness, carbohydrate concentration and temperature

The LT_{50} was related to soluble carbohydrate concentration at both the inter- (multiple regression, $P < 0.001$, $n = 26$) and intraspecific level (Figure 3a). The slopes of the linear regressions of LT_{50} against soluble carbohydrate concentration in January 2004 and January 2005 (Figure 4a) did not differ significantly ($t = 0.31$, $P = 0.76$), suggesting that the relationship between soluble carbohydrates and cold hardiness is unchanged from year to year.

No interspecific relationship between LT_{50} and total carbohydrate concentration was detected. However, when analyzed separately, a relationship was detected in January ($r^2 = 0.62$, $P = 0.02$, $n = 9$, Figure 4b) and in March-April ($r^2 = 0.45$, $P = 0.04$, $n = 9$). The slopes of the linear regressions of LT_{50} against total carbohydrate concentration in January 2004 and January 2005 (Figure 4b) were not significantly different ($t = –1.11$, $P = 0.29$), suggesting that the relationship between maximum

Table 2. Mean temperature causing 50% cell lysis (LT_{50}), soluble carbohydrate concentration (GFS) and total carbohydrate concentration (TC) (± standard error) measured on three dates in three *Quercus* species. Different letters indicate significant differences between species at each sampling date (*t*-tests). Bonferroni corrections were applied to the *t*-tests (significant difference for $P \leq 0.016$). Significant differences between consecutive dates for the same species are indicated by asterisks: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

	October 2003	January 2004	Mar-Apr 2004
LT_{50} (°C)			
Q. ilex	$–11.8 \pm 0.4^{a,**}$	$–27.8 \pm 1.5^{a}$	$–24.6 \pm 0.9^{a}$
Q. pubescens	$–14.6 \pm 0.5^{b,***}$	$–45.4 \pm 3.2^{b,*}$	$–37.6 \pm 1.4^{b}$
Q. robur	$–20.7 \pm 1.3^{c,***}$	$–56.2 \pm 2.9^{c}$	$–46.9 \pm 2.8^{c}$
GFS (mg g^{-1} DM)			
Q. ilex	$12.4 \pm 0.9^{a,*}$	29.8 ± 1.7^{a}	30.6 ± 1.4^{a}
Q. pubescens	$22.3 \pm 0.8^{b,*}$	36.0 ± 1.5^{b}	33.1 ± 1.0^{b}
Q. robur	$23.0 \pm 0.8^{b,**}$	$47.1 \pm 1.7^{c,**}$	24.6 ± 1.5^{c}
TC (mg g^{-1} DM)			
Q. ilex	$20.5 \pm 3.2^{a,*}$	48.5 ± 3.5^{b}	52.9 ± 3.8^{b}
Q. pubescens	$83.8 \pm 3.2^{b,*}$	56.2 ± 4.6^{b}	48.1 ± 3.9^{a}
Q. robur	101.9 ± 3.9^{c}	81.8 ± 5.9^{c}	72.9 ± 4.9^{b}

Table 3. Summary of the analyses of variance of the temperature causing 50% cell lysis (LT_{50}), and soluble (GFS) and total carbohydrate (TC) concentrations in three oak species. Abbreviations: DV, dependent variable; SV, source of variation; S, species; D, sampling date; and P(S), population nested in species.

DV	SV	df	SS	*F*-Ratio	*P*
LT_{50}	S	2	9176.3	35.8	< 0.0001
	D	1	16934	132.2	< 0.0001
	P(S)	3	1033.6	2.7	0.050
	S × D	2	1272.6	5.0	0.008
	P(S) × D	3	54.5	0.1	0.930
TC	S	2	69573	191.5	< 0.0001
	D	1	6615	36.4	< 0.0001
	P(S)	6	8847	8.1	< 0.0001
	S × D	2	32259	88.8	< 0.0001
	P(S) × D	6	6292	5.8	< 0.0001
GFS	S	2	1843.3	10.8	< 0.0001
	D	1	3035.2	35.5	< 0.0001
	P(S)	6	4548	8.9	< 0.0001
	S × D	2	1192	7.0	0.001
	P(S) × D	6	1184	2.3	0.030

cold hardiness and total carbohydrate concentration is also unchanged from year to year.

Both LT_{50} and soluble carbohydrate concentrations were significantly related to the mean monthly temperatures at the location of the sampled population at both the inter- and intraspecific levels (Figures 3b and 3c).

Discussion

Cold hardiness in European oaks

Quercus robur, *Q. pubescens* and *Q. ilex* showed significant differences in cold hardiness (Table 2). Precise quantification of cold hardiness of adult trees of European oak species has not been previously reported. Studies have been conducted on the impact of several factors on cold hardiness in *Q. robur* (Thomas et al. 2004, Cavender-Bares et al. 2005), but they did not provide precise estimates of cold hardiness. Other studies have focused on the cold hardiness of *Quercus petraea* (Matt.) Liebl., a species close to *Q. robur*, but at the seedling stage. These studies reported that seedlings of this species may be hardy to temperatures between –8 °C and –15 °C (Liepe 1993, Konig and Liepe 1996).

Our results are consistent with studies on adult trees of North American *Quercus* species (Sakai and Weisser 1973, Gusta et al. 1983), showing maximum cold hardiness values up to –60 °C. Calmé et al. (1994) assessed the cold hardiness of seedlings of *Quercus rubra* (a species that can be considered ecologically comparable with *Q. robur*) originating from latitude 45° N and found cold hardiness values of between –10 and –20 °C in autumn. These values are consistent with our results for the mid-range and southern populations of *Q. robur*.

The role of carbohydrates in cold hardiness

Total carbohydrate concentration in *Q. robur* and *Q. pubescens* decreased in winter, whereas soluble carbohydrate concentration increased from October to January, resulting in an increase in the ratio of soluble to total carbohydrate concentration (Figures 2d and 2e). We hypothesize that total carbohydrate concentration decreased primarily as a result of respiration in these deciduous species, and that the increase in propor-

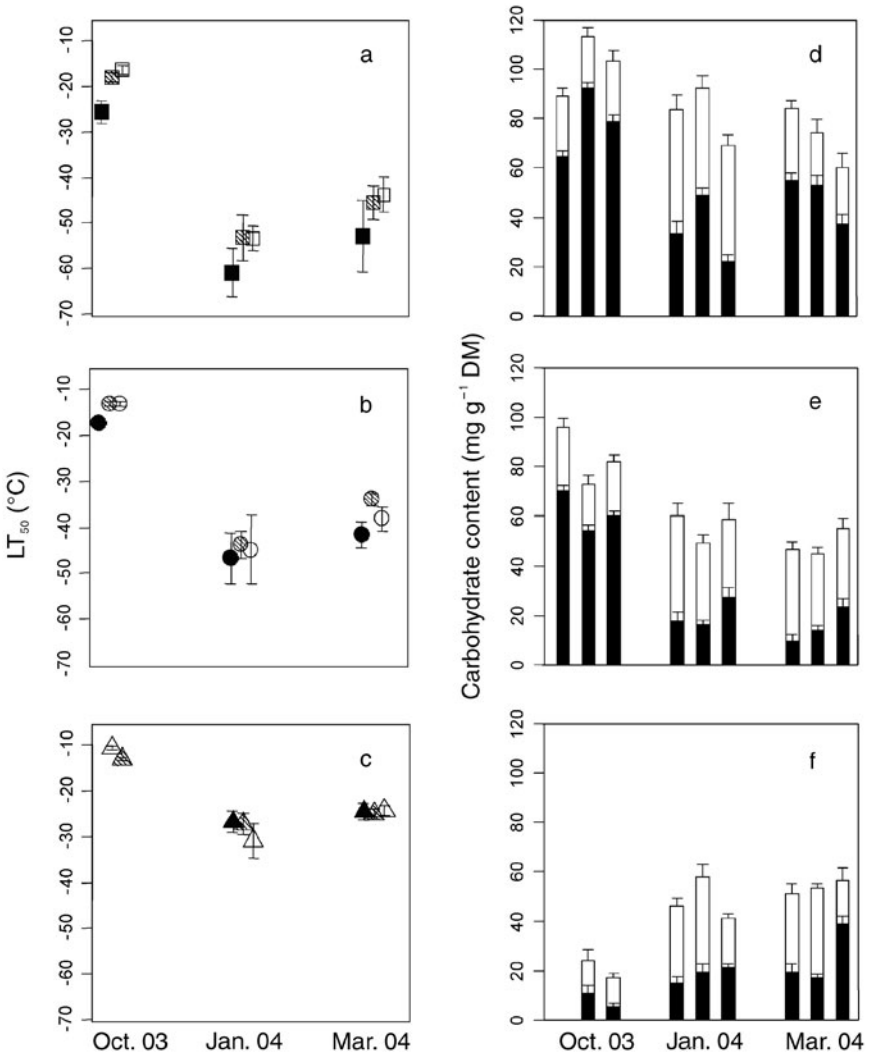

Figure 2. Mean temperature causing 50% cell lysis (LT_{50}) (a, b, c) and mean concentrations of total carbohydrates (d, e, f)—starch (black) and soluble carbohydrates, i.e., glucose, fructose and sucrose (white)—of each population in October 2003, January 2004 and March and April 2004 of *Quercus robur* (a, d), *Q. pubescens* (b, e) and *Q. ilex* (c, f). In figures a, b and c: closed symbols = population 1; hatched symbols = population 2; and open symbols = population 3. In figures d, e and f, for each date, from the left: first bar = population 1; second bar = population 2; and third bar = population 3.

tion of soluble carbohydrates reflected an adaptation that maintains intracellular osmotic concentration. In the evergreen species, *Q. ilex*, the total carbohydrate concentration increased continuously from October to March (Figure 2f), suggesting that winter is a favorable period for evergreen Mediterranean species to accumulate carbon, as shown by Rambal et al. (2003).

Cold hardiness was positively related to soluble carbohydrate concentration (Figure 3a), and the relationship was similar from year to year (Figure 4). This result is consistent with many other studies (Siminovitch et al. 1953, Kramer and Kozlowski 1979, Sauter and Ambrosius 1986, Frossard and Lacointe 1988, Sauter and van Cleve 1994, Améglio et al. 2004, Thomas et al. 2004). The relationship we found, however, was stronger than correlations reported in the literature (e.g., $r^2 = 0.42$ versus $r^2 = 0.27$ in Thomas et al. 2004). Our results are consistent with previous studies indicating that mobilization of starch to sucrose invariably accompanies the development of freezing tolerance (Ogren et al. 1997, Pearce 2001, Klotke et al. 2004), and with studies showing similar dynamics in the changing ratio of starch:soluble sugars in European temperate tree species during winter (Essiamah and Eschrich 1985).

Our results indicate that total carbohydrate concentration is related to maximum cold hardiness. Total carbohydrate concentration represents the pool of carbon that can be used either

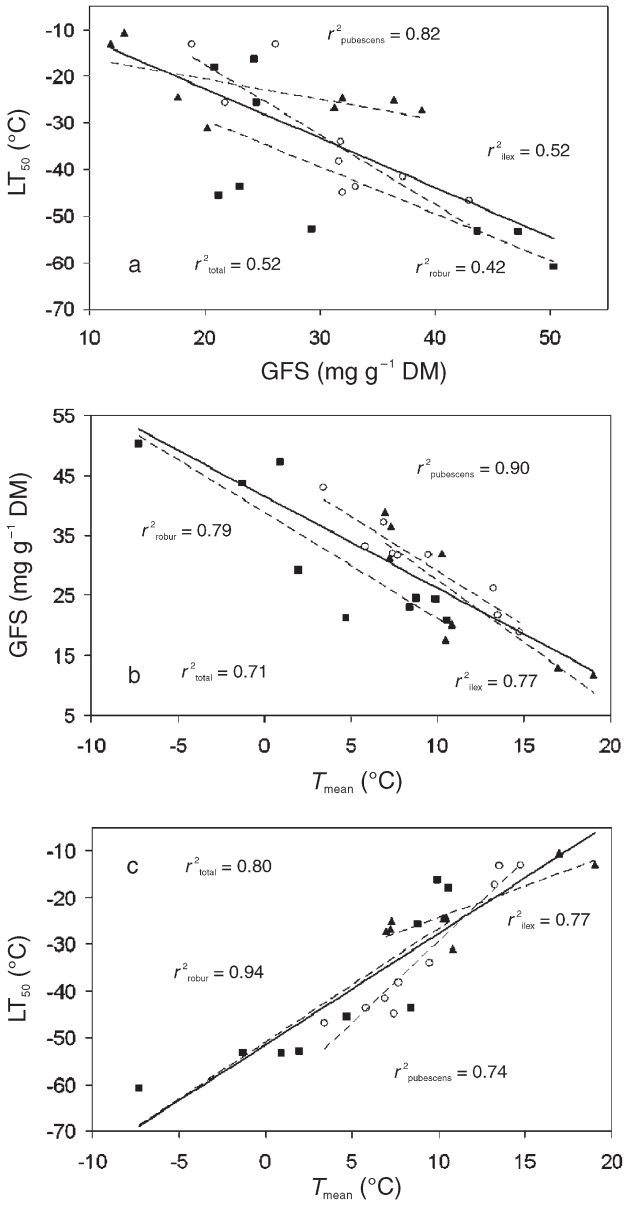

Figure 3. Linear regressions of the population mean temperature causing 50% cell lysis (LT_{50}) against mean soluble carbohydrate concentration (GFS: glucose, fructose and sucrose) on the three sampling dates (a). Linear regressions of population GFS against mean monthly temperature of the sampling month at the location of the sampled population (T_{mean}) (b). Linear regressions of population mean LT_{50} against T_{mean} (c). The solid lines are the all-species regression lines with their associated coefficient of determination (r^2_{total}), and dashed lines are species regressions (■, *Quercus robur*; ○, *Q. pubescens*; and ▲, *Q. ilex*) and associated coefficients of determination (r^2).

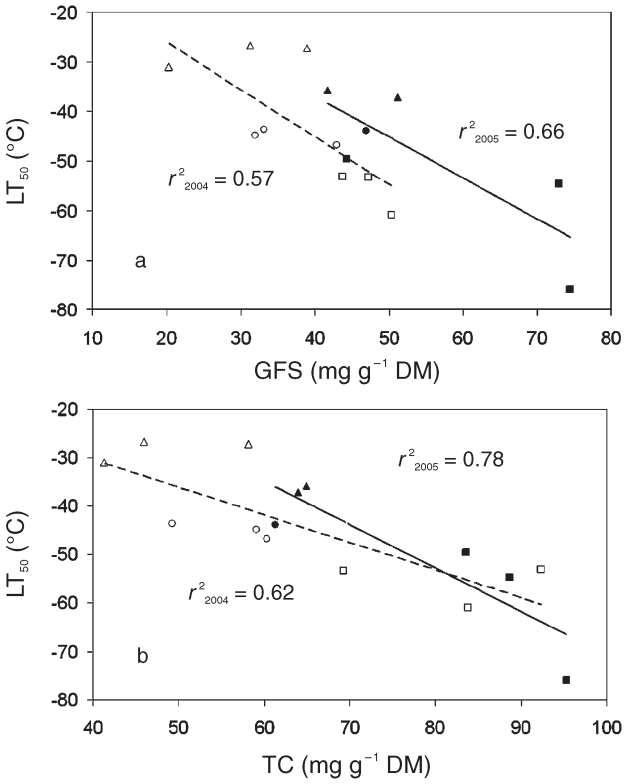

Figure 4. All-species linear regressions of the population mean temperature causing 50% cell lysis (LT_{50}) against (a) mean soluble carbohydrate concentration (GFS: glucose, fructose and sucrose) and (b) mean total carbohydrate concentration (TC) in January 2004 (open symbols) and January 2005 (closed symbols). The dashed lines are regression lines for January 2004 and associated coefficients of determination (r^2), and the solid lines are regression lines for January 2005 and associated r^2 values. Symbols: □, ■, *Quercus robur*; ○, ●, *Q. pubescens*; and △, ▲, *Q. ilex*.

to enhance cold hardiness via hydrolysis to soluble carbohydrates, or to support metabolic processes. At the time of maximum risk (January) of freezing injury, most of the total carbohydrate pool was hydrolyzed to soluble carbohydrates, whereas there was a much smaller proportion of total carbohydrates occurring as soluble carbohydrates at times of lower risk of freezing injury (e.g., October), suggesting that soluble carbohydrates serve in processes other than those associated with growth. According to our results and the studies cited previously, one such role of soluble carbohydrates is in the osmotic adjustment that contributes to the prevention of intracellular freezing. When the risk of freezing injury is low (autumn and spring), total carbohydrate concentration is not necessarily related to cold hardiness because soluble carbohydrates are either allocated to processes such as cell growth or converted to starch. However, in years of exceptional heat or drought, such as 2003 in Western Europe (Schär and Jendritzky 2004, Chuine et al. 2004, Luterbacher et al. 2004), when photosynthetic production is abnormally low, total carbohydrate reserves limit cold hardiness development.

The processes underpinning the relationship between cold hardiness and carbohydrate concentration and composition remain unclear. An increased concentration of soluble carbohydrates lowers the freezing point of the intracellular solution by 1.86 °C per mole of solute dissolved per kg of water (Chang 2001), which means that the measured difference in carbohydrate concentration between hardened and dehardened tissue can explain, at most, only 1–2 °C of the increase in cold hardiness (Sakai 1966, Sauter et al. 1996). However, soluble carbohydrates may have an indirect role in cold hardiness development. If osmotic potential is high in the intracellular solution, freezing occurs first on the surface of the cell wall, in lumens of non-living fibers and vessels or in the extra-cellular spaces (Winget and Kozlowski 1964, Loris et al. 1999, Zweifel and Häsler 2000, Améglio et al. 2001), where osmotic potential is lower, preventing intracellular freezing (Rajashekar et al. 1982, 1983, Hansen and Beck 1988, Guy 1990). This redistribution of water from the symplast to the apoplast protects cells from intracellular freezing but causes dehydration of the protoplast, which is the primary cause of freezing injury in woody plants (Zweifel and Häsler 2000, Améglio et al. 2001).

Cold hardiness, carbohydrates and climate

Although cold hardiness varied significantly among species at all dates, it varied among populations only during the early and late hardening periods (Figure 2). Differences in cold hardiness among populations in autumn and spring at their origin might be caused solely by differences in phenology, which is a highly plastic trait (Larcher 1975), and for which local adaptation has been shown in many species (Rathcke and Lacey 1985). Phenology and cold hardiness are intimately linked. The relationships between LT_{50}, carbohydrate concentrations and temperatures at the population source, during both early and late hardiness, might also be explained by phenological differences among the populations. A warmer climate usually leads to delayed leaf senescence and earlier bud burst so that, in fall and spring, southern populations should exhibit lower cold hardiness than northern populations. However, previous studies (Sarvas 1974, Christersson 1978, Leinonen et al. 1995, Leinonen 1996) indicate that temperature triggers cold acclimation directly as well as indirectly through phenology. Further experiments are therefore required to determine how temperature and changes in carbohydrate concentrations trigger cold hardiness in trees, which is a necessary step to achieve accurate predictions of the impact of climate change on tree frost resistance.

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