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

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Abstract

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

Zusammenfassung

Immergrüne Pflanzen im Unterwuchs temperater Laubwäldern verfügen über ein kurzes Zeitfenster im Frühjahr, während dem sie optimal Photoassimilation betreiben können bevor sich das Kronendach schliesst. Ein Anstieg der Photosynthese-leistung im Frühjahr ist allerdings mit einem Rückgang der Frosthärte verknüpft. Das könnte bei diesen Pflanzen zu Beginn der Saison zu einer erhöhten Anfälligkeit für Frostschäden führen. Das Ziel dieser Studie war es, den zeitlichen Verlauf von Photosynthese und Frostresistenz während des Frühjahres in adulten und juvenilen Blatt-Phänotypen von *Hedera helix* in der Schweiz zu dokumentieren. Die Frostresistenz aller Blätter lag deutlich unterhalb der gemessenen Minimum-Temperaturen auf der Studienfläche, wobei adulte Blätter im Kronenraum eine höhere Resistenz aufwiesen als junge Blätter am Waldboden. Der Anstieg der Photosyntheseleistung erfolgte in beiden Blatt-Phänotypen im Frühjahr erst nach dem Enthärten der Frostre-sistenz. Adulte Blätter zeigten am Winterende und zu Beginn des Frühjahres eine höhere Frostresistenz und entsprechend eine niedrigere Photosyntheseleistung als juvenile Blätter. Allerdings stieg die Photosyntheseleistung adulter Blätter nach den letzten

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stärkeren Frosttagen im Frühjahr stark an und überholte jene juveniler Blättern. Die Frostresistenz blieb aber auch hierbei immer unterhalb der gemessenen minimalen Lufttemperaturen. Das beschriebene zeitliche Muster von Frostresistenz und Photosynthese in *Hedera helix* Blättern verdeutlicht den Konflikt zwischen der Vermeidung von Spätfrostschäden und der Maximierung der Photosyntheseleistung in der kurzen Periode zwischen Winterende und dem Laubaustrieb der kronenbildenden Bäume.

Introduction

Temperate zone deciduous species avoid leaf freezing damage during winter by dropping their foliage and entering into a state of dormancy. Conversely, broad-leaved evergreen species maintain their foliage throughout the year. However, leaves are one of the most freezing sensitive plant tissues and are physiologically costly to maintain, especially during the unfavorable winters of the northern temperate zone (Öquist & Huner, 2003; Sakai & Larcher, 1987). This suggests that the elevational and latitudinal range limits of many broad-leaved evergreen species may be determined by their cold temperature tolerance. Indeed, broad-leaved evergreen distributions in Europe are mostly restricted to the Mediterranean climatic zone, which has relatively mild winters (Sakai, 1980).

The two broad-leaved evergreen species that do exhibit relatively high freezing resistance and thus, inhabit central parts of northern Europe are *Hedera helix* and *Ilex aquifolium* (Iverson, 1944; Sakai, 1980). *H. helix* (common ivy) is unique in that it maintains two phenotypically distinct leaf types on the same plant, adult and juvenile leaves. Adult and juvenile leaf phenotypes may also be referred to as sun and shade leaves. However the terms sun and shade leaves are misleading as leaves are ontogeny-related morphotypes and not a leaf modification in response to light conditions during leaf formation. Therefore we maintain the usage of the terms adult and juvenile leaves, which is consistent with previous similar works (*e.g.* Metcalfe, 2005; Bauer & Bauer, 1980). The names adult and juvenile leaves can be confusing and do not reflect the age of the leaves themselves but rather the life stage of the stem from which the leaves were grown. Adult reproductive stems in *H. helix* are those that are most distant from the basal, non-reproducing juvenile stems (Hoflacher & Bauer, 1982). Both phenotypes may be found in the sun or in the shade but most often *H. helix* changes its leaf phenotype from juvenile to adult with increasing height in the forest, which is due to the change in whole shoot morphology as shoots grow higher in the canopy. As such, in most European forests, *H. helix* leaf phenotypes are strongly stratified within a forest vertical profile. Adult leaves occur in the canopy and juvenile leaves occur on the forest floor or at the base of trees. There is often a transition zone located somewhere in the mid-canopy where leaf phenotypes co-occur but this transition zone is usually relatively small.

Andergassen and Bauer (2002) found that maximum freezing resistance did not vary between the two leaf types but that different temporal patterns of hardening and de-hardening led to higher freezing mortality in juvenile leaves during early or late growing season freezing events. While this study highlighted the similar freezing resistance capacities between the two leaf phenotypes, sampling of leaves occurred at only one height within a botanical garden, which does not reflect the stratified distribution of leaves in a natural forest. Temperature changes significantly within a forest's vertical profile, especially in winter. For example, the canopy may be colder relative to the ground when night-time radiative cooling lowers canopy temperature and the ground vegetation is under snow (Sakai & Larcher, 1987). Conversely, ground cooling may exceed canopy cooling when snow is lacking (Groffman, Hadry, Driscoll, & Fahey, 2006). As the maximum freezing tolerance of leaves is highly correlated with minimum temperatures (Pisek & Schiessl, 1946; Sakai & Weiser, 1973), freezing tolerances of leaves at different heights may vary.

Along with temperature, solar radiation changes considerably with height within a forest (Oberhuber & Bauer, 1991; Poorter et al., 2006). This is particularly relevant for broad-leaved evergreen species in deciduous forests during the early spring and late fall when the canopy remains open and solar radiance is high, even at ground level. During these times most evergreens have large carbon gains relative to other times of the year (Leuzinger, Hartmann, & Körner, 2011; Öquist & Huner, 2003). However, remaining physiologically active when sub-zero temperatures may occur also increases a plant's risk to freezing damage, as active plants tend to be less freezing tolerant than inactive plants (Bauer & Bauer, 1980; Bauer & Kofler, 1987; Weiser, 1970).

Among the many metabolic and physiological changes that occur during the hardening and dehardening process, of particular importance are the alterations to membrane composition and Rubiso activity, which occur simultaneously and often have opposite effects on levels of freezing resistance and photosynthetic activity (Sakai & Larcher, 1987; Sung, Kaplan, Lee, & Guy, 2003). This relationship between photosynthesis and freezing resistance can be readily seen in the fall in *H. helix* plants entering dormancy. As photosynthesis and metabolic activity begin to slow there is a concurrent increase in freezing resistance, which reaches a maximum several weeks after photosynthetic activity has ceased (Steponkus,

1971; Steponkus & Lanphear, 1968). It seems logical then that this process would be reversed in the spring as plants renew metabolic activity but the timing of the dehardening process in the spring may not be synchronized between different leaf phenotypes of *H. helix*, due to variation in light and temperature within the forest's vertical profile. For example, in the spring, juvenile leaves on the ground have only a short window when temperatures are conducive to increased metabolic activity and the canopy remains open allowing for large photosynthetic carbon gains. Yet increasing metabolic activity, including photosynthesis, may expose the plant to increased risk of injury during spring freeze events (Bauer & Bauer, 1980, Bauer & Kofler, 1987).

Deciphering the variable patterns of this freezing resistance *versus* photosynthesis tradeoff is essential for understanding current and future forest dynamics throughout Europe as *H. helix* is often the only liana and can dominate the forest understory. If early or late season freeze events lead to differential mortality among juvenile and adult leaves then our predictions of *H. helix* abundance and distribution under future climate change may be incorrect. *H. helix* is also widespread in North America, where it is often considered an invasive pest, and hence, these questions are also relevant for predicting the further spread of this species outside its natural range.

As such, we specifically wanted to see if the timing of increased photosynthetic activity in the spring of *H. helix* varied among leaf phenotypes as well as highlight any associations between photosynthesis and freezing resistance. We use photosynthesis as a proxy for metabolic activity, as photosynthesis will occur when metabolic processes are active and there is a need for the photosynthetic products (Körner, 2008). We also use freezing resistance as a measure of winter dormancy and dehardening. Our goals were to determine if (1) maximum photosynthesis and freezing resistance were higher in adult phenotype leaves than juvenile leaves and (2) juvenile leaves increased photosynthetic activity and hence reduced freezing resistance earlier in the spring relative to adult leaves.

Materials and methods

This study was conducted at the Swiss canopy crane site near the village of Hofstetten (47°28′N, 7°30′E, 570–580 m a.s.l.), located 12 km southwest of Basel, Switzerland. The site is a mature mixed-hardwood forest but with several coniferous species present throughout the canopy. For a more detailed site description see Pepin and Körner (2002). *H. helix* plants form mats along the forest floor and can be found growing on the trunks of most adult trees. Several individual plants reach the canopy at heights between 25 and 35 m with all leaves in the canopy being of the adult phenotype. *H. helix* does not grow above the canopy and therefore even adult leaves are mostly shaded when canopy closure occurs in deciduous forests. All ground foliage belongs to the juvenile

phenotype. Within an individual plant the switch from adult to juvenile leaves can occur anywhere between 2 and 15 m above the ground but the transition zone is only a few meters long. Sampling of juvenile and adult phenotypic leaves took place at three different heights. Juvenile leaves were sampled at ground level and 1.5 m on the base of trees (hereafter referred to as juvenile ground and trunk leaves). Adult leaves were sampled from the top of the canopy (25–35 m) using the canopy crane. Sampling took place on 22 February, 20 March, 2, 17, and 27 April, and 14 June 2013.

Photosynthetic readings

Gas exchange measurements were taken between 10 a.m. and 2 p.m. on three individual leaves from three different plants from each height class during each sampling period. The time interval at which sampling occurred at each height class was randomized during each sampling period. To minimize effects of altered leaf water relations, during the 22 February, 20 March, and 2 and 17 April sampling a 0.5 m section of each individual plant was cut and recut under water and leaves were measured within a few minutes of being disconnected from the main stem. During the 27 April and 18 June individuals were measured *in situ*. In addition, during the 27 April sampling, three stem sections were cut from three individuals at each height class and gas exchange measurements were performed on leaves after allowing leaves to rest for several minutes. Gas exchange values of these cut individuals did not vary from *in situ* measurements. Therefore, we feel that gas exchange measurements on cut (22 Feb–17 Apr) plants reflect the true measurements of *in situ* individuals. One leaf from each individual was selected and maximum photosynthetic capacity (A_{max}) was measured with a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). Photosynthesis was measured at 400 ppm CO_2, 25 °C, and a saturating light of 500 μmol m^{-2} s^{-1} (Bauer & Bauer, 1980; Carter & Teramura, 1988). Readings were allowed to stabilize and CO_2 gas exchange was recorded after 1–2 min.

Temperature readings

To capture variation of temperature with height, temperature was recorded every 30 min from 22 February to 15 May at 0.5 m and 2.0 m; and 22 February to 14 June at 35 m above the ground. Readings at 0.5 and 2.0 m were recorded with HOBO tidbits (Onset Corp., Bourne, MA, USA) that were shielded from direct sunlight. Temperature at 35 m was recorded from a weather station located at the top of the canopy crane.

Freezing resistance

Intact second year leaves from six individuals at each height (ground juvenile, trunk juvenile, and adult canopy) were collected during each sampling period. One leaf from each individual was cut at the petiole and subjected to one

of seven target freezing temperatures or placed in one of two control chambers at 4 or $-80\,°C$. A detailed description of the freezing system can be found in (Lenz, Hoch, Vitasse, & Körner, 2013). In short, seven different computer controlled freezers were used to lower temperature of samples from $4\,°C$ to the target temperature at a rate of $3\,K\,h^{-1}$. Samples were then held at the target temperature for 4 h before temperature was raised back to $4\,°C$ at a rate of $3\,K\,h^{-1}$. The target temperatures of the freezers were adjusted during each sampling interval to account for seasonal changes in freezing resistance of the leaves (see Appendix A: Table 1). Due to freezer failure, only six target temperatures were used during the 2 and 27 April sampling period. After freezing, samples were kept at $4\,°C$ for 4–6 days to allow tissue necrosis to develop before being visually assessed for survival (Sakai & Larcher, 1987). Following visual observation, samples were placed in distilled water in plastic Falcon tubes and kept at $4\,°C$ for 48 h. Then, samples were assessed using the electrolyte leakage method (Burr, Tinus, Wallner, & King, 1990; Flint, Boyce, & Beattie, 1967).

Statistical analysis

We define freezing resistance as the threshold temperature that kills 50% of sample tissue and is expressed as LT_{50} (Sakai & Larcher, 1987). For each sampling period, LT_{50} was calculated for each individual and then averaged for individuals of the same height class ($n = 6$ for each height class). For visual assessments, LT_{50} was calculated using logistic regression and for electrolyte leakage LT_{50} was calculated using nonlinear Gompertz models (Lim, Arora, & Townsend, 1998). The LT_{50} values for both methods were highly correlated ($R^2 = 0.98$, $p < 0.001$) but overall values were slightly warmer for the visual method. For clarity, only results from the visual estimate will be presented as they represent the more conservative estimate.

Differences in LT_{50} values between different leaf heights over time were analyzed using a linear mixed-effect model using the nlme package in R (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2014) with calendar date, leaf height, and their interaction as fixed effects and the individual plant as a random effect. A significant effect ($p < 0.05$) of the interaction between date and leaf height indicates that leaves occurring at different heights within the forest canopy change LT_{50} values at different rates over time. As such, when the interaction between date and leaf height was significant, *posthoc* Tukey honestly significant difference (HSD) tests were run to look for differences in LT_{50} with leaf height during each sampling period using the multcomp package in R (Hothorn, Bretz, & Westfall, 2008). To look for similar trends in metabolic activity, measured here by photosynthesis, the same analysis was repeated but substituting A_{max} for LT_{50}.

The relationship between A_{max} and LT_{50} values was analyzed using a generalized additive model using the mgcv package in R (Wood, 2011), whereby A_{max} was regarded as

Table 1. Summary of the analysis of variance using a linear mixed effects model for the response of LT_{50} or A_{max}.

| Source of variation	DF$_{num}$	DF$_{den}$	F	P
LT$_{50}$				
Leaf height	2	15	22	**<0.001**
Date	5	75	166	**<0.001**
Leaf height × date	10	75	4.0	**<0.001**
Individual (σ)	0.8			
A$_{max}$				
Leaf height	2	6	3.9	0.08
Date	5	30	98	**<0.001**
Leaf height × date	10	30	17	**<0.001**
Individual (σ)	0.5			

All sources of variation were treated as fixed effects, except for individual that was treated as a random effect. Note that the date was treated as a factor with 6 levels. DF$_{num}$, degrees of freedom of the numerator; DF$_{den}$, degrees of freedom of the denominator; F, F-value from Fisher's F test; P, P-values shown in bold when $P < 0.05$; σ, the estimated standard error for the random effect.

a proxy for metabolic activity in leaves. There was no significant difference among the three different leaf types for the relationship between A_{max} and LT_{50}; therefore only one smoothing function was used. All analyses were carried out using R 3.0.0 (R development core team, 2013).

Results

Lower or more negative LT_{50} values represent higher freezing resistance than the higher or less negative LT_{50} values. Overall, there was a significant effect of leaf height, date, and their interaction on LT_{50} values, indicating a changing effect of leaf height on LT_{50} values over time (Table 1). The lowest LT_{50} values were recorded during the first sampling interval on 22 February and were -19.7, -22.7, and $-29.2\,°C$ for phenotypic ground juvenile, trunk juvenile, and adult leaves respectively (Fig. 1). A light layer of snow covered the ground during this initial sampling period but was absent during subsequent sampling. Adult canopy leaves maintained lower LT_{50} values than ground juvenile leaves throughout the entire sampling period and this difference was significant through 2 April (see Appendix A: Table 2). Differences in LT_{50} values between trunk juvenile and adult canopy leaves were found only through 20 March (see Appendix A: Table 2). Differences in LT_{50} were greatest during the earlier part of the year when ground and trunk juvenile leaves had LT_{50} values approximately 7–10 K higher than adult leaves from the canopy. On 17 April, the merging of LT_{50} values for leaves at all heights was concurrent with an increase in average and minimum temperatures (Fig. 1). LT_{50} values at all leaf heights remained similar after 17 April and through the end of the sampling period (see Appendix A: Table 2). Between the two juvenile height classes, LT_{50} values for ground and trunk
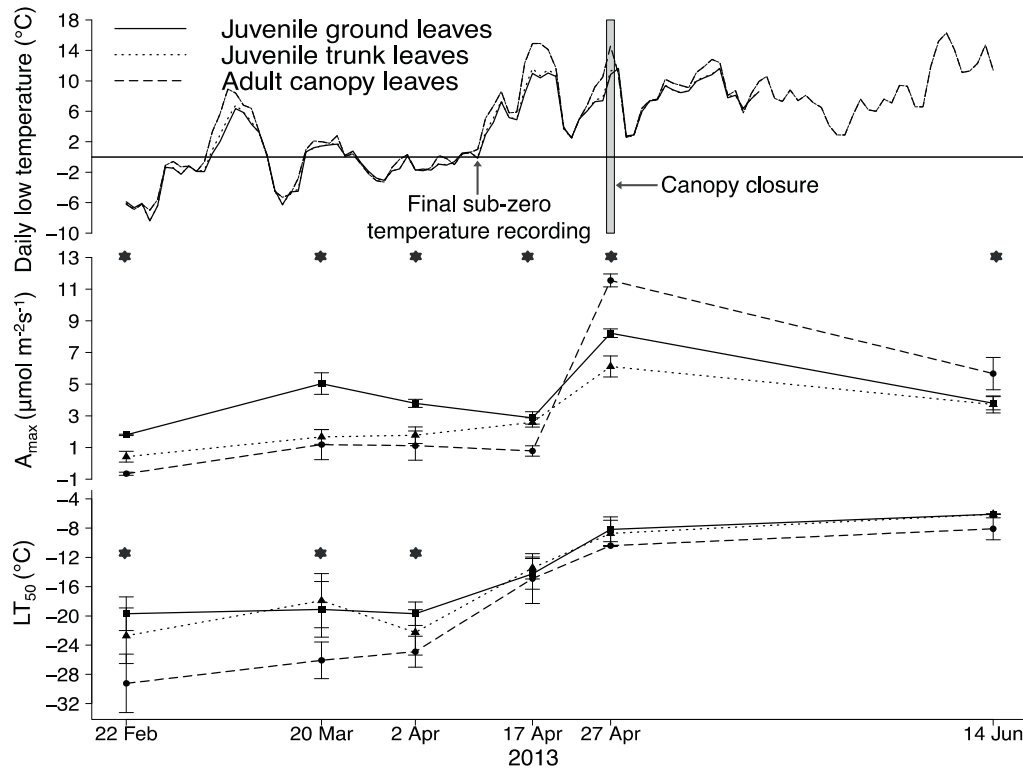
Fig. 1. Seasonal variation of daily low temperature (°C), maximum photosynthetic capacity ($A_{max} \pm$ s.e.) and freezing resistance (LT$_{50}$; °C ± s.e.) as measured by the visual method for two phenotypic leaf types of *H. helix* at three different heights in the forest canopy (during each sampling interval $n = 3$ and 6 for each height/phenotype combination for photosynthetic capacity and freezing resistance respectively). Shaded gray area represents the date of full canopy closure. Asterisks represent significant differences ($p < 0.05$) in freezing resistance or photosynthesis of leaf phenotypes occurring at different heights at different dates according to posthoc Tukey honestly significant difference tests.

leaves were statistically different only during the 2 April and were generally within 1–3 K of each other throughout the entire sampling period.

Photosynthetic values followed a similar pattern as freezing resistance, with all leaves maintaining relatively constant A_{max} through mid-April (Fig. 1). Between the 17 and 27 April, A_{max} values had the largest increase during any sampling interval for all leaf heights. Similar to LT$_{50}$ values, there was a significant effect of date and the interaction between date and leaf height on A_{max} values, indicating a changing effect of A_{max} values at varying leaf height over time (Table 1). The main effect of leaf height on A_{max} was only marginally significant ($p = 0.08$, Table 1). Adult canopy leaves had lower average A_{max} values than ground juvenile leaves from 22 February to 17 April (see Appendix A: Table 3). From 27 April through 14 June, A_{max} in adult leaves increased above values for either type of juvenile leaves (see Appendix A: Table 3). During the entire sampling period, A_{max} values for trunk juvenile leaves generally fell somewhere between adult canopy and ground juvenile leaves, except on 27 April when trunk juvenile leaves had the lowest A_{max} values of any leaf height. Full canopy closure at the site was reached on 27 April (pers. comm. Yann Vitasse). The timing of canopy closure was consistent with a decrease in A_{max} across all height classes after this time

interval, but A_{max} was lower for juvenile leaves than adult leaves.

Absolute coldest temperatures at 0.5 m, 2.0 m and on top of the canopy crane were −8.4, −8.4, and −7.0 °C, respectively. Average daily minimum temperatures were warmer at the canopy (3.7 ± 5.9 °C) than at 0.5 (2.8 ± 5.3 °C) or 2.0 m (2.8 ± 5.3 °C; one-way ANOVA, $p < 0.01$) showing that adult leaves in the canopy experienced overall milder temperatures throughout the sampling period.

General additive models revealed a non-linear relationship between A_{max} and LT$_{50}$ values, which was consistent among leaf phenotypes and height within the forest. The smoothed A_{max} term was a relatively good predictor of LT$_{50}$ (49.7% deviance explained; estimated d.f. = 2.31, $F = 16.48$, $p < 0.0001$) and we used A_{max} as a proxy for leaf activity (*e.g.* hardened or dehardened). However this relationship degrades when LT$_{50}$ values increase to −15 to −10 °C, after which LT$_{50}$ plateaus but A_{max} continues to increase (Fig. 2).

Discussion

Maximum freezing resistances of *H. helix* leaves were well below the coldest temperatures experienced at the field sites for as long as we have records. In fact, over the past 116 years
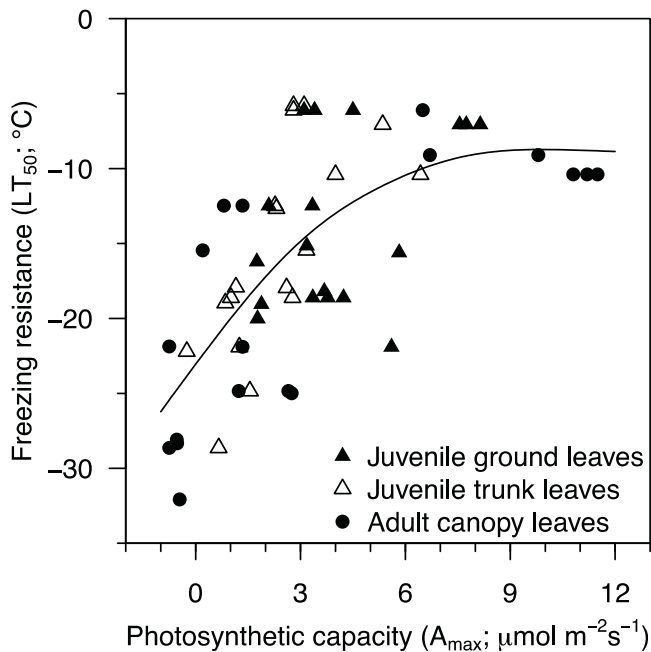
Fig. 2. Relationship between maximum photosynthetic capacity (A_{max}) and freezing resistance (LT_{50}) as determined using generalized additive models for two phenotypic leaf types of *H. helix* at three different heights in the forest canopy ($n = 18$ for each leaf phenotype). There was no significant difference among heights; therefore only one smoother over all heights is shown.

the coldest temperature recorded at Binningen, Switzerland, a weather station approximately 10 km from the field sites, was $-24.1\,^{\circ}C$. This was only 4.4 and 1.4 K colder than the minimum LT_{50} for ground and trunk juvenile leaf phenotypes, respectively, and still 5.1 K warmer than the LT_{50} for adult leaf phenotypes. If we consider that uncut *in situ* leaves may have 1–2 K lower LT_{50} values (Taschler & Neuner, 2004) and that snow cover may buffer against temperature extremes for the ground juvenile leaves, then all leaf phenotypes rarely, if ever, have faced a critical temperature colder than their maximum freezing resistance over the past century. From these results it seems unlikely that the maximum freezing tolerance puts this species at risk of leaf freezing damage at this site, which is away from its range limit. This is in contrast to findings at the species cold range edge, where maximum freezing tolerance is believed to directly limit the species' distribution (Andergassen & Bauer, 2002).

LT_{50} of leaves in our study did vary with leaf phenotype and canopy height, with adult leaves in the canopy having lower LT_{50} values than juveniles closer to the ground through the early spring. Even though temperatures were warmer in the canopy than on the ground, adult canopy leaves in this study maintained lower LT50 values than juvenile leaves. One explanation for this incongruity in *H. helix* is that differences in the anatomical and chemical composition of leaf phenotypes can lead to variation in freezing resistance (Oberhuber & Bauer, 1991). For example, freezing resistance reflects the state of cellular membranes, lipid layers and water channels, with more metabolically active tissues being less tolerant of freezing (Sakai & Larcher, 1987; Weiser, 1970). In low light environments, such as the forest understory, carbon gains *via* photosynthesis are a limiting factor to growth (Hättenschwiler & Körner, 2000) and hence juvenile leaves would need to increase or maintain photosynthetic activity earlier in the spring relative to adult leaves. Our data show higher A_{max} and LT_{50} values in ground juvenile leaves as compared to adult leaves until mid-April (*i.e.* the time of bud break in deciduous trees).

Photosynthesis and freezing resistance are both part of a long and complicated metabolic process that occurs during hardening and de-hardening of plants (Sakai & Larcher, 1987). An increase in metabolic activity, here measured as A_{max}, in the spring should be associated with a decrease in freezing resistance (*i.e.* higher LT_{50} values). Indeed, for ground juvenile leaves LT_{50} and A_{max} values were higher than adult canopy leaves from the start of sampling through 2 April, with values for trunk juvenile leaves occurring between ground juvenile and adult canopy leaves. Furthermore, LT_{50} and A_{max} seem to follow a more or less positive linear relationship until leaves reach an LT_{50} somewhere between -15 and $-10\,^{\circ}C$ (Fig. 2). At this point LT_{50} begins to plateau while A_{max} continues to increase. We interpret this as a signal that the leaf is fully dehardened at this LT_{50} threshold, but metabolic activity and photosynthetic capacity continued to increase through 27 April. This indicates a possible delay from the time plants exit winter dormancy, initiating increased metabolic activity, to when they reach maximum photosynthetic potential.

Such a delay is reasonable considering increases in photosynthetic activity should largely occur after temperatures have warmed past a certain threshold in order to avoid photoinhibition (Bauer & Thöni, 1988). Oberhuber and Bauer (1991) demonstrated that metabolically active juvenile leaf phenotypes of *H. helix* had large increases in photosynthetic capacity shortly after the cessation of freezing nighttime temperatures. It is not surprising then that maximum photosynthesis of leaves in our study had the largest increase in the weeks after freezing temperatures had ended and freezing resistances had plateaued. Similarly, Rylko and Kacperska (1981) found that *H. helix* did not initiate growth in the spring until after the plant dehardened and reduced freezing resistance, a process which took several weeks and was similar in length to many other evergreen species (Ottander & Öquist, 1991; Ottander, Campbell, & Öquist, 1995).

Differences in photosynthetic activity can be explained by differences among phenotypes in mesophyll structure and thickness, associated stomatal conductance, photosynthetic efficiency, and timing of seasonal metabolism (Bauer & Bauer, 1980; Bauer & Thöni, 1988; Hoflacher & Bauer, 1982). Adult leaves maintained lower photosynthetic capacities throughout the end of winter and early spring relative to juvenile leaves but this trend was reversed in mid-April. Presumably, this difference relates to the greater freezing resistance of adult leaves and thus, state of inactivity. Regardless of

leaf phenotype and canopy height, the spike in photosynthetic capacity on 27 April highlights the importance of the brief window that broad-leaved evergreen species have between the end of harsh winter temperatures and full canopy closure. After canopy closure all leaves decreased photosynthetic capacity, but shade adaptation likely enhanced their efficiency of light capture (quantum efficiency), not studied here.

To our knowledge this was the first attempt to capture the physiological changes between maximum photosynthetic capacity and freezing resistance in *H. helix* during the spring, a dominant liana found throughout much of central Europe and, as a neophyte, in oceanic North America. This study showed that low temperature extremes are not likely posing limitations on *H. helix* in the center of its native range and that seasonal patterns of photosynthesis and freezing resistance are similar between different leaf phenotypes while absolute values of these parameters vary among leaf type. Our results also indicate that spring freeze events that limit *H. helix* at more continental or higher elevation locations (Andergassen & Bauer, 2002) are hardly critical in regions where *H. helix* is most abundant. However, the species shows little adjustment (ecotypic differentiation) to the regional climate, given that the mid-winter freezing resistances were similar at our climatically mild site compared to that at the species cold range edge.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2014.07.009.

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