

Annual patterns of xylem embolism in high-yield apple cultivars

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Abstract. Temperate angiosperm species show pronounced annual patterns in xylem embolism. In this study, we investigated whether high-yield cultivars of *Malus domestica* Borkh. growing under optimised soil water conditions follow similar patterns to wild-type plants, and evaluated crucial factors for the formation of winter embolism and the subsequent restoration of the hydraulic system in spring. In five different cultivars growing at three different sites, various hydraulic and microclimatic parameters were monitored over three successive years. In all cultivars on all sites and in all years, the percentage loss of hydraulic conductivity (PLC) increased in autumn with freeze–thaw events and accumulated over winter. Maximum values were reached in late winter and differed significantly among cultivars. In spring, the hydraulic system was restored and PLC remained negligible during summer. Embolism formation in autumn was significantly correlated with the occurrence of freeze–thaw events, whereas further conductivity losses over winter were related to winter desiccation and influenced by climatic and cultivar-specific parameters. Restoration of the hydraulic system in spring was strongly linked to a decrease in the starch content of wood and buds, and soil temperature. Despite high soil water availability, hydraulic recovery took several weeks and was not completed before bud break. Spring is thus a critical phase for temperate angiosperms, especially for high-yield cultivars with risky hydraulic strategies.

Additional keywords: conductance, *Malus domestica*, refilling, starch, xylem pressure.

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Introduction

A functioning water transport system is essential for plant growth and survival, especially in long-lived woody plants. Long-distance water transport is driven by a pressure gradient transduced from leaves to roots and requires hydraulically continuous water columns in xylem conduits (e.g. Tyree and Ewers 1991; Tyree and Cochard 1996; Tyree and Zimmermann 2002). Drought and/or freeze–thaw events can cause breakage (cavitation) of these water columns, resulting in air-filled conduits (embolism) and impaired xylem water transport (Sperry and Tyree 1990; Tyree and Ewers 1991; Tyree and Zimmermann 2002). Drought-induced embolism occurs when the evaporative demand cannot be satisfied by water uptake from the soil, and the xylem pressure thus decreases below species-specific thresholds (e.g. Tyree and Zimmermann 2002). Reduced or inhibited water uptake from the soil is not only provoked by soil drought but also by frozen (frost drought) or cold soils. The latter is related to the increased transfer resistance between soil and roots, the reduced permeability of roots to water as well as the reduced growth of fine roots, which are mainly responsible for water uptake (Kozłowski *et al.* 1991; Larcher 2003; Pallardy 2008; Beikircher *et al.* 2016). In several temperate trees, 6°C was found to be a critical temperature limit for root growth (Alvarez-Uria and Körner 2007). Freeze–thaw-induced embolism develops when air bubbles enclosed in frozen xylem sap expand during thawing and cause cavitation (Sperry and Sullivan 1992; Tyree *et al.* 1994; Hacke and Sauter 1996; Cochard *et al.* 2001; Mayr *et al.* 2007;

Charrier *et al.* 2014). Two critical factors to avoid freeze–thaw-induced embolism are therefore conduit diameter and xylem tension: wider conduits allow the formation of larger bubbles, which shrink more slowly than smaller ones, and nucleate embolism at lower (i.e. less negative) tension (Pittermann and Sperry 2006). Furthermore, freezing velocity and the minimal temperature of the frost cycle also seem to play a role (Sevanto *et al.* 2012; Charrier *et al.* 2014). Thus the extent to which a tree's hydraulic system is impaired during winter strongly depends on anatomical parameters of the xylem (Sperry and Sullivan 1992; Choat *et al.* 2011), the protection against evaporative water losses (leaf shedding, cuticular and peridermal conductance (g_p); Larcher 2003; Beikircher and Mayr 2013) and, with regard to climatic parameters, air and soil temperature, vapour pressure deficit and soil water availability. Previous studies have shown that trees in temperate regions can suffer a considerable loss of hydraulic conductance in winter (Sperry *et al.* 1988b, 1994; Cochard and Tyree 1990; Sperry and Sullivan 1992; Hacke and Sauter 1996; Cochard *et al.* 2001; Ameglio *et al.* 2002; Mayr *et al.* 2003; Christensen-Dalsgaard and Tyree 2014). In deciduous species, though, a full water transport capacity is not required in winter and even a high level of native embolism is not particularly risky for the plant. This changes suddenly in spring, when transpirational water demand increases in response to warmer temperatures, increased daylength and bud break (e.g. Sperry *et al.* 1994; Ameglio *et al.* 2002; Hao *et al.* 2013). In spring, the functionality of the water transport

system has to be restored rapidly to avoid growth limitation or desiccation-related dieback of shoots. Restoration of the hydraulic system in spring is related to the differentiation of new xylem conduits and, in many diffuse-porous angiosperms, to the refilling of embolised ones (Sperry *et al.* 1988b, 1994; Sperry and Sullivan 1992; Hacke and Sauter 1996; Utsumi *et al.* 1998; Cochard *et al.* 2001; Cobb *et al.* 2007; Christensen-Dalsgaard and Tyree 2014). One possible mechanism for hydraulic recovery is the generation of positive xylem pressures in roots or stems that is sufficient to dissolve embolisms before leaf flushing (Sperry *et al.* 1987, 1994; Cochard *et al.* 2001; Ewers *et al.* 2001; Ameglio *et al.* 2002; Cobb *et al.* 2007; Brodersen and McElrone 2013; Hao *et al.* 2013). Refilling has also been observed in the absence of root or stem pressure (Tyree *et al.* 1999; Cochard *et al.* 2001; Salleo *et al.* 2006; Nardini *et al.* 2011; Brodersen and McElrone 2013; Christensen-Dalsgaard and Tyree 2014). The mechanisms involved in refilling without tension are still under debate (see Zwieniecki and Holbrook (2009) and Brodersen and McElrone (2013) for a detailed review). However, there is strong evidence that sugars derived from starch polymerisation in xylem parenchyma generate an osmotic gradient that then drives water inflow from adjacent parenchyma cells into conduits (Salleo *et al.* 2004; Zwieniecki and Holbrook 2009; Nardini *et al.* 2011; Secchi and Zwieniecki 2012). Two important prerequisites therefore are: (I) adequate water supply, with living cells in the xylem and phloem potentially playing a major role (Salleo *et al.* 2004; Brodersen *et al.* 2010; Nardini *et al.* 2011); (II) hydraulic isolation of embolised conduits from water-filled conduits under tension. If air-filled conduits are not isolated, any inflowing water would immediately be drawn away. Hydraulic isolation is probably achieved by special morphological and biochemical features of interconduit pits (Holbrook and Zwieniecki 1999; Nardini *et al.* 2011).

Most studies dealing with annual patterns of xylem embolism have focussed on native species growing in forests under natural conditions or in experimental plots (Sperry *et al.* 1988b, 1994; Cochard and Tyree 1990; Sperry and Sullivan 1992; Hacke and Sauter 1996; Utsumi *et al.* 1998; Cochard *et al.* 2001). Only Ameglio *et al.* (2002) made measurements on orchard-grown walnut (*Juglans* spp.) and peach (*Prunus persica* (L.) Batsch) trees. Artificial systems, such as commercial apple (*Malus domestica* Borkh) orchards are interesting in two aspects: First, the plants are high-yield cultivars, evolved from millennia-long crossing of wild forms as well as specific breeding. Second, plants are usually grown under ideal conditions with regard to nutrient and water supply to minimise hydraulic limitation of growth and productivity (Naor and Girona 2012; Beikircher *et al.* 2013). A recent study found that this combination can lead to risky hydraulic strategies (Beikircher *et al.* 2013).

In this study, we followed the loss of hydraulic conductivity (i.e. native embolism; percentage loss of hydraulic conductivity (PLC)) over three consecutive years in *M. domestica*. We also monitored the water content of bark, wood and buds; the starch content of bark and wood; and g_p . Plant hydraulic aspects were then related to microclimatic conditions and phenology. The main research questions were as follows: (I) Given the optimised water supply throughout the year, do high-yield cultivars show pronounced patterns in native embolism, similar to those observed in native plants at natural sites? (II) Which climatic

and species-related factors are crucial for the level of potential winter embolism as well as refilling in spring? (III) Do cultivars show specific patterns in embolism formation and refilling? To answer these questions, we analysed five different *Malus* cultivars growing in different orchards.

Material and methods

Plant material and study sites

This study was carried out on different apple cultivars in commercial apple orchards in northern Italy. *Malus domestica* Borkh. cv. Golden Delicious specimens were grown on south-exposed orchards near Latsch (643 m above sea level (a.s.l.); 46°37'N, 10°52'E), north-exposed orchards near Tschengls (950 m a.s.l.; 46°37'N, 10°38'E) and near Tarsch (854 m a.s.l.; 46°36'N, 10°53'E). In Latsch, additional cultivars included Braeburn, Gala, Nicoter and Red Delicious. All plants were of similar age and height (for details, see Beikircher *et al.* 2013; Beikircher and Mayr 2013).

The study sites are located in the Vinschgau Valley, which is a dry inner alpine valley with exceptionally high sunshine duration (315 sunny days per year), a high annual mean temperature (9.6°C) and low precipitation (450–550 mm). Elaborate fertilisation protocols and daily irrigation during the growing season ensure optimal plant growth and productivity. At the study sites, air temperature and relative humidity at 250 cm height (measured at the upper crown; EMS 33 sensor); soil temperature (PT 100 sensor) at 5, 15 and 25 cm depths; and soil water potential (GB sensor) at 25 cm depth were recorded at 1-min intervals. Every 15 min, means of these values were stored in a datalogger (Modulog 3029 sensors and datalogger, Environmental Measuring Systems EMS).

Temperature decreases below -2°C and subsequent increases above 2°C within 24 h were defined as freeze–thaw events. From the minimum air temperature (T_{\min}), minimum relative humidity (RH_{\min}), maximum air temperature (T_{\max}) and maximum relative humidity (RH_{\max}), vapour pressure deficit (VPD) was calculated as:

$$\text{VPD} = \left(\frac{e^{\circ}(T_{\max}) + e^{\circ}(T_{\min})}{2} \right) - \frac{(e^{\circ}(T_{\min}) \times \frac{RH_{\max}}{100}) + (e^{\circ}(T_{\max}) \times \frac{RH_{\min}}{100})}{2}, \quad (1)$$

where e° is the saturation vapour pressure at minimum or maximum temperature, respectively (Allen *et al.* 1998).

Sampling and preparation of branches

From June 2010 to June 2013, at irregular intervals throughout the year, five east- or west-exposed branches (orchard rows were aligned north–south) of five different trees per cultivar and site were chosen at random. In the late morning, branches were cut at the base, immediately recut under water and rehydrated for 30 min before they were wrapped in dark plastic bags with wet paper towels and transported to the laboratory. In the laboratory, branches were repeatedly cut from both sites alternatively under water. In an earlier study (Beikircher and Mayr 2015), this harvesting and sampling protocol proved to be appropriate for preserving the hydraulic state and avoid artefactual embolisation

(Wheeler *et al.* 2013) as well as refilling (Trifilò *et al.* 2014). Before June, shoots developed in the previous season were used for measurements, whereas from June onward, we used the respective current-year shoots.

Native embolism, water content, midday leaf water potential and stomatal conductance

For the measurement of native embolism, ~5-cm-long samples were debarked, the ends recut several times with a sharp wood-carving knife (Beikircher and Mayr 2015) and sealed into tubes connected to a 'Xyl'em' hydraulic conductance and embolism measurement system (Bronkhorst). Hydraulic conductance at 4.5 kPa was measured before (k_i) and after (k_{\max}) removal of embolism by repeated high pressure flushes at 95 kPa for 20 min. Percentage loss of hydraulic conductivity was calculated as:

$$PLC = 100 - \left(\frac{k_i}{k_{\max}} \times 100 \right). \quad (2)$$

For measurements, we used distilled, filtered (0.22 μm) and degassed water, containing 0.005% (v/v) 'Micropur Forte MF 1000F' (a mixture containing Ag^+ and NaClO for water sterilisation and preservation; Katadyn Products Inc.) to prevent microbial growth (Sperry *et al.* 1988a; Beikircher and Mayr 2008).

Measurements of water content were made on bark and wood of ~5-cm-long stem samples as well as on terminal buds. Fresh weights and DW (after oven drying at 80°C for 48 h) were measured with an analytical balance (Sartorius BP61S, 0.0001 g precision, Sartorius AG) and the water content (expressed as percentage of DW; $WC_{\%DW}$) calculated as:

$$WC_{\%DW} = \frac{FW - DW}{DW} \times 100. \quad (3)$$

Leaf water potential measurements were made on selected days between 1100 and 1200 hours Central European Time on at least five leaves per cultivar and site, using a pressure chamber (Model 1000 Pressure Chamber, PMS Instrument Co.). Between leaf fall and bud break, measurement of xylem water potential was not possible because of the small portion of living tissues. Stomatal conductance (g_s) was measured between 1030 and 1130 hours CET on sunny days in March and April 2011, using a steady-state leaf porometer (SC-1, Decagon Devices).

Starch contents of wood and bark

Analyses of starch contents were carried out on the cultivars Braeburn, Golden Delicious and Red Delicious in the orchard near Latsch. Therefore, the bark was removed from ~4-cm-long samples and cut into thin strips with scissors. The wood was thinly (~10 μm) sliced using a microtome (Sledge Microtome G.S.L. 1, Schenkung Dapples). After oven drying at 80°C for 48 h, samples were finely ground with a microdismembrator (Braun Biotech). The obtained material was incubated twice in 80% ethanol (v/v) at 75°C with polyvinylpyrrolidone (PVP 40, Sigma Chemicals) added to bind polyphenols during the first incubation. The supernatants were then removed and the solvent evaporated in an oven. The dry residue containing the starch was incubated with NaOH (0.5 N) at 60°C for 1 h, neutralised with hydrochloric acid (0.5 N), treated with amylose (amyloglucosidase, Sigma-Aldrich) dissolved in a

citrate buffer (pH 4.6) to break the starch down to glucose, and incubated for 30 min at 60°C. The supernatants were then mixed with a NADP/ATP and HK/G6P-DH-solution (Enzytec E1210, R-Biopharm) and the absorption of NADPH (dependent on starch content) measured with a UV-visible light spectrophotometer (Lambda 20, Perkin Elmer) at 340 nm before and after the addition of the latter solution. Starch content (SC ; %) was calculated as:

$$SC = c \times 100 \times \frac{V}{m}, \quad (4)$$

where c is the starch concentration (g L^{-1}) in the measured solution, m is the mass of plant material and V is the volume of the solution (L) in the spectrophotometer (see also Mayr *et al.* 2014).

Peridermal conductance to water vapour

For measurement of g_p , eight to 10 end shoots per cultivar and site were harvested in January 2011. Shoots were debarked at the basal end (~1 cm), recut with a sharp wood-carving knife and sealed in tubes connected to a 'Xyl'em' apparatus (see above). The distal part of the shoot with the terminal bud was then cut off under water and the shoot flushed at a pressure of 95 kPa for 45 min to remove embolisms. Afterwards, the wet parts on both sample ends were cut off and the ends were tightly sealed with Parafilm 'M' (Pechiney Plastic Packaging). Immediately after sealing, the sample weight was measured with an analytical balance (Sartorius BP61S, Sartorius). Samples were put in a test tube rack for bench dehydration and FW was measured at 1-min intervals. Simultaneously, air humidity and temperature as well as barometric pressure were monitored with a thermohygrometer (RS Components, Handelsges.mbH.) and a temperature-compensated pressure sensor (SCX15ANC, Honeywell Sensing and Control), respectively. Afterwards, the transpiring surface area was calculated from shoot length and diameter, assuming a cylindrical shape for each shoot. Peridermal conductance for each time interval was calculated from evaporation (E), saturated vapour pressure (SVP ; Pa), actual vapour pressure (VP ; Pa) and barometric pressure (P ; Pa; see Beikircher and Mayr 2013) as:

$$g_p = \frac{E}{(SVP - VP) \div P}. \quad (5)$$

For maximum g_p , the highest values of each shoot were averaged per cultivar and site.

Statistics

Differences in PLC and g_p among cultivars, sites and study years were tested with an ANOVA using the Bonferroni correction (normal distribution, equal variances). Correlation coefficients between embolism levels and number of freeze-thaw cycles experienced were calculated with Pearson's product-moment coefficient. All tests were made at a probability level of 5% using SPSS (ver. 21, SPSS).

Results

Climatic conditions varied considerably between orchards and between study years (Table 1, Fig. 1). The warmest site was Latsch. The coldest one was Tschengls because of the lack of

Table 1. Monthly means of air temperature (T_{air}), vapour pressure deficit (VPD) and soil temperature at 15 cm depth (T_{soil}) during winter and spring, as well as cumulative number of freeze–thaw events (FT) and number of days with soil frost from October to February in the orchards of Latsch, Tschengls and Tarsch in three successive study years

Please note that because of technical problems, no data are available for winter 2010 at Tarsch and spring 2013 in Tschengls (indicated as nd)

Site		Latsch			Tarsch			Tschengls		
Year		2010–11	2011–12	2012–13	2010–11	2011–12	2012–13	2010–11	2011–12	2012–13
T_{air} (°C)	November	3.6	2.2	3.7	nd	3.9	4.0	2.0	–0.6	1.6
	December	–1.4	0.8	–1.3	nd	1.1	–1.2	–2.9	0.0	–2.6
	January	–0.9	0.5	0.5	nd	0.2	0.8	–1.9	0.1	1.0
	February	2.9	0.9	–0.2	nd	–0.3	–0.5	2.2	0.6	0.2
	March	6.6	9.4	4.1	nd	8.6	3.0	5.5	8.8	3.1
	April	12.8	9.5	10.6	nd	8.2	9.5	11.8	8.2	9.6
	May	15.6	14.8	12.8	nd	13.9	11.5	14.6	13.9	11.5
VPD (kPa)	November	0.187	0.324	0.283	nd	0.289	0.242	0.122	0.127	0.128
	December	0.212	0.281	0.224	nd	0.242	0.194	0.168	0.212	0.187
	January	0.272	0.322	0.352	nd	0.299	0.313	0.206	0.323	0.250
	February	0.468	0.468	0.334	nd	0.417	0.296	0.421	0.660	0.328
	March	0.538	0.822	0.323	nd	0.751	0.281	0.505	0.819	0.273
	April	0.963	0.550	0.542	nd	0.455	0.471	nd	nd	nd
	May	0.990	0.962	0.618	0.865	0.831	0.558	nd	nd	nd
T_{soil} (°C)	March	1.6	2.5	–0.7	nd	0.4	0.2	–0.8	–0.8	–0.7
	April	7.5	5.5	3.4	nd	5.3	3.1	5.9	5.1	2.5
FT (<i>n</i>)		55	69	78	nd	27	41	40	57	60
Soil frost (days)		65	44	66	nd	23	20	81	105	87

direct sunshine from mid-November until the end of January. Tarsch is situated ~200 m higher than Latsch. It is characterised by moderate temperatures in winter, caused by frequent temperature inversions, but cold temperatures in spring. The relatively low number of days with soil frost at this site (Table 1) is related to the protection of the soil by a constant snow cover during the winter months. The earliest springtime increase in soil temperature to values above 5°C was observed at the beginning of March 2012 in Latsch. At the other study sites and in the other study years, it occurred in April (Fig. 1).

Percentage loss of hydraulic conductivity and water and starch contents followed pronounced annual patterns. For the sake of convenience, we only present data for the cultivar Braeburn in the main text (Fig. 2). For (comparable) data for other cultivars and other sites, see the figures in the supplementary material (Figs S1–S4, available as Supplementary Material to this paper). With the occurrence of the first freeze–thaw cycles in autumn, embolism levels started to increase and achieved their maxima in late winter. Overall, PLC in November corresponded to the number of freeze–thaw cycles experienced. In Tarsch only, PLC was significantly higher despite having fewer freeze–thaw cycles (Fig. 3).

In mid-winter (January), embolism levels differed significantly between study years, cultivars and sites. Among the three study sites of Golden Delicious, the lowest PLC was found in Latsch (Fig. 3). In Tschengls, significant differences among study years were observed. In January 2012, PLC was about twofold higher than in 2010 and 2013. This was in accordance with a considerably greater number of days with soil frost (105) compared with the other years (~85) and sites (Table 1). Midwinter embolism was also found to vary considerably among different cultivars subjected to similar

conditions in Latsch. In all study years, significantly lower PLC was found in Golden and Red Delicious than in other cultivars. Golden and Red Delicious also showed lower g_p than Braeburn, Gala and Nicoter (Fig. 4).

Despite the differences in winter embolism, all cultivars at all sites were able to fully restore their hydraulic system in spring and levels of native embolism were negligible throughout summer (Fig. 2, Figs S1–S4). Concurrently with the decrease in PLC, a decrease in starch content in wood and bark was observed (Fig. 2, Figs S1–S2). Depending on the study year, considerable differences in restoration speed were found among study sites. In the warm spring of 2012, soil warming over 5°C occurred about 1 month earlier in Latsch. Accordingly, restoration of the hydraulic system was also completed earlier than at the other sites, whereas bud break occurred almost simultaneously at all sites (Table 2). In spring 2011 and 2013, differences in soil temperature and thus also restoration were less pronounced (Fig. 1, Table 2). In contrast to midwinter embolism, no obvious differences in restoration speed and bud break were observed among different cultivars growing at different sites (Table 2).

Discussion

High-yielding cultivars of *M. domestica* growing in optimally-managed orchards showed pronounced annual patterns in embolism, similar to those reported previously for various temperate angiosperm species in natural or seminatural sites. In the following, we focus on two main topics: the formation and the patterns of winter embolism, and restoration of the hydraulic system in spring. Thereby, we first provide a short overview about

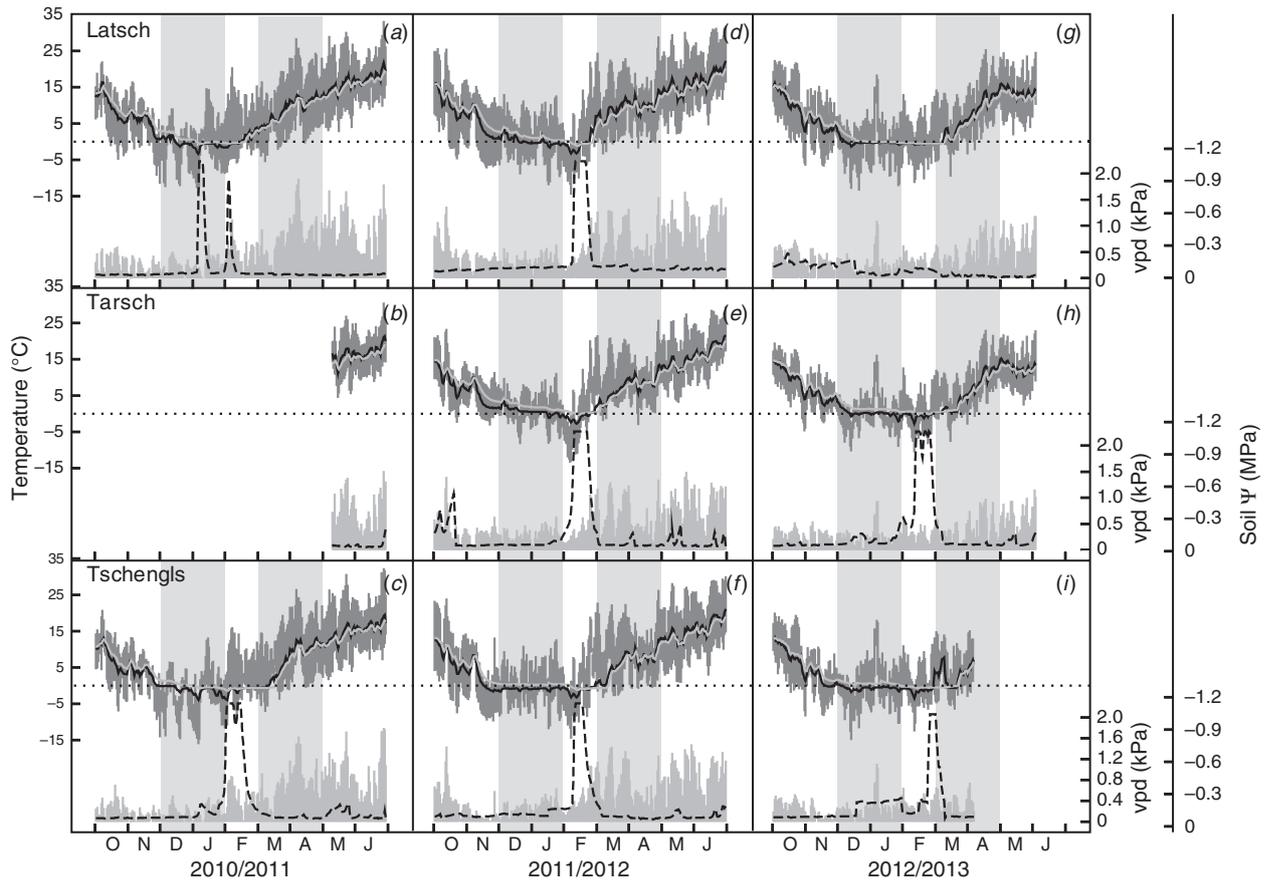


Fig. 1. Daily minimum and maximum air temperature (dark grey area), daily mean soil temperature in 5 cm (solid black line) and 25 cm (solid grey line) depth, daily mean soil water potential (soil Ψ ; dashed black line) in 15 cm depth and daily vapour pressure deficit (VPD; light grey area) from October to June in the apple orchards of (a, d, g) Latsch, (b, e, h) Tarsch and (c, f, i) Tschengls in (a–c) 2010–11, (d–f) 2011–12 and (g–i) 2012–13. Dotted horizontal lines show 0°C. Light grey vertical rectangles indicate midwinter (December, January) and early spring (March, April).

general patterns found and then emphasise the outstanding observations on intra- and intercultivar levels.

Winter embolism

When night temperatures decreased below freezing in autumn, in all cultivars and on all sites, PLC started to increase. During winter, conductivity losses further increased and maximum values were achieved in late winter (Fig. 2, Figs S1–S4). Similar patterns have been reported for several other species growing under natural (Sperry and Sullivan 1992; Sperry *et al.* 1994) or semi-natural (Cochard and Tyree 1990; Hacke and Sauter 1996; Utsumi *et al.* 1998; Cochard *et al.* 2001; Ameglio *et al.* 2002; Cobb *et al.* 2007) conditions. Our findings show that a complex combination of climatic and cultivar-specific factors is decisive for the extent of hydraulic impairment of trees during the cold season. Therefore, winter desiccation plays a major role.

The first PLC increase in autumn can mainly be attributed to freeze–thaw-induced embolism. Accordingly, embolism levels in late autumn (November) differed considerably among sites and years (and thus the respective number of freeze–thaw events). The intracultivar comparison of Golden Delicious growing on

different sites, though, provides evidence that the relationship between embolism and freeze–thaw cycles is complex. For instance, at Tarsch, PLC was significantly higher in the latter two study years despite a much lower number of freeze–thaw cycles compared with Latsch and Tschengls (Fig. 3). An explanation for this finding might be the higher air temperatures in autumn at this site (Table 1), as higher evaporative demands and lower minimal temperatures of frost cycles increase the risk of freeze–thaw-induced cavitation (Sperry and Sullivan 1992; Ameglio *et al.* 2002; Charrier *et al.* 2014). In contrast, in Latsch, where five different cultivars were subjected to similar conditions, within a given study year, no statistically significant differences in PLC among cultivars were observed. Besides similar environmental conditions, this may also be caused by similar xylem anatomy. At least for the cultivars Braeburn, Golden and Red Delicious, a previous study (Beikircher *et al.* 2013) found no significant differences in conduit diameters. Nevertheless, in an annual comparison, PLC in November was significantly correlated with the number of freeze–thaw cycles ($R^2 = 0.762$; Fig. 3).

In midwinter (January), we observed high variations in PLC among sites and among cultivars. Within the three study years, embolism levels were lower overall in the winter season of

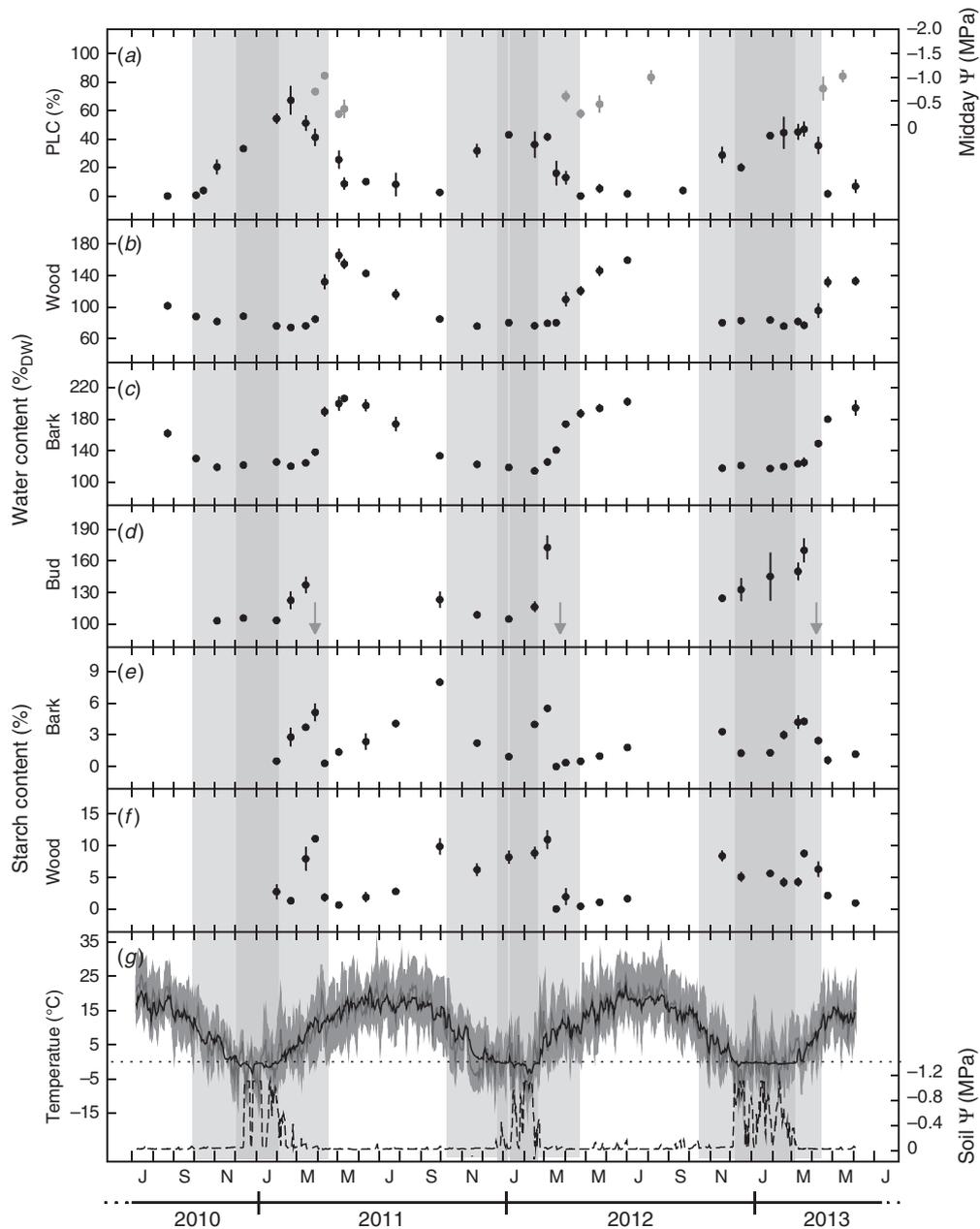


Fig. 2. Mean and s.e. of (a) percentage loss of hydraulic conductivity (PLC, black dots) and midday leaf water potential (midday Ψ ; grey dots); water contents of (b) wood, (c) bark and (d) buds; starch contents of (e) bark and (f) wood; and (g) daily maximum, minimum (grey area) and mean (dark grey line) air temperature, daily mean soil temperature (solid black line) and daily mean soil water potential (soil Ψ , dashed black line) at 15 cm depth in Braeburn apples growing in Latsch. Arrows in (d) show the bud break. Dotted horizontal lines in (g) indicate 0°C. Vertical rectangles indicate subzero air (light grey) and soil (dark grey) temperatures, respectively. ($n = 5$).

2011–12 despite a considerable higher number of freeze–thaw cycles compared with the preceding winter season. The only exception was Golden Delicious in Tschengls, which had significantly more embolism (Fig. 3). The winter season of 2011–12 was remarkably warm with mean monthly air temperatures above 0°C in December and January (Table 1). Accordingly, the soils in Latsch and Tarsch were not permanently frozen until late January. In contrast to those

sites, Tschengls is north-exposed with no direct sunshine from mid-November until the end of January. Therefore, the upper soil layer (5 cm) was constantly frozen from mid-November until late February (Fig. 1). Blocked water supply because of the frozen soil and concurrently high evaporative demands (Table 1) thus explains the observed high conductivity losses in midwinter of 2011–12 in Tschengls. At Latsch and Tarsch, plants profited from the unfrozen soil. Surprisingly, in the following winter season

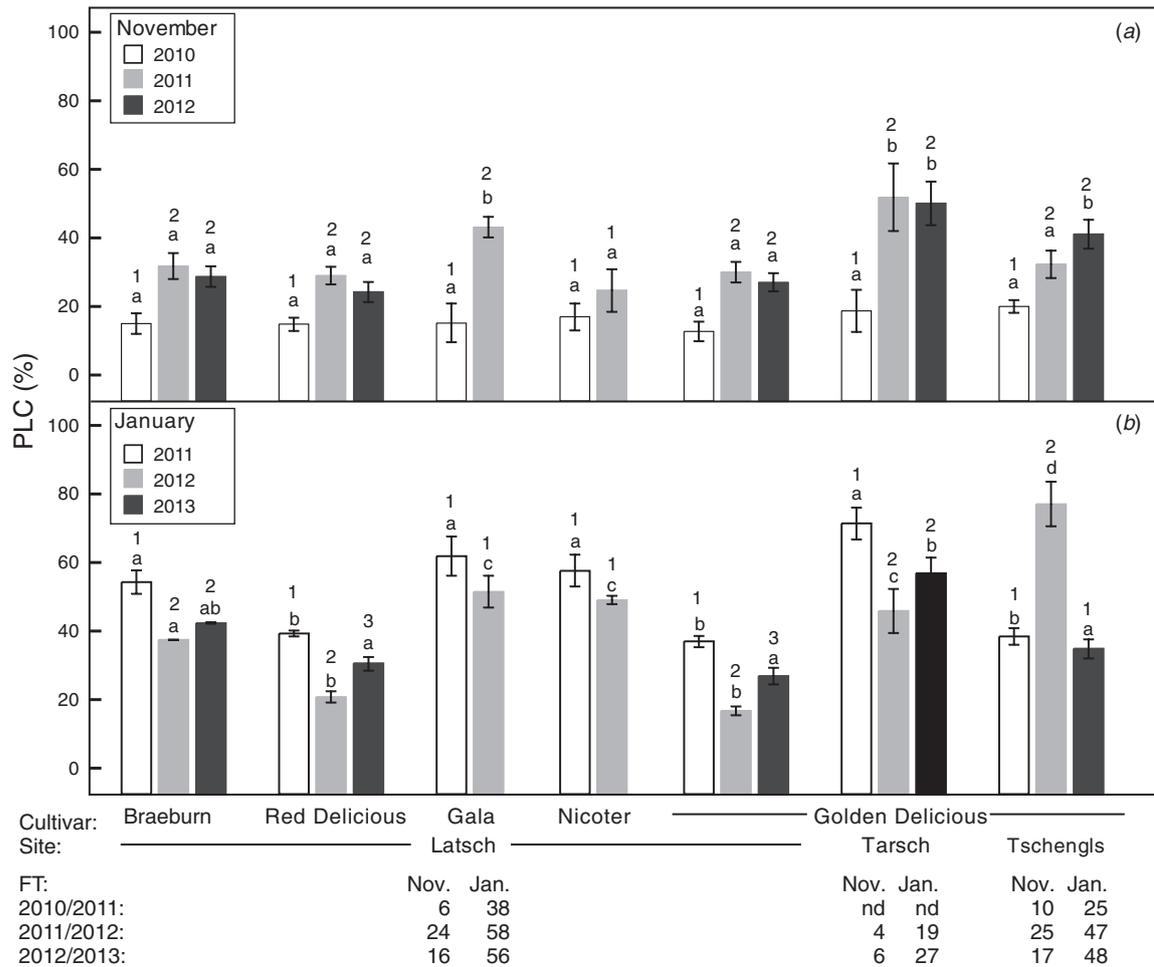


Fig. 3. Mean and s.e. of percentage loss of hydraulic conductivity (PLC) in different cultivars of *Malus domestica* growing on different sites in (a) November and (b) January of the winter seasons 2010–11, 2011–12 and 2012–13. Different letters indicate significant differences among cultivars within a given winter season and different numbers show significant differences among study years within a given cultivar. Numbers below the graph indicate the number of freeze–thaw cycles (FT) experienced until harvesting date of branches used for measurements. Please note that for the cultivars Gala and Nicoter, no data are available for 2012–13. (*n* = 5).

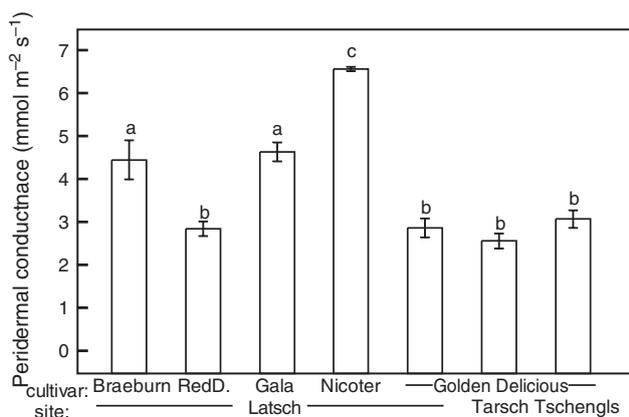


Fig. 4. Mean and s.e. of peridermal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$; *n* = 8–10) in different cultivars of *Malus domestica* growing on different sites in January 2011. Different letters indicate significant differences among cultivars. Red D., Red Delicious.

(2012–13), higher levels of native embolism were found in Tarsch despite the significantly lower number of days with soil frost (Table 1, Fig. 3). This was because the soil in Tarsch was covered with snow from mid-December to mid-February 2013. Therefore, soil temperatures remained around 0°C but water uptake was blocked by the frozen stem base (data not shown). This demonstrates that blockage of water uptake is not simply a matter of soil temperature. Thus, for a detailed analysis of winter hydraulics, soil temperature at different depths as well as other parameters influencing soil and xylem temperature have to be considered.

Midwinter embolism did not only vary significantly among sites but also among cultivars. In January 2012, for instance, PLC in Latsch varied from 16% (Golden Delicious) to 51% (Gala; Fig. 3). Measurements on g_p revealed that cultivars with higher observed PLC were also less protected against water loss through the periderm (i.e. higher g_p ; Figs 3 and 4). Furthermore, Gala and Nicoter are known to develop lammas shoots, which, unlike normal shoots, develop late in the growing season. Because of

the incomplete maturation of the periderm and cuticle, these shoots pose a high risk for uncontrolled water loss during winter (Beikircher and Mayr 2013). Besides protection against water losses, water storage capacity can also play a role in preventing embolism. This was demonstrated in additional measurements in the winter of 2011–12 on young Golden Delicious plants growing directly near the main study plants in Tarsch. In midwinter, embolism values in young trees, which have lower water storage capacity (e.g. Mayr and Charra-Vaskou 2007), were significantly higher than in older trees (Fig. S3).

Spring recovery

Regardless of the amount of winter embolism, in every study year all cultivars on all sites were able to restore their hydraulic system. As early as late winter (February), when soils were still partly frozen, an increase in the water content of buds was observed. As soon as soil temperatures increased above 0°C, the water contents of wood and bark also increased (Fig. 2, Figs S1–S4). Initially, the level of embolism remained high but the remarkably high s.e. in PLC pointed to a high variation among branches and indicated that first refilling processes had already started. Despite optimal soil water conditions, complete restoration of the hydraulic system took several weeks and was not completed before bud break (Table 2, Figs S1–S4). This may be a typical pattern for species in which positive pressure plays no or only a limited role for refilling. In species with pronounced bleeding, refilling is completed before bud break (e.g. Hacke and Sauter 1996). The genus *Malus* is known to develop root pressure in spring but to

a much lesser extent than in, for example, maple (*Acer* spp.), walnut or birch (*Betula* spp.), ceasing before or around bud break (Wiegand 1906). Thus positive pressures might have been involved initially but cannot explain the full recovery, which only occurred several weeks after bud break. If water uptake in spring is still impaired by frozen or cold soils, the resulting late recovery may increase the risk for hydraulic failure, as transpirational water losses can occur already at early phenological stages (Beikircher *et al.* 2016). In March 2011, for instance, we measured midday stomatal conductance values between 78 and 209 mmol m⁻² s⁻¹ on newly developed leaves (phenological stage: ‘half-inch green’, i.e. >10 mm of leaf tissue is protruding from the buds). In Golden Delicious, by April, stomatal conductance was almost in the range of fully developed leaves in summer (see Beikircher *et al.* 2013; Table 2). Because of daily irrigation and thus optimal water supply though, these transpirational losses did not affect our study plants.

Concurrently with the decrease in PLC, we observed a decrease in the starch contents of wood and bark (Fig. 2, Figs S1–S2), which is in accordance with the hypothesis that starch depolymerisation plays a major role in refilling processes (see Introduction). A similar pattern in starch content has been reported for several other species (Essiamah and Eschrich 1985). All cultivars on all sites had restored their hydraulic system by the end of spring in all years. The residual (<10%) loss of hydraulic conductivity in previous-year shoots is probably related to the cavitation or frost fatigue of conduits (Hacke *et al.* 2001; Christensen-Dalsgaard and Tyree 2014). The speed of restoration of the hydraulic system was found to be

Table 2. Date of bud break and completed restoration (defined as the first date of measurement in spring with loss of hydraulic conductivity below 10%) for different cultivars of *Malus domestica* growing at different sites in three successive study years

Mean and s.e. of stomatal conductance (g_s ; mmol m⁻² s⁻¹; $n = 5$) in March and April 2011. Please note that no data are available for the cultivars Gala and Nicoter in 2013 (indicated as nd)

Spring season (year)	Site	Cultivar	Bud break (date)	PLC <10% (date)	g_s (mmol m ⁻² s ⁻¹)	
					29 March	12 April
2011	Latsch	Braeburn	22 March	11 May	136 ± 38	465 ± 47
2012			10 March	19 March		
2013			1 April	24 April		
2011	Latsch	Red Delicious	22 March	3 May	110 ± 32	297 ± 12
2012			10 March	19 March		
2013			1 April	24 April		
2011	Latsch	Gala	22 March	15 May	98 ± 23	411 ± 51
2012			10 March	19 March		
2013			nd	nd		
2011	Latsch	Nicoter	22 March	15 May	102 ± 15	321 ± 9
2012			10 March	19 March		
2013			Nd	Nd		
2011	Latsch	Golden Delicious	15 March	3 May	209 ± 53	614 ± 38
2012			5 March	19 March		
2013			22 March	24 April		
2011	Tarsch	Golden Delicious	20 March	11 May	113 ± 24	322 ± 25
2012			10 March	24 April		
2013			A April	30 April		
2011	Tschengls	Golden Delicious	20 March	11 May	78 ± 32	273 ± 40
2012			10 March	24 April		
2013			1 April	30 April		

strongly related to climatic conditions. As demonstrated below, the crucial factors, therefore, were air and soil temperatures influencing tree phenology and water uptake.

Across all cultivars and all sites, the earliest completion of hydraulic recovery was observed in the spring season of 2012. Following an exceptional mild winter (see above), February and March 2012 were also extraordinarily warm, with mean air temperatures $\sim 3.2^{\circ}\text{C}$ above the long-term average. This led to an early onset of growth and one of the earliest bud breaks ever observed in the study area. In Golden Delicious specimens growing at different sites, bud break occurred more or less simultaneously at the beginning of March 2012, despite considerably differing soil warming and embolism recovery (Fig. 1, Table 2). In the favourably situated orchard near Latsch, soil temperature increased to above 5°C already in early March, but this was delayed by several weeks at Tarsch and Tschengls (Table 1, Fig. 1). Thus in the latter orchards, water uptake could probably not yet cover the demand for refilling and growth processes as well as transpirational water losses. The differences in soil temperature were reflected in restoration speed: In Latsch, PLC decreased below 10% by the end of March, whereas recovery in Tarsch and Tschengls took until the end of April (Table 2). In contrast, in spring 2011 and 2013, soil warming above 5°C on all study sites occurred more or less simultaneously at the beginning of April (Table 1, Fig. 2), and restoration of the hydraulic system occurred around the same time (Table 2). On the intercultivar level, differences in bud break as well as refilling were astonishingly small. Despite significant differences in midwinter embolism among cultivars (see above), in all three study years, leaf flushing and completion of hydraulic restoration occurred more or less simultaneously in all five cultivars. This indicates that these processes are rather environmentally-driven than cultivar-specific.

Summa summarum, high-yield cultivars of *M. domestica* growing under optimised conditions followed similar annual patterns in embolism and related parameters, as have been reported for native species. The formation of winter embolism was related to various climatic and cultivar-specific factors influencing the trees' water status. Restoration of the hydraulic system in spring depended on climatic rather than cultivar-specific parameters, and was not completed before bud break. Although this is common in deciduous plants, it may pose a considerable risk in overbred plants growing in artificial ecosystems like in our study. Three out of the five studied cultivars have been found to exhibit a very risky hydraulic behaviour with high vulnerability to drought-induced embolism and late stomatal closure (Beikircher *et al.* 2013). In combination with a partly impaired water transport system in spring, when water demand for growth and refilling processes as well as transpiration is high, this could lead to aggravation of the trees' water status and subsequently to plant damage. Indeed, in the study area a dieback of plant parts or even whole plants in spring has been observed at irregular intervals in the past, which we assume to be related to winter desiccation and failed recovery in spring (also see Beikircher and Mayr 2013). For the management of such orchards, we thus recommend (I) avoidance of sites with prolonged soil frost, (II) an elaborate irrigation scheme in spring and (III) careful selection of cultivars.

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