Temperature effects on xylem sap osmolarity in walnut trees: evidence for a vitalistic model of winter embolism repair

THIERRY AMÉGLIO,1–3 MÉLANIE DECOURTEIX,3,4 GEORGES ALVES,1,4 VINCENT VALENTIN,1 SOULAIMAN SAKR,4 JEAN-LOUIS JULIEN,4 GILLES PETEL,4 AGNES GUILLIOT4 and ANDRÉ LACOINTE1

1 U.M.R. PIAF (INRA - Université Blaise Pascal), Site INRA de Crouelle, 234 Avenue du Brezet, F-63039 Clermont-Ferrand Cedex 2, France
2 Corresponding author (ameglio@clermont.inra.fr)
3 These authors contributed equally to the paper
4 U.M.R. PIAF (INRA - Université Blaise Pascal), Site Universitaire des Cézeaux, 24 Avenue des Landais, F-63177 Clermont-Ferrand Cedex, France

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Summary  We studied the effect of temperature on the carbohydrate status of parenchyma cells during winter in relation to the efflux and influx of sugars between parenchyma cells and xylem vessels in 1-year-old twigs of walnut (Juglans regia L.). The mechanism of sugar transfer between contact cells and vessels was also investigated. We obtained new insights into the possible osmotic role of sugars, particularly sucrose, in stem pressure formation and winter embolism repair. Accumulation of sucrose in the xylem sap during winter was mainly influenced by: (1) abundant conversion of starch to sucrose in the symplast at low temperatures; (2) sucrose efflux into the apoplast at low temperatures (1 °C); and (3) inefficient sugar uptake at low temperatures, although efficient sugar uptake occurred at 15 °C. We hypothesize that a diethyl pyrocarbonate (DEPC)-sensitive protein mediates facilitated diffusion of sucrose from parenchyma cells to xylem vessels (efflux) in walnut. We discuss the possible occurrence of active H+–sucrose symports and the coexistence of both influx and efflux processes in walnut in winter and the modulation of the relative importance of these flows by temperature.

Keywords: contact cells, facilitated diffusion, stem pressure, sugar transport, water relations.

Introduction

Recently, there has been considerable interest in xylem pressures and their relationship to xylem transport (Salleo et al. 1996, Canny 1997, Holbrook and Zwieniecki 1999, Tyree et al. 1999). Several authors (Sperry et al. 1987, Améglio et al. 1995, Hacke and Sauter 1996, Utsumi et al. 1998, Cochard et al. 2001, Ewers et al. 2001, Améglio et al. 2002) have proposed that positive pressures in the xylem, during winter or spring, have important implications for reversal of frost-induced embolism in temperate woody plants. According to the classical explanation (Pickard 1989, Tyree and Yang 1992, Yang and Tyree 1992), the generation of positive xylem pressure during winter, in the absence of root pressure, involves an osmotic pressure difference between the apoplast and the neighboring compartment, the symplast, i.e., contact cells or vessel-associated cells (VACs) and xylem parenchyma cells.

There have been many studies on winter xylem pressures in Acer spp. and the mechanisms that have been proposed to account for winter stem pressures in this species can be categorized into physical models and vitalistic models. According to the physical models, the winter xylem pressures are attributable strictly to freeze–thaw events (O’Malley and Milburn 1983, Tyree 1983, Milburn and O’Malley 1984). In contrast, according to the vitalistic models, activities of living xylem cells are required for pressure buildup (Wiegard 1906, Johnson 1945, Marvin and Greene 1951). Like Acer, walnut (Juglans regia L.) trees exhibit positive pressures in the xylem sap during winter and spring (Ewers et al. 2001). In this species, winter stem pressure could be associated with freeze–thaw events (Améglio and Cruiziat 1992), low non-freezing temperatures and increased solute concentration of xylem sap (Améglio et al. 2001). Thus, in walnut trees, there are clear correlations between xylem sap osmolarity and xylem pressures.

It is also known from studies with Acer that, at non-freezing low temperatures, starch in stem parenchyma cells is hydrolyzed to sugars, especially sucrose, which are unloaded, in part, into xylem vessels, resulting in increased xylem sap osmotic potential (Marvin et al. 1967, Sauter et al. 1973). Thus, sucrose appears to play a role in the pressure build-up in Acer stems. However, the osmotic role of sucrose in the formation of stem pressures has been questioned (Cortes and Sinclair 1985, Johnson et al. 1987). For instance, Johnson et al. (1987) showed by experimental manipulation, that disaccharides and larger sugar molecules, but not sugar hexoses, can substitute for sucrose in the generation of stem pressure. Based on their findings with hexose sugars, these authors concluded that the positive xylem pressures could not be of osmotic origin and rejected the vitalistic model on this point. However, Johnson et
Sugar influx is considered to be an active H+–hexose co-transport process (Sauter 1980, 1981a). Such a hexose influx could prevent the buildup or maintenance of high osmotic concentrations, and therefore of positive xylem pressure, in the apoplast.

We report on changes both in carbohydrate status in parenchyma cells during winter and in rate of sugar efflux from parenchyma cells to xylem vessels in 1-year-old walnut twigs, and their possible involvement in winter embolism repair. The first objective was to evaluate the impact of carbohydrate mobilization on the efflux process and to determine its temperature dependence. The second objective was to investigate the mechanisms of sugar efflux in the context of the vitalistic model.

Materials and methods

Data were gathered for four winter seasons (1996–1997, 1998–1999, 2000–2001 and 2001–2002) at the INRA PIAF location near Clermont-Ferrand, in south-central France. Measurements were made on excised 1-year-old twigs of walnut (Juglans regia L., cv. Franquette scions on wild walnut rootstocks) from a 15-year-old walnut orchard in 1996. On each date, nine twigs at least 80 cm long were harvested at 0900 h. After removing the apical 10 cm, sap measurements and tissue carbohydrate determinations were made on the upper 30-cm-long segment.

Seasonal xylem sap and tissue carbohydrate evolutions

Xylem sap was extracted from the stem segments, 1 h after twig excision, according to the method of Bollard (1953; see review by Schurr 1998). About 5 cm of bark was removed from the apical end of the stem to prevent contamination with phloem sap. Xylem sap from several stems was extracted simultaneously by applying 0.1 MPa suction in a vacuum distillation system. Sap samples were collected in glass tubes placed on ice and their osmolarity determined with a Roebling 13DR automatic osmometer (Messtechnik, D-1000 Berlin, Germany). Following extraction of xylem sap, the stem samples were frozen in liquid nitrogen and lyophilized. Soluble sugars were extracted from the lyophilized stem tissue with 80:20 (v/v) hot ethanol:water and purified on ion-exchange resins (Bio-Rad AG 1-X8 in the carbonate form (Bio-Rad, Hercules, CA), Dowex 50W in the H+ form (The Dow Chemical Company, Midland, MI), as described by Moing and Gaudillère (1992). Sucrose, glucose and fructose contents were determined spectrophotometrically at 340 nm after enzymatic assays (Boehringer 1984). Starch content was determined by a hexokinase, glucose-6-phosphate linked assays (Kunst et al. 1984) after hydrolysis of the sample with amyloglucosidase (Boehringer 1984).

Efflux studies

The effect of temperature on sugar efflux into xylem vessels in 1-year-old twigs from the same walnut trees was investigated. The ends of 20 shoot segments of 30-cm length were sealed with paraffin film (Parafilm), enclosed in a plastic bag to prevent desiccation and preconditioned for 48 h at 1, 5, 10 or 15 °C in controlled refrigerators (Liebherr 650 l, controlled temperature range: −5 to 15 °C, ± 1 °C). After the 48-h preconditioning period, five of the 20 stem segments were processed as previously described for xylem sap extraction and sugar analysis; sap volume was recorded as the mass of the extracted sap (SM), and the fresh mass of the remaining stem tissues was measured (FM). Stem tissue dry mass (DM) was determined after freeze-drying and the ratio of xylem-contained sap to total stem water volume was approximated as 100(SM/FM – DM)). This ratio was used solely to follow the quantity of sap extracted from vessels over time. Because of air blockages at the ends of the vessels, Bollard’s method extracts sap from some but not all of the vessels.

For the efflux study, tissues outside the cambium were removed from the ends of each of the remaining 15 stem segments to prevent contamination with phloem constituents (Sauter 1982), and a plastic tube was fixed tightly over the basal end. Each segment was then perfused with 3 ml of Control solution A (1 mM KCl, 1mM NaCl, 0.2mM CaCl2 with a 0.5 mM Mes/Tris buffer adjusted to pH 6 with HCl), under moderate pressure (0.1 MPa) that allowed the 3-ml perfusion to be completed within 5 min. The last 0.5 ml of perfusate was collected from the apical end and its osmolarity was checked and compared with that of the original Control solution A: no significant alteration could be detected, showing the effectiveness of substituting xylem sap with Control solution A.

After a 1-h incubation at 1, 5, 10 or 15 °C following substitution of the xylem sap in the stem segments with Control solution A, we used two methods to characterize the efflux process and rate. In the 1996–1997 experiment, the content of xylem vessels was collected by perfusing another 1 ml of Control solution A through the stem segment under a pressure of 0.1 MPa, and the perfusate was assayed for osmolarity and sugar (GFS = glucose + fructose + sucrose) content; this procedure was repeated hourly three times on the same segment to check for measurement repeatability and long-term stem tissue viability. In the 1998–2002 experiments, the content of xylem vessels for each segment was extracted directly according to the method of Bollard (1953) and assayed for osmolarity and soluble carbohydrates. These procedures yield the balance between efflux and influx. Both procedures reflect mainly (but not exclusively) the efflux of sugar because the control solution initially does not contain sugar.

To investigate mechanisms of efflux and the relative importance of influx and efflux in net sugar flux, stem segments were rinsed under pressure (0.1 MPa) with 6 to 8 ml of Control solution B (0.1 mM CaCl2, 0.1 mM KCl, 0.1 mM MgCl2 with a 10 mM MES buffer adjusted to pH 6 with HCl), in the presence and absence of inhibitor. The inhibitors tested were 5 mM DEPC (diethyl pyrocarbonate) and 1 mM PCMBS (p-chloro-
mercuriphenylsulfonic acid), both known to inhibit H⁺-sugar transmembrane symporter activity (Sakr et al. 1993), and 1 mM HgCl₂, a dissipator of the proton transmembrane electrochemical potential difference, which allowed us to evaluate the importance of the energy-dependent component of H⁺-sugar symport processes for influx. Sugar fluxes were quantified by determining the sugar enrichment of Control solution B after 1 h.

**Statistical analyses**

Means and standard errors were computed for all measurements, and the significance ($P < 0.05$) of treatment effect was evaluated by the non-parametric tests of Mann and Whitney (Sprent and Smeeton 1993), or Kruskal and Wallis (Sprent and Smeeton 1993) when more than two treatments were compared. All statistical analyses were performed with XLSTAT 6.0 (Addinsoft, Paris, France).

**Results**

**Changes in stem carbohydrates, xylem sap osmolarity and air temperature during winter**

Significant interconversion between starch and soluble sugars was observed during the winter (Figure 1b). Minimum starch concentrations occurred when air temperature was at its minimum (Figure 1a). Sap osmolarity increased during winter (Figure 1c), but its maximum did not coincide with the maximum glucose + fructose + sucrose (GFS) concentration in stem parenchyma tissues. Maximum sap osmolarity occurred on January 27, at a mean air temperature of 1.2 °C. Between January 27 and February 12, sap osmolarity decreased sharply, in parallel with a sharp increase in mean air temperature (1.2 to 14.1 °C), but there was only a slight decrease in soluble stem sugar concentration.

This immediate response of xylem sap osmolarity to a change in temperature led us to choose the mean temperature of the day that sap was extracted as the reference temperature when investigating the effect of temperature on xylem sap properties. Mean air temperature was computed as the mean of the minimum and maximum air temperatures recorded that day. Negative correlations between mean day air temperature and osmolarity ($r^2 = 0.41; P = 0.01$) and sugar concentration (GFS; $r^2 = 0.59; P = 0.001$) were observed.

**Effects of air temperature on xylem sap osmolarity and sugar effluxes during winter**

To investigate the impact of temperature on sap osmolarity, we measured changes in both sap osmolarity and sap sugar concentrations induced by 48 h of thermal conditioning at 1 or 15 °C for three periods during winter 1996–97. In all cases, sap osmolarity increased in stem segments conditioned at 1 °C for 48 h, whereas it decreased significantly in stem segments conditioned at 15 °C for 48 h (Figure 3a). These differences in sap osmolarity were paralleled by differences in sap sugar concentrations (Figure 3b). After thermal conditioning at 1 °C, sucrose was the main soluble carbohydrate in xylem sap in January and March, whereas hexoses increased significantly in December. Sugars accounted for up to 6.5% of sap weight in January in stem segments conditioned at 1 °C (data not shown), compared with less than 0.2% of sap weight in December in stem segments conditioned at 15 °C (data not shown). In response to 48 h of temperature conditioning (Figure 3c), the ratio of sap volume to total stem water volume was not significantly altered by the 1 °C conditioning, and although the ratio was decreased by the 15 °C conditioning in December and January, it approached the ratio found under natural conditions in March.

To investigate the impact of 1 and 15 °C conditioning on the within-1-h variation in osmolarity of the control solution that
Figure 2. Relationships between xylem sap osmolarity or sap sugar concentration (GFS = glucose + fructose + sucrose) and mean daily air temperature. Abbreviation: $r^2 =$ coefficient of determination.

Figure 3. Time course of xylem sap properties of 1-year-old twigs on three sampling dates (see arrows in Figure 1b). (a) Sap osmolarity; (b) sap sugar concentrations; (c) approximate ratio (%) of xylem vessel-water content to total stem water content under natural conditions (NC) or after 48 h of thermal conditioning at 1 or 15 °C. Values are means ± SE ($n$ = 5).

Figure 4. Sap osmolarity and total sugar concentration (GFS = glucose + fructose + sucrose) of the Control solution A substituted for the original sap after 1 h of temperature treatment at 1 or 15 °C. Substitution (by perfusion) was repeated 3 times, with a 1-h incubation every time. (a) Relationships between osmolarity of Control solution A for the 1st run versus the 2nd and 3rd runs in the same twigs. (b) Mean of sugar effluxes at 1 °C (1st, 2nd and 3rd runs) for three sampling dates (see arrow in Figure 1b). (c) Mean of sugar efflux rates at 15 °C (1st, 2nd and 3rd runs) for three sampling dates (see arrow in Figure 1b). Values are means ± SE ($n$ = 15).
had been substituted for the original xylem sap, perfused Control solution A was collected after 1 h by forcing another 1.0 ml of Control solution A into the stem segment under pressure. This protocol was repeated three times on each stem segment. Figure 4a shows the relationship between osmolarity measured after the first run of each experiment versus the osmolarity after the second and third runs. The apparent efflux rate remained constant for 3 h, indicating that the efflux process in walnut trees remained unaltered over the experimental period. There was a large range of variation in osmolarity for stem segments subjected to thermal conditioning at 1 °C (between 20 to 150 mosmol l⁻¹ h⁻¹), contrasting with a narrow range of variation in osmolarity (< 15 mosmol l⁻¹ h⁻¹) for stem segments thermo-conditioned at 15 °C.

Figure 4b shows the sugar efflux over a 1-h period at 1 °C for three periods in winter 1996–1997 (cf. arrows in Figure 1b). The efflux rate exhibited significant differences among the three periods, ranging from 0.8 to 2% of total sap weight (data not shown). This variation was not explained by changes in parenchyma sugar concentrations. In January and March, sugar efflux rates at 1 °C were similar (0.59 versus 0.56 mg g⁻¹ DM h⁻¹), whereas parenchyma sugar concentrations decreased by more than 50% (73 to 31 mg g⁻¹ DM). In all cases, sucrose accounted for at least 60% of the total sugar fluxes. In contrast, at 15 °C (Figure 4c), the total sugar net efflux rate was one twentieth of that at 1 °C, and represented a maximum of 0.08% of total sap weight, with sucrose (data not shown) not exceeding 40% of the total sugar fluxes. At 15 °C, sugar efflux rate exhibited a wide range of variation among the different periods studied.

During winter 1998–1999, we investigated the effect of temperature (1, 5, 10 and 15 °C) on sugar efflux over 1 h. In this protocol, the content of xylem vessels was extracted directly by the vacuum method. We observed a strong negative relationship (r² = 0.74, P < 0.0001) between temperature and sap osmolarity (Figure 5a), with a sharp increase at 1 °C versus no response at 15 °C. Glucose, fructose and sucrose concentrations of the collected sap showed the same pattern. The net sugar efflux rate decreased with increasing temperature. Figure 5b presents the same results expressed in pressure units by calculating the theoretical osmotic pressure accounted for by the three sugars. Again, the sugar osmotic component explained 80% of sap osmolarity.

**Sugar flux inhibitors**

In February, of the sugar flux inhibitors tested at 1 °C (cf. Table 1), only DEPC caused a significant change in osmolarity of the collected Control solution B during a 1-h hour incubation in xylem vessels. There was a 29% decrease in osmolarity of

<table>
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<th>Treatment</th>
<th>1 °C Osmolarity ± SE</th>
<th>P value</th>
<th>15 °C Osmolarity ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.8 ± 5.9</td>
<td></td>
<td>30.4 ± 1.2</td>
<td></td>
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<tr>
<td>+ DEPC (5 mM)</td>
<td>60.8 ± 7.9</td>
<td>&lt; 0.02</td>
<td>35.3 ± 1.7</td>
<td>&lt; 0.02</td>
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<tr>
<td>+ HgCl₂ (1 mM)</td>
<td>94.2 ± 14.0</td>
<td>ns</td>
<td>45.3 ± 4.8</td>
<td>&lt; 0.01</td>
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<tr>
<td>+ PCMB (1 mM)</td>
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the collected solution in the presence of 5 mM DEPC compared with the control (Table 1), whereas PCMBs had no effect. When stems were kept at 15 °C, the osmolarity of the collected solution was similar to that of the rinsing solution (control). These results confirm that only low temperatures produced high osmolarity. At 15 °C, addition of DEPC (5 mM), PCMBs (1 mM), or HgCl₂ (1 mM) to Control solution B resulted in a significant increase in osmolarity (15, 49 and 160%, respectively) that closely approximated the osmolarity of the control at 1 °C.

Discussion

Previous studies have shown that sucrose accumulation in the xylem sap during winter is mainly influenced by: (1) conversion of starch to sucrose in the symplast at low temperatures; (2) subsequent passive, facilitated efflux of sucrose into vessels; and (3) inefficient resorption of sugars (influx of sugars from vessels into VACs) at low temperatures (cf. Sauter 1988a).

Seasonal changes in carbohydrate content in woody plants have been investigated for a long time (Hartig 1860 in Sauter 1988a) and are well documented (Siminovitch et al. 1953, Ziegler 1964, Kramer and Kozlowski 1979, Sauter and Ambrosius 1986, Frossard and Lacointe 1988, Sauter and Van Cleve 1994). We found significant starch hydrolysis in walnut, resulting in minimum starch concentrations in deep winter, followed by a period of starch resynthesis in late winter, prior to final mobilization of carbohydrates for bud break in spring. Thus, starch degradation occurred at low temperatures during winter, and also as temperature increased in spring. Other studies have shown that the accumulation of sucrose due to winter starch mobilization is an essential step towards freezing tolerance (Sakai 1966, Sauter et al. 1996). For walnut, in addition to the probable role of sucrose in freezing tolerance, sucrose also appeared to be of major importance for embolism repair as a result of starch degradation and the subsequent release of sucrose into xylem vessels.

Améglio et al. (2002) showed that walnut is vulnerable to frost-induced embolism, but the degree of xylem embolism varied during winter and was related to the ability of walnut to generate positive xylem pressure. There may be more than one mechanism involved in pressure generation in walnut tree xylem. In spring and autumn, root pressure is positively correlated with xylem sap osmotic concentration (Améglio and Cruiziat 1992). Likewise, in many temperate trees, such as Betula pendula Roth, Alnus glutinosa L., Fagus sylvatica L., and Quercus robur L., positive xylem pressures are associated with high sugar concentrations in the xylem sap (Essamiah 1980), but only in early spring and not in winter. Moreover, when walnut plants were deprived of stored carbohydrates (starch) by defoliation treatments in late summer and early fall, xylem sap osmolarity and xylem pressure were significantly reduced the following winter (Améglio et al. 2001).

Effect of air temperature on sap sugar concentrations

Because of their location and structure, it has been proposed that vessel-associated cells (VAC) (Czaninski 1968) or contact cells (Sauter 1972) control nutrient exchanges between storage parenchyma tissue and xylem vessels (Czaninski 1977, Sauter and Van Cleve 1992, Alves et al. 2001a). Cytological and metabolic studies on walnut show that VACs are the only cells in the xylem tissues that are connected with the vessels by means of large and numerous pits (Alves et al. 2001b). Consistent with the assumed function of VACs, VACs possess numerous mitochondria and high respiratory enzyme activity, in contrast to regular storage parenchyma cells. In many species, VACs are the sites of sugar secretion into the xylem sap (Sauter et al. 1973, Sauter 1980, 1981a, Braun 1984, Essamiah and Eschrich 1985). The efflux of sugars from parenchyma cells through VACs into the apoplast has been studied in maple (Sauter et al. 1973), willow (Sauter 1980) and poplar (Sauter 1988a). However, the efflux process is still poorly understood, particularly the dynamic relationship between tissue sugar concentration and rate of sugar efflux into the xylem sap. We found that maximum GFS concentration in stem parenchyma did not coincide with maximum sap osmolarity (Figure 1). Between January 27 and February 12, stem soluble sugars remained almost stable whereas a large decrease in sap osmolarity was observed.

As a first approximation, sap sugar concentration was related to the mean temperature of the measurement day (Figure 2), whereas parenchyma sugar concentration was related to longer-term temperature changes (Figure 1b). In poplar, parenchyma starch breakdown (Sauter 1988b) was correlated with low temperature, but required several days (minimum: 1 week) of low temperature conditioning. Similarly, when walnut stem was conditioned for 48 h at 1 °C, high concentrations of sugar were measured in original or perfused xylem sap, contrasting with low concentrations in stem segments conditioned at 15 °C for 48 h. Our results differed from those reported for birch (Sauter and Ambrosius 1986) where net sugar efflux rate was higher in stems preconditioned for 24 h at 21 °C than at 1 °C. In birch, maximum sap sugar concentration occurred in April just before bud break, whereas in walnut maximum sap sugar concentration occurred in deep winter (Figure 1c), even though sugar efflux rate did not change significantly between January and March (0.59 versus 0.56 mg g⁻¹ h⁻¹). Thus, in walnut, low temperature in deep winter could explain high values of sap osmolarity, because sugars accounted for 80% of the sap osmolarity (Figure 5). High sap osmolarity causes water to be driven osmotically into xylem vessels from parenchyma cells or fibers, generating positive pressure (Améglio et al. 2001) that allows embolism repair (Améglio et al. 2002).

Although sucrose accounted for about 60% of the total sap soluble sugars (2% of total sap weight at 1 °C versus 0.08% at 15 °C), hexoses were also present in detectable amounts, and
the mass ratio of sucrose to hexoses (glucose + fructose) was dependent on temperature (1.8, 1, 0.5, 0.25 at 1, 5, 10 and 15 °C, respectively). This suggests either enzymatic hydrolysis of the released sucrose by an extra-plasmic invertase or variable influx of hexoses, or both (Figure 6).

Sugar exchange between VACs and xylem vessels

A third major factor that modulates sucrose accumulation in the xylem sap is influx, i.e., uptake of sugars into parenchyma tissue. Accumulation of sugar in xylem vessels does not occur by simple diffusion, but results from the balance of two opposing fluxes (Figure 6): (1) an efflux of sugars from parenchyma into xylem vessels (Sauter 1980), and (2) an influx (or uptake), of sugars into VACs from the xylem sap (Sauter 1981). In willow (Sauter 1982), sucrose efflux is inhibited by PCMBs (a thiol-reactive compound), suggesting that the efflux is mediated by a facilitated diffusion mechanism. We found that PCMBs had no effect on sap osmolarity during winter at low temperature (1 °C), but DEPC (5 mM) and PCMBs (1 mM) on xylem sap osmolarity in stem segments kept for 48 h at 15 °C, during February 2001 and 2002. Addition of HgCl₂, a dissipator of the proton electrochemical potential difference that drives sucrose influx, which thus indirectly blocks H⁺-sugar symporter activity, resulted in a sharp increase (160%) in osmolarity. Addition of DEPC or PCMBs, both known to block H⁺-sucrose symports (Sakr et al. 1993) caused increases in sap osmolarity of 24 and 34%, respectively. Therefore, we conclude that, when winter temperatures are mild, the influx process coexisted with the efflux process in walnut (Figure 6).

To understand better the balance between sugar influx and efflux, we also assessed the effects of HgCl₂ (1 mM), DEPC (5 mM) and PCMBs (1 mM) on xylem sap osmolarity in stem segments kept for 48 h at 15 °C, during February 2001 and 2002. Addition of HgCl₂, a dissipator of the proton electrochemical potential difference that drives sucrose influx, which thus indirectly blocks H⁺-sugar symporter activity, resulted in a sharp increase (160%) in osmolarity. Addition of DEPC or PCMBs, both known to block H⁺-sucrose symports (Sakr et al. 1993) caused increases in sap osmolarity of 24 and 34%, respectively. Therefore, we conclude that, when winter temperatures are mild, the influx process coexisted with the efflux process in walnut (Figure 6).

This study has provided new insights into the old debate about vitalistic versus physical models to explain winter pressure generation by implicating roles for H⁺-sugar carriers and plasma membrane H⁺-ATPase in xylem tissues. The physiological processes that occur during winter in deciduous trees, particularly the coupled water/carbon fluxes, are clearly important in preparing tree functions for the next vegetative period.

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