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Winter stem xylem pressure in walnut trees: effects of carbohydrates, cooling and freezing

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Summary Pressure transducers were attached to twigs of orchard trees and potted trees of walnut (*Juglans regia* L.) to measure winter stem xylem pressures. Experimental potted trees were partially defoliated in the late summer and early autumn to lower the amount of stored carbohydrates. Potted trees were placed in cooling chambers and subjected to various temperature regimes, including freeze–thaw cycles. Xylem pressures were inversely proportional to the previous 48-h air temperature, but positively correlated with the osmolarity of the xylem sap. Defoliated trees had significantly lower concentrations of stored carbohydrates and significantly lower xylem sap osmolarities than controls. Plants kept at 1.5 °C developed xylem pressures up to 40 kPa, just 7% of the theoretical osmotic pressure of the xylem sap. However, exposure to low, nonfreezing temperatures followed by freeze–thaw cycles resulted in pressures over 210 kPa, which was 39% of the theoretical osmotic pressure. A simple osmotic model could account for the modest positive winter pressures at low, nonfreezing temperatures, but not for the synergistic effects of freeze–thaw cycles.

Keywords: freezing, Juglans regia, pressure transducer, sap osmolarity, stem pressure.

Introduction

During winter, freeze–thaw events can induce embolism (air blockage) and reduce the hydraulic conductivity of temperate woody plants (Cochard and Tyree 1990, Sperry and Sullivan 1992). It has been argued that freezing-induced embolism can limit the growth, survival and geographic distribution of plant species (Sperry and Sullivan 1992, Tyree and Cochard 1996, Pockman and Sperry 1997, Langan et al. 1997). Several authors (Sperry et al. 1987, Sperry et al. 1988, Sperry et al. 1994, Améglio et al. 1995, Hacke and Sauter 1996) have suggested that positive xylem pressures, during spring in particular, can have important implications for the elimination of freezing-induced embolism in temperate woody plants. In *Betula alleghaniensis* Britt., freezing-induced damage to roots caused decreased spring root pressures and recovery from winter embolism, and increased shoot dieback (Zhu et al. 2000).

Although most authors have considered positive pressures in the xylem to be important for eliminating embolisms (Sperry et al. 1987, Holbrook and Zwieniecki 1999, Tyree et al. 1999), the mechanisms underlying winter pressure development remain poorly understood. Xylem pressure development in winter has been studied in many species, particularly *Acer* species, with several hundred papers dealing with the flow of maple sap (see reviews in Marvin 1958, O'Malley and Milburn 1983, Tyree 1983, Johnson et al. 1987, Milburn and Kallarackal 1991). In *Acer*, winter xylem pressures are developed in the stem and not the root, because sap exudation from the stumps of felled trees is negligible in winter, whereas copious exudation occurs from excised shoots (Stevens and Eggert 1945, Marvin and Greene 1951). Proposed mechanisms of winter pressure development in *Acer* can be categorized into either "physical models" or "vitalistic models." According to the physical models, winter pressures are strictly the result of freeze–thaw events (O'Malley 1979, Tyree 1983). In contrast, according to the vitalistic models, activities of living cells in the xylem are required for pressure buildup (Wiegand 1906, Johnson 1945, Marvin and Greene 1951, Marvin 1958). It is known that, at low, nonfreezing temperatures, starch in stem parenchyma cells is hydrolyzed to sugars, especially sucrose (Marvin et al. 1967). Although sucrose appears to play a role in the pressure buildup in *Acer* stems, the osmotic role of sucrose in the development of stem pressures has been questioned because the accumulation of sucrose is apoplastic (Cortes and Sinclair 1985, Johnson et al. 1987). Sauter (1974) suggested that living parenchyma cells are crucial for gas production, leading to temperature-dependent changes in gas pressure in the air spaces within the wood fibers.

Walnut trees (*Juglans regia* L.) have also been observed to display positive pressures in the xylem sap during the winter, autumn and spring. Autumn and spring xylem pressures appeared to be of root origin and were positively correlated with soil temperature (Améglio et al. 2001). Winter stem pressure was associated with low temperatures and with high sugar concentrations in the xylem sap (Améglio and Cruiziat 1992).

In the present study, winter pressure and temperature mea-

surements were made on walnut trees growing in an orchard, and on potted trees subjected to various cooling and freezing experiments, to determine the roles of carbohydrates, temperature, and the freeze–thaw process in stem xylem pressure development. To evaluate the role of stored carbohydrates, potted experimental trees were manually defoliated in the late summer and early autumn to reduce carbohydrate reserves. We tested three hypotheses, each of which is consistent with a vitalistic, osmotically based model of winter xylem pressures. (1) Pressure is positively correlated with sugar concentration in the xylem sap, and inversely correlated with temperature. (2) Compared with control trees, the xylem of defoliated experimental trees will have lower winter osmolarities and lower xylem pressures. Lastly, (3) freeze– thaw cycles will have no effect on xylem pressures beyond their effect on xylem sap osmolarities.

Materials and methods

During the winters of 1997–98 and 1998–99, temperature and pressure measurements were made on 17-year-old walnut trees (*Juglans regia* L. cv. Franquette scions on wild walnut rootstocks) outdoors in an orchard at the INRA PIAF station near Clermont-Ferrand, France. In addition, 5-year-old potted trees of the same variety were used for cooling and freeze–thaw experiments during the winter of 1998–99. Each grafted plant was grown in a 33-liter well-drained container filled with a 1:2 (v/v) mixture of peat and clay soil. The potted trees were fertilized annually with 10 g of NH_4NO_3 and continuously drip-irrigated to field capacity.

Nine pairs of potted trees were matched for similarity in size, branching pattern and leaf area. Based on a coin toss, one tree of each matched pair served as the control and the other tree was repeatedly partially defoliated in late summer and autumn. Defoliation was carried out to reduce the amount of stored carbohydrates, because late summer and fall is the time when trees normally allocate most of their carbohydrates for winter storage (Dickson 1991). Repeated defoliations were used because newly formed leaves can counteract the effect of a single defoliation (Sauter and Neumann 1994). On July 30, 1998 (first defoliation), all but the two distal macroscopic leaves were excised from each branch of the experimental trees with a razor blade. New leaf maturation occurred on five of the eight experimental trees, requiring additional defoliations on August 24 and September 8, 1998. Mean leaf area removed per tree was 1.18, 0.33 and 0.18 m^2 for the first, second and third defoliation, respectively.

The nine pairs of trees were grown outdoors until September 1998, when they were transferred to a frost-protected greenhouse. Greenhouse air temperature normally tracked outside temperatures above 0 °C.

Before the cooling experiments, we covered the soil surface of potted plants with several cm of sand, which resulted in a high soil water content for at least 2 weeks. A cooling chamber was designed to hold up to four potted trees up to 2 m in height. Cooling and warming cycles were computer controlled and copper-constantan thermocouples were used to measure stem, air, and soil temperatures. Data loggers (DL2e, Delta-T Devices, U.K.) recorded temperatures and pressures as 5-min means.

To measure xylem pressures, one or two branches from each tree were excised, the bark removed for 1 cm from the stump and the exposed phloem surface wrapped with teflon tape. The exposed xylem surface was shaved smooth with a razor blade and the end of the branch attached to a length of tubing. A pressure transducer (Model PDCR 1000, Druck LTD, Leicester, England, sensitivity ± 1 kPa) was attached to the other end of the tubing and both it and the tubing were filled with nonfreezing silicone fluid (No. 85409, Fluka Chemika, Buchs, Switzerland; –50 to 200 °C, 100 MPa s viscosity at 20 °C).

Each week, to prevent possible clogging of xylem vessel elements by silicone oil, the surface of the stumps was re-cut by 1 cm and the transducers reconnected. In field-grown trees, based on observations with eight transducers on a single tree, it was necessary to recut the stem surfaces only once every six weeks to prevent loss of positive pressure resulting from vessel clogging.

Plants were exposed to various cooling and warming cycles. In some cases, freeze–thaw cycles were repeated on the same specimens up to four times. Plants were kept at particular resting temperatures for up to 72 h before cooling or warming, but whenever air temperatures were changed in the chambers, the rate of cooling or warming was always 5 °C h^{-1}.

Before and after cooling treatments, xylem sap was extracted from samples of 30-cm stem segments with a vacuum distillation process as described by Bollard (1953) and the sap volume was determined by weight. In some cases, if there was copious exudation of fluid from the cut stem, the bark was peeled back (to avoid phloem sap contamination) and the xylem exudate was sampled with a syringe. Osmolarity of the xylem sap was measured 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 the fresh weight (FW) measured. Stems were lyophilized, and the dry weight (DW) measured. Water content was calculated as (FW – DW)/DW. Soluble sugars were extracted from the stems with hot ethanol:water (80:20, v/v), and purified on ion-exchange resins (Bio-rad AG 1-X8 in the carbonate form, Dowex 50W in the H^+ form), as described by Moing and Gaudillière (1992). Enzymatic assays were used to determine sucrose, glucose and fructose contents as absorbance at 340 nm (Boehringer 1984), and starch content was determined with a hexokinase, glucose-6-phosphate linked assay (Kunst et al. 1984) after hydrolysis with amyloglucosidase (Boehringer 1984).

Results

Orchard trees

In January 1998 when air temperatures were between –5 and 10 °C, orchard trees had positive xylem pressures at 1.5 and

3.5 m above ground. There was a complex relationship between xylem pressures and temperature. Frozen branches showed no pressures on January 8; however, following a thaw, pressures rose quickly. During the subsequent 3 days when air temperature was 4 °C or higher, pressures tended to decrease. Higher xylem pressures were obtained when measurements were made at 3.5 m above ground than at 1.5 m above ground, corresponding to lower temperatures (below 2 °C) at the 3.5 m level (see Figure 1: January 10 at 1200 h).

Defoliated treatment and stem carbohydrates

Carbohydrate reserves, both starch and sucrose, were 30% lower in defoliated plants compared with controls (Figure 2). Concentrations of stored glucose and fructose, which occurred in minor amounts in the stem symplast, were not significantly affected by the defoliation treatment (Figure 2).

Stem osmolarity and xylem pressure

In both defoliated and control plants kept for two days at 18 °C, xylem pressure declined to zero. When air temperatures were subsequently lowered to 1.5 °C for two days, pressures gradually built up to a maximum close to 35 kPa for control and 20 kPa for defoliated plants (Figure 3a, top panel). The inverse experiment gave complimentary results. On December 12 (Figure 3b, bottom panel), defoliated and control plants were kept at 1.5 °C for two days, and their pressures rose to as high as 10 and 25 kPa, respectively. The same plants were then warmed to 18 °C and kept at that temperature for two days, and their pressures dropped to 0 and 5 kPa, respectively. During winter it took up to two days for pressures in stems to equilibrate to a major change in temperature. To analyze the relationship between osmolarity of the stem xylem sap and temperature, we chose the mean temperature for the 48-h period prior to an osmolarity measurement as the independent variable. The mean temperature was determined from the greenhouse data logger at the beginning of an experiment, or from the cooling chamber data logger in the middle or at the end of an experiment.

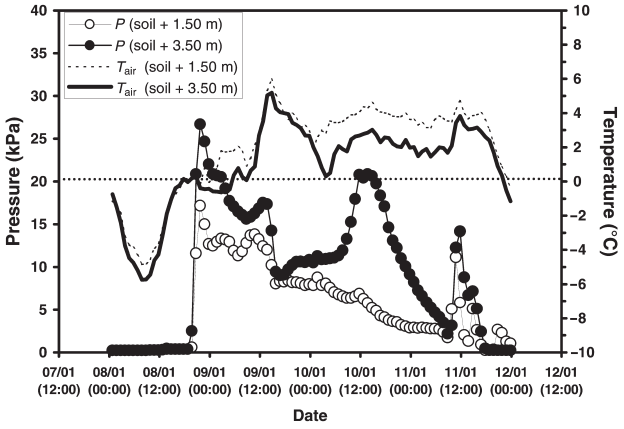

Figure 1. Air temperatures and stem xylem pressures measured at 1.5 and 3.5 m above soil level in an orchard-grown walnut tree in January 1998. Values are 1-h means.

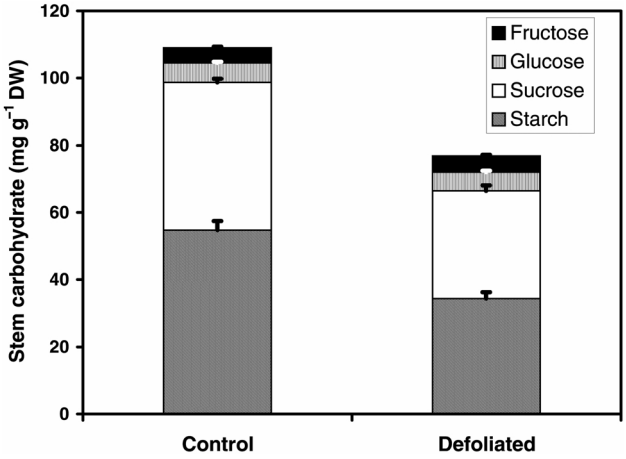

Figure 2. Effect of defoliation on the concentration of stored stem (symplast) carbohydrates for defoliated and control plants. Means + 1 SE are shown, $n = 25$. For the defoliated treatment, leaves were removed during the previous late summer and early autumn.

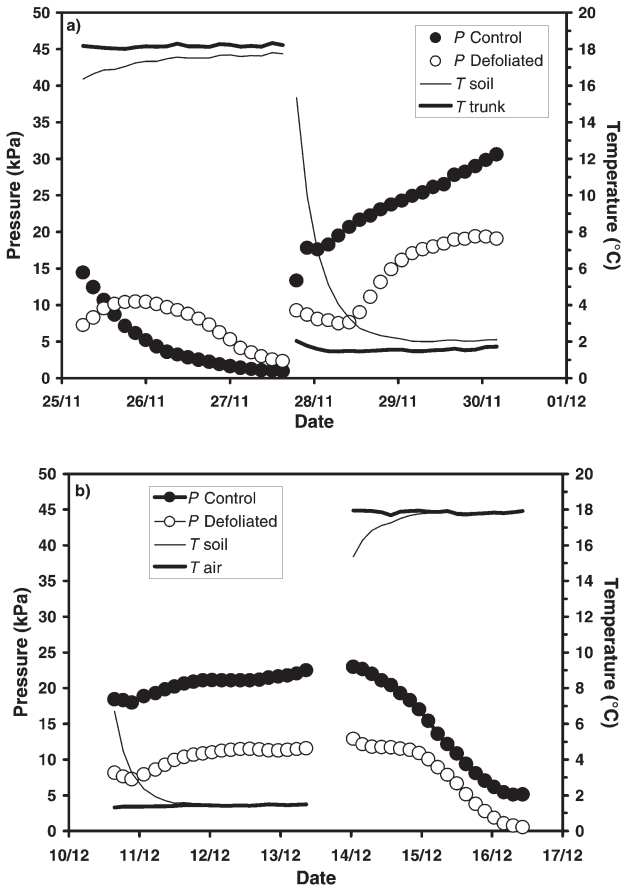

Figure 3. Effect of temperature on osmotically induced stem pressures in late November and December 1999. In the upper panel (a), two control and two defoliated potted trees were kept in the cooling chamber at an air temperature of 18 °C for 2 days, then the air temperature was decreased to 1.5 °C for 2 days. The lower panel (b) shows the inverse treatment; plants were kept first at 1.5 °C for 2 days and then at 18 °C for 2 days. Means ($n = 2$) per treatment are presented. The xylem pressure values are 3-h means.

Osmolarity of the xylem sap was inversely proportional to the mean previous 48-h temperature for both control and defoliated plants. Sap osmolarity was very high in trees subjected to temperatures below 5 °C (Figure 4a). Independently of the temperature treatment, sap osmolarity was about 30% lower in defoliated plants than in control plants (Figure 4a). Although sap osmolarity was sensitive to temperature, the amount of stored (symplastic) carbohydrate was insensitive to the previous 48-h temperature. There was no significant difference in stored carbohydrate for stems kept at 1.5 °C, intermediate temperatures, or 18 °C (results shown for defoliated plants, Figure 4b).

Freeze–thaw cycles

A freeze–thaw cycle increased the osmolarity of xylem sap, and it increased the xylem pressures even more. For example, defoliated and control plants kept at 18 °C for 72 h had xylem pressures close to zero, and xylem sap osmolarities of 25 and 55 mosmol l^{-1}, respectively (data not shown). However, when the plants were subjected to three freeze–thaw cycles with a resting temperature between cycles of 18 °C, the final osmolarities were 101 and 178 mosmol l^{-1} for the defoliated and control plants, respectively (data not shown). These omolarities were higher than expected based on the values obtained at nonfreezing temperatures. With freezing exotherms, and especially with thaw endotherms, there were transient spikes in stem xylem pressure. During the resting phases at 18 °C, pressure tended to dissipate, but with repeated freeze– thaw cycles, the pressure increased to as high as 160 kPa in the control plants, much higher than in control plants that had not experienced a freeze–thaw cycle.

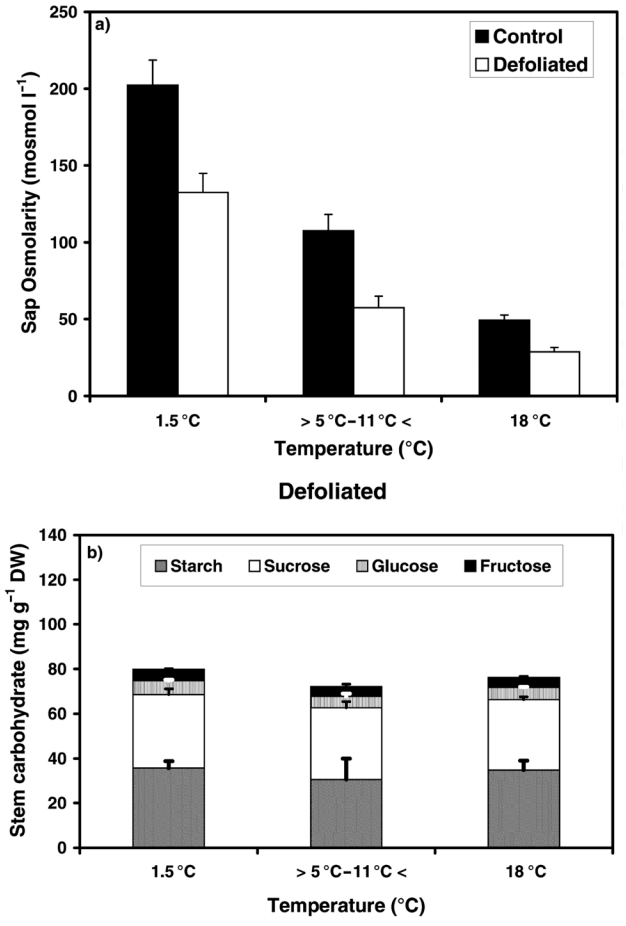

Figure 4. Effects of temperature during the previous 24-h on osmolarity of xylem sap of control and defoliated plants in winter (a) and concentration of stored stem carbohydrates of defoliated plants in winter (b). Means + 1 SE are shown, $n = 8$, 13 and 4 for the 1.5 °C, intermediate temperature, and 18 °C treatments, respectively. For the defoliated treatment, leaves were removed during the previous late summer and early autumn.

Conditions for creating extremely high xylem pressures are shown for two plants in Figure 5b. Defoliated and control plants were kept at 1.5 °C for 72 h, at the end of which the osmolarities were elevated to 129 and 165 mosmol l^{-1} (data not shown), respectively, and xylem pressures were as high as 30 kPa in the controls. However, with the freezing exotherms and thaw endotherms that followed, xylem pressures spiked to the limits of the logger range, which was 219 kPa. Furthermore, with each resting phase at 1.5 °C, pressures continued to rise. At the end of the freeze–thaw cycles, osmolarities were 161 and 219 mosmol l^{-1} for the defoliated and control plants, respectively: these were the highest values recorded.

In response to a freeze–thaw cycle, osmolarity of the xylem sap doubled on average for both control and defoliated trees (Mann-Whitney test, $P < 0.05$). Before the freeze–thaw cycle, defoliated plants had 37% lower osmolarities than controls ($P < 0.05$; Figure 6a), corresponding to a 19% lower amount of stored carbohydrates. In response to a freeze–thaw cycle, stem carbohydrate reserves decreased by 5% in trees in both treatments (Figure 6b).

In response to a freeze–thaw cycle, stem symplast water content decreased by 12% in controls ($P < 0.05$) and by 6% in defoliated plants (NS; Figure 6c). A freeze–thaw cycle increased extracted sap volume by 24 and 14% for control and defoliated plants, respectively (Figure 6d), but the differences were not statistically significant. Both stem water content and xylem sap volume were higher in defoliated plants than in controls; however these differences are confounded, because the values are expressed per dry weight of the stem, and stems of control trees had 19% higher concentrations of stored carbohydrates than stems of defoliated trees.

In terms of freeze–thaw dynamics, there were transient spikes in pressure associated with the freezing exotherms and thawing endotherms. In addition, after a thaw was complete, there were often steady increases in xylem pressure if temperatures were low, or steady decreases in pressure if temperatures were high.

Effects of freeze–thaw cycles on the relationship between xylem sap osmolarity and pressure

There were linear correlations between osmolarity of xylem sap and mean pressure of xylem sap for the previous 12-h period (Figure 7). However, the slope of the regression line for

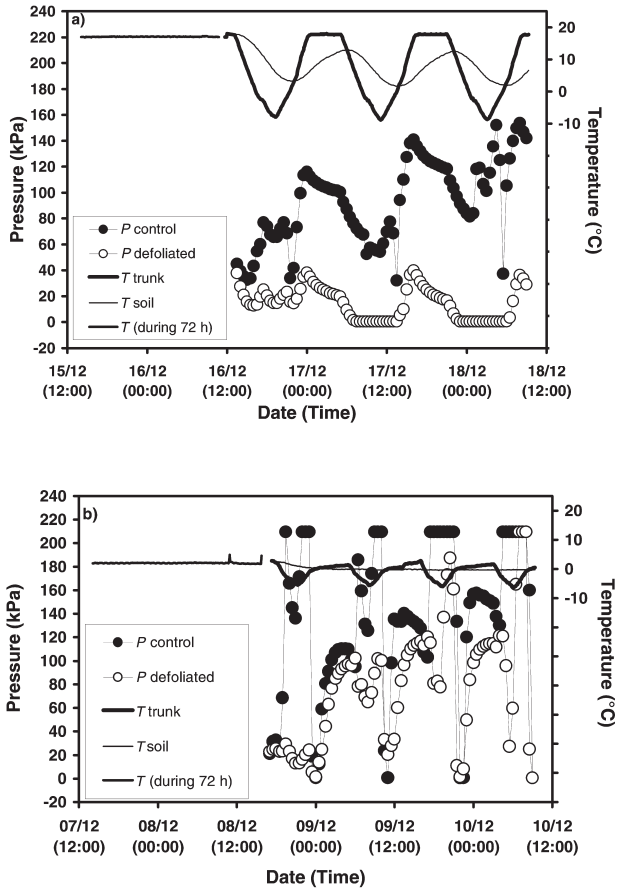

Figure 5. Osmotically induced and freeze–thaw-induced xylem pressures in defoliated and control trees. Plants were kept at 18 °C (a) or 1.5 °C (b) for 72 hours before and after exposure to successive freeze–thaw cycles. Note that the maximum reading the transducers could record was 210 kPa. Pressure values are 30-min means.

plants that had experienced freeze–thaw cycles was 5.8-fold greater than for plants that had not been exposed to a freezing period. Maximum osmolarities and pressures for plants not exposed to a freeze–thaw cycle were about 219 mosmol l^{-1} and 35 kPa, respectively. In contrast, plants subjected to freeze–thaw cycles had osmolarities of up to 250 mosmol l^{-1} and pressures over 210 kPa. The theoretical relationship between osmolarity and osmotic pressure potential (Nobel 1991) is also shown in Figure 7. The predicted slope was much steeper (2.227) than we found for either the non-frozen (slope = 0.151) or the freeze–thawed (slope = 0.879) plants. Thus, the non-frozen plants had pressures of just 6.8% of the theoretical values predicted by their xylem sap osmolarity compared with 39.5% for the freeze–thawed plants.

Discussion

Of our three hypotheses, the first two were supported by the results. Xylem pressures were positively correlated with the osmolarity of the xylem sap, and inversely correlated with temperature, and defoliated trees had lower osmolarities and lower xylem pressures than controls. However, the third hypothesis, that freeze–thaw cycles have no effect on xylem pressures beyond their effect on xylem sap osmolarities, was not supported. Based on the difference in the relationship between xylem sap osmolarity and xylem pressure before and after freeze–thaw events, the freeze–thaw cycle increased pressures almost sixfold over the value directly accounted for by enhanced osmolarity.

By way of interpreting these results, we discuss two mechanisms for generating winter xylem pressures in walnut trees: stem osmotic pressures, and stem freeze–thaw pressures.

Stem osmotic pressures

In the winter, excised shoots exuded sap (positive xylem pressures), and there were clear correlations between xylem sap osmolarities and xylem pressures. In *Juglans regia*, as in *Acer*, most of the osmolarity of the xylem sap in winter can be attributed to sucrose, with increases in sucrose occurring at low temperatures (Johnson 1945, Marvin et al. 1967, Sauter et al. 1973, Cortes and Sinclair 1985, Améglio and Cruiziat 1992). Positive xylem pressures occurred in response to low winter temperatures both in orchard trees and in potted plants that had been stored in a cool greenhouse.

As hypothesized, when *Juglans* plants were deprived of stored carbohydrates by the defoliation treatments, the osmotic concentration of the xylem sap was significantly reduced the following winter. It is well known that defoliation of trees can have deleterious effects on winter survival. In *Juglans*, because winter pressure was related to osmotic concentration, and positive xylem pressures can play a role in reversing embolism, it is possible that one of the deleterious effects of carbohydrate deprivation was a reduced ability to reverse winter embolisms.

Essiamah (1980) showed that, 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 in the early spring, but not in the winter. Perhaps most temperate dicotyledonous trees have a period in the early spring, however brief, when starch is hydrolyzed to sugars and released in the xylem sap.

The mechanism underlying the increase in sugar concentration of the xylem sap during the winter or spring, or both, was not elucidated. The xylem contains contact cells, which are living axial and ray parenchyma cells with large pits connecting them to vessels. In many species, these cells are the sites of sugar secretion into the xylem sap (Sauter et al. 1973, Sauter 1980, 1981, Braun 1984, Essiamah and Eschrich 1985). At least in *Acer* and in *Salix*, there are two competing biochemical processes by living cells of the xylem: secretion (efflux) and absorption (influx) of sugars, with temperature determining which is the dominant process. At low temperatures, efflux dominates. The starch that is stored in parenchyma cells in the xylem is hydrolyzed to sucrose, which is then secreted by contact cells into the xylem sap (Sauter 1980).

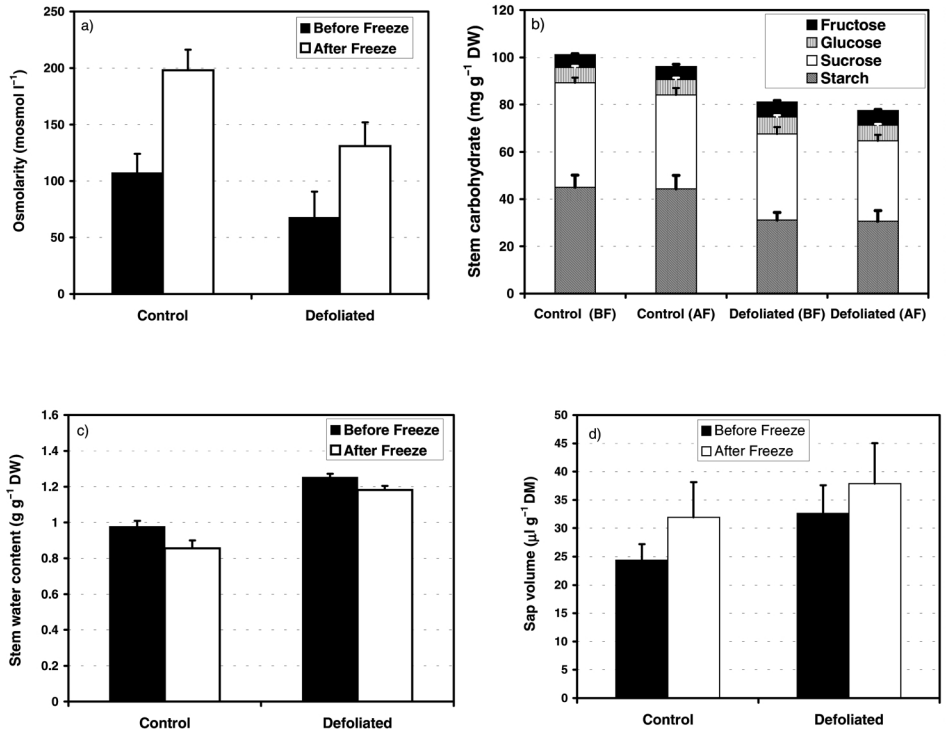

Figure 6. Effects of freeze–thaw cycles on xylem sap osmolarity (a), stem symplast carbohydrate concentrations (b), stem water content of symplast (c) and xylem sap volume (d) of defoliated and control trees. Data are pooled from several experiments with resting temperatures of either 1.5 or 18 °C. Means + 1 SE are shown, n = 7 and 4, before and after freezing, respectively.

Authors of several recent papers have discussed the conditions under which positive pressures could be generated in vessels in the absence of root pressure (Holbrook and Zwieniecki 1999, Tyree et al. 1999). For such pressures to be generated in the apoplast (xylem vessels, fibers, intercellular spaces), the classical explanation (Pickard 1989, Tyree and Yang 1992, Yang and Tyree 1992) involves an osmotic pressure difference between the apoplast and the neighboring compartment, the symplast (contact cells and xylem parenchyma). The semipermeable cell membranes of the symplast separate the two compartments. Such a model would explain why the measured xylem pressures of *Juglans* were well below the theoretical pressure potentials calculated from the osmotic potentials of the xylem sap (Nobel 1991). The osmotic concentration of the xylem sap would be in competition with the osmotic concentration of the contact cells.

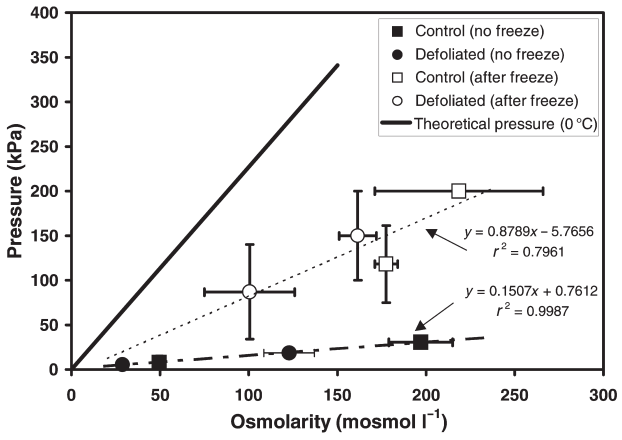

Figure 7. Relationship between xylem pressure and the osmolarity of the xylem sap before (solid symbols) and after (open symbols) freeze–thaw cycles. Experiments were done with resting temperatures of 1.5 and 18 °C, resulting in two values per treatment, with higher and lower osmolarities, respectively. The dashed line shows the relationship when no freezing occurred, the dotted line shows the relationship following a freeze–thaw cycle, and the solid line shows the relationship between osmolarity and the theoretical osmotic pressure. Means ± SE are shown, n = 21 (no freezing) and 4 (after freezing) for each value.

In *Acer*, Johnson et al. (1987) reported that large sugar molecules, disaccharides and larger, could be experimentally substituted for sucrose to generate stem pressure, but sugar hexoses could not. Johnson et al. (1987) claimed that the positive xylem pressures could not have been osmotically generated, because unlike sucrose, hexoses perfused into the xylem were ineffective in generating pressure; however, the authors could not be sure that gradients were maintained between the apoplast and symplast. Sauter (1980) described the movement of hexoses from xylem to contact cells (influx), which he considered to be an active transport process (co-transport H^+-hexoses). Such influx could prevent high osmolarities from being maintained for long in the apoplast.

Low xylem pressures could adversely affect tree health. Defoliation resulted in reductions in carbohydrate reserves, winter osmolarities of the xylem sap, and winter xylem pressures. Perhaps any factor (e.g., insect attack, pathogens, severe summer drought) that results in a reduction of stored carbohydrates can reduce winter xylem pressures, which, in turn, could impact the plant's ability to reverse embolism.

Stem pressures and freeze–thaw cycles

We do not know how the freeze–thaw cycles enhanced pressures beyond those obtained in response to cool, nonfreezing temperatures. The spikes in pressure associated with the freezing and thaw events might be associated with the expansion of water during the phase change from liquid to solid, as has been previously described in conifer xylem (Robson and Petty 1987). The ice within vessels could exert pressure on the surrounding fluid until the ice was completely melted. However, even after a thaw was complete, pressures much higher than those that had occurred before the freeze–thaw cycles were maintained for many hours. It seems that, during a freeze–thaw cycle, additional water was drawn into the stem xylem apoplast, either from the symplast of the stem, or from the lower parts of the stem and roots that had not frozen.

In our experiments, the osmolarity of the xylem sap doubled in response to a freeze–thaw event, and the symplastic stem water content decreased. The increased osmolarity of the xylem sap suggests that there was additional sucrose secretion by contact cells when temperatures were below 1.5 °C, leading to decreased osmolarity of the symplast, which together with the increased osmolarity of the apoplast, drew water from the living cells of the xylem.

We probably underestimated xylem sap weight whenever the xylem sap was under positive pressure. Under such conditions, water must have been lost when the stem was cut prior to sap extraction. This error would have increased with increasing pressures, which may explain why the increases in measured xylem sap volume following the freeze–thaw cycle were not statistically significant. Thus, it would be desirable to measure water uptake or release from stems during freeze–thaw events. In *Acer*, there is uptake of water into the stem during the freezing event, allowing for enhanced pressure with the thaw (O'Malley and Milburn 1983, Tyree 1983, Milburn and O'Malley 1984). This finding explains why, when roots of *Acer* are in frozen soil in the field, sap flow is reduced in the stems, despite elevated sugar concentrations in the stem xylem sap. Apparently the root system must absorb water from the soil in order for substantial pressure buildup to occur (Robitaille et al. 1995).

Aside from enhanced water absorption into the apoplast during the freeze–thaw cycle, another explanation for the increased pressure in xylem vessels could be the expulsion of stored water from xylem fibers. In walnut (authors' unpublished observations) as well as in *Acer* (Sauter 1974), wood fibers contain large gas bubbles during the winter. For *Acer* it has been argued that living parenchyma cells in the xylem are responsible for the gas production, and that temperature changes result in expansion or contraction of the bubbles (Sauter 1974). Similarly, Milburn and O'Malley (1984) concluded that air bubbles in *Acer* fibers stored pressures that were released during a thaw.

To conclude, in *Juglans* as well as in *Acer*, no existing single model explains all of the winter xylem pressure data. A simple osmotic model accounts for the modest positive xylem pressures that occur at low, nonfreezing temperatures. However, one or more physical models may be needed to account for the enhanced xylem pressures that occur during and after freeze–thaw events.

Acknowledgments

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