# Winter embolism, mechanisms of xylem hydraulic conductivity recovery and springtime growth patterns in walnut and peach trees

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**Summary**   Xylem vessels of *Prunus persica* Batsch (peach) and *Juglans regia* L. (walnut) are vulnerable to frost-induced embolism. In peach, xylem embolism increased progressively over the winter, reaching a maximum of 85% loss of hydraulic conductivity (PLC) in early March. Over winter, PLC in walnut approached 100%, but the degree of xylem embolism varied during the winter, reflecting the ability of walnut to generate positive xylem pressures in winter and spring. In contrast, positive xylem pressures were not observed in peach. Controlled freeze–thaw experiments showed that frost alone is insufficient to increase embolism in peach; evaporative conditions during thawing are also required. However, when both species were protected from frost, PLC was zero. At bud break, there was complete recovery from embolism in walnut, whereas PLC remained high in peach. Three mechanisms responsible for the restoration of branch hydraulic conductivity were identified in walnut: the development of stem pressure, the development of root pressure and the formation of a new ring of functional xylem, whereas only one mechanism was observed in peach (new functional ring). The climatic conditions necessary for the manifestation of these mechanisms were investigated.

*Keywords: cambial growth, Juglans regia, Prunus persica, root pressure, stem pressure, water content, xylem sap.*

## Introduction

In temperate woody plants, hydraulic conductivity can be impaired by xylem embolism, which is mainly caused by water stress or frost (Cochard and Tyree 1990, Sperry and Sullivan 1992, Cochard et al. 2001). Frost-induced embolism can occur as a consequence of alternating frost–thaw events (Zimmermann 1983, Cochard and Tyree 1990, Just and Sauter 1991, Améglio et al. 1995, Pockman and Sperry 1997). As the sap freezes, previously dissolved gases escape because of their low solubility in ice (Sperry and Sullivan 1992). On subsequent thawing, these bubbles can either dissolve back into the xylem sap or grow to obstruct the entire xylem conduit (Yang and Tyree 1992), resulting in an embolized conduit that is unable to transport water.

Larger xylem vessels are more vulnerable to frost-induced embolism than smaller vessels and tracheids (Hammel 1967, Sucoff 1969, Ewers 1985, Sperry and Sullivan 1992, Lo Gullo and Salleo 1993, Hacke and Sauter 1996, Davis et al. 1999). This difference is generally explained on the basis that larger xylem vessels produce larger bubbles with a larger radius of curvature, which increases the risk of cavitation (Yang and Tyree 1992).

In coniferous species, freezing events cause little damage to xylem (Sperry and Sullivan 1992), whereas in ring-porous species such as *Quercus* (Cochard and Tyree 1990) and *Fraxinus* (Cochard et al. 1997), a single freeze–thaw event may be enough to impair most xylem conduits. Diffuse-porous species present two cases. In *Acer* (Sperry and Sullivan 1992) and *Fagus* (Borghetti et al. 1993, Cochard et al. 2001), the number of embolized xylem conduits increases progressively over winter, whereas in *Juglans* (Améglio et al. 1995) the embolism rate decreases during winter.

The occurrence of frost-induced embolism has been related to the geographic distribution of plant species (Tyree and Cochard 1996, Langan et al. 1997, Pockman and Sperry 1997). Longer-term consequences can include reduced growth and dieback. Studies on deciduous temperate trees, for example, have shown that growing season values of hydraulic conductivity may be considerably reduced after frost-induced embolism (Sperry et al. 1987, 1988*a*, Cochard and Tyree 1990).

Recovery of xylem function following embolization is necessary for continued transpiration and growth. Such recovery can occur through the replacement of embolized vessels with new functional vessels and refilling of embolized vessels through an active mechanism (Cochard et al. 2001). The first mechanism is common to all plant species with a secondary cambium, but its efficiency during bud break depends on the timing of radial growth resumption. Several authors (Sperry et al. 1987, 1988*a*, 1994, Améglio et al. 1995, Hacke and Sauter 1996) have investigated the second mechanism; i.e., the refilling of embolized vessels through positive pressures in the xylem. Walnut, like maple, exhibits positive pressures in the xylem sap during winter that are associated with a high xylem sap sugar concentration and high osmolarity (Améglio and Cruiziat 1992, Améglio et al. 2001*a*). In contrast, positive xy-

lem pressures during autumn and spring are associated with mineral nutrition and soil temperature (Ewers et al. 2001). In several species, including oak and peach, positive xylem pressures have never been observed.

Our first objective was to investigate and compare frost-induced embolism and the mechanisms of xylem recovery in two diffuse-porous trees: *Juglans regia* L. and *Prunus persica* (L.) Batsch. We investigated xylem pressure and the winter variations of PLC in twigs of mature trees; the progression of embolism in peach trees after controlled freeze–thaw cycles in a freezing chamber; and the timing of embolism recovery in relation to bud break and cambial growth. Specifically, we followed changes in xylem sap osmolarity, xylem sap volume and stem water content in relation to temperature, and variations in stem and trunk diameter.

## Materials and methods

### Natural conditions

Walnut data were gathered for four winter seasons (1994–95 to 1997–98) and peach data for two winter seasons (1996–97 and 1997–98) at the INRA PIAF location near Clermont-Ferrand, in south-central France. Orchard-grown walnut and peach trees were 13 and 5 years old in 1994, respectively. Measurements were performed on 80-cm excised, 1-year-old twigs from orchard- or container-grown trees. After removing the uppermost 10-cm portion of the apex, the stem segment was separated into an upper part used for sap measurements and a lower part used to determine embolism.

### Freeze–thaw cycles

Freeze–thaw cycle experiments were performed on ten 5-year-old, container-grown peach trees previously kept in non-freezing conditions in a controlled temperature greenhouse since September 1998. The greenhouse temperature tracked the outdoor temperature, unless the outside temperature dropped below 0 °C, in which case the greenhouse temperature was maintained at 0–3 °C. The freeze–thaw experiment was performed in March 1999 in a freezing chamber designed to hold two potted trees. Freeze–thaw cycles were computer controlled and repeated on the same specimens up to four times between +19 °C (or +5 °C, according to the experiment) and –9 °C. In some cases, after four initial freeze–thaw cycles between +5 and –9 °C, trees were subjected to an additional three cycles between +19 °C and –9 °C. In all cases, the rate of cooling or warming was 5 °C h$^{-1}$. This rate of temperature change is comparable with that occurring during natural freeze–thaw events. The rate of temperature change that we used was one third the 15 °C h$^{-1}$ used in studies by Davis et al. (1999) for comparison. Relative humidity (RH) and temperature in the freezing chamber were recorded by two Hobo data loggers (Onset Computer, Bourne, MA).

### Xylem embolism

Loss of hydraulic conductivity was measured as described by Sperry et al. (1988*b*). Twig segments were recut to a length of 20 cm (Tyree et al. 1993, cf. Améglio et al. 1998) and manipulated under tap water to prevent air entry into vessels (Cochard et al. 2000). During winter 1995–96, the embolism rate was determined on two branches of each of four orchard-grown walnut trees (*n* = 8 measurements per date). In winter 1996–97 and 1997–98, measurements were performed on three branches from each of three trees (*n* = 9) for walnut and on four branches from each of two trees (*n* = 8) for peach on each date. For the freeze–thaw cycle experiment (1998–99), one branch was sampled before the first freeze–thaw cycle on each of the 10 trees investigated; after each freeze–thaw cycle, three branches per tree were measured.

### Water content, sap volume and osmolarity

Xylem sap was extracted under moderate vacuum (0.06–0.08 MPa) from the apical 30-cm section of the branch used for embolism determination (Bollard 1953). Sap volume was expressed as μl g$^{-1}$ dry mass (DW). Sap osmolarity was measured with a Roebling 13DR automatic osmometer (Messtechnik, D-1000 Berlin, Germany). Following extraction of xylem sap, stem samples were weighed fresh (FW) and after drying (DW) for 48 h at 80 °C and their water content calculated.

Additional determinations of sap volume, sap osmolarity and stem water content were performed during winter 1994–95 for comparable apical segments in walnut trees (three branches from each of three trees: *n* = 9).

### Xylem pressure

During winter 1996–97, continuous xylem pressure measurements were performed on one orchard-grown walnut tree, on each of six branches located at heights ranging from 1.2 to 3.5 m. Sap pressure was recorded on each branch as follows. In fall 1996, the branch was severed 15 cm from its base, a 1-cm band of bark was removed from the cut surface, and the phloem edges were covered with Teflon tape. The transverse stem xylem surface was smoothed with a fresh razor blade, and tubing was tightly connected. A pressure transducer (Model PDCR 1000, Druck, Leicester, U.K.) with a sensitivity of ± 1 kPa was attached to the tubing, and both the tubing and transducer were filled with non-freezing silicone fluid (Améglio et al. 2001*a*). To prevent clogging, 1–2 cm was removed from the cut section every 6 weeks. Xylem pressures were recorded with data loggers (DL2e, Delta T devices, Cambridge, U.K.) as 30-min means.

### Cambial growth

During the winters 1996–97 and 1997–98, the walnut tree measured for xylem pressure and two orchard peach trees were continuously monitored for diameter variations of different organs by means of linear variable differential transformers (LVDTs; model DF2.5, Solartron Metrology, Massy, France). The LVDTs were mounted on a frame made of Invar (Weber métaux, Paris, France), an iron–nickel alloy with a near-zero temperature expansion coefficient (Huguet 1985). For walnut, diameter variations were recorded at seven locations: the trunk, two main branches and four 1-year-old twigs. Diameter variations in peach were measured at five locations:

the trunk, two main branches and two 1-year-old twigs. The LVDT measurements were recorded as 30-min means (10-min means in the freezing chamber experiment).

*Phenological data*

Time of bud burst was determined on five twigs per tree and recorded as the date when 50% of the expected number of buds had broken. Start of elongation was determined on the same twigs as the time when new shoots had reached a length of 2 cm.

*Climatic data and data recorded*

In parallel with cambial growth measurements, trunk, branch and shoot temperatures were measured with thermocouples and recorded with data loggers as 30-min means (10-min means in the cooling chamber experiment). Daily maximum and minimum air temperatures and the daily noon soil temperature at four depths (–10, –20, –50 and –100 cm) were measured at the weather station of Aulnat (Météo France), located less than 1 km from INRA.

## Results

Results are presented geographically for the winters of 1996–97 and 1997–98 only. Data for the winters of 1994–95 and 1995–96 are included in the analysis of relationships between variables presented in Figure 4.

*Winter 1996–97*    Figure 1b shows the time courses of winter embolism in xylem of walnut and peach orchard trees, measured as percentage loss of hydraulic conductance (PLC). In early December, after the first frost event of November (Figure 1a), PLC increased from about 5% to about 50% in both species. In peach, after severe frosts in late December, PLC further increased in January and early February. In early March, a significant decrease in embolism was observed, before PLC increased again in April. The increases in embolism observed during February and April can be related to spring frost events (Figure 1a), whereas the decrease in PLC in early March paralleled the resumption of cambial growth (Figure 1f). In late June, PLC values were significantly higher in old shoots than in new shoots (Figure 1b).

The time course of winter embolism in walnut differed from that in peach, with PLC decreasing in January, late February and April before the resumption of cambial growth (Figure 1f). The decrease in PLC in January paralleled increases in xylem pressure (Figure 1b) and sap osmolarity (Figure 1c). Variation in PLC in February, however, was accompanied by only small changes in xylem pressure. In April, PLC dropped close to zero, concomitant with the occurrence of low but persistent xylem pressures (Figure 1b) that were positively correlated with within-day air temperature variations (Figure 1a). Cambial growth (Figure 1f) resumed in late April, after bud break and total embolism repair.

Walnut xylem pressure in April was related to a sharp increase in total stem water content and soil temperature (Figure 1e), whereas sap osmolarity remained low (Figure 1c).

This contrasts with January, when positive xylem pressure and sap osmolarity were relatively high. Thus, sap osmolarity exhibited large variations in walnut. In contrast, peach exhibited limited variation in sap osmolarity (Figure 1c), which was inversely related to sap volume (Figure 1d). In peach, the volume of sap extracted from twigs during the winter was inversely related to PLC ($r^2 = 0.7$).

Growth dynamics (Figure 1f) and date of bud break differed between species, with bud break occurring a month later in walnut than in peach, whereas shoot elongation growth started at about the same time in both species. Moreover, in both species, cambial growth was concomitant with bud burst; however, the pattern of cambial reactivation was acropetal in walnut, whereas it was basipetal in peach.

Both species exhibited a marked shrinkage in trunk and twig diameters in December and January, when air temperatures remained below 0 °C.

*Winter 1997–98*    At the end of summer 1997, the embolism rate was close to zero in both species. As in the previous year, PLC (Figure 2b) increased in November after the first frosts (Figure 2a). In peach, PLC increased again in February, after another frost period. In contrast, walnut exhibited a continuous decrease in PLC from January through April. In both species, winter PLC variations were inversely related to sap volume (Figure 2d).

In walnut, the decrease in PLC in January and February was accompanied by an increase in sap osmolarity (Figure 2c), whereas maximum PLC occurred when no xylem sap could be extracted (arrow in Figure 2c and Figure 2d). Similar to the previous winter, the final phase of decreasing PLC in March and April occurred without any concomitant change in sap osmolarity (Figure 2c), but with a large increase in stem water content (Figure 2e) as soil temperature increased. In both years, the decrease in PLC in peach in the spring paralleled cambial growth resumption (Figure 2f).

As in winter 1996–97, peach and walnut differed in date of bud break, whereas shoot elongation growth started at about the same time in both species. Again, the cambial reactivation pattern was acropetal in walnut and basipetal in peach (Figure 2f). In peach trunks, cambial growth continued during late summer and autumn until the first frosts.

*Effect of freeze–thaw cycles*

Just before bud break, peach trees that had been maintained in non-freezing conditions throughout the winter exhibited an embolism rate of zero. After four freeze–thaw cycles with temperatures between –9 and +5 °C, PLC increased to about 10% (Figures 3a and 3b). Subsequently, increasing the temperature from +5 to +19 °C led to a further increase in PLC to about 15%. At the same time, relative humidity decreased from 38 to 27% (data not shown). Up to 50% higher PLC values were observed after three additional –9/+19 °C freeze–thaw cycles (Figures 3a and 3b).

A peach tree subjected to four –9/+19 °C cycles, without a prior –9/+5 °C cycle treatment (Figure 3c), reached a PLC of 25% following the third cycle, but decreased below 20%
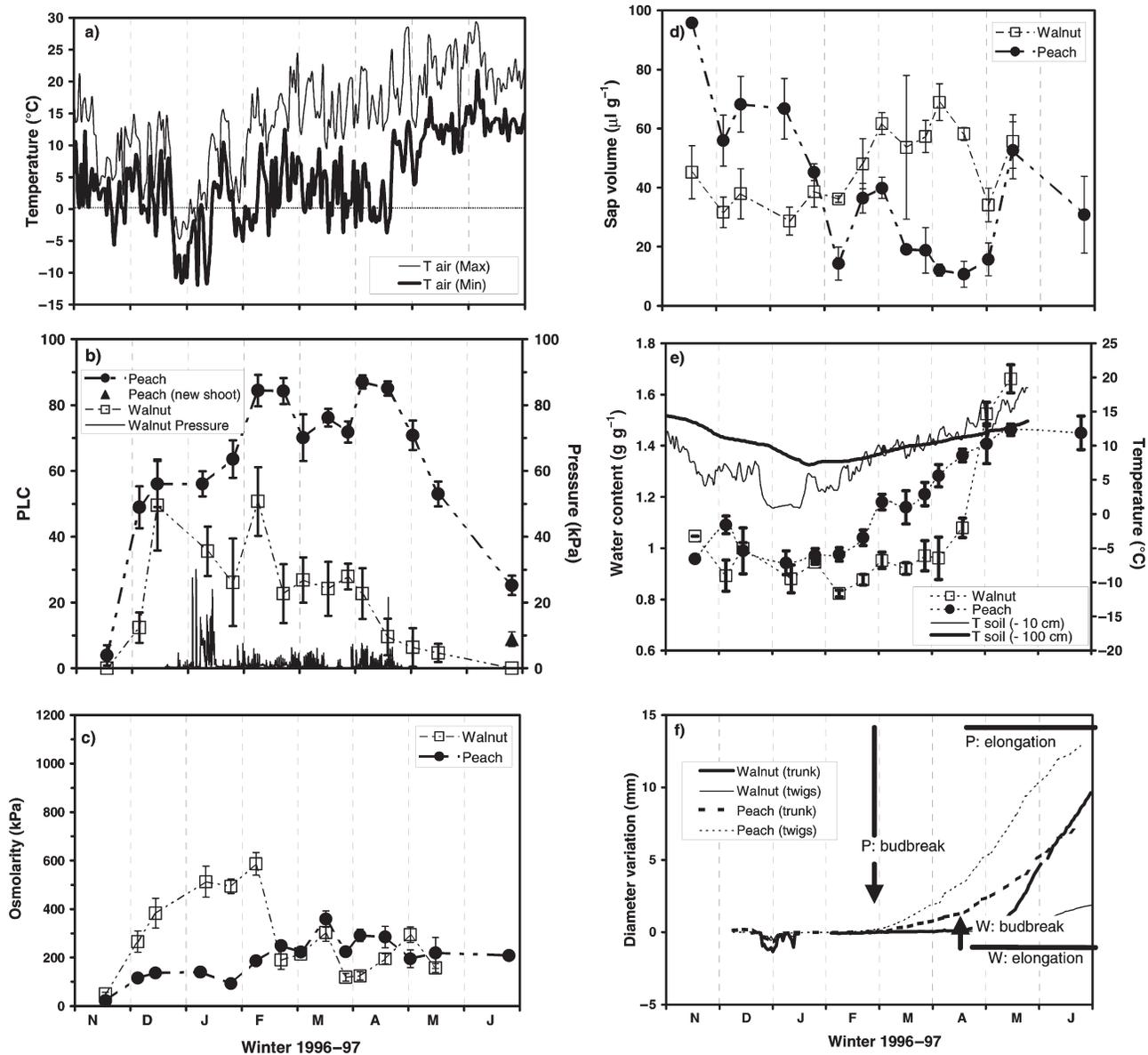
Figure 1. Summary of data for the 1996–97 winter period. (a) Time courses of minimum and maximum daily air temperatures. (b) Percentage loss of hydraulic conductivity (PLC) in the xylem of three 15-year-old walnut and two 7-year-old peach trees and mean hydrostatic xylem pressure measured at different heights ($n = 6$). (c) Sap osmolarity of the same trees. (d) Sap volume extracted from the same trees. (e) Water content of the same trees and daily noon soil temperature at –10 cm and –100 cm depths. (f) Diameter variations for the trunk and four stems (mean is presented) in walnut and for two trunks and four stems (means are presented) in peach. The arrow represents the time of bud burst and the thick line represents the start of elongation (W = walnut; P = peach). Values in panels b–e are means ± SE (walnut: $n = 9$; peach: $n = 8$).

4 days following the last cycle. During this experiment, significant cambial growth was observed. On each cycle, a pronounced trunk constriction was observed when the wood temperature fell below 0 °C.

*Relationships between variables*

There was a correlation between sap osmolarity and mean daily air temperature (mean of maximum and minimum air temperatures) in walnut (Figure 4a: $r^2 = 0.51$), but not in peach (Figure 4d: $r^2 = 0.01$). Increases in osmolarity were related to decreasing xylem sap quantity in peach (Figure 4e: $r^2 = 0.74$),

but not in walnut (Figure 4b: $r^2 = 0.04$). In both species (Figures 4c and 4f), there was a strong correlation between soil temperature at a depth of 50 cm and stem water content during the rehydration phase (January to May). In walnut, however, a strong correlation was observed only for soil temperatures above +8 °C. Table 1 presents correlation coefficient ($r$) and probability ($P$) values between stem water content and soil temperature at different depths (10 cm above ground to 100 cm below ground) and for two periods (the whole period and the January–May period). A good correlation ($r > 0.66$ and $P < 0.01$) was observed for both peach and walnut during
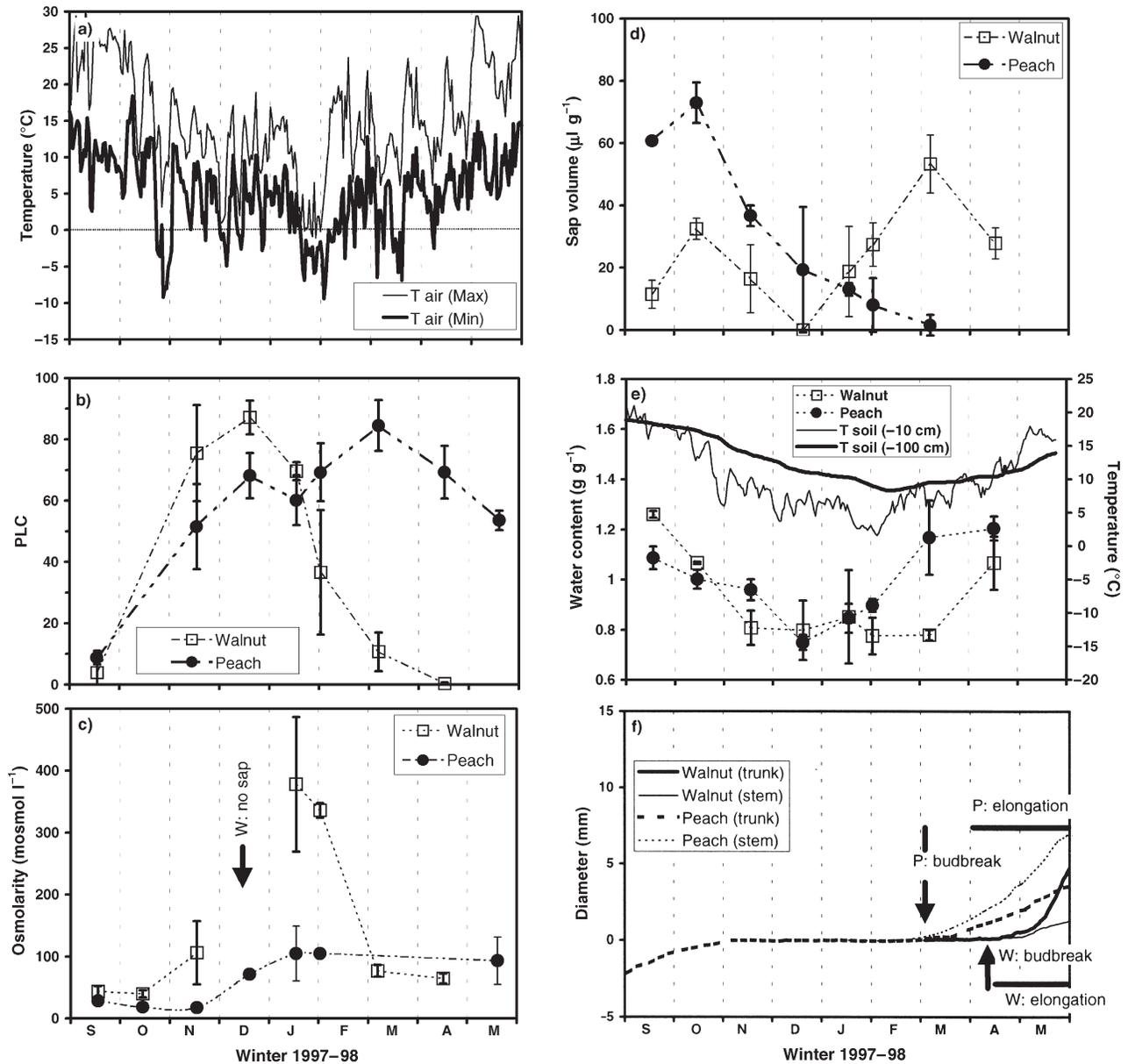
Figure 2. Summary of data for the 1997–98 winter period. (a) Time courses of minimum and maximum daily air temperatures. (b) Percentage loss of hydraulic conductivity (PLC) in the xylem of three 15-year-old walnut and two 7-year-old peach trees and mean hydrostatic xylem pressure measured at different heights (*n* = 6). (c) Sap osmolarity of the same trees. (d) Sap volume extracted from the same trees. (e) Water content of the same trees and daily noon soil temperature at –10 cm and –100 cm depths. (f) Diameter variations for the trunk and four stems (mean is presented) in walnut and for two trunks and four stems (means are presented) in peach. The arrow represents the time of bud burst and the thick line represents the start of elongation (W = walnut; P = peach). Values in panels b–e are means ± SE (walnut: *n* = 9; peach: *n* = 8).

the second period (i.e., the rehydration phase of January to May). The highest correlation was observed at a soil depth of 50 cm.

## Discussion

### Time course of winter embolism

We confirmed that xylem vessels of *Prunus persica* and *Juglans regia* are vulnerable to frost-induced embolism. One-

year-old twigs of both species, when protected from frost (data not shown), exhibited zero PLC. In peach, the degree of xylem embolism increased progressively over the winter, with a maximum of 85% PLC in early March. This gradual increase in xylem embolism during winter is typical of diffuse-porous species (Sperry and Sullivan 1992, Cochard et al. 2001). The degree of embolism increases markedly in ring-porous species after the first winter frosts (Cochard and Tyree 1990). Thus, during winter 1996–97 (Figure 1b), peach exhibited the first increase in PLC in November, and a second increase in Janu-
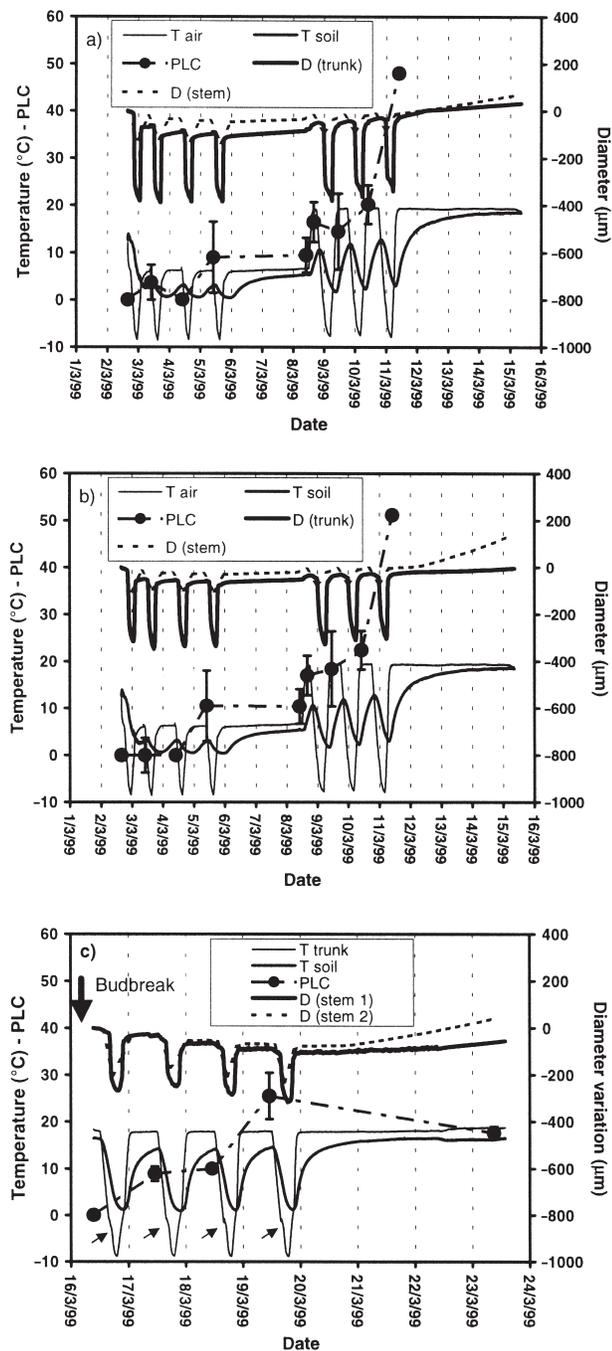
Figure 3. Summary of freeze–thaw experiments for the three study trees. The axis on the left of each panel represents percent of hydraulic conductivity (PLC) and air and soil temperatures (a and b) or stem temperature (c). The axis on the right represents diameter variations. For graph (c), the vertical arrow represents bud break. Oblique arrows represent the exotherms. The PLC values are means ± SE (*n* = 3).

ary, after several episodes of frost. A similar pattern was observed in winter 1997–1998, although the second increase in PLC did not occur until February. The freezing chamber study indicated that frost alone is insufficient to increase embolism; evaporative conditions during thawing are required because four –9/+5 °C freeze–thaw cycles induced a PLC value of

only 10%, whereas the following four freeze–thaw cycles between –9 and +19 °C resulted in a PLC value of more than 50%. We were also able to increase PLC simply by increasing the temperature from +4 to +19 °C (and decreasing the relative humidity from 38 to 27%) in the absence of an additional freeze–thaw cycle (Figures 3a and 3b). Field data yielded similar results. For example, in late January and early February (Figure 1b), the maximum increase in embolism occurred when the diurnal variation in air temperature was maximum.

These results confirm the current theory about winter embolism. On freezing, gases dissolved in the sap are excluded as ice forms and escape as small bubbles (Sperry and Sullivan 1992). On thawing, if the ice melts slowly and no tension develops in the tissue, the air redissolves into the sap, but if tensions develop (e.g., in evaporative conditions), the bubbles expand, causing embolism of the conduit (Pickard 1989, Sperry and Sullivan 1992, Yang and Tyree 1992).

Walnut was more vulnerable to winter embolism than peach, presumably because walnut has larger vessel diameters (60–160 μm) than peach (25–60 μm). Although the PLC of walnut reached nearly 100% (data not shown) during winter 1995–96, the degree of xylem embolism varied during winter (cf. Améglio et al. 1995). Thus, the PLC variation pattern during winter differed in these diffuse-porous species. This difference can be related to the ability of walnut to generate xylem pressure (Figure 1b) in winter (Améglio et al. 2001*a*) and spring (Ewers et al. 2001). Xylem pressure was never observed in peach. As a result, at bud break, embolism recovery was total in walnut, whereas PLC was still high in peach.

*Embolism refilling*

High xylem pressure occurred in walnut during winter (Figure 1b). There has been a recent debate about the conditions under which positive pressures can be generated in vessels in the absence of root pressure (Holbrook and Zwieniecki 1999, Tyree et al. 1999). The classical explanation for positive xylem pressures (Pickard 1989, Améglio and Cruiziat 1992, Yang and Tyree 1992) presupposes an osmotic pressure difference between the xylem sap compartment (the apoplast) and a neighboring symplast compartment, namely vessel-associated cells (VACs; Czaninski 1968) or contact cells (Sauter 1972), in continuity with xylem parenchyma cells. The semipermeable plasma membrane of VACs separates the two compartments, so that an osmolarity gradient between them generates a water flux that can become apparent as sap exudation from excised walnut shoots (Améglio and Cruiziat 1992).

Apparently, this process does not occur in peach under natural winter conditions: no pressure was generated in the xylem, and the increase in sap osmolarity was small (maximum of 150 mosmol l$^{-1}$ versus 380 mosmol l$^{-1}$ in walnut). Furthermore, the limited increase in peach sap osmolarity was concomitant with a sustained sap volume decrease, i.e., it reflected water loss rather than solute gain (Figure 4e). In contrast, walnut sap volume exhibited significant increases during winter, although the increases were not correlated with the quantity of xylem sap (Figure 4b). In peach, the total stem water content increased in January (Figures 1e and 2e) even
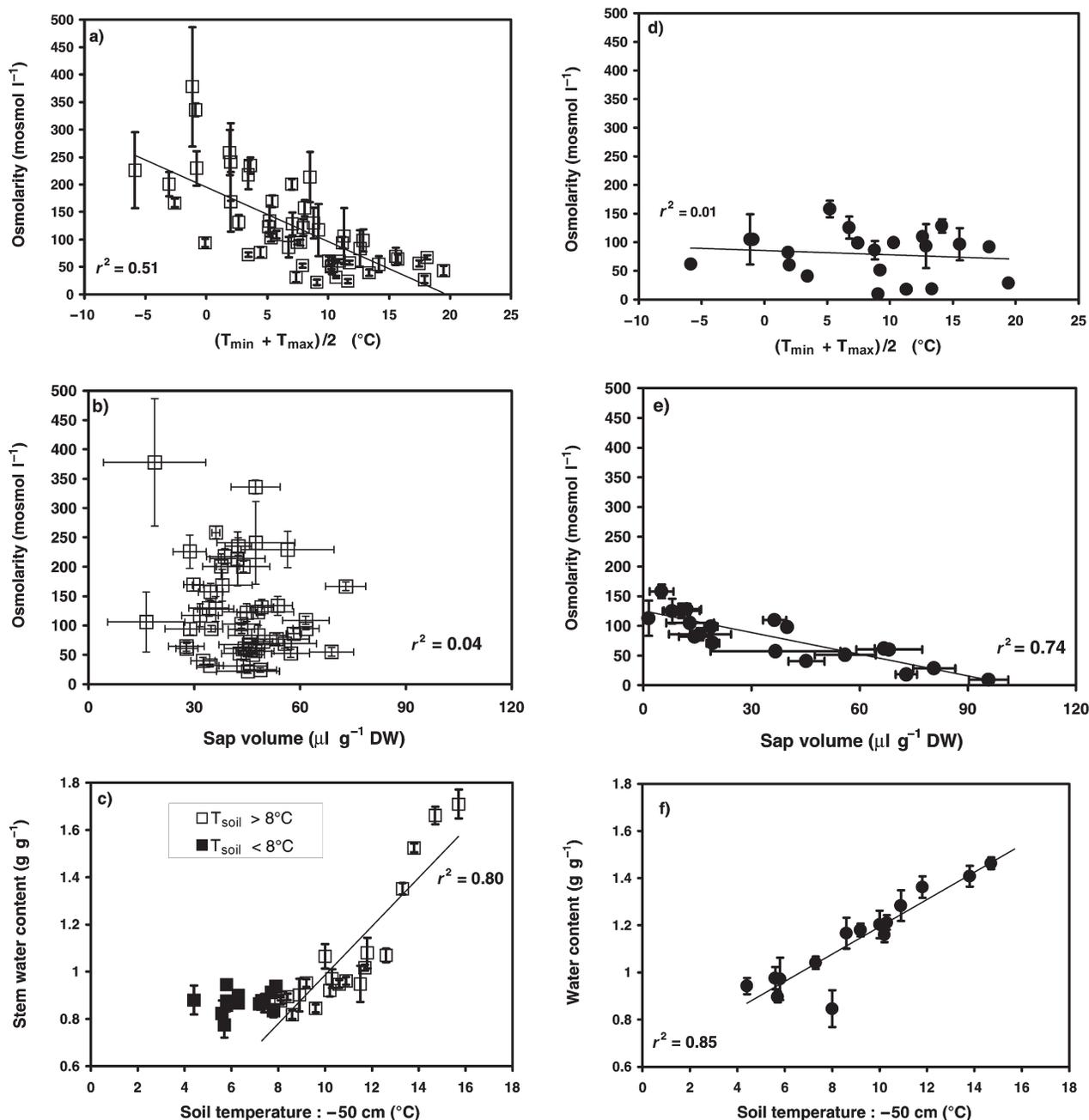
Figure 4. Relationships between variables for walnut (left panels) and peach (right panels). Panels (a) and (d): osmolarity versus mean daily air temperature. Panels (b) and (e): osmolarity versus sap volume extracted. Panels (c) and (f): stem water content versus soil temperature at $-50$ cm depth. For each graph, coefficients of determination ($r^2$) of the linear regression are presented.

though only small amounts of sap could be extracted from the vessels (PLC increased and extracted sap volume decreased). In the study of peach trees, 15–20% of the vessels maintained conductivity at the time of bud break, indicating that rehydration in the absence of leaf transpiration was sufficient to maintain some hydroactive vessels.

In orchard-grown adult walnut trees, sap osmolarity increased when air temperatures fell (Figures 1c, 2c and 4a), confirming a previous observation on potted trees under controlled conditions (Améglio et al. 2001a). Essiamah (1980)

also showed that positive xylem pressures in early spring were associated with high sugar concentrations in the xylem sap of many temperate trees, including *Betula pendula* Roth*, Alnus glutinosa* L.*, Fagus sylvatica* L. and *Quercus robur* L.; however, the phenomenon of positive xylem pressures did not occur in the winter in these species. The VACs are the sites of sugar exchange between the xylem sap and reserve storage parenchyma cells (Sauter et al. 1973, Sauter 1980, 1981, Braun 1984, Essiamah and Eschrich 1985). Apparently, under low temperature conditions, starch is hydrolyzed to sucrose in pa-

Table 1. Stem water content versus soil temperature. Linear correlation coefficient ($r$) and level of significance ($P$) for the relationship between stem water content and soil temperatures at different depths (–10, –20, –50 and –100 cm) for walnut and peach. Values of $r$ were determined based on (1) all the data for the 4 years studied in walnut and the 2 years studied in peach (Total); and (2) only the dates after December of each winter ($\geq$ January).

| Soil depth | Walnut | | Peach | |
|---|---|---|---|---|
| | Total (4 years) | $\geq$ January (4 years) | Total (2 years) | $\geq$ January (2 years) |
| –10 cm | 0.69 ($P < 0.01$) | 0.48 ($P < 0.01$) | 0.61 ($P < 0.01$) | 0.91 ($P < 0.01$) |
| –20 cm | 0.73 ($P < 0.01$) | 0.56 ($P < 0.01$) | 0.60 ($P < 0.01$) | 0.92 ($P < 0.01$) |
| –50 cm | 0.73 ($P < 0.01$) | 0.66 ($P < 0.01$) | 0.46 ($P = 0.02$) | 0.92 ($P < 0.01$) |
| –100 cm | 0.50 ($P < 0.01$) | 0.64 ($P < 0.01$) | 0.10 ($P > 0.1$) | 0.79 ($P < 0.01$) |

renchyma cells (e.g., Ziegler 1964, Kozlowski and Keller 1966, Sakai 1966, Frossard and Lacointe 1988, Sauter 1988), and can be released by way of the VACs into the xylem sap (Sauter 1980).

*Winter xylem pressure in walnut: the source of the water*

The volume of sap extracted from walnut twigs changed significantly during winter, whereas the water content of the twigs did not (see February and March 1997 in Figures 1d and 1e, and January and February 1998 in Figures 2d and 2e). This is consistent with previous observations in controlled chambers (Améglio et al. 2001a), where water content decreased by 12%, whereas extracted sap volume increased by 24%. Because stem water content remained constant or decreased during the winter, we speculate that the additional water found in the vessels is drawn from the adjacent symplast and stem fibers, rather than more distant parts of the tree.

The processes involved in the generation of spring xylem pressures appear to differ from those involved in the generation of winter xylem pressures. We observed that xylem pressure in walnut in the spring (Figure 1b) was positively correlated with air temperature in March and April. In a controlled experiment, warm conditions in spring induced xylem pressures that were related to root pressure (Ewers et al. 2001). This root pressure, which was correlated with the increase in soil temperature, could explain the increase in water content in spring (cf. Figure 1e). The finding that stem water content in walnut was strongly correlated with soil temperature after December supports this interpretation (Figure 4c, Table 1). Stem water content was also correlated with soil temperature in peach (Figure 4d, Table 1), although no root pressure was detected. This point requires further investigation.

*Xylem recovery, bud break, radial growth and shoot elongation*

There are two independent mechanisms involved in the overall increase in hydraulic conductivity in spring (Cochard et al.

2001): (i) production of new functional conduits; and (ii) refilling of air-filled conduits by positive xylem pressure. Our data indicate that the second mechanism is dominant if not exclusive in walnut, whereas only the first mechanism operates in peach.

In peach, xylem pressure was never observed and no significant embolism repair occurred before bud break; it occurred just after bud break with cambial reactivation. The impact of cambial reactivation on embolism recovery was observed under natural conditions in March 1997 (Figures 1b and 1f)—before the increase in PLC in response to spring frosts—and in March 1998 (Figures 2b and 2f). It was also observed in the controlled freeze–thaw experiments (Figure 3c) that, 4 days following the last freeze–thaw cycle, PLC decreased by 8% concomitantly with a diameter increase of 120 µm. In this controlled experiment, reversible shrinkage of bark occurred at temperatures below 0 °C, as previously reported in mature subalpine conifers (Zweifel and Häsler 2000) and walnut (Améglio et al. 2001b). Diameter shrinkage occurred in both species under natural conditions and was associated with severe frost events in late December 1996, early January 1997 and early February 1998 (Figures 1f and 2f).

Because xylem recovery is essential to support transpiration and growth of new leaves, a high PLC could explain the low rates of bud break and subsequent growth (both primary and secondary) of peach in the spring. A peculiarity of early growth after bud break in peach was observed. After breaking, buds developed into a rosette containing only a few expanded leaves and remained in that state for several weeks before the new shoots started elongating. We speculate that the arrested growth of the new shoots is a consequence of high PLC at bud break, and that shoot elongation is possible only after sufficient embolism repair, which, in peach, is dependent on cambial reactivation. This suggestion could be tested by controlling the degree of PLC through controlled freeze–thaw experiments or with a collar pressure chamber to introduce air into the xylem (Améglio et al. 1994, Cochard et al. 2002).

In conclusion, we identified three mechanisms responsible for the restoration of branch hydraulic conductivity in walnut trees, but only one in peach trees. The first mechanism, common to both species, was the formation of a new functional xylem ring. This mechanism has a major impact on hydraulic recovery of young shoots in peach, but only a minor impact in walnut. The second and third mechanisms, which were specific to walnut, involved active refilling of embolized vessels by xylem pressure. We could distinguish (i) winter stem pressure (higher pressure; Améglio et al. 2001a) due to carbohydrate reserve modification that occurred at low air temperatures and (ii) spring root pressure due to solute absorption (Ewers et al 2001) at high soil temperature ($\geq$ 8 °C); together these mechanisms explained most of the xylem hydraulic recovery in walnut during the resting period.

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