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Seasonal variation in xylem pressure of walnut trees: root and stem pressures

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Summary Measurements of air and soil temperatures and xylem pressure were made on 17-year-old orchard trees and on 5-year-old potted trees of walnut (*Juglans regia* L.). Cooling chambers were used to determine the relationships between temperature and sugar concentration ([glucose] + [fructose] + [sucrose], GFS) and seasonal changes in xylem pressure development. Pressure transducers were attached to twigs of intact plants, root stumps and excised shoots while the potted trees were subjected to various temperature regimes in autumn, winter and spring. Osmolarity and GFS of the xylem sap (apoplast) were measured before and after cooling or warming treatments. In autumn and spring, xylem pressures of up to 160 kPa were closely correlated with soil temperature but were not correlated with GFS in xylem sap. High root pressures were associated with uptake of mineral nutrients from soil, especially nitrate. In autumn and spring, xylem pressures were detected in root stumps as well as in intact plants, but not in excised stems. In contrast, in winter, 83% of the xylem sap osmolarity in both excised stems and intact plants could be accounted for by GFS, and both GFS and osmolarity were inversely proportional to temperature. Plants kept at 1.5 °C developed positive xylem pressures up to 35 kPa, xylem sap osmolarities up to 260 mosmol l^{-1} and GFS concentrations up to 70 g l^{-1}. Autumn and spring xylem pressures, which appeared to be of root origin, were about 55% of the theoretical pressures predicted by osmolarity of the xylem sap. In contrast, winter pressures appeared to be of stem origin and were only 7% of the theoretical pressures, perhaps because of a lower stem water content during winter.

Keywords: fructose, glucose, Juglans regia, nutrient uptake, sucrose, temperature, xylem sap.

Introduction

Because water in xylem sap is normally transported under negative pressure (tension) during the growing season, freeze-induced embolism (cavitation) can greatly reduce the hydraulic conductivity of temperate woody plants (Cochard and Tyree 1990, Sperry and Sullivan 1992). There is growing evidence that freeze-induced embolism can limit the growth, survival and geographic distribution of plant species (Sperry and Sullivan 1992, Tyree and Cochard 1996, Langan et al. 1997, Pockman and Sperry 1997, Lemoine et al. 1999). Positive xylem pressures during winter and spring have important implications for vulnerability to freeze-induced embolism in temperate woody plants (Sperry et al. 1988, Sperry et al. 1994, Améglio et al. 1995, Hacke and Sauter 1996, Zhu et al. 2000).

The commercial production of maple syrup has traditionally depended on the occurrence of xylem sap pressures in *Acer saccharum* Marsh. in the late winter, but other species of *Acer* exhibit the same phenomenon. In winter, xylem sap pressures in *Acer* are thought to be derived from the stem, because stems excised in the winter exude copious amounts of sap at low temperatures (Stevens and Eggert 1945, Marvin and Greene 1951). Although positive xylem sap pressures in the winter are derived from stem pressures, *Acer* exhibits root pressures in the spring (e.g., beginning in late March in 1987, Sperry et al. 1988). In addition to *Acer,* root pressures are also reported to result in positive xylem pressures in stems of *Betula* and *Alnus* (Kramer and Kozlowski 1979, Sperry et al. 1994, Zhu et al. 2000) as well as in grapevine (*Vitis* spp., Sperry et al. 1987). In these three species the root pressures are most evident in early spring, before new leaves appear.

In previous work with intact potted plants of walnut (*Juglans regia* L.), we found that summer and autumn defoliations caused a reduction in osmolarity of xylem sap. Furthermore, for both defoliated and control treatments, there were inverse correlations between temperature and winter xylem sap osmolarity and pressure (Améglio et al. 2001). In contrast to winter results, preliminary work with walnut trees suggests that, in autumn and spring, xylem pressures are positively correlated with temperature (Améglio et al. 2000).

Based on the apparent similarity to *Acer*, we hypothesized

that winter xylem pressures in walnut were of stem origin (stem pressures), caused by the production of sugars at low temperatures. Thus we predicted that, in isolated stems in winter, as in intact plants, there would be an inverse correlation between temperature and xylem sap osmolarity. We also predicted a positive correlation between osmolarity and sugar concentration ([glucose] + [fructose] + [sucrose], GFS) of the xylem sap. In contrast, we hypothesized that autumn and spring xylem pressures were of root origin (root pressures). We predicted that there would be positive correlations between temperature and xylem pressure for root stumps and for intact plants, but not for isolated stems. Furthermore, we predicted that, in autumn and spring, xylem pressures would be positively correlated to xylem sap osmolarity, but not correlated to xylem sap GFS.

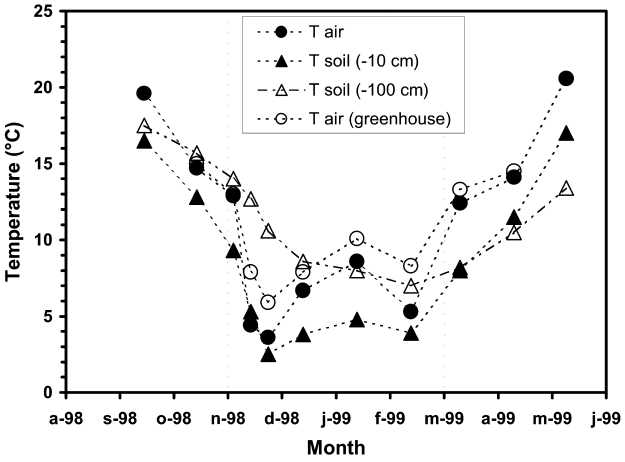

Figure 1. Mean monthly temperatures in Clermont-Ferrand at 1200 h (standard daylight time, France) between September 1997 and May 1998 for air in the orchard (1.2 m above soil), in the greenhouse, and in orchard soil at 10 and 100 cm depth. For November only, each value represents a mean of 10 days. Air temperature in the greenhouse was similar to orchard air temperature, except that a heating system was automatically turned on when temperatures dropped to 0 °C, warming the greenhouse up to 3 °C. Therefore, mean winter temperatures were slightly higher in the greenhouse than in the orchard.

Materials and methods

Temperature and pressure measurements were made on intact plants and excised stems of orchard walnut trees (*Juglans regia* L. cv. Franquette scions on wild walnut root stocks) in the winters of 1997–98 and 1998–99. The trees were grown outdoors at the INRA PIAF station near Clermont-Ferrand, in south-central France, and were 17 years old in 1998.

In addition, 5-year-old potted plants of walnut (*J. regia* cv. Franquette scions on wild walnut root stocks) were used for cooling experiments during the period from October 1998 through May 1999. The grafted plants were grown in individual 33-l well-drained containers filled with a mixture of peat (33%) and clay soil (67%), annually fertilized with 10 g NH_4NO_3 and continuously drip-irrigated to field capacity.

Twelve potted trees were grown outdoors until September 1998, when they were put in a greenhouse, in which the temperature usually tracked the outdoor temperature. However, air temperatures in the greenhouse were continuously recorded and a heating system was automatically turned on when temperatures dropped to 0 °C, increasing the greenhouse temperature to as high as 3 °C. Thus, prior to the cooling experiments, the trees were exposed to low, but not freezing temperatures in the autumn and winter of 1998–99. As a result, mean winter temperatures were only slightly higher in the greenhouse than outdoors (Figure 1), allowing for possible winter acclimation in the greenhouse.

A large cooling chamber was designed to hold up to four potted trees that were 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, Cambridge, U.K.) recorded temperatures and pressures as 5-min means. To measure pressures, one or two branches from each tree were excised and a pressure transducer (Model PDCR 1000; Druck Ltd., Leicester, U.K., sensitivity ± 1 kPa) attached to the branch stump. In other cases, following excision of the main stem, pressure transducers were attached to the root stump or to the base of the excised main stem. In each case, the stem or branch was cut and the stump was tightly connected to silicone oil-filled vinyl tubing that was connected to a transducer (Améglio et al. 2001). As a control, one of the pressure transducers in the cooling chamber was tightly connected to silicone oil-filled vinyl tubing plugged with a rubber stopper.

Because the soil otherwise dried out when plants were kept in the cooling chamber for more than a week, we covered the soil surface of potted plants with several cm of sand to maintain high soil water content for at least 2 weeks, and exposed the plants to various cooling and warming cycles. Plants were kept at particular "resting temperatures" for up to 72 h before cooling or warming at a rate of 5 °C h^{-1}. The range of temperatures that plants were exposed to in the cooling chambers was similar to the range of monthly mean temperatures that occurred in orchard plants during the period from September through May (Figure 1). Unlike plants in orchards, however, the experimental plants did not experience freezing temperatures, which occur periodically during the winter at Clermont-Ferrand.

One-m-long isolated branches from 18 orchard trees were collected in winter 1996–97. One branch per tree was used immediately for measurements of xylem sap osmolarity and sugar concentrations, described below, whereas the other branches were kept at either 15 or 1 °C in a small cooler for 48 h before isolating the xylem sap.

Before and after the cooling treatments in either the large cooling chamber or small coolers, stem segments were sampled for extracting xylem sap. The distal 10 cm of a branch was discarded and the next 20 cm used for sap extraction (Bollard 1953). Osmolarity of extracted xylem sap was measured with a Roebling 13DR Automatic Osmometer (Messtechnik GmbH, Berlin, Germany). Sucrose, glucose and fructose con-

tents were determined spectrophotometrically at 340 nm after enzymatic assays (Boehringer 1984).

Additionally, we observed the dynamics of soil water temperature, nutrient fluxes, and root pressure. Two-year-old, potted hybrid plants of walnut (*J. regia* × *J. nigra* L.) were used for nutrient experiments during the period beginning October 2000. Three plants were individually grown in cylinders (0.20 m diameter × 0.30 m height) filled with chemically inert perlite, covered with a 1-cm-thick layer of silex (2–3-mm diameter beads) pretreated with a chemical waterproofing compound (Mursain®) that limits evaporation and development of algae (Beaujard and Hunault 1997). Six times per day, the plants were drip-irrigated with nutrient solution for 1-h periods (Figure 2). The 70 l of nutrient solution (composition in mol m^{-3}: 1.83 NO_3^-, 0.07 NH_4^+, 0.18 $H_3PO_4^-$, 0.98 K^+, 0.38 Ca^{2+}, 0.35 Mg^{2+}, 0.03 Na^+, micro elements (Kanieltra® 0.2%), pH = 5.1, in deionized water) was replaced each week. A pump situated at plant level ensured homogeneity of the nutrient solution. A second pump returned the surplus solution to the tank (Figure 2). To measure pressure, pressure transducers were attached to the root stumps. Soil temperature in each cylinder, air temperature in the greenhouse and pressure measurements on each root stump were recorded by a data logger. Periodically after mixing, 0.125 l of tank solution was sampled and the nutrient content measured spectrophotometrically (Beaujard and Hunault 1997).

Results

Orchard trees

In January 1998, positive xylem pressures were recorded for orchard walnut trees at air temperatures between –5 and 10 °C. Typically, higher pressures were recorded in stems 3.5 m from the soil than in stems 2.5 or 1.2 m from the soil (Figure 3a). There was a complex relationship between xylem pressure and temperature. Air temperatures between –3 and 3 °C were often associated with sharp increases in xylem pressure, whereas temperatures below –5 °C (data not shown) and above 5 °C were sometimes associated with decreases in xylem pressure (Figure 3a).

Throughout March and April of 1998, positive xylem pressures were recorded for orchard walnut trees at air temperatures between 0 and 25 °C, with the highest pressures consistently occurring in the lower branches (Figure 3b). In contrast to January, in March and April there was a simple relationship between xylem pressure and temperature, with diurnal increases and decreases in air temperature consistently associated with diurnal increases and decreases in xylem pressure, respectively.

Root pressures

Cooling experiments were done with five potted trees in October and early November 1998, and with four potted trees in May 1999. A silicone oil-filled pressure transducer was attached to either a root stump (R1 in Figure 4a, R19 in Figure 4b), to an excised shoot (R19 (stem), Figures 4b and 4c), to a control, which was a silicone oil-filled vinyl tube with a sealed end (Figures 4a and 4c), or to a whole potted plant (P2) with a shoot tip removed to attach the transducer (Figure 4c). The control plants showed no effect of temperature on pressure except for a transient increase of about 3 kPa when air temperatures were increased from 22 to 26 °C (Figure 4a). Therefore, the pressure readings at temperatures from 22 to 26 °C may be less reliable than those made at lower temperatures.

A summary of the autumn and spring temperature-step experiments is given in Table 1. For all plants, there was a positive linear correlation between soil temperature and xylem pressure (r^2 = 0.83 to 0.96) at temperatures between 3 and 21 °C (Figure 4, Table 1). During those periods when air temperatures were changed in a step-like manner, xylem pressures tracked soil temperatures more closely than air temperatures. This was true both for plants with low positive pressures (up to 40 kPa; Figure 4a) and for plants with high positive pressures (up to 100 kPa; Figure 4b). Xylem pressures appeared to peak at soil temperatures of about 21 °C (Figure 4b). In addition, the temperature–pressure correlations for branch stumps on intact trees (Table 1; P2, P3) were similar to those for the root stump (R1–R5, R16–R19 in Table 1). In contrast, excised shoot sys-

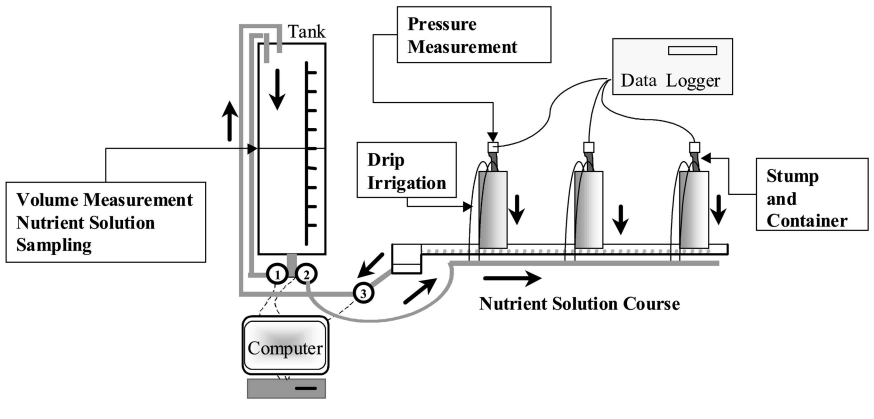

Figure 2. Diagram of drip irrigation system for measuring and recirculating nutrient solutions. Pressure transducers were connected to root stumps while nutrient uptake and soil temperature were monitored.

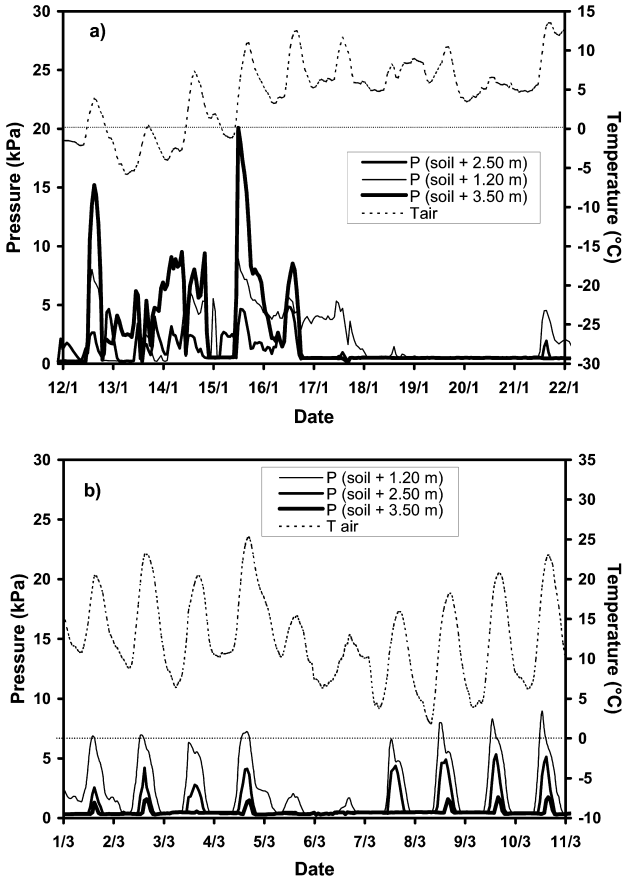

Figure 3. Orchard air temperatures and xylem pressures of a representative orchard walnut tree for 10 days in January (a) and April (b) 1998. Pressure transducers were attached to stems at heights of 1.2, 2.5 and 3.5 m from soil level.

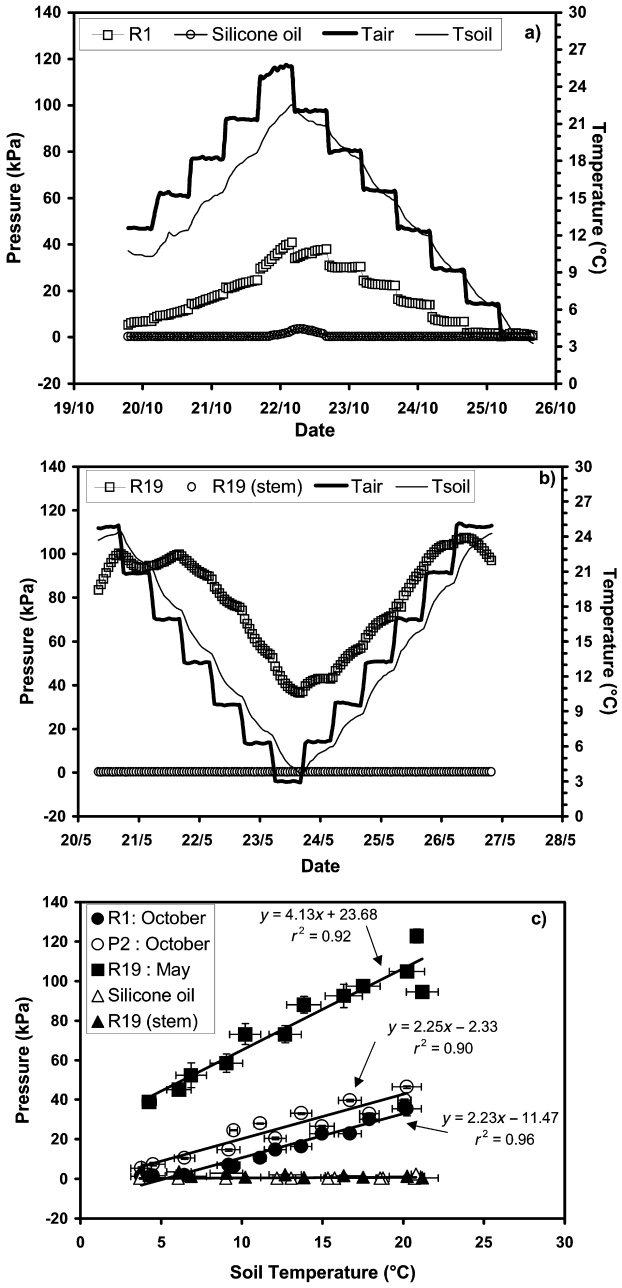

Figure 4. Effects of cooling chamber temperature on xylem pressure of potted walnut trees in October and May. Air temperature was modified in a step-like manner. At each step, air temperatures were maintained for 12 h before warming or cooling at a rate of 5 °C h^{-1}. Panels (a) and (b) show results for autumn and spring, respectively, with the pressures given as 1-h means. Panel (c) shows xylem pressure as a function of soil temperature, with 12-h means (± SE) given for pressure and temperature.

tems showed no positive xylem pressures during the same periods, regardless of temperature (e.g., R19 (stem) in Figure 4b).

The slope of the regression line for xylem pressure versus temperature varied among plants from 1.33 to 4.13 kPa $°C^{-1}$, and tended to be higher in spring (overall mean ± SE: 3.63 ± 0.41) than in autumn (2.20 ± 0.60), and greater for root stumps than for intact plants (Figure 4c, Table 1). In autumn, when experiments were repeated on the same plants, the slopes were greater for the second experiment than for the first experiment (Table 1).

On November 8, a change in the xylem pressure–temperature relationship became evident. Starting on November 8, when the temperature was decreased from 5.5 to 1.5 °C, there was an initial sharp drop in xylem pressure followed by a steady increase in xylem pressure. When the temperature was then raised to 5.5 °C on November 9, there was a transient increase in xylem pressure followed by a steady decline. The temperature was then alternated between 1.5 and 5.5 °C on a 24-h cycle (Figure 5a). For each of four such cycles between November 9 and 16 there were gradual pressure increments at the lower temperature and pressure decrements at the higher temperature. With each cycle the pressure maximum at 5.5 °C decreased, suggesting a reduction in the ability of the plant to maintain root pressure.

Stem osmolarity and xylem pressures

During late November and December, it took up to 2 days for

Table 1. Linear regression analysis of xylem pressure as a function of soil temperature in autumn and spring, based on temperature-step experiments as shown in Figure 3. The plant code for each individual indicates whether the root stump (R#) or whole potted plant (P#) was used. The slope (kPa $°C^{-1}$) and r^2 values are shown for each experiment. In one instance (*) the same root system (R1) was used for two successive experiments. In two instances (**), the pressure transducer was first attached to a whole potted plant (P2 and P3) with only a shoot tip removed to attach the transducer, then, for the following experiment, the main stem was excised and the transducer was attached to the root stump (R2 and R3).

Date	Plant code	Slope of regression	r^2
Autumn			
Oct 19–26	R1*	2.23	0.96
	P2**	2.25	0.90
	P3**	1.33	0.85
Oct 2–27	R1*	3.71	0.93
	R2**	4.13	0.95
	R3**	4.19	0.88
Nov 2–16	R4	2.98	0.89
	R5	2.49	0.91
Spring			
May 19–27	R16	3.79	0.87
	R17	3.22	0.83
	R18	3.38	0.89
	R19	4.13	0.92

stem pressures of potted trees to equilibrate to changes in temperature in the cooling chamber. Therefore, to analyze the relationship between osmolarity of stem xylem sap and temperature, we chose the mean temperature from the 48-h period prior to an osmolarity reading as the independent variable. The mean temperature was determined from the data logger in the greenhouse at the beginning of an experiment, or from the data logger in the cooling chamber in the middle or at the end of an experiment.

In one set of representative experiments, on November 16, plants were moved from the greenhouse (where the previous 48-h mean air temperature was 9.7 °C) to the cooling chamber and kept at 1.5 °C for 2 days (Figure 5b). Their xylem pressures rose from 12.9 kPa to as high as 35 kPa. When the same plants were then warmed to 18 °C and kept at that temperature for 2 days (Figure 5b), their xylem pressures dropped to 0.4 kPa.

The inverse experiments yielded complementary results. Plants were kept in the greenhouse with a 48-h mean air temperature of 5.6 °C, then, starting on November 25, plants were moved to the cooling chamber and kept at 18 °C for 2 days (Figure 5c). Their xylem pressures dropped from 14.4 kPa to zero (Figure 5c). When temperatures were then dropped to 1.5 °C for 2 days, xylem pressures gradually built up to 30.6 kPa (Figure 5c).

Combining results from several winter experiments in which xylem sap osmolarity was measured at the beginning and end of the temperature-controlled period, the results of experiments with intact potted plants were similar to the results

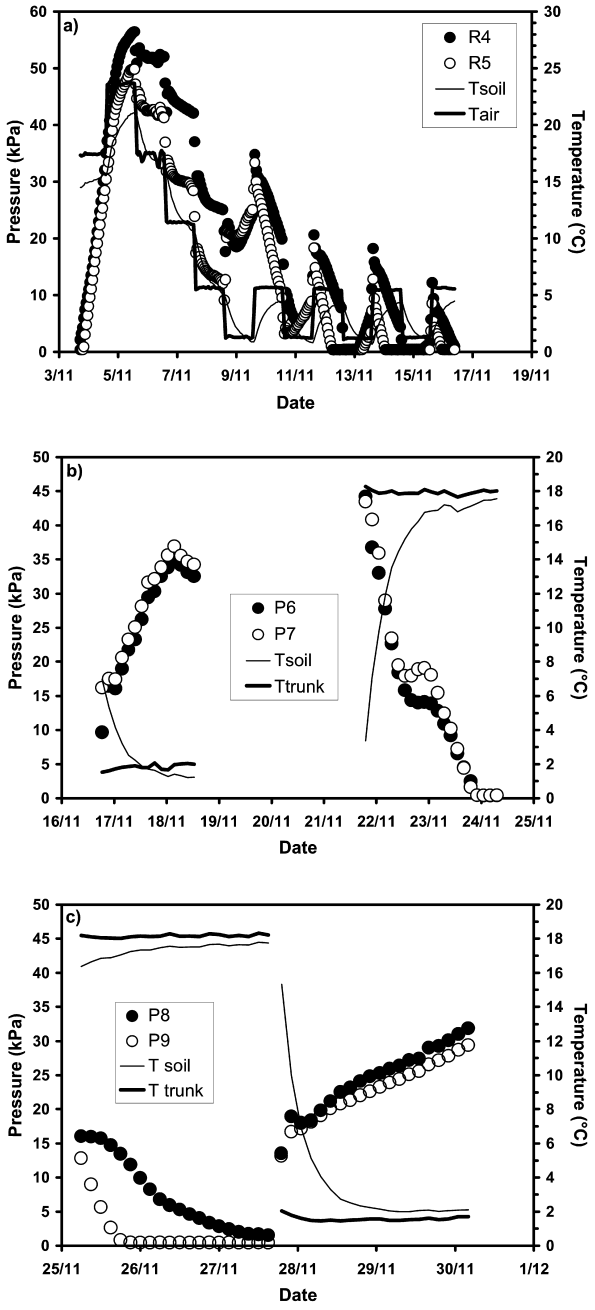

Figure 5. Effect of cooling chamber temperature on xylem pressures of potted walnut plants in November 1999. Thermocouples were inserted in the soil (Tsoil), placed in the center of the chamber (Tair), or placed on the surface of the trunk (Ttrunk). In (a), for two root stumps (R4, R5), air temperature was initially modified in a step-like manner, then, starting on November 8, air temperature was repeatedly alternated between 5.5 and 1.5 °C. Air temperatures were kept at particular values for 24 h before warming or cooling at a rate of 5 °C h^{-1}. In (b) (cool/warm experiment), two potted trees (P6, P7) were kept in the cooling chamber at a resting temperature of 1.5 °C for 2 days, then the air temperature was raised and maintained at 18 °C for more than 2 days. Panel (c) (warm/cool experiment) shows the inverse treatment for two plants (P8, P9): air temperature was first kept at 18 °C for 2 days, then at 1.5 °C for 2 days. The pressures are 1-h means (a) or 3-h means ((b) and (c)). Plants were sampled for xylem sap osmolarity and symplast carbohydrates at the beginning and end of the experiments.

of experiments with excised branches of orchard trees (Figure 6). In particular, osmolarity of xylem sap was inversely proportional to the previous mean 48-h temperature, with high osmolarity (about 205 mosmol l^{-1}) when temperatures were maintained below 5 °C, and low osmolarity (about 50 mosmol l^{-1}) when temperatures were maintained above 15 °C. The initial sampling, which was done when temperatures were intermediate between 5 and 15 °C during the previous 48 h, showed intermediate values of osmolarity (Figure 6).

Seasonal osmolarity, xylem pressures and temperature

Based on results from many experiments, we found a different relationship between temperature and xylem pressure in winter than in autumn or spring. In October and May, plants showed positive correlations between temperature and xylem pressure. There were high xylem pressures at temperatures above 15 °C, but low pressures at temperatures below 5 °C. In contrast, in late November–December, there were no longer positive correlations between temperature and pressure. Instead, high xylem pressures were found at temperatures below 5 °C, and low xylem pressures at temperatures above 15 °C (Figure 7).

In winter, autumn and spring, there were positive correlations between xylem sap osmolarity and xylem pressure. However, the slope of the linear regression line was much steeper for root stumps in autumn + spring (Root) than for intact plants in winter (Stem in Figure 8a). Furthermore, root pressure values were much closer to theoretical pressures calculated from osmolarity (Nobel 1991) than were the stem pressure values (Figure 8a). Based on differences in the slopes of the regression lines, the root pressure values were 55% of theoretical values, whereas stem pressures were 7% of theoretical values.

Osmolarity of stem xylem sap in winter correlated well with sugar concentration (GFS). At high osmolarities GFS concen-

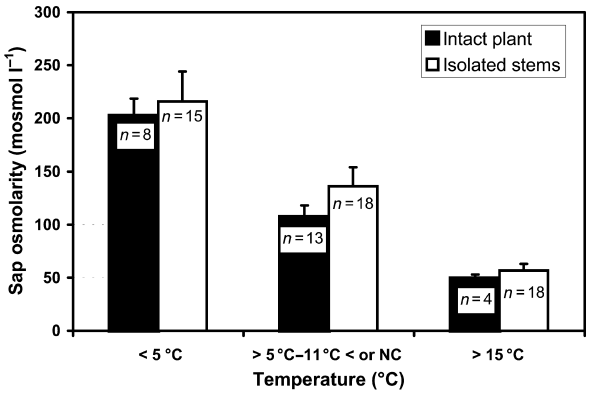

Figure 6. Winter experiments: effect of temperature on osmolarity of stem xylem sap of intact plants and isolated stems. Osmolarity was measured first for intact potted plants taken from the greenhouse (temperature from 5 to 11 °C) or from orchard branches in their natural condition (NC). Then the potted plants or isolated branches of the orchard trees were put in coolers kept below 5 °C or above 15 °C for at least 48 h before measuring xylem sap osmolarity.

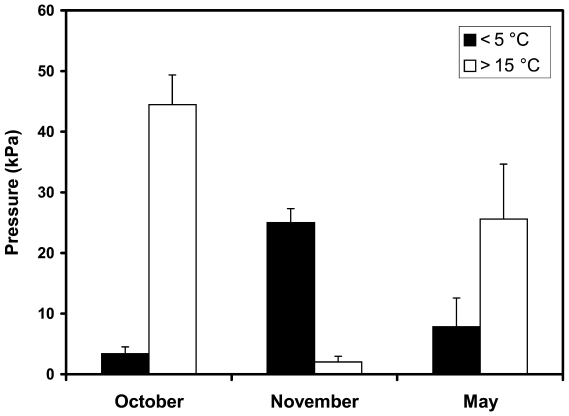

Figure 7. Summary of the relationship between mean air temperature during the previous 24 h (< 5 °C versus > 15 °C) and experimental xylem pressure (previous 12-h mean) in October, November–December and May. Means ± SE are shown; n = 8, 8 and 4 for October, November–December and May, respectively. The October and May pressures were interpreted as root pressures and the November–December pressures were interpreted as stem pressures.

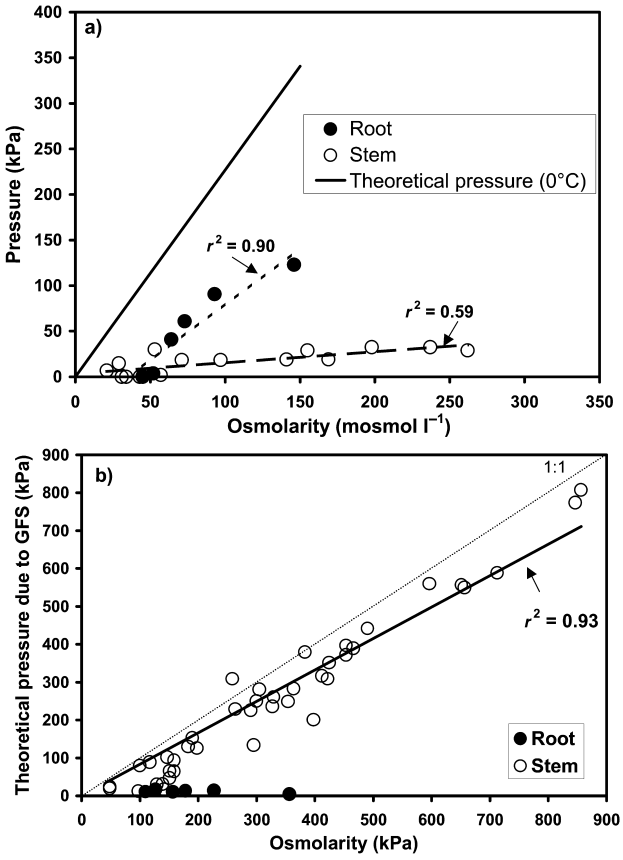

Figure 8. Relationship between osmolarity of xylem sap and pressures during the winter (Stem pressure) and during the autumn and spring (Root pressure). Panel (a): measured xylem pressure (kPa) as a function of osmolarity (mosmol l^{-1}). The solid line indicates the theoretical pressure potential derived from osmotic concentration (Nobel 1991). Panel (b): the theoretical potential due to glucose + fructose + sucrose (GFS) as a function of the total osmolarity. The dotted line indicates the GFS value representing 100% of total osmolarity.

trations reached 70 g l^{-1} (data not shown). The GFS concentrations accounted for 83% of the osmolarity in winter (Stem, Figure 8b). On a weight basis (g l^{-1}), sucrose was the most important of the three sugars measured (46%, SE = 3%). In autumn and spring, no relationship was found between osmolarity of xylem sap and sugar concentration (Root, Figure 8b). Instead, GFS concentrations ranged from 0.5 to 2.0 g l^{-1} and accounted for only 0.05% of the osmolarity of xylem sap.

Nutrient experiments

Table 2 shows the concentrations of the nutrient solution during September and October 2000; a fresh nutrient solution was introduced each week. During the first 2 weeks, the aerial parts of intact plants transpired at a rate of 0.7 l day^{-1} per plant (data not shown), which was about 1% day^{-1} of the total volume of nutrient solution in the system. During this time the nutrient concentrations decreased each week by 99% for NO_3^- and NH_4^+, by about 50% for $H_3PO_4^-$ and Ca^{2+} and by 10% for Mg^{2+} and K^+. At the beginning of the third week (October 11), the aerial part was removed to leave the root stump, causing transpiration to cease. Nevertheless, the nutrient concentrations still decreased by approximately 93% for NO_3^-, 35% for $H_3PO_4^-$, 19 and 10% for Ca^{2+} and Mg^{2+} respectively, and 0% for K^+. Thus, despite lacking aerial parts, the root stumps continued to absorb nutrients, principally NO_3^-.

Figures 9c and 9d relate xylem pressure and concentration of NO_3^-. We observed a reduction in [NO_3^-] during the week and xylem pressure values of between 60 to 100 kPa. With the application of fresh nutrient solution on October 17 (Figure 9d), there was a rapid increase in xylem pressure from about 90 to 130 kPa in the first 4 h and up to 160 kPa within 24 h. During that 24-h period, the concentration of NO_3^- in the nutrient solution dropped from 1.74 to 0.92 mol m^{-3} (Figure 9d, Table 2), indicating that there was considerable uptake of mineral nutrients, especially NO_3^-, associated with the enhanced root pressures.

Root pressures were not correlated with greenhouse air temperature, but with each 1-h drip irrigation period there were immediate, short-term changes in xylem pressure (Figure 9). These pressure variations were positively correlated with soil temperature. Small increases in soil temperature during an irrigation period (usually about 1 °C) resulted because the nutrient solution in the mixing tank was at a slightly higher temperature than the soil water temperature.

Discussion

We obtained evidence of two mechanisms for generating positive xylem pressures in walnut trees: root pressures, which were operative only at higher temperatures in autumn and spring, and stem pressures, which were operative only at low temperatures in winter. There was a disparity between measured pressures and theoretical pressures calculated from the xylem sap osmolarities, especially when comparing winter stem pressures with autumn and spring root pressures.

Root pressures

Clark (1874) was the first to report root pressures in *J. nigra* (black walnut), although at that time workers did not clearly distinguish between xylem exudation due to root pressure versus that due to stem pressure. We assume that positive xylem pressures observed in *Juglans* spp. in autumn and spring were of root origin for four reasons. First, during autumn and spring, xylem pressures occurred in stumps of decapitated root systems as well as in branches of intact plants, but not in stems of excised shoots. Second, during spring, unlike in winter, xylem pressures in orchard trees decreased with increasing tree height, implying that pressures originated in the root and dissipated with height. Third, in autumn and spring, xylem

Table 2. Nutrient concentrations (mmol l^{-1}) in the drip-irrigation experiment depicted in Figure 2. A fresh supply of nutrients was introduced once a week and subsequent reductions in nutrient concentrations indicated uptake by the roots. For the first 2 weeks, three whole plants were used, then the shoots were excised (October 11, 2000, 0900 h). Figure 9 shows the root pressures and soil temperatures for parts of Weeks 3 and 4.

Measurement date	Mineral nutrient					
	[NO_3]	[NH_4]	[P]	[K]	[Ca]	[Mg]
Week 1 (whole plant)						
Sept 26 (1000 h)	1.739	0.078	0.171	0.924	0.401	0.340
Oct 3 (0700 h)	0.023	0.001	0.084	0.867	0.178	0.303
Week 2 (whole plant)						
Oct 3 (1000 h)	1.734	0.063	0.172	0.987	0.362	0.346
Oct 10 (0700 h)	0.017	0.001	0.079	0.970	0.215	0.314
Week 3 (root stump)						
Oct 10 (1000 h)	1.723	0.068	0.177	1.005	0.359	0.343
Oct 11 (0900 h)	0.955	0.001	0.150	0.969	0.323	0.364
Oct 17 (0700 h)	0.070	0.001	0.098	0.974	0.263	0.329
Partial Week 4 (root stump)						
Oct 17 (1000 h)	1.741	0.066	0.177	1.005	0.367	0.350
Oct 18 (1100 h)	0.923	0.009	0.154	0.987	0.347	0.366

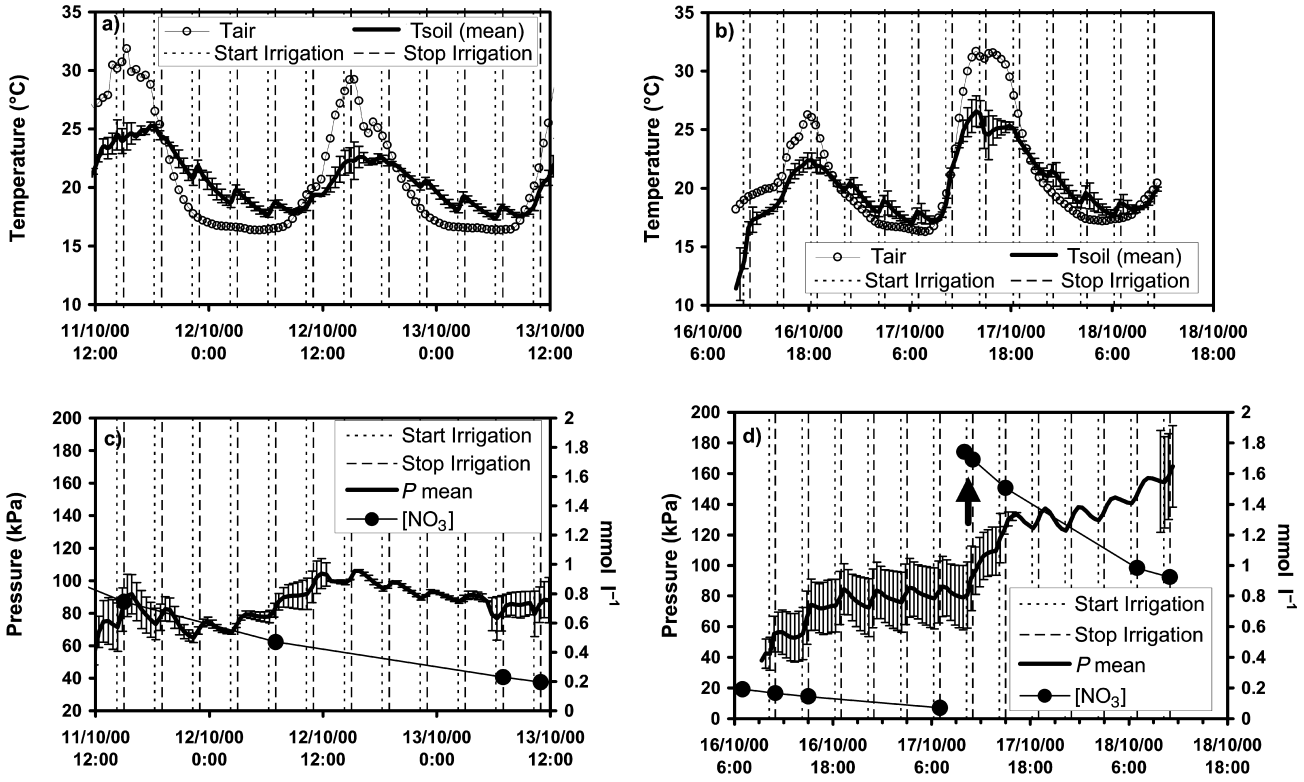

Figure 9. Effects of temperature and nutrient solution NO_3^- concentrations on xylem pressures during the course of several days in October 2000, from the system shown in Figure 2. Panels (a) and (b) show soil temperature (Tsoil mean ± standard deviation, $n = 3$) and greenhouse air temperature (Tair). The dashed lines indicate when irrigation began and ended. Panels (c) and (d) show the pressure values. Each value is a 30-min mean of three pressure measurements with standard deviation. In Figure 9d we omitted standard deviations when one of the three sensors failed. The arrow in Figure 9d represents the time when fresh nutrient solution was applied (cf. Table 2, October 17, 2000, 1000 h).

pressures were positively correlated with soil temperature. This is consistent with results for many woody plants, showing that the development of root pressures is dependent on warm moist soils, humid air and low transpiration (Kramer and Kozlowski 1979, Cochard et al. 1994). Our nutrient experiments showed that root pressures are extremely sensitive to soil temperature and to the nutrient content of soil water. Fourth, although autumn and spring xylem pressures in walnut trees were not associated with high sugar concentrations in the xylem sap, the uptake of nitrates may be particularly important for the formation of root pressures. In *Acer* (a well studied system for stem pressures), stem pressures are associated with sugars in the xylem sap and with low winter temperatures (Johnson 1945, Marvin 1958, Johnson et al. 1987).

In potted plants, there was a close relationship between soil temperature and xylem pressure in both autumn and spring, suggesting the involvement of root activity in the generation of xylem pressure. Presumably, root pressures depend on fine roots actively taking up mineral nutrients and secreting these solutes into the apoplast of the root steles. Active uptake of minerals is positively correlated with temperature. For potted plants, the slope of the regression line between soil temperature and xylem pressure was steeper in spring than in autumn. Possibly, growth and metabolism of fine roots were more rapid in spring, allowing for enhanced mineral uptake.

In October, the slopes of regression lines between soil temperature and xylem pressure were steeper for the second 7-day warming/cooling cycle than for the first cycle. This result may indicate that new fine roots were produced during the first warming cycle, allowing greater mineral uptake during the second warming cycle. It is not known what becomes of the root pressure response during winter. Perhaps during late autumn fine roots are shed, or there is some other dormancy mechanism whereby the root system no longer generates positive pressures in response to warm soil temperature. The drip irrigation nutrient experimental system should offer opportunities to examine dormancy mechanisms as well as the specific mineral requirements for the generation of root pressures.

The orchard trees showed a positive correlation between air temperature and xylem pressure. At shallow depths (< 10 cm), soil temperature tends to track diurnal air temperature in spring at Clermont-Ferrand. However, at depths of 1 m or more, there is little diurnal variation in soil temperature (data not shown). Thus, the diurnal variations in spring root pressures are probably associated with the activities of relatively shallow roots.

Seasonal differences in root pressures will influence xylem embolism in the field. At Clermont-Ferrand, orchard walnut trees normally drop their leaves in mid-November, with bud break occurring in April. By the time leaves drop in Novem-

ber, soil temperatures are low and dormancy mechanisms, such as shedding of fine roots, may have started. Because leaf transpiration would counter the buildup of root pressure, we predict that orchard trees would have the greatest root pressures in March and April, when soils start to warm, but before the maturation of new leaves. Such root pressures would be well timed to remove any winter embolism that may have occurred in vessels.

Stem osmotic pressures

Winter stem pressures in *J. regia* are similar to those reported for *Acer* spp. (Johnson 1945, Marvin et al. 1967, Sauter et al. 1973, Cortes and Sinclair 1985). As in *Acer*, most of the osmolarity of the xylem sap in *Juglans* in winter can be attributed to sucrose and its component simple sugars, glucose and fructose. The increases in GFS occurred at low temperatures, corroborating earlier findings (Améglio and Cruiziat 1992). We found that even excised walnut stems develop sugars in their xylem sap at low winter temperatures.

We did not determine to what extent these phenomena apply to other woody plants besides *Acer* and *Juglans*. In many temperate dicotyledonous trees such as *Betula pendula* Roth., *Alnus glutinosa* L., *Fagus sylvatica* L. and *Quercus robur* L., there is a period in early spring when starch is broken down to sugars and released in the xylem sap, resulting in positive xylem pressures (Essiamah 1980). The ability to produce positive, sucrose-based xylem pressures throughout the winter may be peculiar to *Acer* and *Juglans*. However, within the xylem of many temperate trees there are "contact cells," which are living axial and ray parenchyma cells with large pits connecting them to vessels. Contact cells are the sites of sugar secretion (efflux) into the xylem sap (Sauter et al. 1973, Sauter 1980, 1981, Braun 1984, Essiamah and Eschrich 1985).

Measured versus theoretical pressures

To generate a positive hydrostatic pressure in xylem sap, there must be a difference in the osmotic potential of the xylem sap compartment (the apoplast) and the neighboring compartment (contact cells and xylem parenchyma, or symplast). The semipermeable membranes of contact cells separate the two compartments. Presumably, the osmotic potential of the apoplast becomes greater than the symplast, resulting in water uptake and positive pressures. The hydrostatic pressure of the xylem sap would not be equal to the theoretical osmotic pressure, however, unless the symplast component was composed of pure water.

Root pressures were equal to 55% of theoretical pressure potentials, whereas stem pressures were equal to only 7% of theoretical pressure potentials. The roots were in immediate contact with water-saturated soil, providing a means to increase the total water content of the plants. We conclude that, for winter stem pressure, access to a water supply may limit the generation of positive hydrostatic pressures.

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