

Water storage in the wood and xylem cavitation in 1-year-old twigs of *Populus deltoides* Bartr.

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ABSTRACT

The possible role of water expelled from cavitated xylem conduits in the rehydration of water-stressed leaves has been studied in one-year-old twigs of *populus deltoides* Bartr. Twigs were dehydrated in air. At desired values of leaf water potential (ψ_l) (between near full turgor and -1.62 MPa), twigs were placed in black plastic bags for 1–2 h. Leaf water content was measured every 3–5 min before bagging and every 10 min in the dark. Hydraulic conductivity and xylem cavitation were measured both in the open and in the dark. Cavitation was monitored as ultrasound acoustic emissions (AE). A critical ψ_l value of -0.96 MPa was found, at which AE increased significantly while the leaf water deficit decreased by gain of water. Since the twigs were no longer attached to roots, it was concluded that water expelled from cavitated xylem conduits was transported to the leaves, thus contributing to their rehydration. Xylem cavitation is discussed in terms of a 'leaf water deficit buffer mechanism', under not very severe water stress conditions.

Key-words: *Populus deltoides*; poplar; xylem cavitation; leaf rehydration.

INTRODUCTION

It has been known for years that trees can store water in their wood with a seasonal or even diurnal rhythm of storage and depletion (Reynolds 1965; Landsberg, Blanchard & Warritt 1976; Waring, Whitehead & Jarvis 1979). For some trees such as *Pseudotsuga menziesii* (Mirb.) Franco, it has been suggested that the major reservoir of water is the sapwood (Waring & Running 1978). Waring *et al.* (1979) reported that 64% of the stored water available for transpiration in *Pinus sylvestris* L. was in the stem sapwood, and less than 5% in the cortex and foliage.

The amounts of water that can be extracted from the storage compartment and used for rehydrating leaves, as well as the conditions under which water is made available to leaves, are of theoretical and practical importance for a better understanding of plant water

economy. In particular, water can be held in the wood by capillarity, e.g. in the fibres (Zimmermann 1983) and enter the xylem conduits as their internal pressure potentials decline, or it can be expelled from embolized xylem conduits (Edwards & Jarvis 1982; Dixon, Grace & Tyree 1984).

Whether the amount of water stored in the wood may rehydrate leaves (Dixon *et al.* 1984), or is too little to account for a significant increase in leaf water potential (Roberts 1976), is currently a matter of controversy.

During a brief period of joint work at the Universität für Bodenkultur of Vienna, Austria, experiments were performed with the purpose of testing whether the mechanism proposed by Zimmermann ('air seeding') is the cause of xylem cavitation (Zimmermann & Milburn 1982; Zimmermann 1983).

When 3-year-old detached twigs of *Salix viminalis* were left drying in air and tested for leaf water potential (ψ_l) and xylem cavitation, ψ_l dropped less than expected, thus suggesting that water, either stored or released from cavitated xylem conduits, had become available to maintain a ψ_l higher than expected on the basis of the transpiration measurements.

The present investigations were designed with the purpose of checking whether a significant amount of water could be released from the wood of 1-year-old twigs and contribute to the rehydration of leaves that were allowed to transpire only stored water. The relations between water cavitation in xylem and leaf water content were also studied.

MATERIALS AND METHODS

All samples were collected from two 10-year-old plants of *Populus deltoides* Bartr. growing at sea level in the Botanical Garden of Messina, Sicily. One-year-old twigs bearing leafy shoots of the current year growth were tagged in April 1989. Each one-year-old twig (over 70 cm long) bore at least six shoots and each shoot bore 10–12 leaves. All experiments were performed between May and July 1989.

Two groups of experiments were designed: the first was mainly devoted to measurements of water loss and water potential changes in leaves (still attached to shoots) put in the dark at different water potential values, as well as to measurements of xylem cavitation

on twigs both outside and inside the dark chamber. In the second group, the reduction in xylem hydraulic conductivity was measured before and after twigs had been in darkness.

Changes in leaf water content, water potential and xylem cavitation

The following variables were measured:

Leaf water potential

Leaf water potential (ψ_l) was measured by the pressure chamber technique (Scholander *et al.* 1964; Tyree & Hammel 1972). All the ψ_l measurements were performed at 20°C in a constant temperature room.

Leaf fresh weight

Leaf fresh weight (W_f) was measured by weighing detached leaves within about 5 s after ψ_l had been measured.

Leaf turgid weight

Leaf turgid weight (W_t) was obtained by placing leaves, with their petioles immersed in water and covered with plastic bags, in the dark for 20 min to 1 h depending on the initial value of ψ_l . Leaves were then reweighed for W_t , and ψ_l was remeasured so as to check that it was near zero and that leaves were not oversaturated with water. At full turgor, ψ_l was in every case between 0.01 and 0.02 MPa.

Leaf surface area

Leaf surface area (A_l) (one side only) was measured on paper replicas of the leaves at full turgor.

Leaf water deficit

Leaf water deficit (D) was expressed as:

$$D = \frac{W_t - W_f}{A_l} \quad \text{g m}^{-2}$$

i.e. as the amount of water lost per unit leaf surface area with respect to the reference point of full turgor.

Acoustic emissions

Acoustic emissions (AE) were detected in the ultrasound range (Tyree & Dixon 1983, 1986; Dixon *et al.* 1984; Sandford & Grace 1985; Tyree *et al.* 1986; Salleo & Lo Gullo 1986, 1989a,b; Sperry, Tyree & Donnelly 1988; Tyree & Sperry 1989). Acoustic emissions were counted using an AE transducer (R15, Physical Acoustics Corp., Princeton, USA) connected to a 'drought stress monitor' (Model 4615, Physical Acoustics Corp.). The transducer was clamped to exposed wood (about

1 cm²) of 1-year-old internodes at about one third of the twigs length. A thin layer of silicon grease was interposed between the transducer and wood to secure good acoustic contact and prevent wood dehydration. Acoustic emissions in the frequency range of 100–300 kHz were picked up by the transducer, amplified by 52 dB and counted with the microprocessor of the drought stress monitor (Tyree & Sperry 1989).

Leaf water potential isotherms

Leaf water potential isotherms of five to seven leaves at increasing symplastic water losses were measured at 20°C by the pressure chamber technique (Scholander *et al.* 1964; Tyree & Hammel 1972). This permitted estimation of ψ_{ltp} (i.e. ψ_l at the turgor loss point). The procedure employed has been described in detail elsewhere (Salleo 1983; Lo Gullo, Salleo & Rosso 1986).

The two *P. deltooides* plants were irrigated the evening preceding the experiments and, at the same time, the intact twigs to be studied were enclosed in black plastic bags so as to allow the leaves to reach full turgor.

The twigs were cut off in the field under filtered tap water and brought to the laboratory while still in the plastic bags and with their cut ends immersed in water. After the bags were removed, ψ_l , W_f , W_t and AE were measured. Once the condition of no AE and near-zero ψ_l was confirmed, the water container was removed and thence ψ_l , W_f and W_t were measured every 3–5 min until ψ_l reached pre-determined values (between –0.3 and –1.6 MPa). The entire twigs were then enclosed in a black plastic bag equipped with a zip fastener to enable access. After the twigs had been bagged ψ_l , W_f and W_t were measured every 10 min for 1 h while AE were recorded continuously. This procedure allowed measurement of the amount of water lost by the leaves while inside the black bags, starting from different ψ_l values, and evaluation of whether eventual recovery of ψ_l and leaf water content occurred at the reduced transpiration rate. This would have indicated that water stored in the twig was used to rehydrate leaves.

The two extreme cases of twigs still remaining in contact with water and twigs left drying in the open were also tested.

Measurements of the loss of xylem hydraulic conductivity

In a second group of experiments, the twigs already treated and measured as described above were also tested for loss of hydraulic conductivity before or after they had been in black plastic bags for 1 h. This allowed evaluation of the impact of any cavitation on hydraulic conductivity.

The procedure used to measure xylem hydraulic conductivity has been described by Sperry *et al.* (1988) and was only slightly modified.

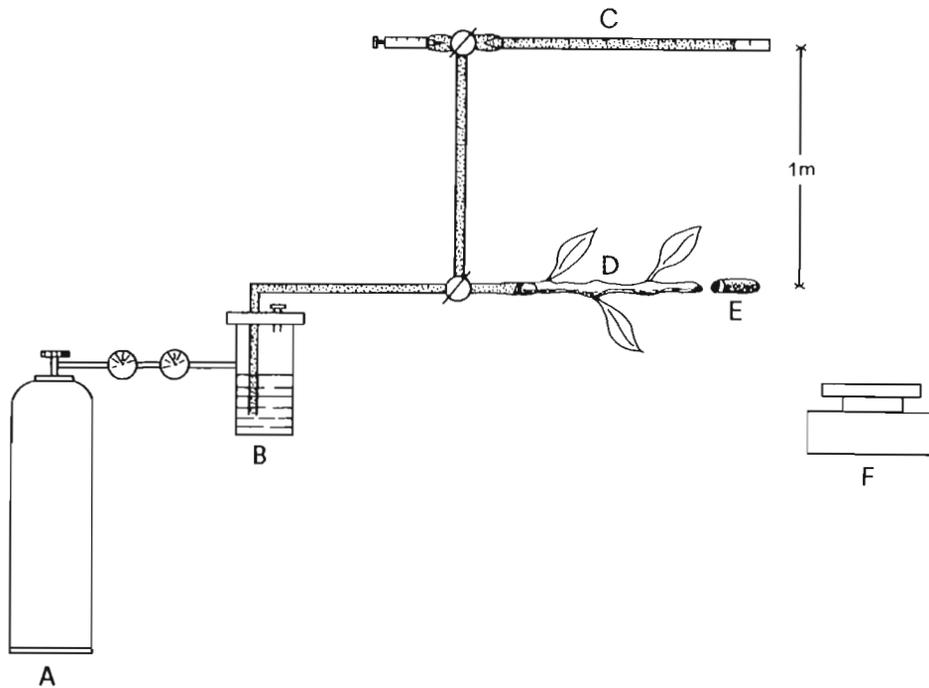


Figure 1. A diagram (not to scale) of the equipment used for measuring hydraulic conductivity of twig segments. The stem segment (D) is perfused at low pressure (10 kPa) using a pipette (C). The flow rate is measured by weighing small tubes (E) filled with plastic sponge, with a balance (F). Emboli are removed by flushing the sample with a solution (B) under a pressure of 175 kPa supplied by compressed air from a cylinder (A).

Preliminary measurements of the longest xylem conduits were performed using the method of Zimmermann & Jeje (1981). In 1-year-old twigs of *P. deltoides* the longest xylem conduits were no longer than 28 cm. However, these were only about 3% of the total number of xylem conduits. The other 97% were 15 cm long or less.

The twigs were cut into 35-cm-long segments and connected to the apparatus shown in Fig. 1. The perfusion solution was 100 mol m^{-3} KCl (Zimmermann 1978) which has previously been shown to sustain a constant flow for several hours (S. Salleo and M. A. Lo Gullo, unpublished data). The solution (prepared using twice-distilled water) was repeatedly filtered under vacuum through $0.2\text{-}\mu\text{m}$ filters. The flow rate, driven by a pressure difference of 10 kPa, was measured by collecting the water in small plastic tubes (1.5 cm long) attached to the distal cut end of the twigs. The tubes, filled with plastic sponge, were weighed every 60 s. After at least four approximately constant values of flow rate had been obtained, a pressure difference of 175 kPa was applied and the twigs were freely flushed for 20 min. The pressure was then released in steps of about 18 kPa min^{-1} and a new measurement of the flow was made at a pressure difference of 10 kPa. Flushing and measuring were continued until the flow rate became constant. This last value was taken as the maximum flow rate through

completely filled conduits and the first value was converted to a percentage of this maximum.

RESULTS

From leaf water potential isotherms, the value of leaf water potential at the turgor loss point (ψ_{tlp}) was found to range between -1.92 and -2.00 MPa. Thus, we decided to test leaves at ψ_l values between -0.1 and -1.6 MPa, i.e. well within the 'turgid region'.

In Figs 2 and 3, time courses of D, ψ_l and cumulative AE (cAE), as obtained in some typical experiments, are shown. When twigs very near to full turgor ($\psi_l = -0.04$ MPa) were tested (Fig. 2A, B) while in contact with water, ψ_l decreased only by 0.03 MPa and leaf water deficit increased from 0.4 to 1.28 g m^{-2} . No AE were counted.

Twigs dehydrated to ψ_l of -0.3 MPa (Fig. 2C, D) reached an initial leaf water deficit of 3.4 g m^{-2} . During the first 5–7 min in the dark, the water loss increased to 4.3 g m^{-2} and ψ_l decreased accordingly. Acoustic emissions began to be produced at $\psi_l = -0.2$ MPa (i.e. when the twigs were still in the open) and reached a total of 55, 60 min after bagging the twigs. At this time, a loss of hydraulic conductivity of 11.1% was recorded.

When twigs were enclosed in black bags at $\psi_l = -0.72$ MPa (Fig. 2E, F), the leaf water deficit increased

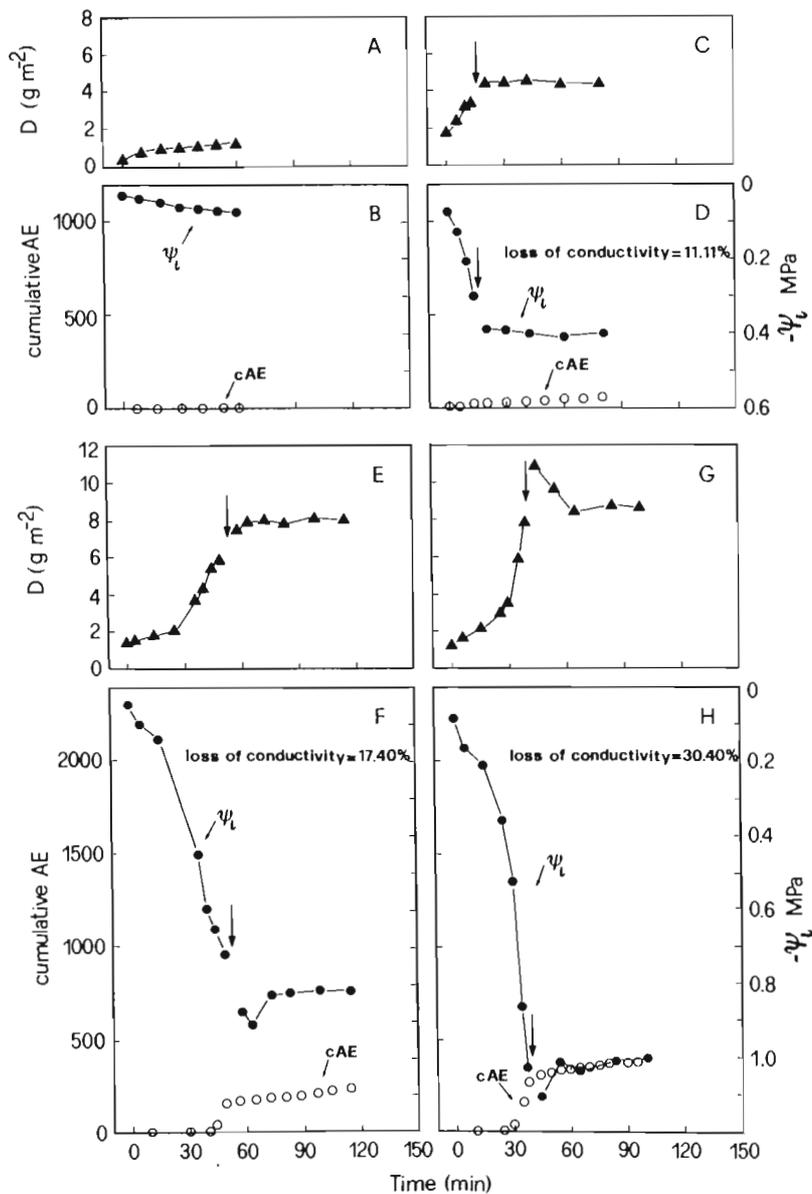


Figure 2. Time course of the leaf water deficit per unit leaf surface area $D = (W_i - W_t)/A_1$ (C, E, G), cumulative acoustic emissions (cAE) from the wood (D, F, H), recorded before and after twigs were put in black bags. The arrows indicate when the twigs were put in the bags. Changes in the same variables in twigs left in water (A, B) are also reported.

significantly during the first 5–7 min (from 6 to 7.8 g m⁻²) and ψ_t decreased from -0.72 to -0.91 MPa. The initial increase in leaf water deficit (and decrease in ψ_t), as recorded in both experiments (Fig. 2C, D, E, F), is likely to result from transpiration before the stomata closed. After putting the twigs in the dark, the AE rate decreased significantly: 158 AE occurred during the first 30 min in open air, corresponding to an average AE rate of 5.3 AE min⁻¹. A total of 230 AE were recorded 60 min later, corresponding to an AE rate of only 1.2 min⁻¹. The loss of conductivity after 1 h in the dark was 17.4%.

The leaves put in the dark at $\psi_t = -1.02$ MPa (Fig. 2G, H), had an initial increase in water deficit from 7.8 to 10.9 g m⁻², followed by a decrease to 8.4 g m⁻² within 20 min so that the final water deficit was only 7.7% and recovers of ψ_t from -1.1 to -1.00 MPa, indicating that water had been transferred to the leaves from the twig xylem.

Again, the AE rate decreased significantly while the twigs were in the dark. In the open air, cumulative AE (cAE) increased in number to 270 in 15 min, corresponding to an AE rate of 18 min⁻¹. Thereafter, AE continued to increase to 390 in the next 60 min, i.e. with

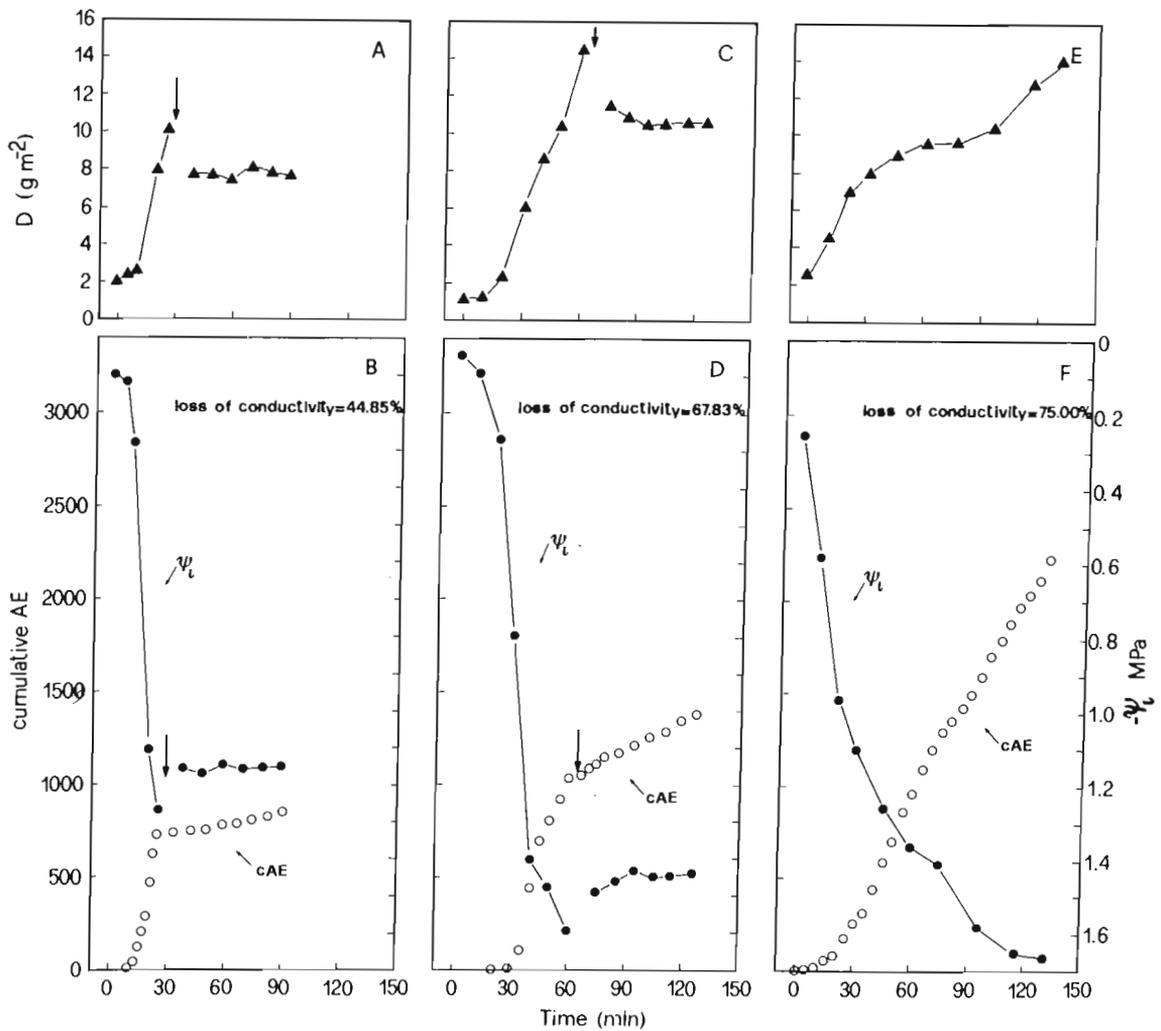


Figure 3. Time course of the leaf water deficit per unit leaf surface area $D = (W_i - W_f)/A_1$ (A, C), cumulative acoustic emissions (cAE) from the wood (B, D), recorded before and after twigs were put in black bags. The arrows indicate when the twigs were put in the bags. Changes in the same variables in twigs left drying in air (E, F) are also reported.

an average AE rate of only 2 min^{-1} . At this time, the loss in conductivity was as high as 30.4%.

Twigs put in the dark at $\psi_l = -1.27 \text{ MPa}$ (Fig. 3A, B), showed an immediate decrease in D from 10.2 to 7.8 g m^{-2} , corresponding to a gain in the leaf water content of 2.4 g m^{-2} , within 10 min. At the same time, ψ_l recovered from -1.27 to -1.15 MPa , thus confirming that water had entered the leaves and rehydrated their living cells.

The 730 cAE produced by the twigs in the dark (with an average AE rate of 29 min^{-1}), increased to 850, 60 min later, with an AE rate of only 2 min^{-1} . A loss of conductivity of 44.85% was recorded under these conditions.

A quite similar behaviour was shown by leaves put in the dark at $\psi_l = -1.62 \text{ MPa}$ (Fig. 3C, D). The AE rate (about 35 AE min^{-1}) declined to 5.7 AE min^{-1} . When twigs were left drying in air (Fig. 3E, F), the total leaf

water deficit after 120 min was about 12.6 g m^{-2} , ψ_l dropped to less than -1.65 MPa , cAE were about 2000 and conductivity loss was about 75%.

Figure 4 shows the changes in D inside the black plastic bags in relation to the ψ_l at which the twigs were put in the dark. Positive changes in D represent a decrease in leaf water content, negative changes a gain in leaf water content, with respect to the initial water deficit. It can be seen that, when twigs were put in the dark at values of ψ_l between -0.1 and -0.95 MPa , the leaf water deficit inside the bags increased as ψ_l decreased (up to 2.3 g m^{-2}). At more negative ψ_l values, the leaf water deficit became less and less, demonstrating a gain in the leaf water content.

Table 1 gives the total number of AE recorded (both in the open and in the dark), together with the corresponding loss of hydraulic conductivity of the twigs. It can

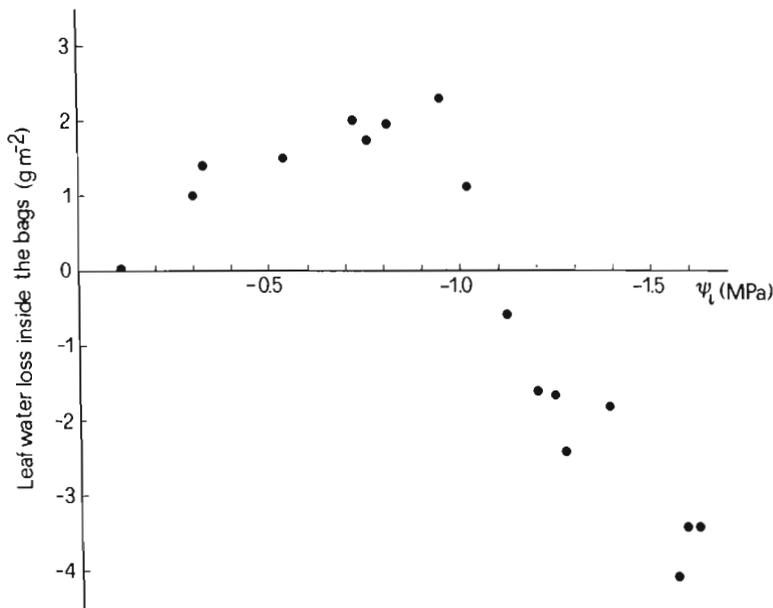


Figure 4. Changes in the leaf water deficit (D) of twigs put for 1 h in black bags at different initial leaf water potential (ψ_i) values.

be seen that the higher the number of AE produced in the xylem, the larger was the measured conductivity loss which reached about 75% in twigs left drying in the open.

This:

- (1) confirms that the AE recorded were the result of water cavitation occurring in xylem; and
- (2) suggests that a significant part of the AE counted were produced in the xylem conduits, since other wood compartments such as fibres do not contribute to the conduction of water.

When the loss of hydraulic conductivity was plotted versus the corresponding ψ_i value (Fig. 5), a 'vulnerability curve' (Sperry *et al.* 1988) was obtained with a flex point, between $\psi_i = -0.94$ and $\psi_i = -1.02$ MPa, beyond which the loss in conductivity increased substantially to over 30% for a reduction in ψ_i of less than 0.1 MPa. It is of interest to note that this critical ψ_i interval corresponded exactly to that at which the leaf water deficit inside the bags began to diminish (Fig. 4).

DISCUSSION

At a critical ψ_i interval between -0.94 and -1.02 MPa, water deficit of leaves in darkness began to recover and beyond this critical ψ_i range, this recovery increased in relation to the initial water stress until about 27% of the previous water deficit was eliminated.

That 80–100% of the recovery occurred during the first 10 min suggests that water entered the leaves via the apoplast (symplastic water transport would have required a much longer time).

The increase in number of AE with applied water

stress indicates that an increasing number of xylem conduits was cavitating and this was confirmed by the decrease in hydraulic conductivity recorded at increasing levels of water stress.

The correspondence of the ψ_i interval (-0.94 to -1.02 MPa) at which the leaves began to gain water with that at which the loss in conductivity increased critically for small decreases in ψ_i provides good evidence that water expelled from cavitated xylem conduits entered the still functioning ones and was transported to the leaves, thus contributing to their rehydration.

Table 1. Mean number of cumulative acoustic emissions (cAE) as recorded after the twigs were confined in the dark for 1 h (column 1). Hydraulic conductivity loss (L_{loss}), expressed as a percentage of the maximum, recorded after the twigs had remained 1 h in the dark (column 2)

cAE ($n = 5$)	L_{loss} (%) ($n = 5$)
49.2±4.61 **	11.44±0.71 **
234.8±19.16 *	19.49±1.00 **
410.0±31.42 *	30.99±1.44 *
880.8±74.32 *	44.43±1.79 **
1392.8±96.13 *	68.42±3.24
2426.4±199.96	78.00±4.15

* $P < 0.05$; ** $P < 0.01$.

The number of samples is given in parentheses. Means are given plus or minus the standard error of the mean. Lines are drawn connecting values significantly different from each other.

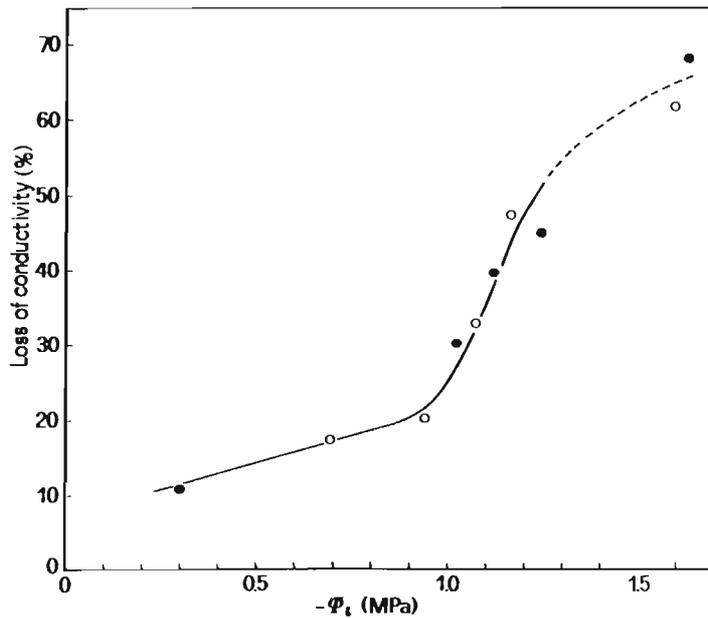


Figure 5. Vulnerability to cavitation of *P. deltoides* twigs measured as the percentage loss of conductivity in relation to leaf water potential (ψ_l). Solid and open circles represent twigs left drying in air and after remaining in the dark for 1 h, respectively.

The purpose of the present study was to investigate whether a significant amount of water could be released from the wood of young twigs and rehydrate the leaves of plants under water stress conditions. The second question was whether xylem cavitation would provide a significant water source for the maintenance of leaf water content. Our data suggest that the answer to both questions is yes.

Thus, under short term and not very severe water stress conditions, xylem cavitation can be regarded as a not entirely disadvantageous event in plants, since: (1) the loss of some xylem conduits increases the resistance to vertical water flow to leaves, thus inducing stomatal closure and the decline of transpiration; (2) water expelled from cavitated xylem conduits enters the still-functioning ones, reduces their internal tension and contributes to rehydration of leaves. Within limits, therefore, xylem cavitation could act as a 'water deficit buffer' mechanism. Tyree & Yang (1990) have recently made similar suggestions. Analysis of 'vulnerability curves', i.e. of the relation between loss of hydraulic conductivity and leaf water potential, is of ecological significance as well as of practical usefulness for detecting the range of ψ_l within which xylem cavitation can be regarded as a self-protective mechanism rather than as destructive of the functional integrity of the water conducting system.

ACKNOWLEDGMENTS

We thank Professor T. Hinckley, University of Washington, USA, Professor H. Richter, Universität für Bodenkultur, Vienna, Austria, and Professor M.T.

Tyree, University of Vermont, USA, for stimulating discussion and helpful suggestions.

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Received 13 June 1991; received in revised form 23 September 1991; accepted for publication 14 November 1991

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