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# Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism

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SUMMARY

Xylem recovery from cavitation-induced embolism was studied in 1-yr-old twigs of *Laurus nobilis* L. Cavitation was induced by applying pre-established pressure differentials ($\Delta P_{cv}$) across the pit membranes of xylem conduits. $\Delta P_{cv}$ were 1·13, 1·75 and 2·26 MPa, corresponding to about 50, 77 and 100% of the measured leaf water potential at the turgor loss point. $\Delta P_{cv}$ were obtained either by increasing xylem tensions or by applying positive pressures from outside, or by a combination of the two. The percentage loss of hydraulic conductivity (PLC) did not change, regardless of how the $\Delta P_{cv}$ were obtained. This confirmed that xylem cavitation was nucleated by microbubbles coming from outside the vessels. Positive pressures, however, amplified (up to 75%) and sped up the xylem refilling (20 min) in comparison with that measured in unpressurized twigs (*c.*50% in 15 h). Twigs girdled proximally to their pressurized segment 1 min after the desired pressure value had been reached, did not recover from embolism. The later the twigs were girdled with respect to when they were tested for PLC, the higher was their recovery from embolism, suggesting that some messenger was transported in the phloem which stimulated xylem refilling. Indol-3-acetic acid (IAA) applied to the exposed cortex of both pressurized and unpressurized twigs, induced an almost complete recovery from PLC. We hypothesize that the refilling of cavitated xylem might be a result of an auxin-induced increase in the phloem loading with solutes. This would cause radial transport of solutes to cavitated xylem conduits via the rays, thus decreasing their osmotic potential and making them refill. No positive xylem pressure potentials were measured during xylem recovery from PLC.

Key words: *Laurus nobilis* L. (Laurel), xylem embolism, xylem recovery, phloem loading, hormones.

## INTRODUCTION

Water cavitation in xylem has been recognized as the primary cause of damage to plants subjected to drought and freezing stress as well as to pathogenic stresses like tracheomycoses (e.g. Newbanks, Bosch & Zimmermann, 1983; Dixon, Grace & Tyree, 1984; Sperry & Tyree, 1988; Tyree & Sperry, 1989; Just & Sauter, 1991; Sperry & Sullivan, 1992; Lo Gullo & Salleo, 1993). The vulnerability of plants to cavitation has been measured in about 60 different species so far, in terms of the relationship between the loss of hydraulic conductivity of stem and the xylem pressure potential (e.g. Tyree & Dixon, 1986; Salleo & Lo Gullo, 1989; Cochard & Tyree, 1990; Cochard, 1992; Cochard *et al.*, 1992; Tyree & Yang, 1992; van Doorn & Jones, 1994).

The onset of cavitation in the case of drought stress appears to depend on the pressure differential across the pit membranes connecting xylem conduits with each other or with other wood compartments (Crombie, Hipkins & Milburn, 1985; Sperry & Tyree, 1988, 1990; Cochard & Tyree, 1990). This finding is in agreement with the 'air seeding' hypothesis first advanced by Zimmermann (1983), according to which air-water menisci at the pit membrane pores reduce their radius until air microbubbles enter the conduits at a critical pressure difference, thus nucleating cavitation and causing reduction in xylem hydraulic conductivity (Edwards & Jarvis, 1982).

By contrast, not much is known about the

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mechanism(s) by which plants can repair cavitation-induced damage to their water conducting system. This is crucial, because according to the Cohesion Theory most plants transport water from roots to leaves at negative pressures close to their cavitation-threshold. As a consequence, plants are likely to experience xylem cavitation very frequently during their life (Tyree & Sperry, 1988).

Until now, the only mechanism of xylem recovery from embolism which has been fully recognized, involves positive pressures developing in the root system (root pressure) e.g. in herbs (Milburn, 1979). Root pressure has proved to cause refilling of embolized xylem conduits in *Vitis labrusca* and *V. riparia* after winter rest (Sperry *et al.*, 1987), in a vine-like bamboo during the wet season (Cochard, Ewers & Tyree, 1994), and has been hypothesized to play a similar role in *Zea mays* (Tyree *et al.*, 1986) and in some lianas (Ewers, Fisher & Fichtner, 1991) but until now there has been no proof that root pressure develops on a shorter than seasonal time scale.

A different mechanism involving ion secretion by living wood cells into *Pinus sylvestris* L. embolized tracheids was hypothesized by Grace (1993) but excluded by Borghetti *et al.* (1991) who advanced an alternative hypothesis of xylem refilling based on chemical activity of tracheid walls. No direct proof of this has been presented so far.

In the course of our previous work, some of us had measured substantial nocturnal recovery from xylem embolism in pre-stressed plants of *V. vinifera* (Salleo & Lo Gullo, 1989), *Ceratonia siliqua*, *Laurus nobilis* and *Olea oleaster* (Salleo & Lo Gullo, 1993). Rapid reversal of embolism had been also reported by Waring, Whitehead & Jarvis (1979) in *P. sylvestris*. Tyree & Yang (1992) and Yang & Tyree (1992) presented models and experimental work showing that no xylem refilling can take place in plants with xylem water under tension unless positive pressures or at least pressures close to atmospheric develop in xylem, and suggested that data by Salleo & Lo Gullo (1989) as well as data by Waring *et al.* (1979) should have resulted from some error. However, the same authors were aware of the possibility that some other mechanism could be responsible for the observed reversal of embolism, and in a recent paper (Lewis, Harnden & Tyree, 1994) it was admitted that more experiments are needed better to define the biophysical conditions during the reversal of xylem embolism.

The present paper aims to investigate possible mechanisms of xylem refilling on a diurnal time scale. In particular, the possibility that xylem recovery from embolism might involve either root pressure developing at low transpiration and high air relative humidity (e.g. at night) or through some direct phloem action was examined. This approach was based on the idea that the only known sources of positive pressure in a plant are the root and the phloem, and that the xylem-phloem exchange via the rays has been described as an efficient and rapid pathway (Van Bel, 1990).

*Laurus nobilis* L. (laurel), an evergreen sclerophyll growing in all the Mediterranean Basin region (Pignatti, 1982) was used. This species belongs to the group of sclerophylls termed 'laurel-type trees' (Lausi, Nimis & Tretiach, 1989) which are typical components of the Laurisilva forest, a plant community whose members grow only in high relative humidity, especially in the summer (Kaemmer, 1974). Laurel has been shown to resist both drought and freezing provided the stress level is not too severe and its duration short (Larcher, 1981; Salleo & Lo Gullo, 1993).

## MATERIALS AND METHODS

One hundred 7-yr-old plants of *L. nobilis*, derived from a restricted number of mother plants by root suckers, were selected. This was expected to assure a better structural homogeneity of plants and hence, a more homogeneous response of plants to stress. Plants were grown in open air in 0·6 m-diameter and 0·6 m-height containers and were regularly irrigated. When used they were 1·5–1·8 m tall and had 10–12 1-yr-old twigs per plant. Before experiments, plants were transferred to a room at temperature of 15–18 °C with artificial lighting at an irradiance of 175 W $m^{-2}$ and photoperiod of 9 h. All the experiments were made during autumn and winter 1994–5 when plants were not actively growing.

Before plants were subjected to experiments, 15 pressure-volume curves were measured for leaves from different plants, using the pressure chamber technique (Scholander *et al.*, 1964; Tyree & Hammel, 1972; Salleo, 1983). This had the aim of measuring the leaf water potential at the turgor loss point ($\Psi_{tlp}$) which was taken as a reference point for applying given stress levels. $\Psi_{tlp}$ turned out to be $-2{\cdot}26 \pm 0{\cdot}31$ MPa. Pressure differentials of 1·13, 1·75 and 2·26 MPa were applied to conduit pit membranes (see below), corresponding to *c.* 50, 77 and 100 % $\Psi_{tlp}$.

### *Inducing xylem cavitation*

Pre-established pressure differentials across pit membranes of xylem conduits were achieved either by increasing xylem tensions (assuming that air in the intercellular spaces was at atmospheric pressure) or by applying positive air pressures to intact twigs from outside or by differently combining xylem tensions and positive pressures.

Xylem pressure potential ($\Psi_x$) was measured in each plant by covering three leaves from different twigs with tinfoil and black plastic bags for 2 h so that leaf water potential ($\Psi_l$) could equilibrate with

$\Psi_x$. Then, $\Psi_l$ was measured using the pressure chamber. Xylem tensions were increased by suspending irrigation to plants until the desired $\Psi_x$ value was reached.

Positive air pressures were applied using a 'pressure collar' designed by H. Richter (Universität für Bodenkultur, Vienna, Austria) and described in a previous paper (Salleo *et al.*, 1992). A new version of this pressure collar has dimensions reduced to those of a postcard so that the pressurized twig segment was about 0·08 m long. Compressed air was applied to intact twigs at about two-thirds of their length (total twig length = 0·55 ± 0·15 m), at pressures which were complementary to the pre-measured xylem tensions so as to reach the desired pressure differential across the pit membranes. The pressure was increased at a rate of about 70 kPa $min^{-1}$, maintained at the established value for 20 min and then decreased at the same rate. Such slow rates were expected to allow pressure to become uniform within the twig and to prevent any additional cavitation during depressurization. In plants at full turgor (as reached by putting repeatedly irrigated plants in the dark for 24 h), the pressure differential was obtained by applying positive pressures of 1·13, 1·75 and 2·26 MPa.

*Hydraulic measurements*

Pressurized stems were either cut off and tested for hydraulic conductivity 2 min from pressure release, or tightly covered (with the entire plant) with black plastic bags and maintained in the dark for 20 min or 15 h after the complete pressure release. Plants with unpressurized stems were treated in the same way after the desired $\Psi_x$ value had been reached. Stems were cut off under filtered distilled water at their junction plane with older stems and re-cut at both sides using new razor blades. Excised twigs were connected to the equipment for measuring their hydraulic conductivity using the technique first described by Sperry, Donnelly & Tyree (1988).

The perfusion solution was 50 mol $m^{-3}$ KCl, filtered through 0·1 $\mu$m filters. The hydraulic conductivity measurements were performed at a pressure of 10 kPa and alternated with 'flushes' at 175 kPa (Lo Gullo & Salleo, 1991) in order to remove emboli, until the conductivity ceased to increase and became constant ($K_{max}$). The initial measurement ($K_i$) was expressed in percentage of $K_{max}$ and the percentage loss of conductivity (PLC) was calculated as $(1 - K_i/K_{max}) \times 100$.

The impact of each pressure differential and of different times (2 min, 20 min and 15 h) after pressure release was measured using two twigs per plant from four different plants. Replication was the same for unpressurized twigs.

To check whether phloem transport was involved in xylem refilling, twigs from different plants were girdled before testing them for hydraulic conductivity. A ring of bark $3 \times 10^{-3}$ m wide was removed at a distance of $6 \times 10^{-2}$ m from pressure collar either on its proximal or distal side. The exposed wood was immediately covered with silicone grease to prevent dehydration. Twigs were girdled at different times during and after pressurization, i.e. 1 min after the desired pressure value had been reached, 1 min before dropping pressure and 2, 10 and 19 min after the complete pressure release, i.e. 19, 37, 58, 66 and 75 min after pressurization had started (Fig. 3*a*).

Each experiment with twigs girdled proximally or distally to the pressure collar was repeated on two twigs per plant from three different plants.

*Experiments with hormones*

To check whether xylem refilling could be caused by phloem via radial transport, hormones believed to stimulate or inhibit phloem loading were used: indol-3-acetic acid (IAA) as the phloem loading stimulator (Sturgis & Rubery, 1982) and abscisic acid (ABA, in the racemic form) as the phloem loading inhibitor (Malek & Baker, 1978). Solutions were prepared at concentrations of 1 and 0·1 mol $m^{-3}$ of IAA and ABA, respectively. Both hormones were first dissolved in $1{\cdot}5 \times 10^{-3}$ l ethanol and then in a 50 mol $m^{-3}$ KCl solution (to a total volume of 0·1 l) prepared with distilled filtered water.

Three small areas of *c.* 6 $mm^2$ each per twig were prepared within the stem segment to be pressurized, by peeling off the epidermis. These areas were 0·02 m apart from each other, at different angles with respect to the stem's vertical axis. Pieces of thin blotting paper, *c.* 50 $mm^2$ in surface were wetted with *c.* $6 \times 10^{-5}$ l of hormone solution and applied to the exposed areas. Plastic sheets were then fixed to these areas to maintain the paper *in situ* and prevent evaporation. Control twigs were supplied with KCl at the same concentrations without IAA or ABA.

Hormone solutions were applied to unpressurized twigs in the same way at equal twig lengths. In this case, however, all 1-yr-old twigs of a pre-stressed plant were supplied with hormones as described above, except for those used as controls.

The effect of each solution tested was measured in terms of changes in the hydraulic conductivity in the treated stems with respect to that recorded in the untreated ones, 20 min and 15 h after pressure release (pressurized stems) or 15 h after solutions were supplied (unpressurized stems). Each solution was tested in at least two twigs per plant from three different plants.

*Root pressures*

Two stems per plant of 2 yr age, bearing at least four 1-yr-old twigs each were selected from five different plants. Two proximal twigs were used for measuring xylem pressure potentials, simultaneously. Two

other twigs, inserted on the same 2-yr-old stem were pressurized to a pressure differential of 1·75 MPa, starting from plants near full turgor. Plants remained covered with black plastic bags during measurements. Experiments were performed with twigs supplied or not with IAA, as described above.

Twigs used for measuring xylem pressures were cut off under distilled filtered water, at about 0·1 m from their junctions and connected to pressure transducers (Omega Engineering, Inc. Stamford, CT, USA mod. 102, accuracy $7 \times 10^{-5}$ MPa or 0·01 psi). The transducers were tightly fixed into a rigid plastic tubing, about $3 \times 10^{-3}$ l in volume, filled with distilled water previously filtered through 0·1 $\mu$m filters. The cut stems were fixed under water at the opposite side of the tubing. The xylem pressure readings were made every 5 min, using a digital pressure-indicator (Omega, mod. 350). Long-term measurements (15 h after pressure release) could not be performed because water in the tubing underwent sufficient tension for air bubbles to appear, thus altering the measurements.

## RESULTS

Figure 1 records PLC for the three stress levels tested. The PLC was about 15–20 %, 25–28 % and 55–62 % in twigs subjected to pressure differentials of 1·13, 1·75 and 2·26 MPa, respectively. At equal pressure differentials applied to the pit membranes PLC did not change, whether they were obtained by increasing xylem tensions or by applying positive pressures or both, differently combined.

Positive pressures, however, exerted a significant influence on both the kinetics and amount of xylem refilling (Fig. 2). Recovery from embolism in pressurized twigs as indicated by the reduction in PLC (Fig. 2, dashed columns with respect to white columns), was recorded only 20 min after the complete pressure release. By contrast when the pressure differentials across the pit membranes were only a result of xylem tensions, no rapid recovery was recorded although it occurred more slowly (15 h after a given $\Psi_x$ was reached) in plants at $\Psi_x = -1{\cdot}13$ and $-1{\cdot}75$ MPa. In other words, positive pressures applied to intact stems hastened xylem refilling.

The recovery from xylem embolism in unpressurized twigs was about 50 % (PLC was reduced from *c.* 20 % to *c.* 10 % in plants at $\Psi_x = -1{\cdot}13$ MPa and from *c.* 28 % to 15 % in those at $\Psi_x = -1{\cdot}75$ MPa). Pressurized twigs, on the contrary, showed recoveries from PLC up to 80 % (see e.g. black column with respect to white column in Fig. 2 for twigs subjected to a pressure differential of 1·75 MPa as obtained starting from $\Psi_x = -1{\cdot}5$ MPa plus a positive pressure of 0·25 MPa). In other words, positive air pressures increased the amount of xylem recovery from embolism in comparison with that recorded in water-stressed plants.

A third observation was that the amount of xylem recovery from embolism in pressurized twigs was especially large where a 'native' xylem tension existed ($\Psi_x = -0{\cdot}5$ to $-1{\cdot}5$ MPa, Fig. 2). Such tensions corresponded to those measured by some of us and which trigger cavitation in *L. nobilis* (Salleo & Lo Gullo, 1993).

Figure 3*a* shows the time course of twig pressurization and in Figure 3*b* PLC is reported for twigs with a pressure differential applied of 1·75 MPa as obtained starting from $\Psi_x = -0{\cdot}5$ MPa plus a positive pressure of 1·25 MPa. The PLC of twigs cut off 2 min after pressure release (white unlabelled column, Fig. 3*b*) was about 28 % but 18 min later it was reduced to about 9 % (dashed column, same Fig). However, if twigs were cut off 20 min after pressure release, but previously girdled proximally to the pressure collar 1 min after the desired pressure

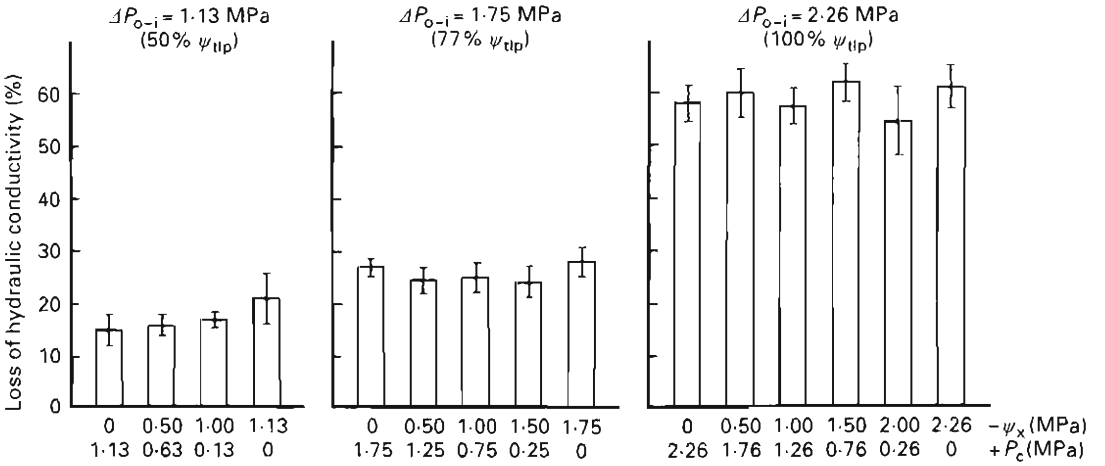


**Figure 1.** Percentage loss of hydraulic conductivity (PLC) ± SD ($n = 8$) vs pressure differentials ($\Delta P_{o-i}$) applied to 1-yr-old stems. $\Delta P_{o-i}$ resulted from different xylem pressure potentials ($-\Psi_x$) and/or positive air pressures ($+P_c$) applied from outside (see the abscissa). $\Delta P_{o-i}$ are also reported as % of the leaf water potential at the turgor loss point ($\Psi_{tlp}$).

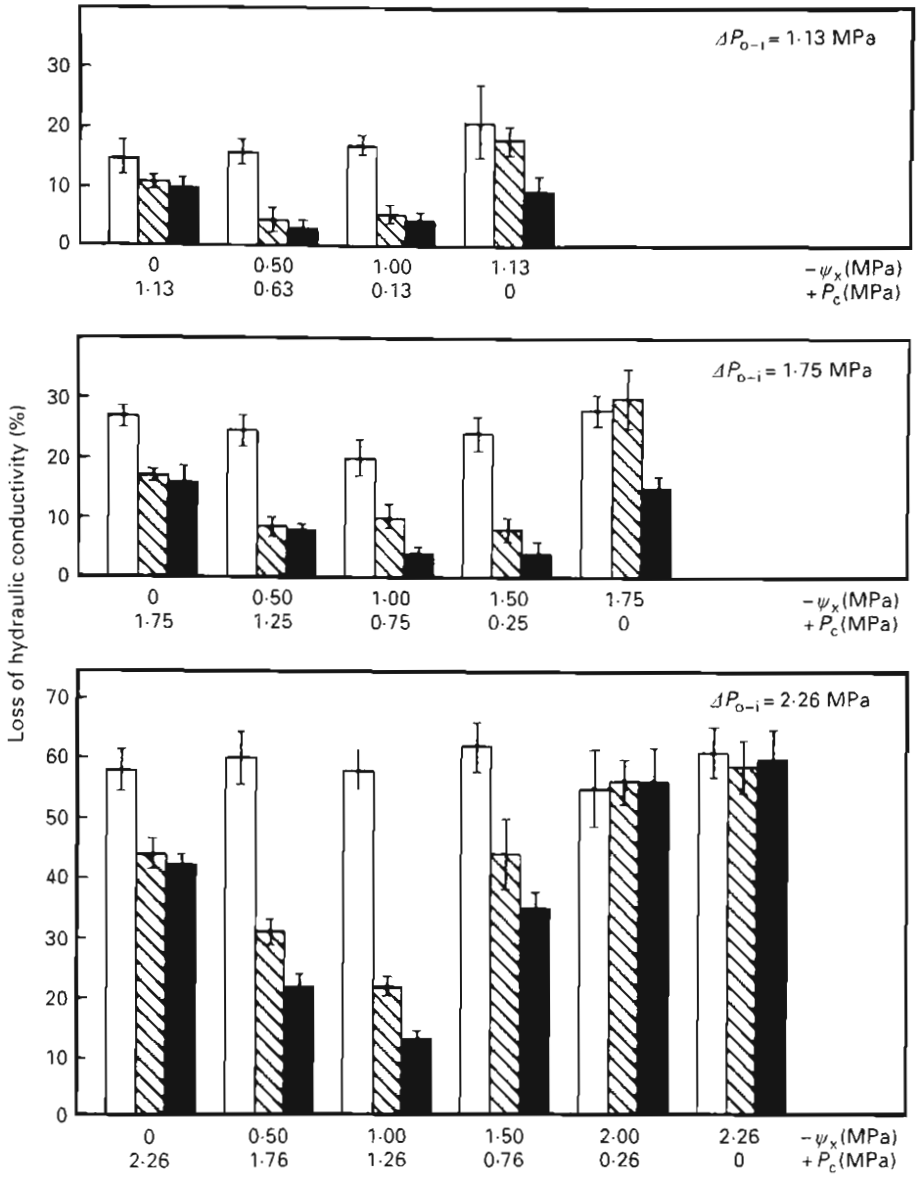


**Figure 2.** Percentage loss of hydraulic conductivity (PLC) ± SD ($n = 8$) vs. pressure differentials ($\Delta P_{o-i}$) applied to 1-yr-old stems. For values on the abscissa, see Figure 1. □, PLCs as measured 2 min after pressure release; ▨, PLCs as measured in plants put in the dark for 20 min after pressure release; ■, PLCs as measured in plants put in the dark for 15 h after pressure release.

value had been reached (arrow A, Fig. 3*a*), the xylem recovery from embolism was suppressed (column A, Fig. 3*b*). The PLC began to reduce to 22 and 24 % (columns B and C, Fig. 3*b*) in twigs girdled proximally to the pressure collar 1 min before dropping the pressure (arrow B, Fig. 3*a*) and 2 min after the complete pressure release (arrow C, Fig. 3*a*), respectively. An even better recovery was recorded in twigs girdled 10 min after pressure release (arrow D, Fig. 3*a*) causing PLC to reduce to 14 % (column D, Fig. 3*b*). Twigs girdled 1 min before cutting them off (i.e. 19 min after pressure release, arrow E, Fig. 3*a*) showed PLC values not different to those recorded in ungirdled twigs (column E, Fig. 3*b*).

By contrast, if twigs were girdled distally to the pressure collar (columns A, B, C Fig. 3*c*), xylem recovery from embolism was equal to that of ungirdled twigs (dashed column, Fig. 3*b*), regardless when they were girdled, i.e. xylem recovery was not altered by the interruption of the vertical phloem pathway, distal to the cavitated twig segment.

### *Effect of hormones*

Figure 4 shows the effect of two hormones (IAA and ABA) on the reduction in PLC in twigs under a pressure differential across the pit membranes of 2·26 MPa (corresponding to 100 % $\Psi_{ulp}$). This was achieved starting from $\Psi_x = -1{\cdot}5$ MPa plus a positive pressure of 0·76 MPa.

Compared with a PLC of *c.* 55 % measured in twigs cut off 2 min after pressure release (Fig. 4) and reductions in PLC to 45 % and 30 % in twigs cut off

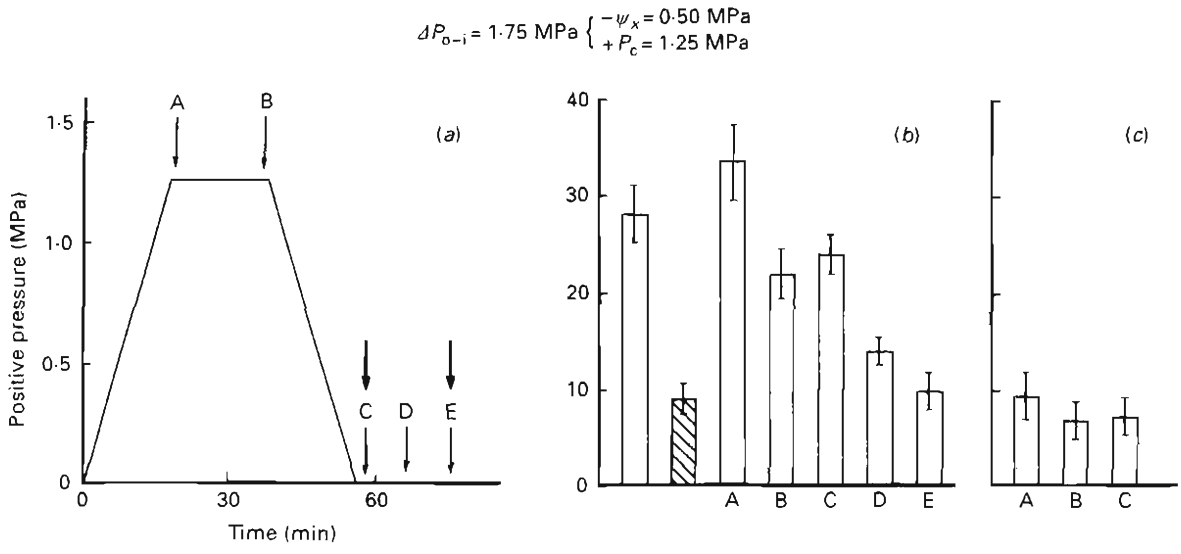


**Figure 3.** (*a*) Time course of stem pressurization. Thin labelled arrows indicate the time of twig girdling. Thick arrows refer to the time when twigs were cut off for hydraulic measurements. (*b*) Percentage loss of hydraulic conductivity (PLC) ± SD ($n$ = 6) as measured in twigs 2 min after pressure release (white unlabelled column) and in plants put in the dark for 20 min after pressure release (dashed column). Other columns labelled with capital blocks refer to PLCs as measured in twigs cut off 20 min after pressure release and previously girdled proximally to the pressure collar at the times indicated by thin arrows in (*a*) (same capitals). (*c*) Labelled columns as in (*b*) for twigs girdled distally to the pressure collar. The pressure differential applied ($\Delta P_{o-i}$) to twigs is reported as total value and as partial values in terms of xylem pressure potential ($-\Psi_x$) and/or positive pressure ($+P_c$).

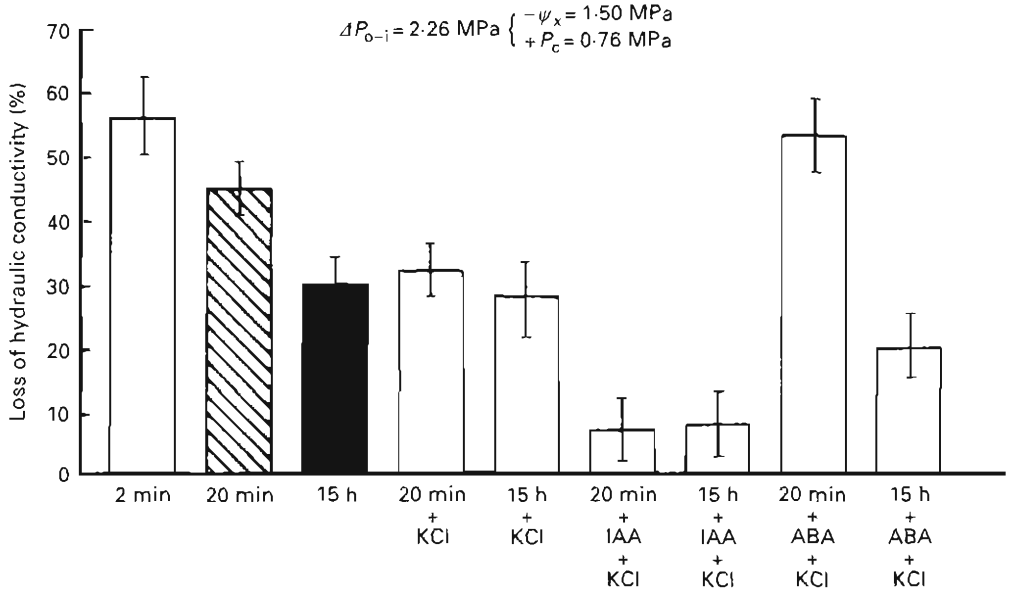


**Figure 4.** Percentage loss of hydraulic conductivity (PLC) ± SD ($n$ = 6) as measured in pressurized twigs cut off at 2 min, 20 min and 15 h after pressure release. Treatments of twigs with KCl solutions with or without indol-3-acetic acid (IAA) or abscisic acid (ABA) are reported under each column.

20 min and 15 h after pressure release respectively, IAA promoted a much larger recovery. The PLC in IAA-treated twigs was reduced to 6 and 7 %, at the same test times (i.e. a recovery of 85–90 % was recorded). Part of this effect was thought to be owing to water pushed into the twig by pressurization. Therefore, 50 mol $m^{-3}$ KCl solutions without hormones were given (Fig. 4), causing partial recoveries as recorded 20 min and 15 h after pressure release (PLC was reduced to 32 and 28 %, respectively). These PLCs were not different from the PLC measured in twigs without any external water supply 15 h after pressure release and, in every case, were much higher than those induced by IAA.

The PLC of ABA-treated twigs (Fig. 4) was not different from that measured in the corresponding untreated twigs. Surprisingly, PLC (as measured 20 min after pressure release) was higher in ABA-

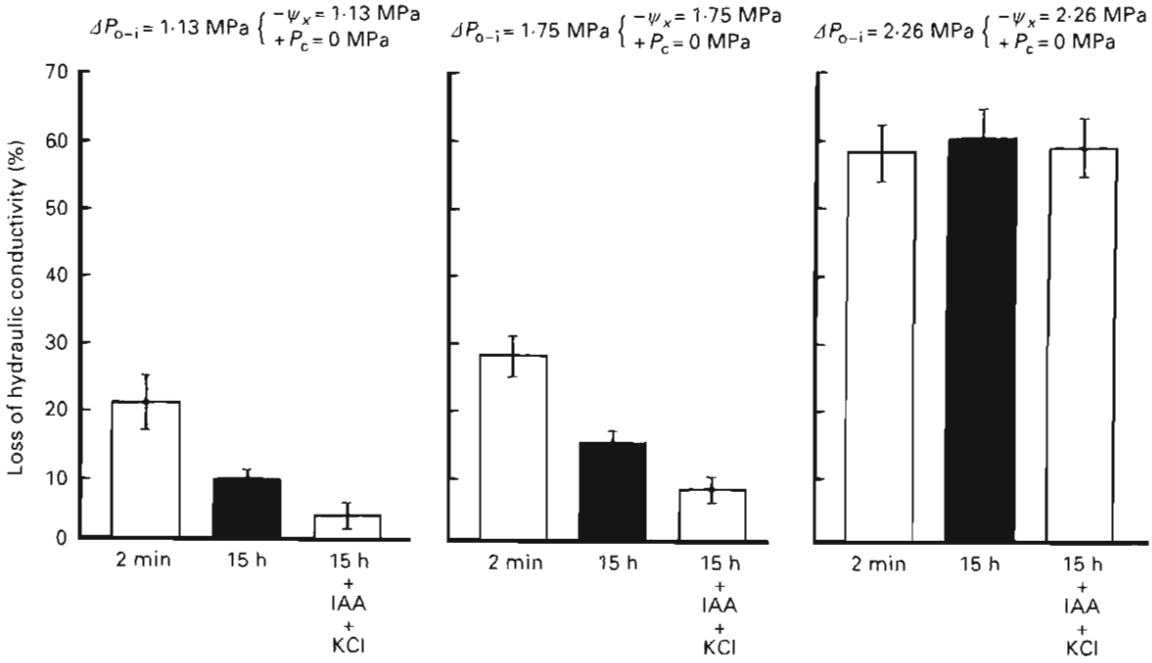


**Figure 5.** Percentage loss of hydraulic conductivity (PLC) ± SD ($n = 6$) as measured in unpressurized twigs from plants pre-stressed to different $\Psi_x$ values. The PLC was measured of twigs cut off 2 min or 15 h after a given $\Psi_x$ had been reached, with or without external indol-3-acetic acid (IAA) supply (as indicated under each column).

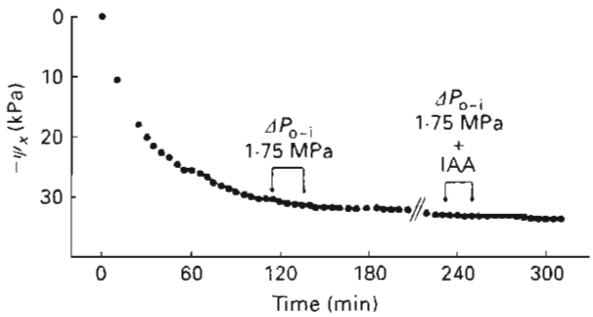


**Figure 6.** A typical experiment showing the time-course of the xylem pressure potentials ($-\Psi_x$) measured in 1-yr-old twigs before, during and after stem pressurization. No changes in xylem pressure potentials were recorded in response to pressure-induced xylem embolism, even in twigs supplied with 1 mol $m^{-3}$ indol-3-acetic acid (IAA).

treated twigs than in those receiving KCl solution. In other words, ABA had no effect or, maybe, a small inhibiting effect on xylem recovery from embolism.

Figure 5 shows the effect of IAA on xylem refilling in unpressurized twigs from pre-stressed plants with $\Psi_x = -1{\cdot}13$, $-1{\cdot}75$ and $-2{\cdot}26$ MPa. Auxin promoted a substantial recovery from loss of conductivity, i.e. PLC was reduced from 21 and 28% to *c.* 4 and 8% in plants with $\Psi_x = -1{\cdot}13$ and $-1{\cdot}75$ MPa, respectively. These reductions in PLC were significantly greater than those measured at the same time (15 h) in untreated stems (10 and 15%, respectively). Plants subjected to the severest water stress tested ($\Psi_x = -2{\cdot}26$ MPa) did not recover from embolism even if supplied with IAA (Fig. 5).

*Xylem pressure potentials*

Figure 6 shows a typical experiment measuring the xylem pressure potentials before, during and after stem pressurization. After a period of about 2 h, during which the transducers recorded increasing tensions up to about 30 kPa below the atmospheric pressure (set equal to zero, Fig. 6), one stem was pressurized for 20 min. During this time, xylem tensions increased slightly with the same slope as before. After pressure release, xylem tensions did not tend to be released and the same happened when a second stem was pressurized and supplied with IAA (Fig. 6).

## DISCUSSION

Positive air pressures applied to intact stems in presence or not of xylem tensions did not affect the measured PLC. This was also reported by Cochard, Cruiziat & Tyree (1992) but they did not combine positive and negative pressure as in the present study. Our data further confirm that xylem cavitation is unlikely to be nucleated by stable microbubbles at hydrophobic cracks or coming out of sap. If this were the case, tensions would be needed to cause bubbles to expand whereas positive pressures applied to twigs at full turgor should cause bubbles to dissolve or leave them undisturbed. The 'air-seeding' mechanism of nucleation (Zimmermann, 1983), is probably the only one causing cavitation in xylem conduits.

Moreover, positive pressures caused a more rapid and larger xylem refilling than that recorded in unpressurized twigs. We attribute both effects to the compression of the twig's phloem and cortex tissues which might have caused water to move radially to xylem. Since even unpressurized twigs recovered from embolism (within 15 h) under simulated nocturnal conditions (Fig. 2), positive pressures are a useful tool for studying xylem dysfunction and recovery.

'Native' xylem tensions of 0·5–1·5 MPa as combined with complementary positive pressures (Fig. 2) seemed to increase both rapid and slow xylem recovery. This cannot be attributed to artefacts due to positive pressures because the amount of recovery increased as native xylem tensions increased (between $\Psi_x = -0{\cdot}5$ and $-1{\cdot}5$ MPa), i.e. at decreasing complementary positive pressures.

Values of $\Psi_x$ of $-0{\cdot}8$ to $-1{\cdot}2$ MPa have been found to be the cavitation-threshold in 1-yr-old twigs of *L. nobilis* plants (Salleo & Lo Gullo, 1993) so that it is not unreasonable to hypothesize that such xylem tension values might have acted like signals. Hydraulic signals in response to external stimuli like wounding have been reported to propagate in the vertical direction (Malone, 1992; Boari & Malone, 1993). One possibility is that a signal generated within the wood might be transported via phloem to roots. Our experiments with girdled twigs support this idea. In fact (Fig. 3), twigs induced to cavitate did not recover from loss of hydraulic conductivity if they were girdled proximally to the cavitated segment, about 19 min after starting pressurization (or 1 min after the established pressure value had been reached, Fig. 3*a*). The later the twigs were girdled after they were cut off, the larger was their xylem recovery from embolism (or their reduction in PLC). Since the recovery itself appeared to occur 20 min from pressure release, it was thought that some sort of messenger travelled via phloem either in the proximal direction to reach an effector organ like the root or in the opposite direction to reach the cavitated twig segment where it promoted xylem recovery.

Measurements of xylem pressure potentials, however, were not consistent with the former view. Both in hormone-treated and in untreated stems, the xylem pressure potentials as measured on cut stems were never above atmospheric (Fig. 6). A xylem pressure of 30 kPa below the atmospheric value as measured at 0·4 m from the ground suggests that the xylem sap in the root was under tension too and that, therefore, no positive root pressure had developed in response to xylem cavitation. In other words, $\Psi_x$ in the stem was too negative to account for the reversal of emboli (Yang & Tyree, 1992).

Experiments with hormones gave better results. Solutions of IAA applied on the cortex parenchyma of pressurized twigs caused xylem conduits to recover from embolism, almost completely (Fig. 4*b*). This effect cannot be attributed to KCl solutions pumped into the twigs during pressurization, for in the controls without IAA, PLC was reduced from *c.* 55 to 30 % and complete recovery was not recorded.

Solutions of IAA also promoted xylem recovery from embolism in unpressurized twigs of prestressed plants (Fig. 5). Here, the time needed for auxin to show its effect was much longer than in pressurized stems (15 h instead of only 20 min), probably because auxin diffusion is much slower than movement under pressure. Results with hormone-treated unpressurized stems confirm that the action of IAA on xylem recovery from embolism is measurable even in plants under physiological conditions.

Auxins are known to stimulate cell proton pumps to result in an increased proton efflux coupled with active secondary $K^+$ influx and a cotransport of sugars into cells (Marré, 1979; Davies, 1987). Additional influx of ions and organic molecules into the sieve elements is expected to cause, in turn, an increase in their internal pressure and a larger concentration gradient between the sieve elements and the neighbouring cells that might enhance the radial transport of solutes into the xylem via ray cells (Van Bel, 1990). This view was also supported by the contrary data recorded in ABA-treated twigs. Although the action mechanism of this hormone is still not clearly understood, especially as regards its effects in the root, we know that ABA reverses auxin action and inhibits phloem loading (Malek & Baker, 1978). In our case, ABA had no effect on xylem refilling but inhibited the short-term recovery (20 min) induced by externally applied KCl solutions. This is consistent with our hypothesis that xylem refilling is related to an increased phloem loading. Xylem-phloem solute exchange has been found to occur along both symplastic and apoplastic paths (Van Bel, 1990). We hypothesize that solutes might be secreted by phloem, move radially along the ray cell walls, enter the embolized xylem conduits and increase the solute concentration of the residual water within them, thus promoting xylem refilling.

Twigs girdled distally to the cavitated segment recovered like the ungirdled ones (Fig. 3*c*). This suggests that IAA was transported in the apical direction, coming from leaves proximal to the cavitated twig segment. The time needed for IAA to be transported to the cavitated segment can be estimated on the basis of the experiments reported in Figure 3*b*. The suppressed recovery from PLC as measured in pressurized twigs, girdled 1 min after the established pressure value had been reached, together with the increasing reduction in PLC, the later these twigs were girdled, suggests that IAA first reached the twig's cavitated segment in about 10 min. Since our twigs were about 0·55 m long and the cavitated segment was at about two-thirds of their length, i.e. about 0·30 m from their junctions,

the time needed for IAA to reach the phloem of the cavitated twig segment suggests a distance from the hormone source of about 0·17 m, if we assume an average transport rate of 1 m $h^{-1}$. This suggests that the hormone source might be the leaves inserted on 1-yr-old twigs.

Our data tend to exclude any role played by root pressure in xylem refilling, on a short-term time scale. However, we feel that a more refined equipment should be used to measure eventual positive pressures that might develop transiently in the root, in response to water stress-induced xylem cavitation.

Although more experiments are needed better to clarify the possible role played by the phloem-to-xylem exchange in xylem recovery from embolism as well as the role played by auxins, our tentative conclusion is that the hypothesis advanced by Grace (1993) of a 'vitalistic' theory of xylem recovery from embolism, should be revisited and that xylem refilling might be a rather complex phenomenon under metabolic control.

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