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Ultrasound acoustic emissions from dehydrating leaves of deciduous and evergreen trees

S. B. Kikuta, M. A. Lo Gullo, A. Nardini, H. Richter & S. Salleo

Introduction

Although xylem tensions, that is pressures below atmospheric in the xylem conduits, have been questioned (Zimmermann et al. 1993), the cohesion theory of sap ascent in plants (Boehm 1893; Dixon 1914) is generally supported by recent papers (Grace 1993; Milburn 1996; Richter 1997). A corollary of this theory is that the water columns can be interrupted at critical tension values by xylem cavitation, giving rise to embolism and the consequent blockage of water transport to leaves.

An ingenious method for detecting xylem cavitation was developed by Milburn & Johnson (1966). Audible acoustic emissions (AAE) from cavitating xylem were recorded with a simple record-player pickup arm connected to an amplifier and clamped to the petiole of a detached Ricinus leaf. Later (Milburn 1973; Milburn & Cromptie 1984; West & Gaff 1976), the equipment for detecting low-frequency acoustic signals was greatly improved. Measurements were further facilitated when Tyree & Dixon (1983, 1986) registered ultrasound acoustic emissions (UAE) in the range of 0.2–2 MHz with UAE transducers clamped to the stem wood and connected to UAE counters. Ultrasound acoustic signals are strongly attenuated in wood and bark over relatively short distances (about 1–20 dB cm⁻¹, Tyree & Sperry 1989a). Therefore, the current protocol for wood is to remove about 100 mm² of the bark and clamp UAE transducers to the exposed wood surface, with a thin layer of silicon grease interposed to prevent desiccation (Salleo & Lo Gullo 1986; Sandford & Grace 1985; Tyree et al. 1984). In spite of this precaution, the fragile superficial xylem conduits are likely to become embozilized when wounded during peeling (Milburn 1993). This artifact may favour embolism under the transducer, as air–water menisci between functional and non-functional conduits would become more numerous, thus increasing the statistical probability of bubbles entering xylem conduits under critical tensions (Zimmermann 1983).

Milburn (1973) measured cavitation in blades and petioles with audio methods but, to our knowledge, no ultrasound measurements in leaf blades have been described. UAE data have been published for node-to-petiole junctions only (Salleo & Lo Gullo 1989). Therefore, little is known about cavitation in the leaf midrib and minor veins, although they are in direct contact with the living mesophyll cells experiencing the most negative water potentials in a plant.

The purpose of the present study was to test the relevance of ultrasound acoustic signals to leaf dehydration, to...
evaluate measurement procedures, and to compare data from several forest species with other information on how they function under drought stress.

MATERIALS AND METHODS

Plant material

Preliminary experiments were conducted on *Ilex aquifolium* L. (holly) and *Citrus aurantium* L. (bitter orange). Holly is a typical understory shrub of deciduous forests at high air humidity, while the bitter orange requires irrigation for active growth.

The study was later extended to include seven more species from different environments: *Ceratonia siliqua* L. (carob) and *Phillyrea angustifolia* L. (narrow-leaved phillyrea) are both adapted to xeric areas of the Mediterranean Basin (Pignatti 1982). *Quercus ilex* L. (holm oak) and *Viburnum tinus* L. (laurestinus) are characteristic of warm areas of the Mediterranean Basin but require a higher soil-water content and lower summer temperatures than carob or narrow-leaved phillyrea. Moreover, *V. tinus* is a typical understory species in holm-oak forests (Pignatti & Nimis 1995), both of these species growing at low elevations in the north of Italy and at up to 1800 m in Sicily (Pignatti 1982). *Laurus nobilis* L. (laurel) and *I. aquifolium* (holly, see above) are 'laurophylls', that is, sclerophylls growing in areas with high air humidity (Kaemmer 1974; Lausi, Nimis & Tretiach 1989). Finally, two deciduous trees were studied: the thermophilic *Prunus mahaleb* L. (rockcherry) and *Acer campestre* L. (hedge maple), both species found in Italy between sea level and 800 m and in Sicily at up to 1600 m.

Methodological experiments

Preliminary studies were conducted in a garden-grown old tree of *I. aquifolium* and 4-year-old potted greenhouse plants of *C. aurantium* in the Botanical Garden, Universität für Bodenkultur, Vienna. One-year-old leaves were resaturated in a closed vessel in the dark for 12–15 h with their petioles in distilled water. Near full turgor (leaf water potential, \( \Psi_t \), \( -25 \) kPa) was found with the pressure chamber (Scholander et al. 1964). Turgescence leaves were allowed to dry in air under light provided by the halogen lamp (24 W, 150 V) of a slide projector positioned with its front lens 0.3 m from the sample. The quantum flux at the leaf surface was 550 \( \mu \text{mol} \text{cm}^{-2} \text{s}^{-1} \).

Two UAE transducers (RI151, Physical Acoustics Corp., Princeton, NJ, USA) with a diameter of 18 mm were connected to two different UAE counters (4615 Drought Stress Monitor, Physical Acoustics Corp.) and clamped to the opposite sides of a leaf at the proximal third of the midrib. At first, a thin layer of silicon grease was interposed between the transducers and the leaf to improve contact. Later, this measure was discontinued, as it was found to be unnecessary.

In another series of experiments conducted between October and December 1996 and facilitated by automatic data acquisition with high resolution, the measurements were repeated over prolonged periods. This permitted a comparison of coincidences between signals sensed on both sides of the midrib. Signals were often emitted in bursts, where 2 s of high activity were preceded and followed by more or less 'silent' periods. It was analysed how bursts were registered by the sensors on the two sides.

In addition, two UAE transducers were clamped on the adaxial side of the leaf, one at the proximal, the other at the distal third of the midrib, for a comparison of signal numbers produced in different positions. To distinguish between signal production by veins and by mesophyll cells, two transducers were mounted on opposite sides near the leaf margin and without contacting the midrib; thus only minor veins were close to the transducers.

UAE were amplified by 72 dB (52 dB by the main amplifier and 20 dB by the built-in transducer preamplifier). At this gain level, the background count was only about 20 events per day. There were two ways of recording UAE signals. In most of the experiments, cumulative numbers of UAE were recorded every 2–5 min using a stopwatch (accuracy \( \pm 1 \) s). Later, a dedicated computer program permitted on-line recording for up to 80 h, storing the number of events during periods of 2 s.

Dehydration was interrupted by putting petioles in water. UAE were recorded during both dehydration and subsequent rehydration. To relate UAE to known levels of dehydration, \( \Psi_t \) was measured with an L-51 leaf hygrometer connected to an HR-33T Microvoltmeter (Wescor, Logan, UT, USA). The hygrometer sensor was clamped near the tip of a resaturated and weighed leaf, following the procedures outlined by Richter (1978). During equilibration the blade was covered with clingfilm to avoid transpirational water loss. When \( \Psi_t \) values were stable, the cover was removed and an ultrasound sensor attached to the leaf base.

Experiments on eight woody forest species

Field measurements and leaf water potential isotherms

Individuals over 15 years of age were selected in the Botanical Garden of Trieste (north-east Italy), about 100 m above sea level. *C. siliqua* trees grew only in the Botanical Garden of Messina (Sicily) at sea level, where additional studies of some of the other species were made. Leaves of the current year’s growth were first measured for their surface area \( A_L \) while still attached, using a Leaf Area Meter (Li-Cor 3000, Li-Cor, Lincoln, NE, USA), at 0800 h.

Leaves were then collected and oven-dried at 70 °C for 3 d to obtain their dry mass \( M_D \). The leaf specific mass was calculated in terms of ‘degree of sclerophylly’ \( S \) from the expression: \( S = M_D / A_L \) (Cowling & Campbell 1983). Information was expected from \( S \) on the possible relation between sclerophylly (high \( S \)) and vulnerability to cavitation, as sclerophylly is generally considered...
Ultrasound acoustic emissions from leaves (Mooney 1982) an adaptation typical of drought-resistant plants. Fifty leaves per species were tested for S.

To establish reference values for leaf dehydration, at least five leaf water potential isotherms (pressure–volume curves; Tyree & Hammel 1972) were measured for each species, using a pressure chamber. This allowed determination of the leaf water potential at the turgor loss point ($\Psi_{tp}$) and the bulk modulus of elasticity at full turgor ($\epsilon_{max}$; Tyree & Jarvis 1982), the later parameter being calculated as $\frac{d\Psi_p \times W}{dW}$, where $\Psi_p$ is the turgor potential and $W$ is the actual symplastic water content in a leaf. Therefore, $\epsilon_{max}$ should express the ‘rigidity’ of the walls of living cells. The larger the value of $\epsilon_{max}$ the more substantial is the drop in $\Psi_p$ and hence the change in $\Psi_1$, in response to reductions in cell water content (Tyree & Karamanos 1981).

The minimum diurnal water potential ($\Psi_{min}$) at 1400 h of at least five leaves per species from the south-facing part of the crown was measured with the pressure chamber in July 1995. Leaves in the laboratory could then be dehydrated within the range of the $\Psi_1$ changes the species was likely to experience in natural conditions.

Leaf cavitation

Cavitation was measured by clamping a UAE transducer to the adaxial side of excised leaves illuminated with incandescent light to enhance water loss. Cumulative ultrasound acoustic emissions (cUAE) were recorded every 5 min using a stopwatch (accuracy ± 1 s). Each experiment was repeated seven to 10 times per species and lasted up to 6 h. Forty leaves per species were resaturated as described above. One leaf was used for counting UAE while on another 39 leaves $\Psi_1$ was measured every 10 min with the pressure chamber, until $\Psi_1$ reached $\Psi_{tp}$ or until UAE stopped.

To estimate the specific sensitivity to cavitation of leaf conduits, the ratio of the number of UAE to that of xylem conduits was calculated. Three to five leaves were cross-sectioned at the proximal third of their midribs, that is, where the transducer was placed in cavitation experiments. Microtome sections 10 $\mu$m thick were stained with Safranin and Fast Green and mounted in Canada balsam. The number of conduits per section in the midrib as well as in the major (first- and second-order) veins was counted under a microscope.

All experiments in this series were conducted between September 1995 and May 1996.

RESULTS

Methodological experiments

Figure 1 shows the time course of UAE in detached leaves of I. aquifolium during dehydration. The cumulative number of emissions from each side, sensed by two transducers clamped over the midrib at the proximal third, was very different. Sixty to 90 min after the start of the measurement there was a strong increase, which was more pronounced on the adaxial side. Within 120 min, 2234 UAE came from the adaxial side but only 1553 from the abaxial side. Signal production declined after 180–210 min. At the end of the measurement, after 25 h, 3930 versus 2624 UAE were recorded from the adaxial and abaxial side, respectively. Mounting the transducer adaxially over the midrib at the distal third revealed a dramatic reduction in UAE compared with the proximal third (Fig. 2). In some (but not all) leaves UAE started somewhat later distally.

Figure 3 presents the UAE time course for opposite sides of the blade in a region without major veins. The low level of UAE was similar for both sides. After 28 h, 341 adaxial UAE were recorded, compared with 395 abaxial signals.

Figure 4 compares signal numbers simultaneously sensed on both sides of the midrib. Whenever five or more signals in 2 s were recorded on one side, the corresponding
Experiments on eight woody forest species

Anatomical measurements and leaf water potential isotherms

The degree of sclerophyll (S) of the eight species is reported in Table 1 together with the mean leaf surface area (one side only). Cowling & Campbell (1983) suggested an S of 0.75 g dm⁻² as a border value between sclerophylls and soft-leaved plants. According to their definition, which was based on measurements in numerous species growing in different environments, all our study trees are sclerophylls, except for A. campestre and Pr. mahaleb.

numbers for both sides were plotted. Such multiple events or bursts were never sensed on one side only, and there was a stronger attenuation on the abaxial side.

Figure 5 shows \( \Psi \) values for C. aurantium measured during dehydration and rehydration with a hygrometer attached to the blade. Leaf water potential decreased from -0.23 MPa at the first acoustic signals to -0.34 MPa 20 min later (30 min after starting dehydration) and further to -0.59 MPa immediately before the petioles were put in water (+ W). At \( \Psi = -0.34 \) MPa, activity increased sharply from 20 to more than 250 cUAE (adaxial side) and from 5 to 160 cUAE (abaxial side) in only 12 min. This abrupt increase in UAE did not stop until about 7 min after petioles were put in water again.
The number of xylem conduits per section in the midrib and the major veins as well as conduit diameters (Table 1) varied widely between species. The first parameter ranged from 196.3 ± 17.6 in A. campestre to 1070.8 ± 130.9 in I. aquifolium, the second from 8.39 ± 3.14 μm in Ph. angustifolia to 16.92 ± 6.57 μm in L. nobilis.

Höfler diagrams relating changes in Ψ" (Ψ', Table 2) and osmotic potential (Ψ"') to the leaf symplastic water loss were plotted to determine the leaf water potential at the turgor loss point (Ψ'_t, Table 2). It varied from -1.68 MPa in I. aquifolium to -3.20 MPa in Ph. angustifolia. At the turgor loss point, the leaf had lost less than 6% of the water in C. siliqua but as much as 25% in A. campestre. The range of ε_{max} values for the leaves (Table 2) was from 13.36 MPa for A. campestre to 34.22 MPa for C. siliqua.

The minimum diurnal Ψ'_t (Ψ'_min; Table 2) was in every case higher (less negative) than Ψ'_t but in some plants it was close to Ψ'_t, thus indicating that these specimens were significantly dehydrated on summer days. Ψ'_min recorded in L. nobilis, I. aquifolium and C. siliqua was 91.1, 86.9 and 89% Ψ'_t, respectively, while in Ph. angustifolia, Q. ilex and V. tinus Ψ'_min was only 68.2, 62.0 and 66.8% Ψ'_t, respectively, and thus well above the turgor loss point.

Leaf cavitation

Figure 6 shows cUAE in air-drying leaves versus time. Cumulative ultrasound acoustic emissions increased up to an inflection point in the curve, then the increase slowed down rather suddenly. We shall call the cUAE at this point the ‘plateau value’. It ranged from 240 cUAE in Ph. angustifolia to 2040 cUAE in I. aquifolium. Cumulative ultrasound acoustic emissions during leaf dehydration were normalized by expressing them as a percentage of the plateau in each species. In turn, the percentage UAE was plotted against the Ψ'_t measured during dehydration (Fig. 7).

Only about 10% of the plateau value was emitted from leaves of C. siliqua and 20% from those of V. tinus when they were dehydrated to the minimum field Ψ'_t recorded in the summer. Moreover, C. siliqua leaves started to produce UAE at Ψ'_t = -1.75 MPa, which was the most negative cavitation threshold among the species studied.

At the minimum diurnal leaf water potential (Ψ'_min), a percentage of UAE as large as 80% of the plateau value was recorded in the two laur-ophylls (L. nobilis and I. aquifolium) as well as in Ph. angustifolia. The cavitation threshold (the Ψ'_t value at which UAE increased sharply) was very different in the three species: it was about -1.4 MPa in L. nobilis, about -0.9 MPa in Ph. angustifolia and only -0.4 MPa in I. aquifolium. The surprisingly high Ψ'_t at which I. aquifolium started to cavitate massively was in agreement with the findings of previous studies (Nardini, Lo Gullo & Tracanelli 1996), underlining the extreme vulnerability to cavitation of this species.

Table 2. Minimum diurnal leaf water potential (Ψ'_min) at 1400 h in July 1995, leaf water potential at the turgor loss point (Ψ'_t) and bulk modulus of elasticity at full turgor (ε_{max}). Values are means ± SD; n = 5.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ψ'_min (MPa)</th>
<th>Ψ'_t (MPa)</th>
<th>ε_{max} (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurus nobilis L.</td>
<td>2.65 ± 0.04</td>
<td>2.91 ± 0.09</td>
<td>24.99 ± 2.13</td>
</tr>
<tr>
<td>ilex aquifolium L.</td>
<td>1.46 ± 0.03</td>
<td>1.68 ± 0.04</td>
<td>17.82 ± 2.70</td>
</tr>
<tr>
<td>Viburnum tinus L.</td>
<td>1.29 ± 0.03</td>
<td>1.93 ± 0.07</td>
<td>34.22 ± 2.14</td>
</tr>
<tr>
<td>Ceratonia siliqua L.</td>
<td>1.80 ± 0.03</td>
<td>2.02 ± 0.07</td>
<td>32.20 ± 3.45</td>
</tr>
<tr>
<td>Phillyrea angustifolia L.</td>
<td>1.88 ± 0.06</td>
<td>2.90 ± 0.06</td>
<td>32.54 ± 0.86</td>
</tr>
<tr>
<td>Quercus ilex L.</td>
<td>1.75 ± 0.05</td>
<td>3.20 ± 0.05</td>
<td>27.57 ± 4.12</td>
</tr>
<tr>
<td>Acer campestre L.</td>
<td>1.61 ± 0.05</td>
<td>1.94 ± 0.03</td>
<td>13.36 ± 1.82</td>
</tr>
<tr>
<td>Prunus mahaleb L.</td>
<td>2.25 ± 0.06</td>
<td>2.62 ± 0.01</td>
<td>15.32 ± 3.83</td>
</tr>
</tbody>
</table>

Figure 6. Time course of cumulative ultrasound acoustic emissions (UAE) from the adaxial leaf sides of eight forest species. Vertical bars are SD of the means.

The ratio of the plateau value of UAE to that of the conduits per section at the proximal third of the leaves where the transducers were positioned (Table 1), was as high as 2.71 for Pr. mahaleb and 6.38 for A. campestre and quite low for C. sitiqua (0.20), Ph. angustifolia (0.25) and V. tinus (0.4).

As the plateau value of cUAE (Fig. 6) was reached at different $\Psi_l$ values in the specimens studied, the specific vulnerabilities were compared in terms of the ratios of the number of conduits to that of the cUAE (UAE/conduit) reached at three reference potentials: $-0.5$ MPa was near the cavitation threshold of I. aquifolium, A. campestre and Pr. mahaleb; $-1.0$ MPa corresponded to the threshold of Q. ilex, V. tinus, and Ph. angustifolia; $-1.3$ MPa was near the threshold of L. nobilis.

In turn, the UAE/conduit ratios at the three pre-established $\Psi_l$ values were plotted against $S$ and $\varepsilon_{\text{max}}$, the two parameters closely related to the hardness and rigidity of the leaf blade (Lo Gullo & Salleo 1988). When $S$ was plotted versus UAE/conduit, no correlation was found between the two parameters at any of the three pre-established $\Psi_l$ values. On the contrary, analogous plots of $\varepsilon_{\text{max}}$ versus UAE/conduit (Fig. 8) showed correlation coefficients ($r^2$) of 0.40 for $\Psi_l = -0.5$ MPa, 0.57 for $\Psi_l = -1.0$ MPa and 0.84 for $\Psi_l = -1.3$ MPa. In all three cases, a higher $\varepsilon_{\text{max}}$ corresponded to smaller numbers of UAE/conduit. These data suggest that no correlation, or at most a weak one, existed at higher leaf water potentials, while the correlation of $\varepsilon_{\text{max}}$ with UAE/conduit became closer at more negative $\Psi_l$, that is, in severely dehydrated leaves.

**DISCUSSION**

The main advantage of measuring xylem cavitation acoustically is that the experimental procedure is non-destructive and acoustic emission measurements can be performed continuously for an extended period on the same plant organ. By contrast, the main disadvantages are that (1) listening distances are variable and depend on plant material, signal energy and amplifier gain, so that it is uncertain how much tissue is being monitored; (2) the cavitations of different elements (conduits, fibres and perhaps even living cells) are hard to distinguish (Sandford & Grace 1985) in terms of signal energy or frequency (Tyree & Sperry 1989b); and (3) cavitation effects on water transport are difficult to estimate without additional measurements of hydraulic conductance.

Figure 7. Ultrasound acoustic emissions (UAE) as a percentage of the plateau value reached in 360 min (Fig. 6) versus the leaf water potential ($\Psi_l$) measured during dehydration. Vertical broken lines indicate minimum diurnal leaf water potential ($\Psi_{\text{min}}$), unbroken lines indicate leaf water potential at the turgor loss point ($\Psi_{\text{lp}}$).
differently on the two sides, and that we are not seeing events (Fig. 4) is proof that the same signals are registered to explain the observations. The response during multiple suites as depicted by Tyree & Sperry (1989a, b), seems to attain few fibres at either side of the bundle. This anatomy, with the strong attenuation of acoustic signals in plant tissues as reported by Tyree & Sperry (1989a, b), seems to explain the observations. The response during multiple events (Fig. 4) is proof that the same signals are registered differently on the two sides, and that we are not seeing completely separate events in tissues close to each epidermis: in most cases, a high number of adaxial signals coincides with somewhat fewer abaxial ones.

It is surprising that marked differences in the number of signals sensed can occur over distances of less than 2 mm. Obviously, the concept of ‘listening distance of sensors’ is meaningless when applied to signals of unknown energy distribution: strong signals will be counted over a longer distance or through tissue layers of higher attenuation than weak ones. As the decibel scale is logarithmic, even a small numerical loss in signal strength will cause weak events to go uncounted.

Fewer signals were recorded over the distal third of the midrib than over the proximal third (Fig. 2). The explanation again is based on anatomy. The number of xylem conduits and fibres along the midrib declines rapidly towards the leaf tip. Also, cavitation in the proximal third may transiently release the tension more distally. The time-lag that sometimes occurs before the onset of distal signals could be the result of this or of the reduced number of conduits. Regions without major veins produced few signals (Fig. 3), suggesting that the signals were produced by deeply imbedded minor veins and not by the abundant mesophyll cells. Approximately the same number of signals arrived at sensors on each side. All these data suggest that routine UAE measurements on leaf blades are preferably made over the midrib, at a position close to the base, and on the adaxial side.

The plateau values (Fig. 6) varied widely both within a single species (as indicated by the large SD of the means) and between species. Such differences probably reflected different numbers of cavitating elements in the specimens studied. The number of tracheae and tracheids under the transducer will be different, but they need not be the only structures susceptible to cavitation. In principle, cavitation can take place in conduits and fibres (Ritman & Milburn 1988, 1991; Tyree & Sperry 1989b) and is said to occur even in living parenchyma cells (Oertli 1993; Oertli, Lips & Agami 1990).

Nevertheless, acoustic measurements have already considerably increased our insight into the precarious process of water transport in the stem xylem. An extension of these investigations to the leaf seems of interest.

Methodological experiments on *L. aquifolium* and *C. aurantium* confirmed that UAE from the leaf were attributable to water cavitation. Acoustic signals started when leaves were dehydrated and stopped when they again took up water. In *C. aurantium* we measured a $\Psi_i$ value ($-0.34$ MPa, Fig. 5) at which UAE critically increased in number, and a slightly higher value ($-0.30$ MPa) when UAE stopped during leaf rehydration.

In every case (Figs 1 & 5, and other measurements), more UAE were sensed on the adaxial than on the abaxial side of the midrib. We believe that this is because of the smaller amount of tissue between xylem conduits and the adaxial epidermis. In fact, the xylem in the leaf bundles is almost always turned upwards. In *L. aquifolium*, the abaxial (lower) surface is shielded from the xylem by a layer of phloem and a well developed spongy parenchyma. There are few fibres at either side of the bundles. This anatomy, with the strong attenuation of acoustic signals in plant tissues as reported by Tyree & Sperry (1989a, b), seems to explain the observations. The response during multiple events (Fig. 4) is proof that the same signals are registered differently on the two sides, and that we are not seeing...
old \((\Psi_{\text{cav}})\) of the leaves. \(\Psi_{\text{cav}}\) was lowest in \textit{C. siliqua} \((-1.75\ MPa)\) and highest in \textit{L. aquifolium} \((-0.4\ MPa)\). This agrees with previous findings that carob is very resistant to drought \((\text{Lo Gullo \& Salleo 1988; Lo Gullo, Salleo \& Rosso 1986})\) and to cavitation \((\text{Salleo \& Lo Gullo 1989, 1993}).\) By contrast, holly is very sensitive to cavitation \((\text{Nardini, Lo Gullo \& Tracanelli 1996}).\)

Cavitation activity in terms of UAE as a percentage of the plateau value versus time showed that \textit{C. siliqua} leaves were most resistant at the minimum diurnal \(\Psi_t\) in the field \((\Psi_{\text{min}}, \text{Table 2}).\) In fact, at \(\Psi_{\text{min}}\) cUAE for this species was only about 10% of the plateau value. \textit{V. tinus} recorded one hundred per cent UAE was reached at about \(-3.2\) and \(-2.5\ MPa\) in \textit{C. siliqua} and \textit{V. tinus}, respectively, which is well beyond \(\Psi_{\text{min}}\) in both species \((-2.02\) and \(-1.93\ MPa,\) respectively). \(\Psi_{\text{tip}}\) is not reached under common field conditions by \textit{C. siliqua} (Lo Gullo \& Salleo 1988) or \textit{V. tinus} \((\text{Chiudina Piaceri, Salleo \& Lo Gullo 1994}).\) Therefore neither species is likely to suffer leaf cavitation to a large extent, even during summer.

Leaves of \textit{Q. ilex} were more prone to cavitate, as 30–50% UAE were recorded at \(\Psi_{\text{min}}\) while 100% UAE were reached at \(\Psi_t = -3.0\ MPa\), that is near \(\Psi_{\text{tip}}.\) \textit{Salleo \& Lo Gullo} \((1993)\) reported that 1-year-old holm-oak twigs lost more than 50% of their hydraulic conductivity at \(\Psi_t = \Psi_{\text{tip}}.\) All these data agree with the suggested arcto-tertiary origin of \textit{Q. ilex} \((\text{Raven 1973; Kyriakopoulos \& Richter 1991})\) preceding its adaptation to warmer and drier climates.

Much higher percentages of the plateau value \((80\%)\) were recorded in all the other species. In particular, \textit{A. campestris} \((\text{Fig. 7})\) showed a large variability in the number of UAE \((\text{see also Fig. 6})\) so that the percentage UAE at \(\Psi_{\text{min}}\) varied between 40 and 80%. The high percentage of UAE for leaves of \textit{Ph. angustifolia} was surprising because this species is typical of arid environments. An interesting similarity exists between \textit{Ph. angustifolia} and another member of the Oleaceae, \textit{Olea oleaster} \((\text{Hoffmng. et Link. (wild olive), whose 1-year-old twigs undergo xylem cavitation and tolerate its effects (Salleo \& Lo Gullo 1993)})\).

Our initial hypothesis was that sclerophyll might confer a higher resistance to cavitation, corresponding to the higher resistance to drought claimed by different authors for sclerophylls \((\text{Mooney 1982; Pignatti 1984; Pigott \& Pigott 1993}).\) In fact, no correlation was found between \(S\) and the cUAE from the leaves of different species \(\Psi_{\text{min}}.\)

The degree of sclerophyll \((S)\)—the dry weight per unit leaf surface area—is governed by wall density and dimensions, lumen size and spacing of all the cellular elements. Previous studies \((\text{Chiudina Piaceri et al. 1994; Lo Gullo \& Salleo 1988; Salleo \& Lo Gullo 1990})\) had questioned that sclerophyll \textit{per se} might play a role in overall drought resistance. Our data suggest that sclerophyll is not closely related to cavitation resistance, one of the components of drought resistance.

In turn, the bulk modulus of elasticity should describe the ‘rigidity’ of the walls of living cells only \((\text{Tyree 1981; Tyree \& Karamanos 1981})\), which presumably increases with wall diameter and cellulose content. The correlation between \(\varepsilon_{\text{max}}\) and UAE/ conduit, which increased at a more negative \(\Psi_t\) \((\text{Fig. 8})\), were therefore unexpected. Conduit cavitation should not be related to \(\varepsilon_{\text{max}}\) if this parameter is indeed a property of living cells as stated by the theory of pressure–volume curves. The rather high correlation coefficient \((r^2 = 0.84)\) between \(\varepsilon_{\text{max}}\) and UAE/conduit at \(\Psi_t = -1.3\ MPa\) suggests that other elements than conduits were cavitating at this water potential, or that stiff walls of living cells inhibit sound propagation when they become flaccid. If this is not the case, \(\varepsilon_{\text{max}}\) needs to be interpreted differently.

In conclusion, we feel that the acoustic method for detecting cavitation deserves further study and is likely to provide more than only qualitative data, as suggested previously \((\text{Lo Gullo \& Salleo 1991; Tyree \& Sperry 1989a})\). Cavitation measurements in the leaf blade were experimentally correct, as they produced only minimal alterations of leaf structure and function. They also gave reasonable results: leaf cavitation data were in good agreement with other published ecophysiological measurements for the same species, as well as with the available information on adaptation to their habitats.

A great advantage of measuring UAE in the leaf is that little injury is caused, so that field measurements could probably be prolonged for several weeks. By contrast, the current protocol for the stem implies that UAE transducers must be repositioned after 1–2 days, before the wound-induced phellogen produces cicatricial tissues (mainly cork) that prevent further recordings. Furthermore, cavitation in the leaf may be directly compared with other parameters measured in this organ, where many physiological investigations are carried out. Measurements on leaves may also prove valuable for the study of xylem vulnerability in herbaceous plants.

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