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COMPARISON BETWEEN ULTRASONIC ACOUSTIC EMISSION (UAE) AND HYDRAULICALLY MEASURED LOSS OF HYDRAULIC CONDUCTANCE IN *Eucalyptus* spp. CLONE GU210

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ABSTRACT

This study was undertaken to compare and validate the Ultrasonic Acoustic Emission (UAE) method with that of traditional hydraulically assessed method of cavitation detection in *Eucalyptus* spp. clone (GU210) grown for 12 months in pots. Vulnerability of xylem cavitation to the main stem was assessed as the leaf water potential corresponding to the maximum rate of acoustic emissions per hour (ψ_L, EPH_{max}), and as the critical water potential triggering cavitation events, calculated as the mean of the water potentials of data points lying between 5% and 10% of the total accumulated ultrasonic acoustic emissions (ψ_{CAV}, cUAE%). When measured hydraulically the water potential corresponding to 50% loss of conductivity (PLC_{50}), or the water potential corresponding to the initiation of conductivity loss was used.

The hydraulic vulnerability curve was not sigmoidal in nature; rather it showed an increase in stem conductance loss with decreasing leaf water potential that could be described by a second order polynomial. But, cUAE% curve was almost sigmoidal in shape. Vulnerability curves of UAE% vs. water potential, and of PLC vs. water potential did not overlay, and the water potentials corresponding to PLC_{50} and $cUAE\%_{50}$ differed. One possible reason for this is that hydraulically measured PLC_{50} generally occurs at lower water potentials than estimates from acoustic methods and therefore, it is clear that the acoustic data cannot be used directly to assess the degree of conductivity loss. Acoustic methods do not directly measure the influence of cavitation events on hydraulic conductance and therefore, assessment of vulnerability using these methods is more subjective. However, the relationship

established between percentage loss in hydraulic conductivity (PLC) and cumulated UAE (cUAE) values corresponding to common water potentials showed a linear relationship between PLC and cUAEs, with the total cUAEs corresponding to about 80% of the PLC.

Keywords: Eucalyptus, stem xylem cavitation, ultrasonic acoustic emissions, hydraulic method

INTRODUCTION

In plants, transpiration pulls the water column upward under tension through the xylem tissue of the roots to the leaves. The high tensions in the xylem cause dissolved gases to come out of solution into the vapour phase. Because the water is under tension, this micro-void will expand explosively to fill the conduit, a process known as cavitation. The cavitated conduit is at a pressure close to vacuum, and air will come out of neighbouring wet tissue (and diffuse from the outside of the stem) to fill the conduit with air at atmospheric pressure, forming an embolism. An embolized conduit will not conduct water until water potential (ψ_P) returns to near-atmospheric pressure and refills the conduit cavitation (Tyree & Sperry, 1989a; Tyree & Ewers, 1991; Tyree *et al.*, 1994). Cavitation, predominantly induced in the xylem as a result of drought (Zimmermann, 1983; Sperry & Tyree, 1988; Sperry *et al.*, 1996; Cochard, 2002) and freezing (Sperry, 1993; Utsumi *et al.*, 1999; Jaquish & Ewers, 2001) temporarily reduces xylem water transport. Loss in hydraulic conductivity is frequently observed among the different growth forms (conifers, angiosperms, shrubs, and lianas). This loss in conductivity is strongly related to cavitation events in the xylem and subsequent embolism formation (Schultz & Mathews, 1988; Alder *et al.*, 1996; Hacke & Sauter, 1996; Lo Gullo *et al.*, 1998). In response to xylem embolisms steeper water potentials in the xylem exist which can lead to stomatal closure. Such closure reduces both loss of water and uptake of carbon dioxide (Becker *et al.*, 2000) and hence reduces growth.

Several techniques have been described to measure the xylem caviations. Cavitation events in the tissues can be monitored microscopically, anatomically, acoustically and hydraulically. Renner and Ursprung (1915) (*loc. cit.* Zimmermann, 1983) first observed the phenomenon of cavitation in the fern sporangia under the microscope and Milburn (1970) (*loc. cit.* Zimmermann, 1983) observed cavitations in fungal ascospores. However, Pierce (1936) (*loc. cit.* Tyree & Sperry, 1989a) may have been the first person to demonstrate the occurrence of cavitation in the stem

segments. Salleo & Lo Gullo (1989) quantified xylem embolism under the microscope for stained and unstained xylem conduits.

Milburn & Johnson (1966) introduced an acoustic technique to allow the detection of cavitation in whole organs. Tyree & Dixon (1983) developed a more powerful technique to detect acoustic emissions using ultrasonic frequencies and to measure the energy and frequency range of the acoustic emissions. The advantage of ultrasonic acoustic emission (UAE) over the low frequency acoustic technique is that the UAE technique selectively amplifies high frequency vibrations and simultaneously filters out the lower range frequencies associated with audible sound that can be produced by various experimental manipulations (Tyree & Sperry, 1989b). Ultrasound acoustic emissions associated with cavitation events (Tyree & Dixon, 1983; Tyree & Sperry, 1989a ; 1989b) are presumably produced by the vibrating walls of cavitating xylem conduits (Tyree *et al.*, 1984). Ultrasound is propagated for shorter distances in air and the problem of extraneous noise is therefore minimized, and thus it became possible to use this technique in the natural environment (Jackson & Grace, 1996). Also, *in situ* measurements have been demonstrated in gymnosperms (Nardini & Salleo, 2000) and angiosperms (Vander Willigen *et al.*, 2000). Detected UAE data available for various plant organs such as internodes, node to petiole junctions (Salleo & Lo Gullo, 1989; Lo Gullo & Salleo, 1991) and leaf blades (Salleo *et al.*, 2000; Nardini *et al.*, 2001).

Very specialized, nondestructive and costly methods have been developed by various researchers to detect xylem embolisms. These include the use of a beam of gamma rays (Dixon *et al.*, 1984), use of X rays (Habermehl, 1982), NMR studies (Ratkovic & Bacic, 1993), computer tomography (Raschi *et al.*, 1995) and cryo-scanning electron microscopy (McCully, 1999; Utsumi *et al.*, 1999).

Sperry *et al.* (1988) introduced a direct but destructive method to quantify the extent of xylem embolisms using excised plant segments in a low-pressure hydraulic conductivity apparatus. The hydraulic conductivities are measured when the segments are perfused with perfusing solution at a low pressure before and after the removal of air embolism by a flush at a high pressure. The hydraulic conductivity of the plant segment is computed as the quotient of mass flow rate and pressure gradient. The percentage difference in the initial and maximum conductivity is the percentage loss of hydraulic conductivity. Kolb *et al.* (1996) and Nardini *et al.* (2001) have modified the design of the technique of Sperry *et al.* (1988) to facilitate hydraulic studies in densely branched shoots, root systems and leaves. Comparative studies made by Lo Gullo & Salleo (1991) showed similar results among the acoustic emission, low-pressure hydraulic conductivity and anatomical methods (Hacke & Sauter, 1995;

1996). Nevertheless, all these methods have advantages and disadvantages, so they are not alternative to one another (Lo Gullo & Salleo, 1991). As pointed out by Salleo *et al.* (2000) an UAE technique does not provide information about the impact of xylem cavitation on wood hydraulic conductance. However, the acoustic emissions technique is nondestructive and allows continuous monitoring (Lo Gullo & Salleo, 1991; Jackson & Grace, 1996; Salleo *et al.*, 2000).

According to Tyree & Sperry (1989a) hydraulically measured vulnerability curves generate a useful gauge of the impact of embolism on plant water relations. However, such xylem vulnerability measurements are usually made in the laboratories, on either excised stems or root segments, and these segments cannot sense hydraulic or chemical signals from the roots that would influence stomata and hence possibly avoid cavitation events. Therefore, little is known about daily cavitation events occurring in living plants in the field, except those measured by Jackson *et al.* (1995a ; 1995b); and Jackson & Grace (1996) using an UAE sensor.

In the present study, the daily courses of xylem cavitation events were detected using an ultrasonic acoustic emission instrument concurrently with leaf water potential measurements. These are non-destructive methods, which have an advantage in time-based experiments with limited numbers of replicates. These studies were undertaken to compare the UAE and hydraulic techniques, and to assess whether the data provided by the UAE technique could be used as a measure of the effect of cavitation events on hydraulic conductance. Measurements of acoustic emissions and corresponding water potentials made on a plant on one day were compared with vulnerability to cavitation measured hydraulically on the same plant on the following day.

MATERIAL AND METHODS

Plant materials and study site

Eucalyptus grandis x *E. urophylla* hybrid (GU210) (Family Myrtaceae) was selected for the present study on the basis of the drought susceptibilities of mother plants, which are indigenous to the Australia mainland, Tasmania and Papua New Guinea. The drought response of GU210, a recently developed hybrid, is not known. Planting material (rooted macrocuttings) was obtained from Mondi Forests Tree Improvement Research Unit, Hilton, South Africa. Potted material was grown outdoors in the greenhouse complex of the School of Life and Environmental Sciences, University of Natal, Durban, South Africa.

Six replicate plants of the clone GU210 maintained in 25 l pots and randomized with other clones were used for different studies. GU210 clone was subjected to high water treatment for 12 months. Plastic sheeting was placed under the pots to restrict root penetration into the ground. The potting soil used was a mixture of sand, loam and compost (4:4:3). Pots were filled with soil according to a technique described by Bohm (1979). Ramets were potted on 24th February, 2000 and initially watered daily.

The 'high water' treatment was designed to mimic the annual rainfall (1280 mm) in the region where the selected clones were grown. The surface area of each pot was calculated, and this area X 1280 mm was the total volume of water added to the high water treatment over a year. The rate of application varied over the year according to mean monthly rainfall, and the water for one month was added in eight to nine equal amounts, twice every week, generally on Mondays and Thursdays. Nutrients were applied from six months after planting. 1 g prochloraz manganese chloride (Sporgon; Hoechst Schering AgrErvo, South Africa) and 1.25 ml tebuconazole (Folicur, Bayer, South Africa) per litre were added to soil, and foliar fungicides (2 g mancozeb (Dithane; Efekto, South Africa) and 1 ml chlorothalonil (Bravo; Shell, South Africa) per litre were sprayed on Thursdays and Mondays in the first week of each month. Soil macronutrients (1 g Agrofert Mondi Orange N: P: K 13.5:18:11.4 per litre) and foliar trace elements solutions [2.5 ml Trelmix (18 g Fe, 4 g Cu, 2 g Zn, 1 g B and 0.4 g Mo; (Hubers, South Africa) per litre] were also added.

Vulnerability to xylem cavitation, Leaf water potential (Ψ_L) and Thermocouple psychrometry

Leaf water potential was non-destructively measured concurrently with main stem xylem UAE counts on potted plants using L-51 leaf hygrometer chambers and an HR-33T Dew Point Microvoltmeter (Wescor Inc., Logan, Utah, U.S.A) in the dew point mode. Leaves were randomly clamped into leaf chambers at two different levels for each measurement point, one from the upper and one from the middle part of the canopy. Taking of measurements began at 0600 h and continued at 20 min intervals until 1400 h or until xylem recovery was observed from the necessary leaf water potential. The chambers were clamped onto the leaves in the evening before the measurements were taken to ensure equilibration of more than 12 h, even though vapour equilibration had probably occurred before this time.

Diffusive resistance to water vapour movement from the leaf to hygrometer leaf chambers was reduced by gentle abrasion of the adaxial leaf surface using aluminium oxide. Then the abraded leaf surface area was cleaned with deionized

water, allowed to dry for ten minutes, and the psychrometer leaf chambers were sealed against the abraded area (Savage *et al.*, 1984). Vertical supports from the ground were used to hold the psychrometers in position in the leaf canopy. The aluminium housing of psychrometers were insulated with small blocks of polystyrene and white plastic covers to minimize temperature fluctuations. Leaf shading was minimized to allow adequate vapor pressure equilibration between leaf and the thermocouple psychrometer (Oosterhuis *et al.*, 1983). The leaf chambers were calibrated after five sets of measurements with NaCl solutions at laboratory temperature (approximately 25°C).

Daily xylem cavitation events detected by ultrasonic acoustic emissions (UAE)

Daily courses of xylem cavitation events were counted in the main stem by the ultrasonic acoustic emission method, using a calibrated ultrasonic sensor (Model 1151; Physical Acoustic corp., Princeton, NJ, USA) and a preamplifier (model 4615; Physical Acoustic Corp., Princeton, NJ, USA), sold as a drought stress monitor (DSM). A 20 mm-long strip of main stem bark was carefully removed from 0.2 m above the soil surface by a sharp razor. A thin layer of water-soluble KY lubricant jelly was smeared between the ceramic made head of the UAE sensor and the area of exposed xylem to facilitate the easy transmission of acoustic events to the clamped sensor as well as to avoid surface evaporation. The sensor was mounted directly on the exposed tissue portion.

Measurements were made on clear days only when transpiration rates and xylem tensions would be high. An UAE sensor was clamped on the main stem at the height of 0.2 m above the soil surface around 1730 h and the drought stress monitor (DSM) collected cavitation events until 1400 h of the following day. During the day, xylem water potential was assessed by measuring the leaf water potential of two to three matured leaves from the middle and upper layers of the canopy that were well exposed to environmental conditions using L-51 leaf chambers. Leaf chambers were clamped onto the leaves at the same time of clamping the UAE sensor to the stem, in order to give adequate time to equilibrate overnight before the measurements were taken. Cumulated ultrasonic acoustic emission events (cUAE) and events per minute (EPM) were collected by the DSM at 20 min intervals during this period. Cumulative emissions were expressed as a percentage of the plateau or maximum number of events detected (UAE %), and plotted against the water potential measured at the corresponding time.

Daily xylem cavitation events were measured at 1min intervals from 1800 h to 1400 h of the following day. A dead time of 400 μs with amplification gain of 72

decibels were selected as these settings reduced to a minimum background noise in the experimental area. The ultrasonic acoustic emission (UAE) sensor was usually installed 30 min prior to commencement of measurements to ensure equilibration. An UAE sensor was suspended in the air for a few days at the pot trial site at different gain settings, and settings minimizing extraneous noise was chosen as described by Jackson & Grace (1996). During the day, xylem water potential was assessed by measuring the leaf water potential. A software package written for use with a personal computer was used to download the record of cumulated events (CE) and events per minute (EPM) during the measured time at 20 min intervals. EPM were plotted in relation to time, and as concurrent leaf water potentials were measured, EPM could be plotted as a function of leaf water potential. Also, cumulated UAE were expressed as a percentage of the first plateau maximum corresponding to the cumulative number of UAE recorded at the time as described by Salleo *et al.* (2000). The percentage of cumulated UAE (cUAE in %) was also plotted against water potential (vulnerability curve), which was recorded concurrently.

Low pressure flow meter: hydraulic vulnerability curve

The hydraulic conductivity and xylem vulnerability to embolisms were measured according to the methods described by Sperry *et al.* (1988) and later modified by Vander Willigen & Pammenter (1998). The hydraulic conductivity of the plant segment is computed as the quotient of mass flow rate and pressure gradient (Sperry *et al.*, 1988). Vulnerability of xylem to cavitation and embolism was measured as the percentage loss of hydraulic conductivity with decreasing xylem water potential of the plant segments.

After the UAE measurements on a plant had been completed, the same plant was watered to field capacity and harvested on the following morning. The whole shoot was cut close to the soil surface and immediately brought to the laboratory. The terminal portion of the shoot was removed and, using a low-pressure flow meter, an initial hydraulic conductivity was measured and the stem was allowed to dehydrate, while conductivity and corresponding water potential were measured at intervals. Conductivity and corresponding water potential were measured at 5 – 6 points on each plant. Finally, the stem was flushed with water at high pressure and maximum conductivity was measured, and percent loss of conductivity was calculated for each point.

The main stems of some other plants were also cut at these soil surface and the shoots were also brought immediately to the laboratory. To prevent stem

embolism and evaporation, the cut end surface was sealed with parafilm and the entire shoot was wrapped in black plastic bags.

The proximal cut end of the stem was perpendicularly re-cut while submerged in water. The whole shoot was covered and the proximal end was connected with PVC tubing to a reservoir of filtered, distilled and degassed 0.01M HCL, which prevented long term decline of xylem conductivity caused by microbial growth within the xylem conduits (Sperry *et al.*, 1988; Cochard *et al.*, 1992). The distal end was then cut and clamped under water to PVC tubing that connected to a glass tube with the tip dipping into a beaker of water placed on an analytical balance sensitive upto four decimal places. Water was allowed to flow from the reservoir, through the shoot to the balance. The amount of water flowing through the branch at a given head pressure was recorded as a mass at every 30 seconds for 5 minutes using a programmed computer. This measurement constituted the initial hydraulic conductivity.

After measuring the initial conductivity, shoot xylem pressure was measured from two leaves, one each from the distal and middle portions of the shoot, using a Scholander pressure chamber (Scholander *et al.*, 1965). The shoot was then dehydrated on the bench top for 3 - 4 days while both distal and proximal ends were tightly sealed with parafilm. During this period 5 - 6 measurements of xylem water potential and concurrent hydraulic conductivities were taken for each sample. Before taking each measurement the shoots were fully covered with black plastic bags and allowed for equilibration of tissue water potential throughout the shoots for about 70 minutes. Since the required number of samples was not available, a single vulnerability curve was produced from a shoot that had been subjected to several measurements instead of taking individual measurements from numerous shoots as described by Pammenter & Vander Willigen (1998). Once the shoot flow had virtually ceased, the maximum conductivity was measured after the shoots were fully perfused with water at 375 kPa for 60 minutes until water dripped from the leaves.

Hydraulic conductivity

The formula given below was used to calculate the hydraulic conductivity (K_h) of xylem lumina in kg m $MPa^{-1}s^{-1}$.

$$K_h = \frac{\text{Rate of flow (kg } s^{-1}\text{) x Length of shoot (m)}}{\text{Pressure head (MPa)}}$$

Percentage loss of conductivity

The percentage loss of conductivity (PLC) was calculated as,

$$PLC = \frac{(K_h \text{ max} - K_h)}{K_h \text{ max}} * 100$$

where K_h and K_h max are the actual and maximum hydraulic conductivities of the shoots, respectively.

Vulnerability curve was plotted as the percentage loss of hydraulic conductivity against the xylem water potential as described by Sperry *et al.* (1988).

RESULTS AND DISCUSSION

The accumulation of acoustic emissions, the rate of emission detection, and the corresponding decline in water potential over the course of the day are given in Figures 1A, 1B and 1C respectively. These are the combined data for all plants. Acoustic emissions were continued to be detected when the experiment was terminated at 1400 h, and therefore, a plateau value was not observed. However, maximum cUAE was estimated by fitting an exponential sigmoid curve to the data (Fig. 2), and the fitted value of maximum cUAE was used to calculate cUAE% for each plant at every 20 minutes. UAEs accumulated in a sigmoidal manner and the rate of emissions peaked (EPM_{max}) at about 1100 h. cUAE % was plotted against leaf water potential (Fig. 3A) to yield a 'vulnerability' curve based on acoustic data. The vulnerability curve determined using the conventional hydraulic method is shown in Fig. 3B Unlike the accumulation of emissions over time (Fig. 1A) and the plot of cUAE % against water potential, the hydraulic vulnerability curve was not sigmoidal in nature; rather it showed an increase in stem conductance loss with decreasing leaf water potential that could be described by a second order polynomial.

The relationship between PLC and cUAE

The vulnerability of xylem to cavitation can be assessed by a number of criteria. When measured hydraulically, the water potential corresponding to 50% loss of conductivity (PLC_{50}), or the water potential corresponding to the initiation of conductivity loss can be used. Because acoustic methods do not directly measure the influence of cavitation events on hydraulic conductance, assessment of vulnerability using these methods is more subjective. The water potential corresponding to the accumulation of 50% of total acoustic emissions ($cUAE_{50}$) would not necessarily be a good measure, as cUAE will depend on the water potential developed by the

transpiring plant. For example, the minimum water potential developed by whole plants in pots in this study was higher (about -2.5 MPa, Figs.1C and 3A) than those achieved by dehydration of excised shoots during hydraulic measurements (about -5 MPa, Fig. 3B). Consequently, the curves of cUAE % vs. water potential and of PLC vs. water potential do not overlay, and the water potentials corresponding to PLC_{50} and $cUAE_{50}$ differed. The same argument would apply for using the maximum rate of acoustic emissions (EPM_{max}) as a measure of vulnerability. A method has been proposed (Salleo *et al.*, 2000) to determine the threshold water potential corresponding to the initiation of rapid cavitation events, the 'initiation' being judged as the mean of the water potentials for all data points lying between 5% and 7.5% of total cumulative emissions (ψ_{CAV},cUAE%). The data points between 5 and 10% were used in this study.

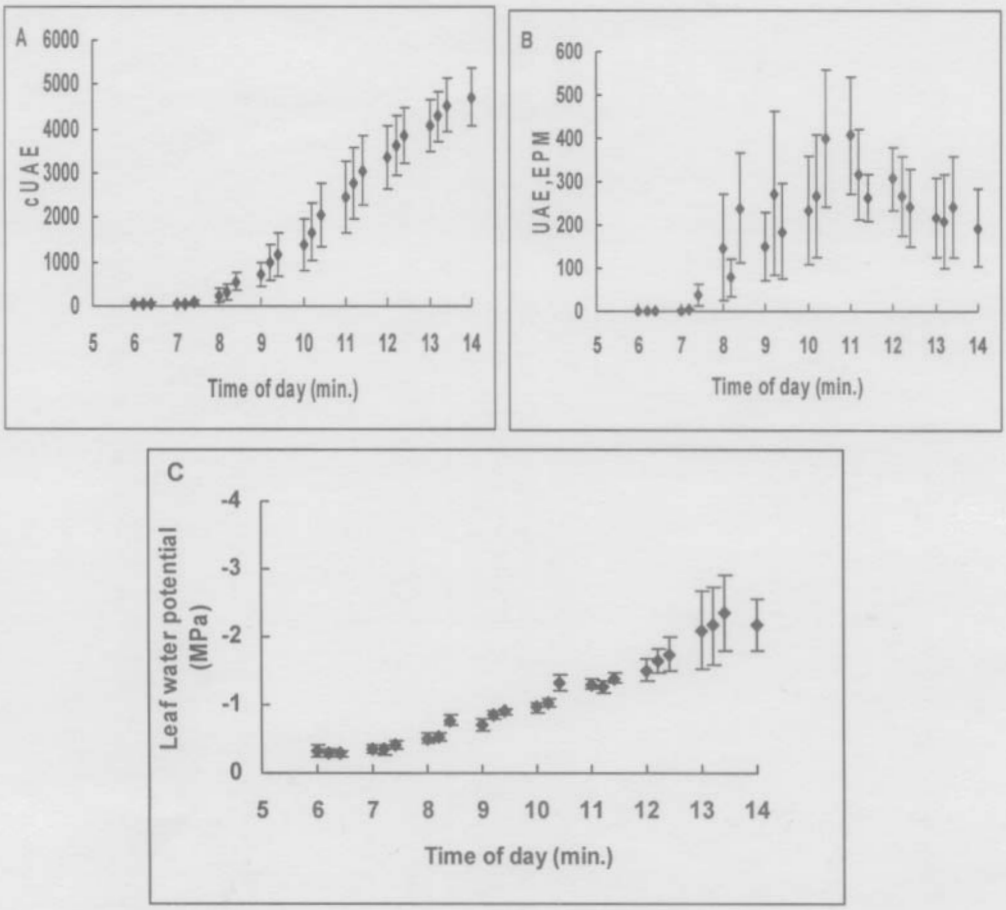

Fig. 1: A - Cumulated ultrasonic acoustic emission (cUAE), B - Events per minute (EPM) from the main stem, and C - concurrently measured leaf water potential for 12 months old plants of clone GU210 subjected for high watering treatment. Error bars represent the ± standard error of the mean (n=6). Note that the six replicates were measured on separate days.

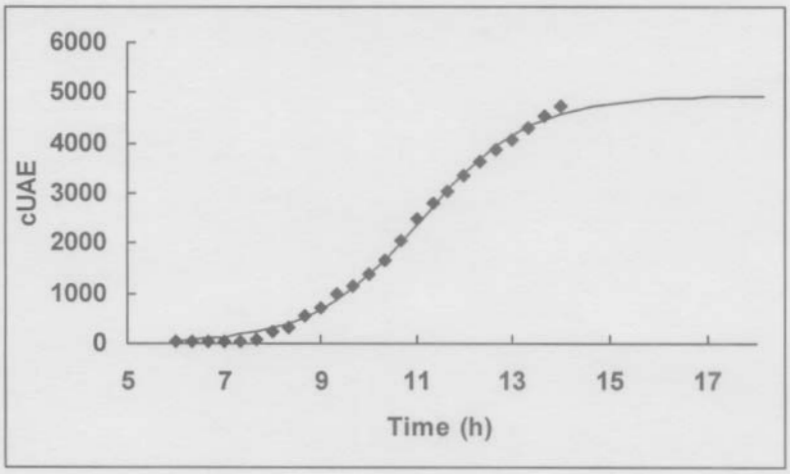

Fig. 2 : Maximum cUAE estimated by fitting an exponential sigmoid curve to the cUAE data. The sigmoidal equation is cUAE=4944/ [1+exp{-0.87*(time-11.1)}].

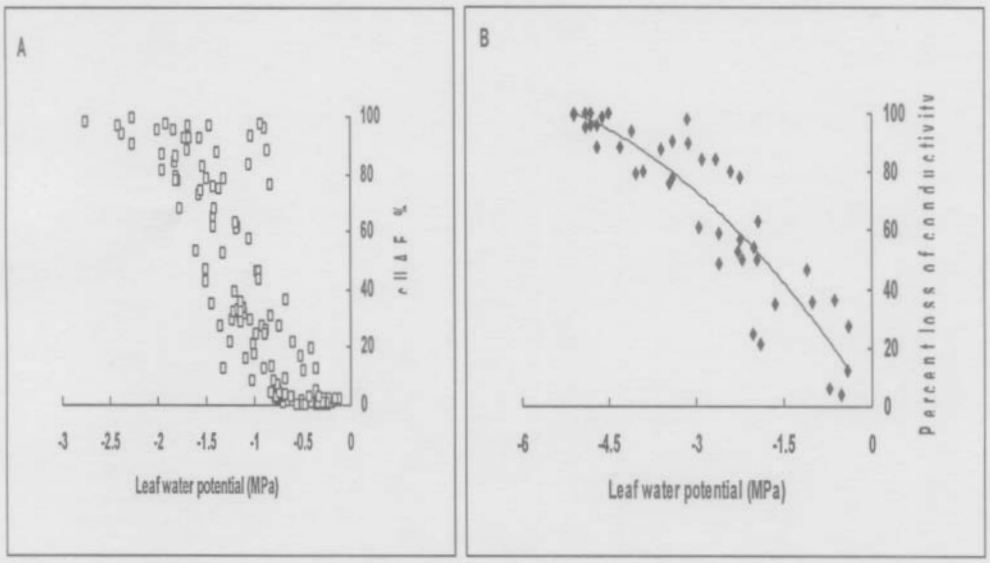

Fig. 3: A - Cumulated ultrasonic emission (cUAEs) from the main stem, expressed as percentage of the maximum cumulated ultrasonic emission (cUAE,%), as a function of xylem water potential in the main stem of 12 months old plants of clone GU210 subjected for high watering treatment and B - vulnerability of xylem to cavitation illustrated as the percentage loss of hydraulic conductivity as a function of water potential for the same plants (B). All data points from each replicate are shown (n=6).

The water potentials corresponding to different assessments of 'vulnerability' are shown in Table1.

Table 1: The leaf water potential corresponding different assessments of vulnerability to xylem cavitation using ultrasonic acoustic emission (UAE) and hydraulic methods in *Eucalyptus* spp. hybrid (n=6)

Methods detected Cavitations	Measuring scales	Critical xylem water potential (MPa)
Hydraulic method	PLC_{50}	-1.95 MPa
Acoustic method	$cUAE_{50}$	-1.30 MPa
	EPM_{max}	-1.30 MPa
	ψ_{CAV},cUAE,%	-0.73 MPa

From these values it is clear that the acoustic data cannot be used directly to assess the degree of conductivity loss. Similar lack of correspondence of the two methods has been reported by Cochard (1992) and Jackson *et al.* (1995 a) in two different studies on *Pinus sylvestris* L. Hacke & Sauter (1995) and Nardini *et al.* (2001) showed that hydraulically measured PLC_{50} generally occur at lower water potentials than those estimated using acoustic methods. One possible reason for this is that the acoustic method may sense emissions from tracheids and fibres, which would have no influence on conductivity (Cochard & Tyree, 1990).

However, it can be demonstrated that the hydraulic and acoustic techniques are measuring the same phenomenon for quantifying xylem cavitation. The data sets of PLC vs. water potential and cUAE vs. water potential were searched to find points of the same water potential in the two sets. The PLC and cUAE values corresponding to these common water potentials were then used to relate PLC to cUAEs. A plot of these data pairs showed a linear relationship between PLC and cUAEs (Fig. 4; R^2 = 0.62, P=0.000), with the total cUAEs corresponding to a PLC of about 80%. These data suggest that acoustic emissions were in fact an expression of cavitation events occurring in the xylem conduits. Similar conclusions have been drawn by Lo Gullo & Salleo (1991); Salleo & Lo gullo (1993) and Salleo *et al.* (2000).

The slopes of vulnerability curves determined using the hydraulic technique are generally sigmoidal in shape (Tyree & Sperry, 1988; Lo Gullo & Salleo, 1991; Pammenter & Vander Willigen, 1998; Kavanagh *et al.*, 1999). However, the clone GU210 yielded a more gradual increase in conductivity loss with decreasing water

potential, and similar shaped curves were reported in three coffee cultivars (Tausend *et al.*, 2000).

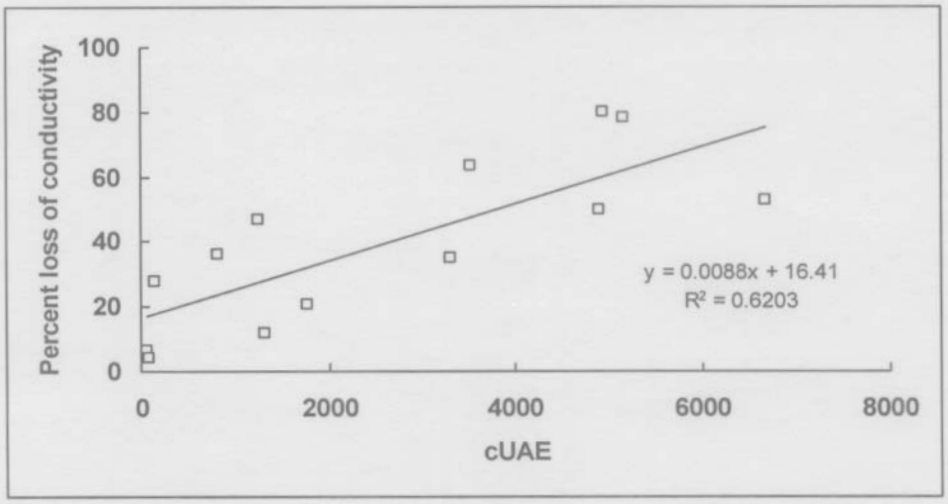

Fig. 4: The relationship between cumulated ultrasonic emission (cUAEs) and percent loss of conductivity (PLC) of stems of 12 months old plants of the clone GU210 subjected to high watering.

Interestingly, the cUAE % curve for GU210 was almost sigmoidal in shape. The water potential corresponding to PLC_{50} for GU210 (-1.95 MPa) was lower than that of field grown *Eucalyptus* spp. clones (-1.50 MPa), one of which was also a GU clone (Vander Willigen & Pammenter 1998), but was higher than that of *E. calmuldulensis* (-3.0 MPa) (Franks *et al.*, 1995), although there is no doubt that vulnerability to cavitation does vary among genotypes (e.g. Sperry & Tyree, 1990; Tausend *et al.*, 2000; Vander Willigen *et al.*, 2000). Jackson *et al.* (1995b) and Vander Willigen and Pammenter (1998) have shown that there are no differences in the vulnerability among the same genotypes grown on mesic or xeric sites. However, Sperry & Saliendra (1994), Alder *et al.* (1996), Franks *et al.* (1995), Kavanagh *et al.* (1999) and Sparks and Black (1999) showed that the growing conditions affect the vulnerability. Further, Nardini & Salleo (2000) suggested that some xylem cavitation-induced reduction in shoot hydraulic conductances is the signal for stomatal closure preventing runaway embolism. The mechanism linking xylem cavitations and stomatal response remains uncertain and requires further investigations in the field using a tool like an UAE in living plants.

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REFERENCES

Alder, N.N., J.S. Sperry & W.T. Pockman 1996. Root and stem embolism, stomatal conductance and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. Oecologia 105: 293-301.

Becker, P., F.C. Meinzer & S.D. Wullschleger 2000. Hydraulic limitation of tree height: a critique. Functional Ecology 14: 4-11.

Bohm, W. 1979. Methods of Studying Root Systems. Ecological studies 33. Springer-Verlag, Berlin, Heidelberg, New York. pp 105-107.

Cochard, H. 1992. Vulnerability of several conifers to air-embolism. Tree Physiology 11: 73-83.

Cochard, H. 2002. Xylem embolism and drought-induced stomatal closure in maize. Planta 215: 466-471.

Cochard, H. & M.T. Tyree 1990. Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. Tree Physiology 6: 393-407.

Cochard H, N. Breda, A. Granier & G. Aussenac 1992. Vulnerability to air embolism of three European oak species (*Quercus petraea* (Matt) Liebl, *Q. pubescens* Willd, *Q. robur* L). Annales Des Sciences Forrestieres 49: 225-233.

Dixon, M.A., J. Grace & M.T. Tyree 1984. Concurrent measurements of stem density, leaf and stem water potential, stomatal conductance and cavitation on a sapling of *Thuja occidentalis* L. Plant, Cell and Environment 7: 615-618.

Franks, P.J., A. Gibson & E.P. Bachelard 1995. Xylem permeability and embolism susceptibility in seedlings of *Eucalyptus camaldulensis* Dehnh. from two different climatic zones. Australian Journal of Plant Physiology 22: 15-21.

Habermehl, A. 1982. A new non-destructive method for determining internal wood condition and decay in living tree: results and further developments. Arboricultural Journal 6: 121-130.

Hacke, U. & J.J. Sauter 1995. Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in *Fagus sylvatica, F. purpurea* and *Populus balsamifera*. Journal of Experimental Botany 46: 1177-1183.

Hacke, U. & J.J. Sauter 1996. Drought induced xylem dysfunction in petioles, branches and roots of *Populus balsamifera* (L.) and *Alnus glutinosa* (L.) Gaertn. Plant Physiology 111: 413-417.

Jackson, G.E. & J. Grace 1996. Field measurements of xylem cavitation: Are acoustic emissions useful? Journal of Experimental Botany 47: 1643-1650.

Jackson, G.E, J. Irvine and J. Grace 1995a. Xylem cavitation in Scots pine and Sitka spruce saplings during water stress. Tree Physiology 15:783-790.

Jackson G.E., J. Irvine & J. Grace 1995b. Xylem cavitation in two mature Scots pine forests growing in a wet and a dry area of Britain. Plant, Cell and Environment 18: 1411-1418.

Jaquish, L.L. & F.W. Ewers 2001. Seasonal conductivity and embolism in the roots and stems of two clonal ring-porous trees, *Sassafras albidum* (Lauraceae) and *Rhus typhina* (Anacardiaceae). American Journal of Botany 88: 206-212.

Kavanagh, K.L., B.J. Bond, S.N. Aitken, B.L. Gartner & S. Knowe 1999. Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. Tree Physiology 19: 31-37.

Kolb, K.J., J.S. Sperry & B. Blamont 1996. A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems. Journal of Experimental Botany 47: 1805-1810.

Lo Gullo, M.A & S. Salleo 1991. Three different methods for measuring xylem cavitation and embolism: A comparison. Annals of Botany 67: 417-424.

Lo Gullo, M.A., A. Nardini, S. Salleo & M.T. Tyree 1998. Changes in root hydraulic conductance of *Olea oleaster* seedlings following drought stress and irrigation. New Phytology 140: 25-31.

McCully, M.E. 1999. Root xylem embolisms and refilling: relation to water potentials of soil, roots and leaves and osmotic potentials of root xylem sap. Plant Physiology 119:1001-1008.

Milburn, J.A. & R.P.C. Johnson 1966. The conduction of sap. II. Detection of vibrations produced by sap cavitation in *Ricinus* xylem. Planta 66:43-52.

Nardini, A. & S. Salleo 2000. Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? Trees 25: 14-24.

Nardini, A., M.T. Tyree & S. Salleo 2001. Xylem cavitation in the leaf of *Prunus laurocerasus* and its impact on leaf hydraulics. Plant Physiology 125: 1700-1709.

Oosterhuis, D.M., M.J. Savage & S. Walker 1983. Field use of *in situ* leaf psychrometers for monitoring water potential of a Soybean crop. Field Crops Research 7: 237-248.

Pammenter, N.W. & C. Vander Willigen 1998. A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. Tree Physiology 18: 589-593.

Ratkovic, S. & G. Bacic 1993. NMR and water transport in plants. In: Water Transport in Plants Under Climatic Stress. (Borghetti, M. A. Raschi, J. Grace Eds). pp 129-139. Cambridge University Press.

Raschi, A., R. Tognetti, H.W. Ridder & C. Beres 1995. Water in the stems of sessile oak (*Quercus petraea*) assessed by computer tomography with concurrent measurements of sap velocity and ultrasound emission. Plant, Cell and Environment 18: 545-554.

Salleo, S. & M.A. Lo Gullo 1989. Different aspects of cavitation resistance in *Ceratonia siliqua*, a drought-avoiding mediterranean tree. Annals of Botany 64: 325-336.

Salleo, S. & M.A. Lo Gullo 1993. Drought resistance strategies and vulnerability to cavitation of some Mediterranean sclerophyllous trees. In: Water Transport in Plants under Climatic Stress (Borghetti, M., J. Grace & A. Raschi Eds). pp. 99-113. Cambridge University Press, Cambridge.

Salleo, S., A. Nardini, F. Pitt & M.A. Lo Gullo 2000. Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.). Plant, Cell and Environment 23: 71-79.

Savage, M.J., H.H. Wiebe & A. Cass 1984. Effect of cuticular abrasion on thermocouple psychrometric *in situ* measurement of leaf water potential. Journal of Experimental Botany 35: 36-42.

Scholander, P.G., H.T. Hammel & E.D. Bradstreet 1965. Sap pressure in vascular plants. Science 148: 339-346.

Schultz, H.R. & M.A. Matthews 1988. Resistance to water transport in shoots of *Vitis vinifera* L. relation to growth at low water potential. Plant Physiology 88: 718-724.

Sparks, J.D. & R.A. Black 1999. Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. Tree Physiology 19: 453-459.

Sperry, J.S. 1993. Winter embolism and spring recovery. In: Water Transport in Plants under Climatic Stress (Borghetti, M., J. Grace & A. Raschi Eds). pp. 86-98. Cambridge University Press, Cambridge.

Sperry, J.S. & M.T. Tyree 1988. Mechanism of water stress-induced xylem embolism. Plant Physiology 88: 581-587.

Sperry, J.S. & M.T. Tyree 1990. Water stress induced xylem embolism in three species of conifers. Plant, Cell and Environment 13: 427-436.

Sperry, J.S. & N.Z. Saliendra 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant, Cell and Environment 17: 1233-1241.

Sperry, J.S., J.R. Donnelly & M. T. Tyree 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell and Environment 11: 35-40.

Sperry, J.S., N.Z. Saliendra, W.T. Pockman, H. Cochard, P. Cruiziat, D. Davis, F.W. Ewers & M.T. Tyree 1996. New evidence for large negative xylem pressures and their measurement by the pressure chamber method. Plant, Cell and Environment 19: 427-436.

Tausend, P.C., G. Goldstein & F.C. Meinzer 2000. Water utilization, plant hydraulic properties and xylem vulnerability in three contrasting coffee (*Coffea arabica*) cultivars. Tree Physiology 20: 159-168.

Tyree, M.T. & F.W. Ewers 1991. The hydraulic architecture of trees and other woody plants. New Phytology 119: 345-360.

Tyree, M.T. & J.S. Sperry 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Plant Physiology 88: 574-580.

Tyree, M.T. & J.S. Sperry 1989a. Vulnerability of xylem to cavitation and embolism. Annual Review of Plant Physiology and Molecular Biology 40: 19-38.

Tyree, M.T. & J.S. Sperry 1989b. Characterization and propagation of acoustic emission signals in woody plants: towards an improved acoustic emission counter. Plant, Cell and Environment 12: 371-382.

Tyree, M.T., S.D. Davis & H. Cochard 1994. Biophysical perspectives of xylem evolution: Is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? IAWA Journal 15: 335-360.

Tyree, M.T. & M.A. Dixon 1983. Cavitation events in *Thuja occidentalis* L.? Plant Physiology 72:1094-1099.

Tyree, M.T., M.A. Dixon, E.L. Tyree & R. Johnson 1984. Ultrasonic acoustic emissions from the sapwood of Cedar and Hemlock. Plant Physiology 75: 988-992.

Utsumi, Y. Y., Sano, R. Funada, & J. Ohtani 1999. The progression of cavitation in early wood vessels of *Fraxinus mandshurica* var *japonica* during freezing and thawing. Plant Physiology 121: 897-904.

Vander Willigen, C. & N.W. Pammenter 1998. Relationship between growth and xylem hydraulic characteristics of clones of *Eucalyptus* spp. at contrasting sites. Tree Physiology 18: 595-600.

Vander Willigen, C., H.W. Sherwin & N.W. Pammenter 2000. Xylem hydraulic characteristics of subtropical trees from contrasting habitats grown under identical environmental conditions. New Phytology 145: 51-59.

Zimmermann, M.H. 1983. Xylem Structure and the Ascent of Sap. Springer-Verlag, Berlin. pp 1-99.