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Ultrasound emission after cycles of water stress in *Picea abies*

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Summary

The relationships among rate of ultrasound acoustic emission (AE), xylem water potential and transpiration rate were investigated in 5-year-old potted saplings of *Picea abies* Karst. after cycles of water stress. Water-stressed plants displayed minimum xylem water potentials of -3.9 MPa, near-zero transpiration rates and up to 45 AE counts per minute. After rewatering, water-stressed plants no longer produced AEs. Well-watered control plants produced only a small number of ultrasonic AEs. After three cycles of water stress (lasting 24 days in total) it was estimated that about two-thirds of the functional tracheids were embolized. The concomitant reduction in hydraulic conductance was about 70%.

Introduction

It has been proposed that cycles of water stress predispose plants to drought as a result of cavitation of the xylem water columns, leading to loss of hydraulic conductivity (Milburn 1979, Peña and Grace 1986). Cavitation may be detected acoustically with a suitable detector. Milburn's original detector operated at audio frequencies and so was susceptible to interference from a variety of external sound sources (Milburn and Johnson 1966). Recently, sensors operating at ultrasonic frequencies have been employed to overcome this problem; and it is generally held that each acoustic emission (AE) is produced by the breakage of a water column in one tracheid (Tyree and Dixon 1983, Tyree et al. 1984*a*, Sandford and Grace 1985). Production of ultrasonic AEs has been demonstrated in several conifers, but the technique has rarely been used under field conditions.

This paper reports on ultrasonic acoustic emissions from the stems of potted saplings of *P. abies* Karst. growing near Firenze, Italy, during the summer, and their relationship with conductance, transpiration rate and plant water potential. Water stress may severely limit production of *P. abies* in a Mediterranean climate, and it is hoped that the detection of cavitation by the recording of ultrasonic AEs will, in the future, be useful in studies on trees in the forest.

Materials and methods

Plant material

Seedlings from an alpine seed source (Val di Fiemme, Province of Trento) were grown in the nursery "La Piana" at Camporgiano (province of Lucca, Tuscany) since 1982. In 1986, they were transplanted to 3-liter plastic pots filled with a mixture of acid brown soil, peat and fine gravel. In 1987, twenty 5-year-old plants were chosen for uniformity. The mean plant height ± SD was 0.50 ± 0.05 m, the mean overbark and the mean underbark stem diameter were 1.14 ± 0.18 cm and 0.90 ± 0.12 cm, respectively, and the mean projected leaf area was 0.14 ± 0.03 m^2. Ten control plants were irrigated daily. Another 10 plants were irrigated daily except during the periods July 15–21, August 8–14, and August 16–25, 1987, when no water was supplied.

Measurements

Xylem water potential was measured with a pressure chamber (Ditta Giudice, Pisa, Italy) approximately every two hours on a single excised shoot taken from each of four plants of each treatment. The remaining plants in each treatment were used to measure acoustic emissions (AE) and transpiration rates. Stomatal conductances were measured at irregular intervals with a Li-Cor 1600 porometer (Li-Cor, Lincoln, NE). To measure acoustic emission rates, a Bruel and Kjaer (DK-2859, Naerum, Denmark) model 8312 broad-band sensor was pressed against the stem of each plant for 5 minutes in turn with a spring-loaded holder that applied a constant force. The stem had previously been prepared by removing a small "window" of bark to expose the xylem, and coating this exposed area with petroleum jelly to prevent water loss, and then with ultrasound gel (Parker, New Jersey, USA) to improve acoustic transmission from the xylem to the sensor. The signals were counted with an instrument similar to that described by Sandford and Grace (1985). However, a four-pole Chebyshev high-pass filter was used to obtain a sharper reduction of the response below 0.1 MHz. The threshold setting was adjusted so that background counts did not occur even over many hours.

Transpiration rate was measured gravimetrically by weighing the plants to the nearest 0.1 g. The pots were enclosed in plastic bags to prevent water loss from the soil, the bags were tied around the stems, and weighed every 2 hours. Leaf area was determined at the end of the experiment on a subsample of needles with a Li-Cor 3000 area meter (Li-Cor, Lincoln, NE).

Wood anatomy was investigated using fresh material from the experimental plants. Tangential slices, 0.3 mm thick, were cut with a microtome and macerated in a (1/1 v/v) solution of 10% aqueous nitric acid and 10% aqueous chromic acid, for about 2 weeks at room temperature. After this treatment, individual tracheids were readily observed in the suspension. The length and width of the tracheids were measured with a light microscope, after staining with methylene blue.

Site

The experiment took place at the nursery site of the Forestry Faculty, Firenze (Lat.

43°47′ N, Long. 11°40′ E, altitude 40 m). The meteorological data for the days during which intensive measurements were made came from a meteorological station located on the same site.

Results

All measurement days were bright and warm, with shortwave radiation exceeding 600 W m^{-2}, vapor pressure deficit up to 2 or 3 kPa and daytime air temperatures that were seldom less than 20 °C. Wind speeds (2 m aboveground) were variable but never particularly high (Figure 1).

A comparison was made between results obtained with the apparatus described above and that used in Edinburgh by Sandford and Grace (1985). At intervals throughout the day, 5-min counts were made first with the Firenze equipment and then with the Edinburgh equipment. The instruments did not agree in absolute count rate (Figure 2), which is probably explained by a difference between sensors in spectral response (Figure 3); although they differed also in sensitivity, background noise level, and in the pressure exerted by the spring-loaded holder used for clipping

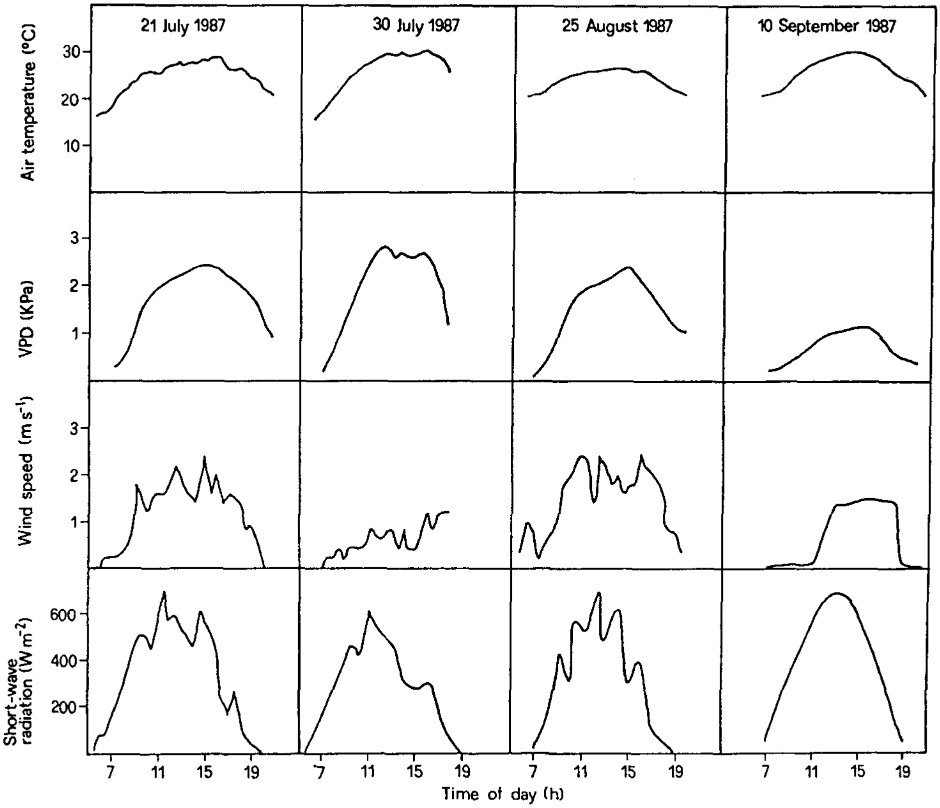

Figure 1. Meteorological conditions during the days of measurement.

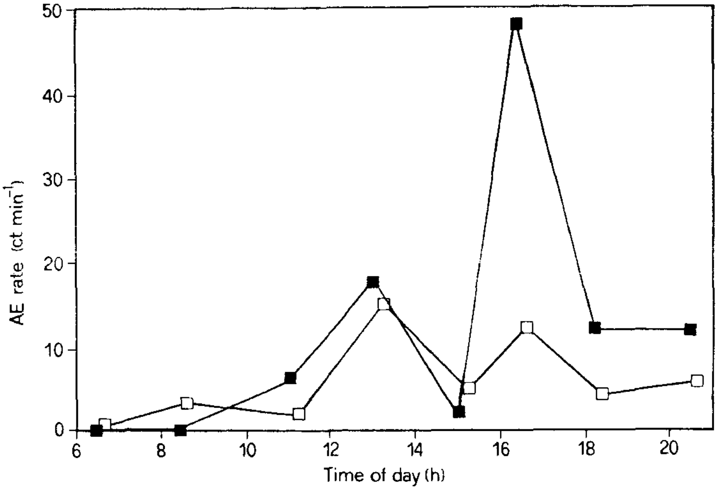

Figure 2. Ultrasonic acoustic emissions recorded by the Firenze (■) and the Edinburgh (□) equipment on July 21, 1987.

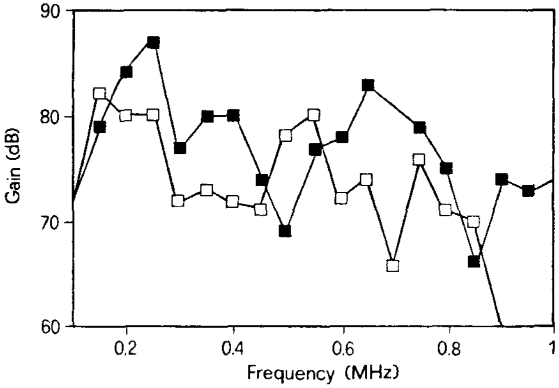

Figure 3. Spectral responses of the Bruel and Kjaer broad-band sensor used in the Firenze (■) and in the Edinburgh (□) equipment.

the sensors to the stem. The Firenze equipment usually gave the highest AE count rates, but coincident peaks and troughs were evident. During the diurnal time course (Figure 2), the correlation coefficient between the results with the two sensors was 0.73, $P < 0.05$.

On July 21, 1987, when the water-stressed plants had experienced seven days without irrigation, the minimum water potential of the control plants was −2.0 MPa, whereas that of the water-stressed plants was less than −2.0 MPa for the entire day, with predawn readings of −2.5 MPa (Figure 4a). Stomatal conductance was too low to measure with the porometer (Figure 4b), and transpiration was very low (Figure 4c). Acoustic emissions from the water-stressed plants continued from 0900 to 1700 h

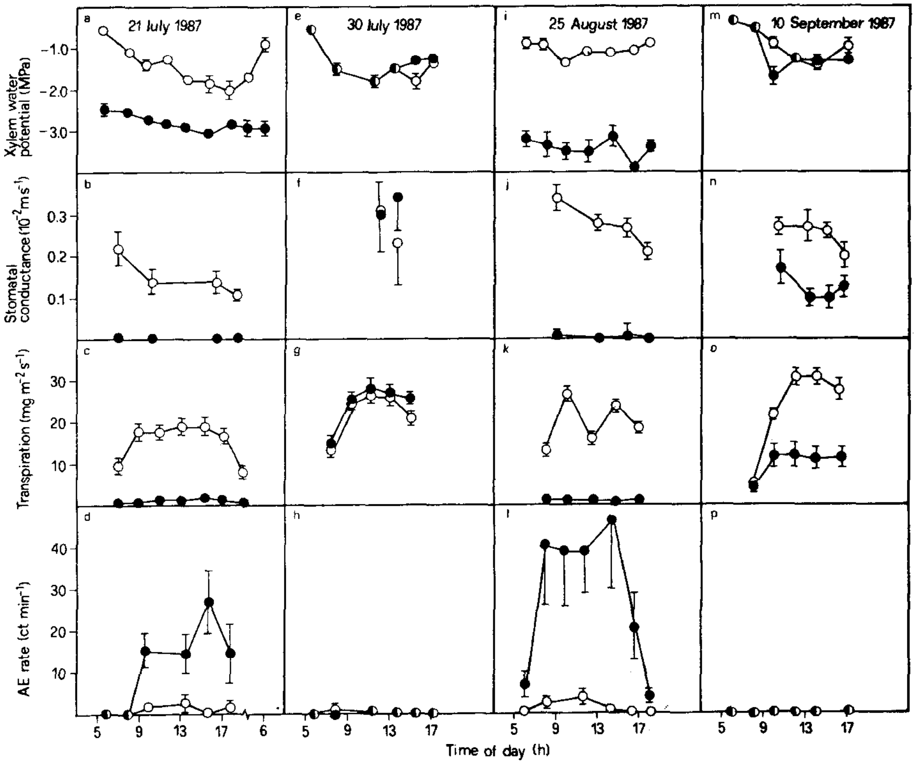

Figure 4. Diurnal variation in xylem water potential, stomatal conductance, transpiration rate and ultrasonic acoustic emissions; ○, well-watered plants; ●, water-stressed plants (following a drought, July 21 and August 25, and after rewatering, July 30 and September 10); ◐, overlapping data points. Bars are standard errors.

with maximum rates of up to 26 counts per minute, whereas control plants produced less than 5 AEs per minute (Figure 4d).

The water-stressed plants were then watered until July 30, 1987 by which time they had recovered from the previous withholding of water as indicated by their water potentials, stomatal conductances and transpiration rates which did not differ statistically from those of control plants (Figures 4e–g). There were only a few acoustic emissions in both treatments (Figure 4h).

Between July 30, 1987 and August 25, 1987 water-stressed plants were subjected to two more cycles of drought before being remeasured on August 25, 1987. At this time, the predawn water potential of water-stressed plants was −3.2 MPa (Figure 4i). Stomatal conductance and transpiration rates were near-zero and acoustic emissions were apparent as early as 0530 h and were as high as 45 per minute (Figures 4j–l). The control plants had acoustic emission rates of less than 5 per minute.

After the last drought treatment, all plants were watered daily until September 10, 1987, when highly significant differences remained in water potentials, stomatal conductances, and rates of transpiration between control plants and those previously water-stressed, even though the "recovery period" was of the same duration as that following earlier water-stress treatments. Previously water-stressed plants differed from control plants in diurnal pattern of water potential and had lower transpiration rates and stomatal conductances (Figures 4m–o). Very few acoustic emissions were detected in either water-stressed or control plants (Figure 4p).

If acoustic emissions are produced when xylem water columns cavitate, a relationship might be expected between xylem potential and acoustic emission rate, because low water potential will tend to cause water columns to break more rapidly (Tyree and Dixon 1983, Tyree et al. 1984*b*, Sandford and Grace 1985). Such a relationship, although it was only a weak one, was observed on July 21 and August 25 (Figure 5).

To assess the magnitude of change in hydraulic resistance of the water-conducting system, transpiration rate was plotted against water potential (Figure 6) (Whitehead and Jarvis 1981, Landsberg 1986) and hydraulic resistance estimated from the slope of the curve.

The data did not fit straight lines, as would be expected if the stem were acting as a simple hydraulic resistor. Instead, there was much scatter, implying considerable withdrawal of water from stored reserves located in the xylem or bark tissues (Figure 6). During the drought on July 21 and August 25, the transpiration rates of the water-stressed plants could not be measured accurately, and so no conclusions could be drawn from this case. In the other cases, difficulty arose in applying a capacitance model (cf. Jones 1978) because the transpiration data are not spot readings but are obtained as 2-h integrals. Consequently, we chose to fit a straight line to the data of September 10 to obtain an estimate of the hydraulic conductance. The line of best fit suggests that the hydraulic conductance of the plants which had experienced three cycles of drought was only 28% of that of control plants (the previously water-stressed plants had a conductance ± SE of $0.77 \pm 0.19 \times 10^{-8}$, whereas the control plants had a conductance of $2.73 \pm 0.62 \times 10^{-8}$ m MPa^{-1} s^{-1}).

The mean length and outside diameter of tracheids were 1.10 and 0.017 mm, respectively.

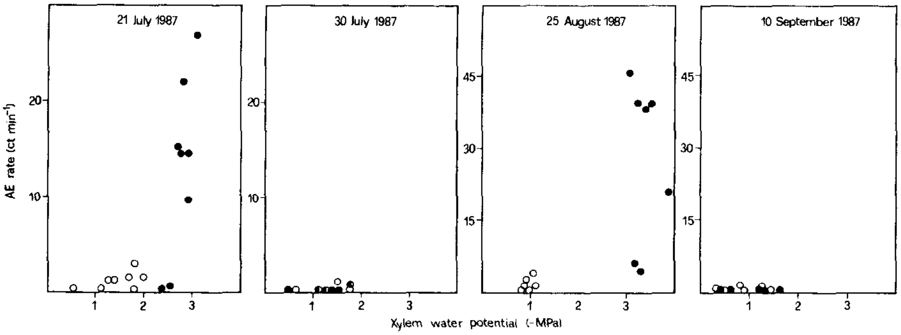

Figure 5. Relationship between ultrasonic acoustic emissions and xylem water potential; O, well-watered plants; ●, water-stressed plants (following a drought, July 21 and August 25, and after rewatering, July 30 and September 10); ◐, overlapping data points.

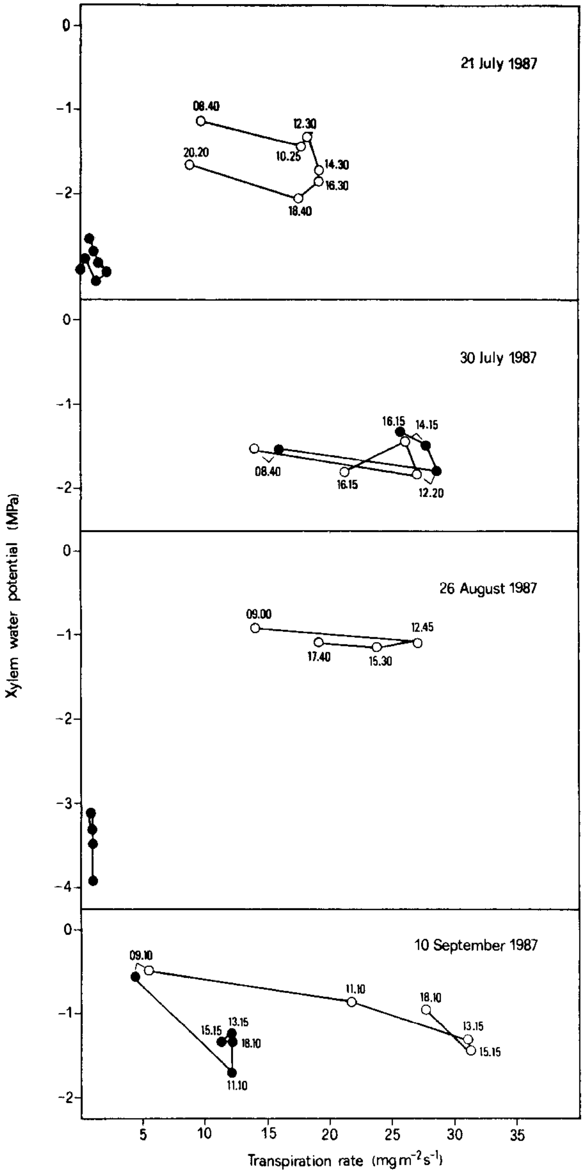

Figure 6. Relationship between transpiration rate and water potential; ○, well-watered plants; ●, water-stressed plants (following a drought, July 21 and August 25, and after rewatering, July 30 and September 10).

Discussion

The interpretation of acoustic emissions has been discussed elsewhere (Milburn and Johnson 1966, Milburn 1973, Milburn and McLaughlin 1974, Dixon et al. 1984, Tyree et al. 1984*a*, 1984*b*, Sandford and Grace 1985, Crombie et al. 1985, Jones and

Peña 1986, Salleo and Lo Gullo 1986, Tyree and Dixon 1986, Tyree et al. 1986, Ritman and Milburn 1988). Most data are consistent with the view that a single emission represents the cavitation of water in one tracheid. However, the counting efficiency is not always high, because the sensor may not detect all acoustic events, some of which may be either too weak, or of a frequency outside the sensor's range. Sandford and Grace (1985) estimated the counting efficiency of the Edinburgh equipment to be about 10%, whereas Tyree and Dixon (1983) have obtained values of 100%.

The rate of acoustic emissions detected in *P. abies* is similar to that found in young *Pinus sylvestris* L. also growing in pots (Peña and Grace 1986). It is much less than the rate obtained in debarked xylem because the latter dehydrates very rapidly (Dixon et al. 1984). In interpreting the extent of cavitation represented by the observed rate, one major unknown is the listening distance of the sensor. It is known that ultrasound is propagated moderately well in wood, but the attenuation varies according to the direction (i.e., anisotropic propagation). In tests on small pieces of wood, Sandford and Grace (1985) came to the conclusion that the sensor detected events occurring within 2 cm of it. If we assume this value, and knowing the dimensions of the tracheids, we estimate that the listening volume contains about 8 million tracheids. On a typical sunny day, such as August 25, 1987, we measured about 0.023 million events. If the counting efficiency is only 10%, this represents 0.23 million cavitations. Thus, quite a significant proportion of the tracheids may be embolized and unavailable for water flow following a drought lasting seven days. An approximation suggests that seven days without water reduces the number of functional tracheids by about 20% if there is no nocturnal refilling. Thus, three cycles of drought, lasting 24 days in total, might reduce the hydraulic conductance by about 69%. An estimate based on the data in Figure 6 suggests a reduction in conductance of 72%.

We did not assess the extent of xylem refilling, but in a similar study, Peña and Grace (1986) found that refilling occurred over 3 days following irrigation. We assume that some refilling must have occurred in the present experiment as well, although the environmental conditions at Firenze were more severe than those during the Edinburgh experiment, with higher VPDs and radiation loads.

The ultrasound sensor is potentially valuable for studying water stress of field plants, and has been used successfully with herbaceous plants (Tyree et al. 1986). Further development is, however, desirable if the data are to be used in a quantitative manner. We have shown that the two sensors produced different count rates. Although we have been unable to make a comprehensive analysis of the cause of the variation, we suspect that the main sources of variation are the spectral responses of the sensors, the levels of background noise and the clamping pressure exerted by the holder. Further work is required to clarify these points, but in the meantime it seems that the development of ultrasound sensing in woody plants has progressed to a point where it might be used to control irrigation.

Acknowledgments

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References

Crombie, D.S., M.F. Hipkins and J.A. Milburn. 1985. Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. Austr. J. Plant Physiol. 12:445–454.

Dixon, M.A., J. Grace and M.T. Tyree. 1984. Concurrent measurements of stem density, leaf and water potential, stomatal conductance and cavitation on a shoot of *Thuya occidentalis* L. Plant Cell Environ. 7:615–618.

Jones, H.G. 1978. Modelling diurnal trends of leaf water potential in transpiring wheat. J. Appl. Ecol. 15:613–626.

Jones, H.G. and J. Peña. 1986. Relationships between water stress and ultrasound emission in apple. J. Exp. Bot. 37:1245–1254.

Landsberg, J.J. 1986. Physiological ecology of forest production. Academic Press, New York and London, 198 p.

Milburn, J.A. 1973. Cavitation studies on whole *Ricinus* plants by acoustic detection. Planta 112:333–342.

Milburn, J.A. 1979. Water flows in plants. Longman, London, 225 p.

Milburn, J.A. and R.P.C. Johnson. 1966. The conduction of sap. II. Detection of vibrations produced by sap cavitations in *Ricinus* stem. Planta 69:43–52.

Milburn, J.A. and M.E. McLaughlin. 1974. Studies of cavitation in isolated vascular bundles and whole leaves of *Plantago major* L. New Phytol. 73:861–871.

Peña, J. and J. Grace. 1986. Water relations and ultrasound emissions of *Pinus sylvestris* before, during and after a period of water stress. New Phytol. 103:515–524.

Ritman, K.T., and J.A. Milburn. 1988. Acoustic emission from plants: ultrasonic and audible compared. J. Exp. Bot. 39:1237–1248.

Salleo, S. and M.A. Lo Gullo. 1986. Xylem cavitation in nodes and internodes of whole *Chorisia insignis* plants subjected to water stress. Relations between xylem conduit size and cavitation. Ann. Bot. 58:431–442.

Sandford, A.P. and J. Grace. 1985. The measurement and interpretation of ultrasounds from woody stems. J. Exp. Bot. 36:298–311.

Tyree, M.T. and M.A. Dixon. 1983. Cavitations events in *Thuya occidentalis*? Ultrasonic acoustic emissions from the sapwood can be measured. Plant Physiol. 72:1094–1099.

Tyree, M.T. and M.A. Dixon. 1986. Water stress induced cavitation and embolism in some woody plants. Physiol. Plant. 66:397–405.

Tyree, M.T., M.A. Dixon and R.G. Thompson. 1984*a*. Ultrasonic acoustic emissions from the sapwood of *Thuya occidentalis* measured inside a pressure bomb. Plant Physiol. 74:1046–1049.

Tyree, M.T., M.A. Dixon, E.L. Tyree and R. Johnson. 1984*b*. Ultrasonic acoustic emissions from the sapwood of cedar and hemlock. An examination of three hypothesis regarding cavitations. Plant Physiol. 75:988–992.

Tyree, M.T., E.L. Fiscus, S.D. Wullschleger and M.A. Dixon. 1986. Detection of xylem cavitation in corn under field conditions. Plant Physiol. 82:597–599.

Whitehead, D., and P.G. Jarvis. 1981. Coniferous forest and plantations. *In* Water Deficits and Plant Growth, Vol. VI. Woody Plant Communities. Ed. T.T. Kozlowski. Academic Press, New York, pp 49–152.