

## Formation and seasonal occurrence of xylem embolism in *Alnus cordata*

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### Summary

We investigated the vulnerability of xylem to embolism and the seasonal occurrence of xylem embolism in Italian alder (*Alnus cordata* Loisel.) by acoustic and hydraulic methods. Wood anatomy was also studied. More than eighty percent of the vessels were less than 50 mm long and no vessels were longer than 120 mm. Mean vessel diameter was 48  $\mu\text{m}$ . Ultrasound acoustic emissions from root and branch segments dehydrating in air followed a similar pattern: in both tissues, emission peaks were recorded when the relative water content of the xylem was around 0.2. In branches dehydrating in air, xylem embolism increased linearly as water potential decreased. In trees in the field, more than 80 percent of hydraulic conductivity was lost in the tree crowns during winter. Recovery from winter embolism occurred mostly before bud burst. In summer, xylem embolism was low (< 30%) and acoustic emissions from roots, stem and branches of trees in the field were also low.

*Keywords:* acoustic emissions, cavitation, hydraulic conductivity, Italian alder.

### Introduction

The processes of cavitation and embolization of xylem conduits in woody plants have been widely studied (see review by Tyree and Sperry 1989). Hypotheses have been advanced to explain the formation and spreading of xylem embolism (Robson et al. 1988, Sperry and Tyree 1988). Non-destructive acoustic techniques have been developed to monitor cavitation processes in the xylem, under both laboratory and field conditions (Milburn and Johnson 1966, Tyree et al. 1984, Sandford and Grace 1985), and methods to measure the extent of xylem embolism in woody stems have been assessed (Sperry et al. 1988a).

Wide consensus now exists that xylem can be considered a vulnerable pipeline (Milburn 1969, Zimmermann 1983) and that xylem embolism is a common condition in woody plants. Results suggest that woody plants often operate near the point of catastrophic xylem failure (Tyree and Sperry 1988), with potentially heavy consequences for plant productivity and survival.

Further information is required about the role of environmental factors on the formation of xylem embolism, its seasonal patterns, and inter- and intra-specific variations in the vulnerability of xylem to embolism, because this feature may represent an important trait that can be used to select for drought and frost resistance in trees.

We have used acoustic and hydraulic methods to investigate the vulnerability of xylem to embolism and the seasonal occurrence of xylem embolism in Italian alder (*Alnus cordata* Loisel.). Wood anatomy was also investigated. Italian alder is a deciduous tree species that is native to a small area in South Italy and Corsica. Because it is considered an important species for afforestation on poor and nitrogen-deficient soils, it is currently the subject of a genetic improvement program in Italy.

## Materials and methods

### *Study site and plant material*

Experiments were performed on 9-year-old Italian alder trees growing in an experimental plantation near Firenze, Italy (43°47' N, 11°40' E; 40 m above sea level).

### *Measurement of vessel lengths and diameters*

The paint infusion method described by Zimmermann and Jeje (1981) and Ewers and Fisher (1989) was used to determine vessel lengths in branches of *Alnus cordata*. Four apical branches, 1 to 1.5 m long, were excised from one tree and their cut ends immersed in water. A green latex paint was diluted (50/50) in water and centrifuged at 1300 g for 2 min. The supernatant was filtered through Whatman No. 3 filter paper to remove all particles larger than 6 µm in diameter and then filtered through a Millipore filter paper to exclude all particles less than 0.2 µm in diameter. The resulting latex emulsion contained particles small enough to pass through the vessel lumen but too large to pass through the inter-vessel pit membrane.

The emulsion was fed to the apical branches at a pressure of 150 kPa for two days. Afterward, 15-mm long segments were cut at regular distances from the point of application and embedded in Histo-resin for 20 h at 50 °C. From each segment, five 7-µm thick sections were cut with a microtome. Paint-filled vessels were counted, with an optical microscope, at 10 different positions across each section, over a surface of 0.4 mm<sup>2</sup>. The diameters of 20 vessels were measured on each of five sections taken from different positions on each branch. In total, 400 vessels were measured.

### *Measurement of acoustic emissions and xylem embolism*

Ultrasound acoustic emissions (UAE) from the xylem were recorded by ultrasonic transducers (PAC I15I) that were clamped on the excised or intact plant organs. Signals from the transducer were amplified by 75 decibels, and logged with a 4615 Drought Stress Monitor (Physical Acoustic Corporation, Princeton, NJ, USA).

Xylem embolism was determined by comparing the hydraulic conductivity of branch segments before and after the removal of air emboli by the flushing method described by Sperry et al. (1988a). Apical branches, 4 to 10 mm in diameter, were cut from trees, wrapped in plastic bags and brought to the laboratory. Segments, 100- to 150-mm long, were excised by cutting the apical branches under water. Anatom-

ical measurements indicated that only 5.6% of the vessels were more than 100 mm in length (Figure 1a).

The apical branch segments were shaved underwater, mounted in rubber tubes and connected to a water reservoir on one side and to an analytical balance on the other side. A solution of ascorbic acid ( $20 \text{ mol m}^{-3}$ ) in distilled, degassed water was allowed to flow through each segment under a positive pressure of 6–10 kPa. Flow rates were recorded gravimetrically by means of the balance and readings were logged continuously by a computer. Hydraulic conductivity ( $k_h$ , mass flow rate per pressure gradient) was calculated as the average of five 1-min measurements after steady state had been reached. Maximum conductivity ( $k_m$ ) was determined after flushing out air emboli from the segments at a pressure of 170–190 kPa for 45–90 min. Percentage loss of hydraulic conductivity was computed as  $100(k_m - k_h)/k_m$ .

#### *Acoustic emissions from dehydrating wood segments*

Three branches and three root segments, each 0.2 m long, were excised from one tree. In the laboratory, they were recut under water to a length of 0.1 m, stripped of their bark, allowed to rehydrate in degassed water for one night and weighed (initial weight,  $W_{in}$ ). The segments were then placed on a laboratory bench and allowed to dehydrate in air. Acoustic emissions from the dehydrating segments were measured at regular intervals over 24 h. In turn, the same ultrasonic transducer was clamped on each segment for five min. At the same intervals, the segments were weighed

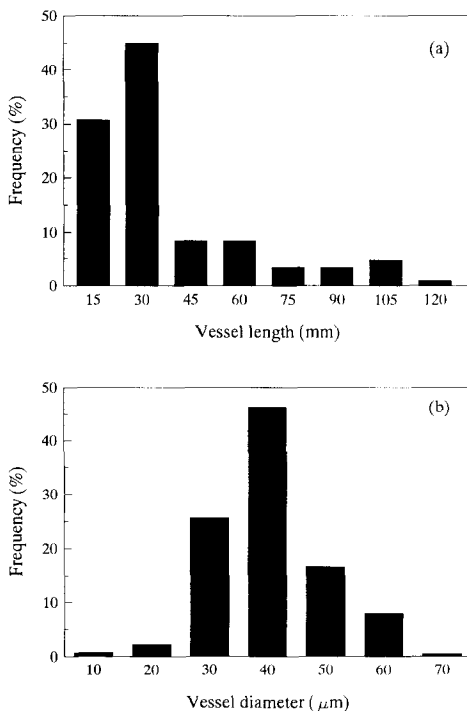


Figure 1. Vessel length distribution (a) and vessel diameter distribution (b) in *Alnus cordata*.

(fresh weight,  $W_f$ ). When acoustic emissions stopped, the segments were oven dried at 80 °C for 48 h, and weighed again (dry weight,  $W_d$ ). The ratio  $(W_f - W_d)/(W_{in} - W_d)$  was defined as relative water content (RWC).

#### *Xylem embolism in dehydrating branches*

The vulnerability of xylem to embolism was determined by measuring the extent of xylem embolism as a function of xylem water potential ( $\Psi$ ) of a dehydrating branch. By this method a vulnerability curve was obtained (see Tyree and Ewers 1991).

Two branches, 2 m long and 30–50 mm in diameter at their base, were cut in the field during the growing season. Branches were recut under water and flushed with distilled degassed water at 150 kPa for 1.5 h. Successively, they were allowed to dehydrate on the laboratory bench. During dehydration,  $\Psi$  was measured at regular intervals with a pressure chamber on three excised shoots from each branch. At the same intervals, the extent of xylem embolism was measured on another two excised shoots from each branch. Ultrasound acoustic emissions were recorded throughout the experiment by clamping four ultrasonic transducers to the main branch axis.

#### *Xylem embolism, acoustic emissions and water flow in the tree*

In the early morning on several dates between March 1991 and September 1992, lateral branches were excised from one “edge” tree in the experimental plantation. Three lateral branches (hereafter defined as external) were excised from the competition-free crown side, and another three lateral branches (hereafter called internal) were excised from the side of the crown in competition with a neighboring tree. Branches were wrapped in plastic bags and immediately brought to the laboratory to measure xylem embolism.

Ultrasound acoustic emissions from the xylem of the stem, roots and branches of the same “edge” tree were also recorded throughout the season. Four ultrasonic transducers were clamped to the stem at a height of 1 m, and two transducers were clamped to the roots. A transducer was also clamped to each of four branches, two in the internal part of the crown and two in the external part of the crown. A small portion (100 mm<sup>2</sup>) of bark was removed to expose the xylem in the area where the transducers were applied. The xylem was coated with silicon grease to prevent water loss from the tissue.

At the time of bud burst, March 16–19, 1992, xylem flow through the trunk and two branches of the same “edge” tree was measured by the thermoelectric heat pulse method, with a custom heat pulse velocity recorder (Soil Conservation Centre, P.O. Box 8041, Palmerston North, New Zealand) (see Borghetti et al. 1993).

## **Results**

Based on the distribution frequency of paint-filled vessels from the point at which the paint was fed to branches, we computed vessel length distribution (Ewers and Fisher 1989). Eighty-three percent of vessels were less than 50 mm long, and only 5.6% of vessels were more than 100 mm in length. No vessels longer than 120 mm













