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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 µm. 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. 1988*a*).

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 Historesin 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^2. 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. (1988*a*). 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. Anatomical 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 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

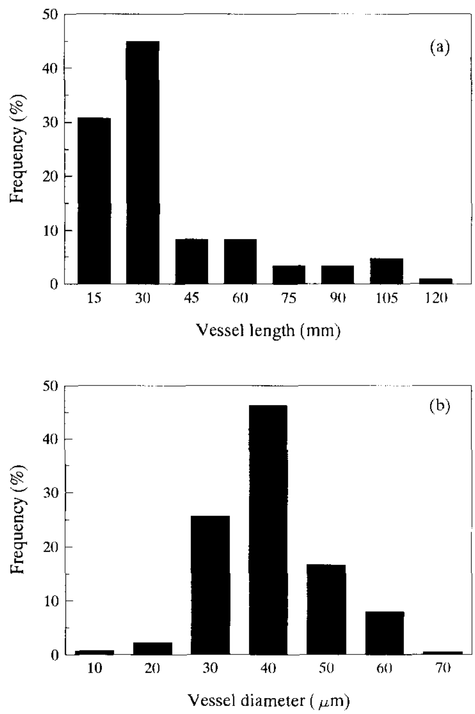

Figure 1. Vessel length distribution (a) and vessel diameter distribution (b) in *Alnus cordata*.

(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 (Ψ) 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, Ψ 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^2) 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 were observed (Figure 1a). Ninety-seven percent of the vessels had a diameter between 30 and 60 μm, and mean vessel diameter was 48 μm (Figure 1b).

Ultrasound acoustic emissions (UAE) from root and branch segments dehydrating in air followed a similar pattern (Figure 2). Ultrasound acoustic emissions started when relative water content (RWC) dropped to 0.7 and 0.6, in branch and root segments, respectively. In both tissues, emission peaks were recorded when RWC was around 0.2, but emissions stopped at RWC ≅ 0.1.

In branches dehydrating in air, xylem embolism, measured as loss of hydraulic conductivity, increased linearly as water potential (Ψ) decreased (Figure 3a). There was a 4% loss of hydraulic conductivity at Ψ = −0.1 MPa and this increased to 80% at Ψ = −2.0 MPa. Acoustic emissions increased in parallel with decreasing water potential (Figure 3b).

Large variations in xylem embolism were observed throughout the season in the tree crown (Figure 4). Before bud burst in 1991, xylem embolism was between 20 and 30%, but it declined after bud burst and remained below 10% until June. In July, xylem embolism increased to 18–19%, but at the beginning of November it was low (10%). After this date, however, there was a rapid and large increase in xylem embolism from 30% at the time of leaf abscission, in mid-November, to 82% on December 10, 1991. During this period, air temperatures were frequently below 0 °C

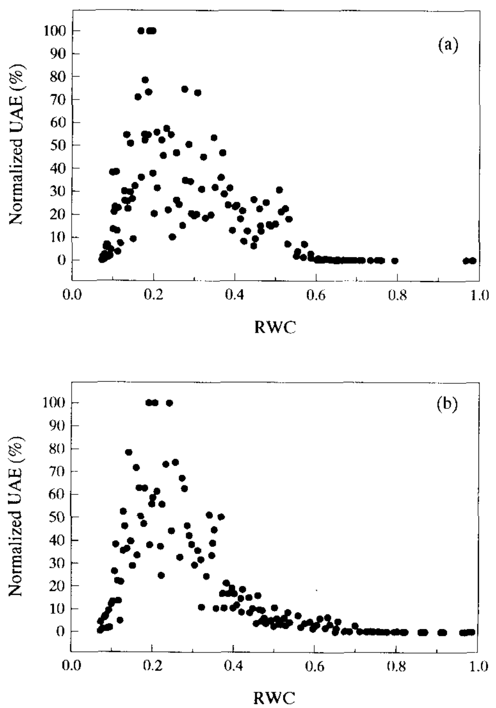

Figure 2. Normalized values of ultrasound acoustic emissions (rate of UAE at a given RWC/peak rate of UAE) as a function of relative water content (RWC), from (a) root and (b) branch segments dehydrating in air.

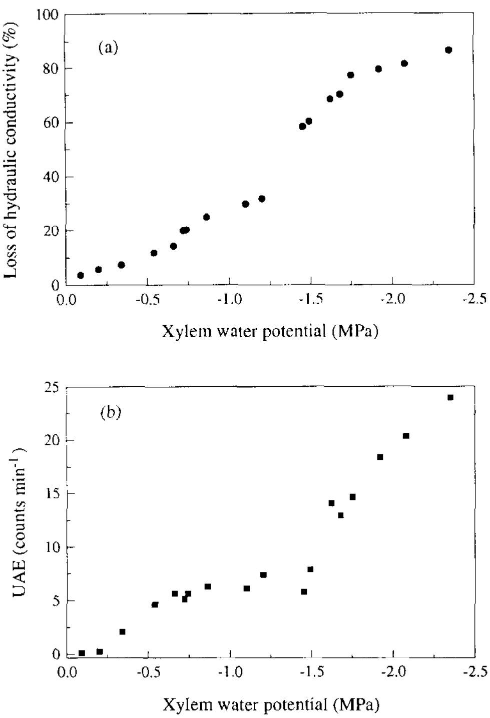

Figure 3. Relations between (a) xylem embolism, expressed as percentage loss of hydraulic conductivity, and xylem water potential; and between (b) ultrasound acoustic emissions (UAE) and xylem water potential, in branches dehydrating in air. Each point represents the average of two xylem embolism and four UAE measurements, for cases (a) and (b), respectively.

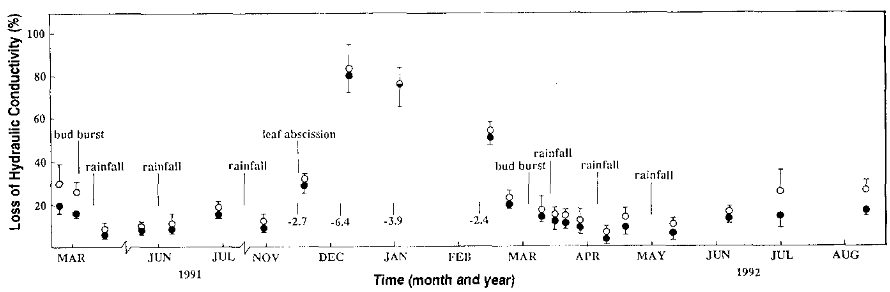

Figure 4. Seasonal changes in xylem embolism, expressed as percentage loss of hydraulic conductivity, in branches located in the internal (●) and external (O) part of the crown. Numbers in the figure indicate minimum air temperature during some cold days in the winter 1991–1992. Dates of bud burst, leaf abscission and main rainy events are indicated. Bars are standard errors (n = 3).

at night (Figure 4). From January to February 1992 embolism declined, but it was still higher than 50% in mid-February. Afterward, a rapid decline was observed, and a few days before bud burst, xylem embolism was about 20%. During the spring of 1992, xylem embolism was low (10%) with a minimum of 5% in April 1992. We observed a slight increase in July and August (Figure 4). In most cases, no significant differences in the extent of xylem embolism were observed between branches sampled from the internal and external parts of the crown.

Daily acoustic emissions from roots, stem and branches in the field were generally low. Different organs were characterized by different emission rates, and in most cases followed the order: roots < stem < branches (Figure 5).

During four days in March 1992 at the time of bud burst and early leaf development, acoustic emissions from the stem and water flow through the stem and one branch were recorded continuously. Water flow ranged between 2 and 9 g m^{-2} s^{-1}, reaching a maximum around midday. The peaks of UAE corresponded with the highest values of water flow (Figure 6).

Discussion

Vessel length distribution in *Alnus cordata* was similar to that found in other diffuse-porous species. No vessels longer than 100 mm were found in *Betula occidentalis* or *Populus tremuloides* (Sperry and Sullivan 1992), whereas vessels up to 200 mm in length were observed in *Acer saccharum* (Zimmermann and Jeje 1981).

The observed patterns of acoustic emission were similar to those found in previous studies (Sandford and Grace 1985, Borghetti et al. 1991). In the RWC range between 1 and 0.7, no acoustic emissions were recorded. This may be because the cavitations produced ultrasound acoustic emissions that were outside the range of the transducer, or because water evaporated first at the cut ends of the severed conduits or from capillary spaces in the xylem.

In excised branches, both xylem embolism and ultrasound acoustic emissions increased linearly with decreasing xylem water potential. No threshold of water potential for the onset of cavitation of xylem conduits was observed.

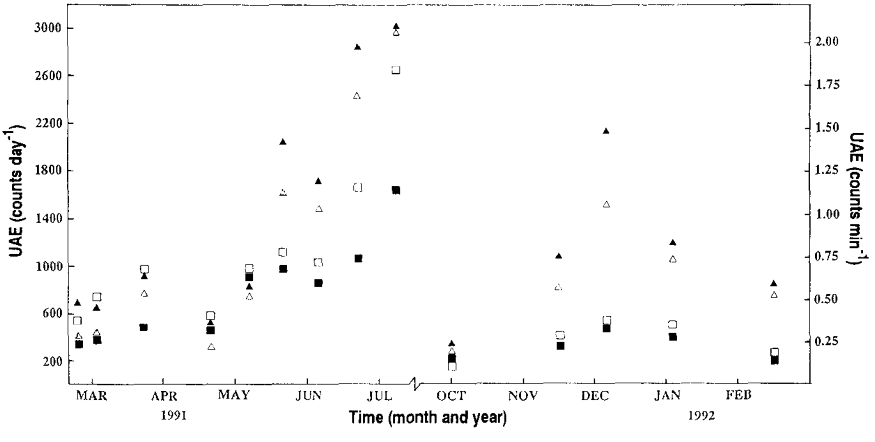

Figure 5. Seasonal patterns of daily ultrasound acoustic emissions (UAE) from branches located in the internal (△) and external (▲) crown part, stem (□) and roots (■).

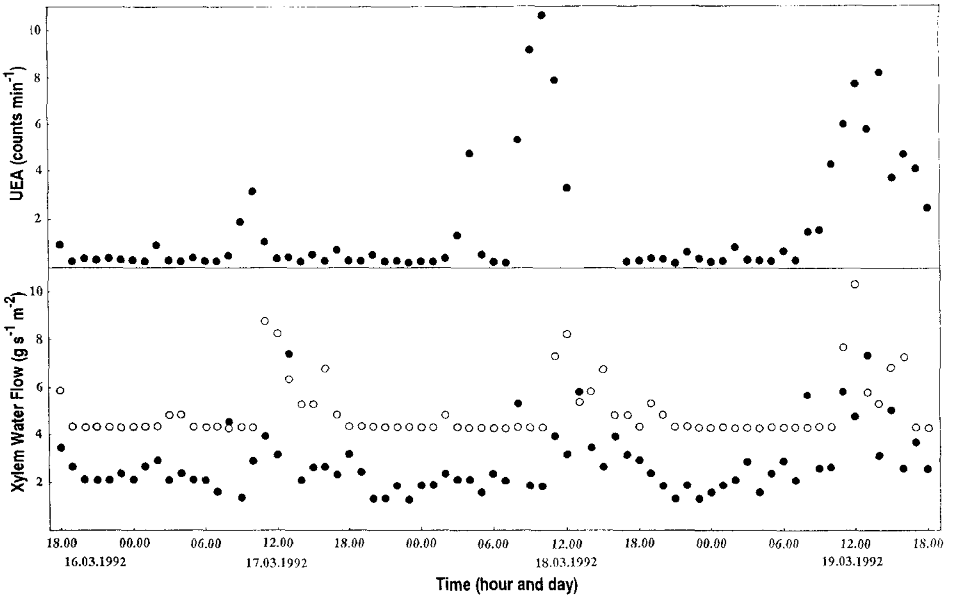

Figure 6. Daily course of xylem water flow through stem and branches and ultrasound acoustic emissions (UAE) in the period March 16–19, 1992.

There is general consensus that a critical pressure difference across the intervessel pit membrane is required to cause aspiration of a gas bubble from an adjacent and already embolized vessel into a water-filled and still functional vessel, causing the vessel to cavitate. The magnitude of this pressure difference is considered to be a function of the interconduit pit membrane structure, namely the dimensions of pores through the membrane (Sperry and Tyree 1988).

The linear relationship between xylem embolism and water potential suggests that vessels cavitating at different water potentials, and therefore showing different vulnerabilities to embolism, coexist in *Alnus cordata*. The degradation of pit membranes as a result of ageing may be responsible for the different vulnerabilities to embolism of vessels produced at different dates throughout the season or in different years (Sperry et al. 1991). Localization of xylem vulnerability may allow cavitation to be a less sudden and catastrophic phenomenon. Cavitation of a proportion of the vessels may cause a localized release of tension in surrounding xylem (Dixon et al. 1984).

Alnus cordata was highly vulnerable to embolism, and more than 80% of hydraulic conductivity in detached branches was lost at a water potential of −2.0 MPa. These results agree with previous findings on the response of *A. cordata* to drought (Borghetti et al. 1989). Sperry and Sullivan (1992) demonstrated that the vulnerability of xylem to water stress-induced embolism is not correlated with conduit volume, indicating that, small vessels, like those found in *Alnus*, do not necessarily assure a good trade-off between xylem efficiency and vulnerability to cavitation.

High levels of xylem embolism were observed during winter in *A. cordata*. Sperry et al. (1988*b*), Sperry and Sullivan (1992) and Sperry (1993) measured similar levels of winter xylem embolism in species with similar xylem structure, e.g., *Betula occidentalis, Betula cordifolia, Fagus grandifolia, Acer saccharum* and *Populus tremuloides*. Freeze-thaw cycles in the xylem and ice sublimation from xylem vessels may both contribute to the formation of xylem embolism during winter.

In 1992, Italian alder recovered quickly from winter embolism before the onset of bud burst. The capacity for early recovery of xylem functionality, ensuring a prompt bud burst, may be an important evolutionary feature (Wang et al. 1992). It may be a consequence of the production of new vessels, because cambial activity can precede bud burst and spring growth (Kramer and Kozlowski 1979); or it may involve the development of root pressure as a result of increases in soil temperature and soil water in late winter due to heavy rainfall and large radiation loads.

A low (< 30%) level of xylem embolism was found throughout the summer, which coincided with the low rates of ultrasound acoustic emission recorded in the field during the growing season. The lateness of leaf senescence in this tree may be a consequence of the maintenance of functional xylem during the summer. Wang et al. (1992) also observed a close relationship between xylem embolism and foliar phenology.

Acoustic emission rates recorded in the field suggest that vulnerability of xylem to embolism may differ among the different plant organs. As expected (Zimmermann 1983), apical branches seem more vulnerable to water stress-induced embolism than the stem or roots.

In trees in the field, the peak rate of sap flow through the xylem coincided with the peak rate of ultrasound acoustic emissions. Because acoustic emissions are diagnostic of cavitation, xylem tension, which controls sap flow rate, can be considered the critical variable linking the two events (cf. Borghetti et al. 1993).

Acknowledgments

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