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Drought-Induced Xylem Dysfunction in Petioles, Branches, and Roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn.

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Variation in vulnerability to xylem cavitation was measured within individual organs of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. Cavitation was quantified by three different techniques: (a) measuring acoustic emissions, (b) measuring loss of hydraulic conductance while air-dehydrating a branch, and (c) measuring loss of hydraulic conductance as a function of positive air pressure injected into the xylem. All of these techniques gave similar results. In *Populus*, petioles were more resistant than branches, and branches were more resistant than roots. This corresponded to the pattern of vessel width: maximum vessel diameter in 1- to 2-year-old roots was 140 μm, compared to 65 and 45 μm in rapidly growing 1-year-old shoots and petioles, respectively. Cavitation in *Populus* petioles started at a threshold water potential of −1.1 MPa. The lowest leaf water potential observed was −0.9 MPa. In *Alnus*, there was no relationship between vessel diameter and the cavitation response of a plant organ. Although conduits were narrower in petioles than in branches, petioles were more vulnerable to cavitation. Cavitation in petioles was detected when water potential fell below −1.2 MPa. This value equaled midday leaf water potential in late June. As in *Populus*, roots were the most vulnerable organ. The significance of different cavitation thresholds in individual plant organs is discussed.

Zimmermann (1983) reported that trees show a considerable resistance to water flow in branch junctions and petioles. He concluded that pressures are significantly lower (more negative) in leaves and small distal branches than in the main stem, which would confine cavitation to the expendable organs of a tree. Leaf shedding would limit xylem tension and would protect the trunk from serious embolism. This assumption is true if (a) distal plant parts are more vulnerable to cavitation than proximal parts, or (b) the vulnerability to cavitation is about the same in petioles, twigs, and stems and there is a large pressure gradient caused by a high transpiration rate (Tyree et al., 1993).

The vulnerability of different plant organs to cavitation has only recently been investigated, and results are still contradictory. Whereas Tyree et al. (1993) found that petioles of *Juglans regia* were clearly more vulnerable than 1-year-old shoots, Sperry and Saliendra (1994) reported that petioles of *Betula occidentalis* were more resistant to cavitation than branches and trunks. In *Acer saccharum*, small, leaf-bearing stems were more vulnerable than larger (>6 mm diameter) stems (Tyree et al., 1991). Finally, there were no intraspecific differences in the cavitation response of petioles and 1-year-old twigs of three European oak species (Cochard et al., 1992a).

The aim of the present study was to compare vulnerability of petioles, branches, and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. We also measured vessel diameters and in some instances vessel lengths in different plant organs of *Populus* to determine if there is any relationship between vessel volume and vulnerability to cavitation.

MATERIALS AND METHODS

Plant Material and Site

Experiments were carried out on different individuals of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. in the Botanical Garden of Kiel University (Kiel, Germany). Trees of one species were of similar size and age (at least 15 years old). Some *Populus* shoots were collected from plants whose trunk had been cut at breast height in late winter. These trees produced rapidly growing shoots (>1.5 m long and 1 to 1.3 cm in basal diameter) that carried much larger leaves than normally growing twigs. Root segments in *Populus* were cut from shallow roots that occasionally produced new sprouts.

Ψ Measurements

Ψ was measured on leaves (*Populus*) or small twigs (*Alnus*) using a pressure chamber. To estimate the Ψ of 1-year-old twigs in the field, leaves were sealed with reflective aluminum tape 1 d before the measurements to prevent transpiration (Sperry and Saliendra, 1994). Root Ψ was assumed to equal predawn Ψ.

Measurement of AEs as a Function of Ψ (Acoustic Vulnerability Curves)

Branches were collected in the morning and brought to the laboratory for rehydration. An ultrasonic transducer (model I15I, Physical Acoustics, Princeton, NJ) (see Tyree

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Abbreviations: AEs, acoustic emissions; k_h, hydraulic conductivity; Ψ, water potential.

and Sperry, 1989) was attached with a spring-loaded clamp to petioles and exposed wood of branches. Transmission of ultrasound was facilitated by a water-soluble acoustic couplant (Dunegan, Irvine, CA). Ultrasonic AEs were monitored using a model 4615 drought stress monitor (Physical Acoustics) with the total amplifier gain set at 55 dB. Branches were dehydrated on the laboratory bench while the cumulative number of AEs and Ψ were periodically recorded. The detection of AEs was stopped when Ψ reached approximately -3 MPa. This value is associated with an 80 to 90% embolism rate in branches of *P. balsamifera* (Hacke and Sauter, 1995) and *Alnus* (see "Results"). Lower Ψ values were difficult to measure because of the inaccuracy of the pressure chamber as the xylem reached 100% embolism (Sperry et al., 1996). Previous work (Hacke and Sauter, 1995) had shown that AEs are predictive of loss of k_h in *P. balsamifera*.

Measurement of Native Embolism Rate

Branches were harvested in the morning, brought to the laboratory, and recut under water. Segments from these branches were cut under water to avoid the inclusion of vessels embolized during collection. Roots from 1-year-old root sprouts were cut under water by bending the roots into a water-filled tray to minimize the induction of embolism during collection (Alder et al., 1996). Vessels in roots are much larger than in twigs (Zimmermann and Potter, 1982).

Native state embolism rate was expressed as the percent loss of k_h, referring to a maximum value obtained after a series of 100-kPa flushes of measuring solution through segments (Sperry, 1993). We used deionized water (degassed and filtered through 0.2-μm filters) as our measuring solution. Root segments and segments of rapidly growing *Populus* shoots were 12 to 15 cm long, segments of normally growing 1-year-old twigs had a length of 4 to 6 cm, and petiole segments were 4 to 5 cm long.

It was necessary to use a small pressure head (3 kPa) when k_h of root segments was determined, because air may easily be displaced from large vessels open at both ends (Alder et al., 1996).

Hydraulic Vulnerability Curves

The percent loss of k_h can be expressed as a function of the minimum Ψ reached during a dehydration ("vulnerability curve"). We used the dehydration method (Tyree et al., 1992) in excised *Alnus* branches to determine xylem vulnerability. Branches were air-dehydrated for different periods. When a certain Ψ was reached, branches were wrapped in plastic bags that contained wet towels. Shoots were left overnight to allow air to diffuse into cavitated conduits and to promote pressure equilibration. Ψ was re-measured using the pressure chamber, and percent loss of k_h was determined in 3- to 5-year-old and 1-year-old branch segments, as described above.

The same vulnerability curve is obtained whether embolism is induced by dehydration or by positive air pressure injected into the xylem of hydrated stems that are at atmospheric xylem pressure (Cochard et al., 1992b; Sperry and Saliendra, 1994; Jarbeau et al., 1995; Pockman et al., 1995; Alder et al., 1996; Sperry et al., 1996). The air-pressure method, which has been described in detail by Sperry and Saliendra (1994), was used in roots because Ψ of roots could not be measured with the pressure chamber. Briefly, a root segment 21 to 26 cm long and 0.45 to 0.55 cm in basal diameter was inserted into a steel chamber with both ends protruding, and the basal end attached to a supply of filtered (0.2 μm), degassed, and deionized water. k_h through the segment was measured after the portion of segment in the chamber had been subjected to a 10-min pressure treatment. Segments had been flushed prior to the embolism measurements, so the initial value of k_h refers to maximum k_h.

Anatomical Measurements

Vessel lengths were measured following the paint-infusion method (Zimmermann and Jeje, 1981). A 1000:1 water: paint suspension (Royal Sovereign Graphics, London, UK) was filtered through 7-μm filters and was gravity fed into a segment from a 2-m column for >24 h. At the beginning and the end of the paint infusion, a partial vacuum (-60 kPa) was applied to the efflux end of the segment for 5 min to facilitate particle flow. After completion of the paint infusion, the axis was cut into segments of equal length. These were dried, the ends were cut smooth, and paint-filled vessels were counted. In petioles, counting was restricted to the largest vascular bundle. Paint-filled xylem elements that were closer than 5 mm to the influx end of the petiole axis were not counted in order to exclude tracheids. Therefore, vessels shorter than 5 mm are not represented in the distribution.

Vessel diameters were determined using a microscope (Reichert, Vienna, Austria) with a projection screen. Measurements were done in sectors reaching from pith to cambium on $\geq$150 vessels per segment. It was difficult to identify tracheids in cross-sections of petioles. Therefore, measurements were made in cross-sections used in the paint-infusion experiments, and diameter determination was restricted to paint-filled conduits located 5 mm from the influx end of an axis. This excluded tracheids and vessels shorter than 5 mm. Thus, the diameter distribution of petioles may be an overestimation to some extent, i.e. there may be more narrow vessels.

RESULTS

Figure 1 shows variation in vulnerability curves within different organs of *Populus*. AEs were detected when Ψ fell below -0.5 MPa in rapidly growing 1-year-old shoots (0.8-0.85 cm diameter), below -0.8 MPa in normally growing 1-year-old twigs (0.4-0.45 cm diameter), and below -1.1 MPa in petioles. Lowest leaf Ψ observed in the 1994 growing season was -0.9 MPa (Hacke and Sauter, 1995). Based on these data, there was a safety margin of 0.2 MPa between minimum leaf Ψ and the cavitation threshold in petioles. On one sunny afternoon a value of branch Ψ was found that was 0.18 MPa higher than leaf Ψ (Table I).

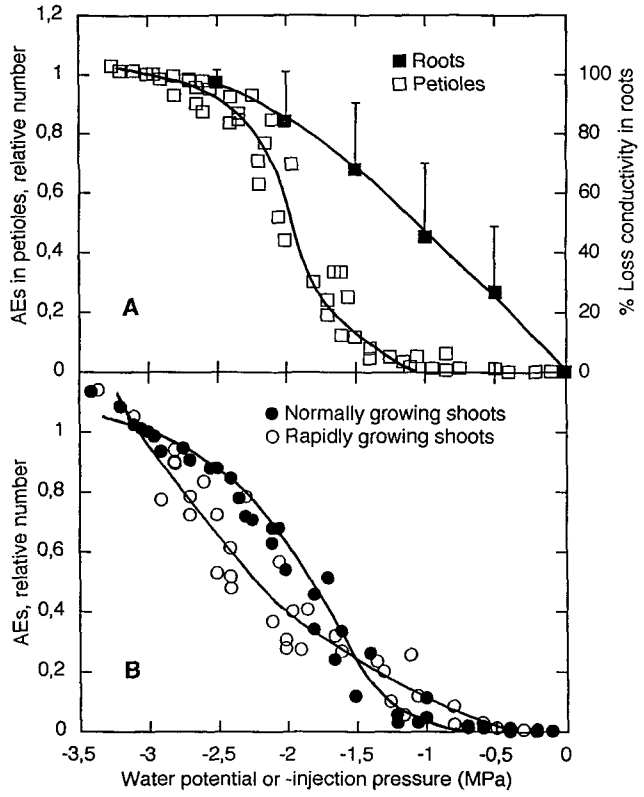

Figure 1. Vulnerability curves of petioles, 1- to 2-year-old roots (A) and 1-year-old branches (B) of *P. balsamifera*. The hydraulic vulnerability curve in A shows the percent loss of k_h as a function of the negative of air pressure injected into the xylem of hydrated root segments. Injection pressures are shown as negative values for comparison with other methods. Means for seven segments are shown + SD. Acoustic vulnerability curves of petioles and twigs express the relationship between relative number of AEs and Ψ. A relative value of 1.0 corresponds to the sum of AEs recorded at a Ψ of −3 MPa. Data are for five replicates.

Assuming minimum branch Ψ to be −0.7 MPa in *Populus*, we predicted a very low embolism rate in branches. Indeed, native embolism rate was <10% in both twigs and petioles (Table I).

Vessels in roots embolized at very low air pressures (Fig. 1A). Despite the high vulnerability, the native embolism rate of roots was only 11%. This was probably due to the high soil Ψ (Table I).

To some degree, vulnerability curves in *Populus* correlated with vessel anatomy. Roots having the widest vessels (140 μm, Fig. 2) were extremely vulnerable to cavitation, whereas higher xylem tensions were necessary to induce cavitation in petioles and normally growing 1-year-old twigs that had a maximum vessel diameter of 45 μm. In rapidly growing 1-year-old shoots, conduit diameter (Fig. 2) as well as the cavitation-inducing Ψ (Fig. 1B) were intermediate. As expected, vessels were significantly shorter in petioles than in long, 1-year-old twigs (Fig. 3). The shape of the vulnerability curves reflected the ranges of vessel diameters, i.e. steep vulnerability curves occurred in petioles and small, 1-year-old twigs, which showed a narrow range of vessel diameters.

Vulnerability curves of *Alnus* branches were obtained by three different techniques. The air-injection technique, the dehydration method, and the acoustic method all gave similar results (Figs. 4, A–C). As in *Populus*, roots were the most vulnerable plant organ in *Alnus* (Fig. 4A). Vulnerability curves of 1-year-old and 3- to 5-year-old branches were indistinguishable (Fig. 4B). Although maximum vessel diameter was only 15 μm in petioles, compared to 40 μm in 3- to 5-year-old internodes, petioles were more vulnerable to cavitation than branches (Fig. 4C). AEs in petioles started at Ψ = −1.2 MPa. Midday Ψ in this species was −1.18 ± 0.04 MPa (mean ± SD, n = 4 d). Midday Ψ was measured on sunny days in June (28-30°C), but it is possible that Ψ was even lower later in the season. This small safety margin from critical cavitation levels in petioles corresponds to the observation that *Alnus* trees at drier locations lost many of their leaves during a summer drought in July and August.

Table I. *Ψ and native embolism rate in different plant organs*

Ψ of leaves, 1-year-old twigs, and roots (= predawn−Ψ) on a sunny day (September 18, 20°C in the afternoon), and native embolism rate in different organs of *P. balsamifera*. Number of measurements is shown in parentheses; values are means ± SD.

Plant Organ	Ψ	Loss of k_h
	MPa	%
Leaves	−0.66 ± 0.05 (4)	8.0 ± 12.9 (6)[a]
One-year-old rapidly growing twigs	n.d.[b]	3.2 ± 5.5 (6)
One-year-old normally growing twigs	−0.48 ± 0.07 (4)	3.3 ± 8.2 (6)
Roots	−0.1 ± 0 (3)	11.1 ± 10.7 (6)

[a] In petioles. [b] n.d., Not determined.

DISCUSSION

Three methods were used to characterize the susceptibility of different plant organs to water-stress-induced cavitation. The air-injection technique, the dehydration

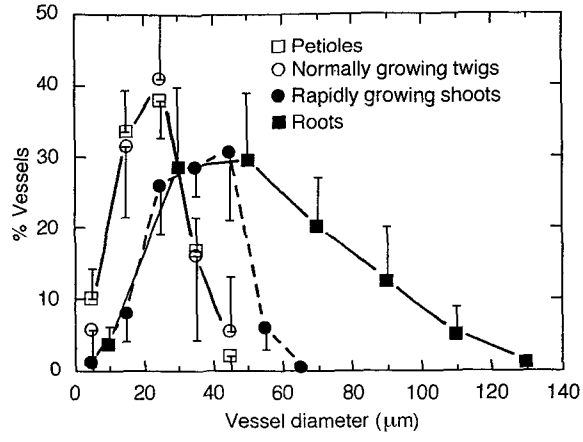

Figure 2. Vessel-diameter distributions in petioles, 1-year-old twigs (10-μm size classes), and 1- to 2-year-old roots (20-μm size classes) of *Populus*. Error bars show ± SD, n = 5 segments.

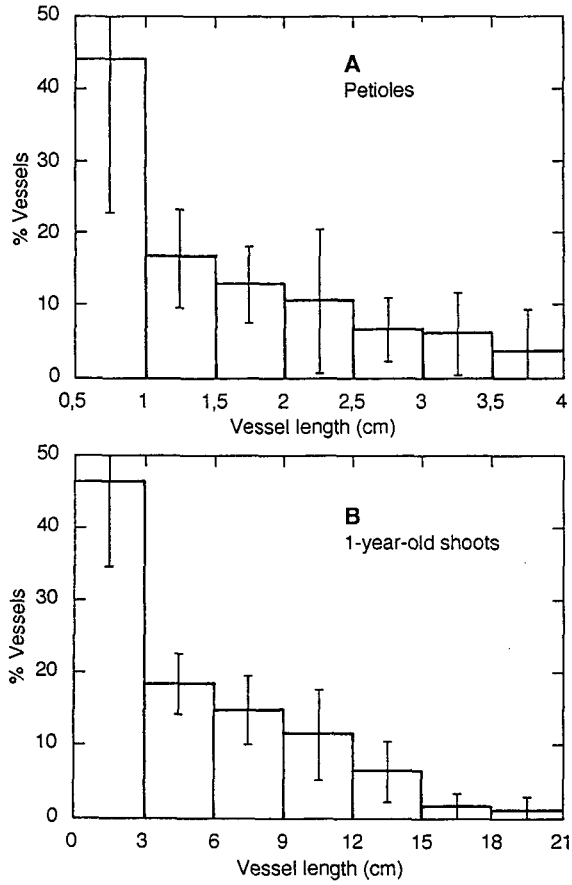

Figure 3. Vessel-length distributions in petioles (A) and rapidly growing 1-year-old shoots (B) of *Populus*. The branch diameter was between 0.8 and 0.85 cm. Error bars show ± SD, $n = 5$.

method, and the acoustic method gave similar results, proving the reliability of the presented vulnerability curves. In both *Populus* and *Alnus*, roots were strikingly more vulnerable to cavitation than branches (Figs. 1 and 4). This is in agreement with previous studies on *Betula occidentalis* (Sperry and Saliendra, 1994), *Acer grandidentatum* (Alder et al., 1996), and *Acer negundo* (U. Hacke and J.S. Sperry, unpublished data). Roots were the most vulnerable plant organ and had the widest vessels (Fig. 2). The typical habitat of the *Populus* and *Alnus* species investigated, however, is characterized by high soil Ψ values. Therefore, high embolism rates are not likely to occur in roots of these species. At our study site, the native embolism rate in *Populus* roots was only 11% (Table I). Moreover, the results of Alder et al. (1996) suggest that embolism reversal does occur in roots of *A. grandidentatum* when conditions are favorable, e.g. during prolonged rain. It is possible that embolism reversal also occurs in roots of *Populus* and *Alnus*, even though root pressure was not observed in *Populus* during the monitoring period in an earlier investigation (Hacke and Sauter, 1996).

In contrast, serious embolism may occur in the root system in drier locations (Alder et al., 1996), which then may limit growth noticeably. When stomata are closed during static (soil drought-induced) water stress, Ψ tends to equilibrate within a plant. Under these conditions, vessels in roots would cavitate first. The segmentation hypothesis (Zimmermann, 1983), which predicts that cavitation will be confined to small, expendable twigs and leaves due to the lower Ψ in these most distal parts of a tree, would not be valid in this case.

Under conditions of dynamic (transpiration-induced) water stress, vessels in petioles of *Alnus* would cavitate prior to conduits in branches, because xylem pressure would be lowest in leaves and because petiole xylem of *Alnus* was more

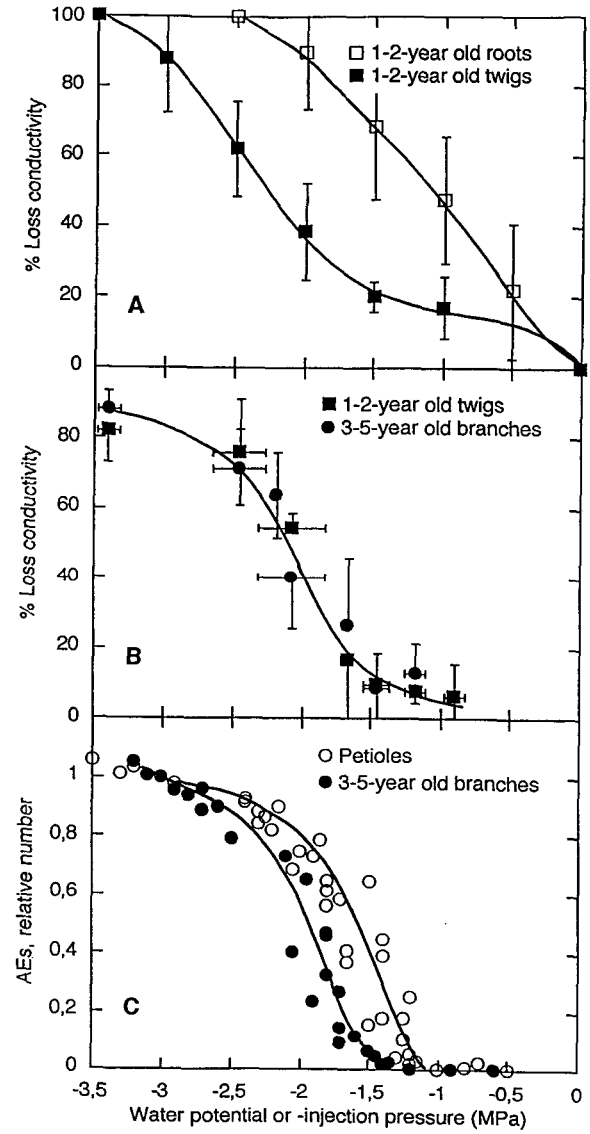

Figure 4. Vulnerability curves of petioles, branches, and roots of *A. glutinosa*. Hydraulic vulnerability curves were obtained by air injection (A) and dehydration (B). Bars denote SD values. Means in A refer to eight root segments and five branch segments. Vertical error bars in B are based on means of four to six segments, and horizontal error bars are based on three to four pressure-chamber measurements. Acoustic vulnerability curves are shown in C. Data are for five replicates. A relative value of 1.0 corresponds to the cumulative number of AEs recorded at a Ψ of −3 MPa.

vulnerable to cavitation than branch xylem (Fig. 4C). This is in agreement with the segmentation hypothesis. In *Populus*, petioles were slightly more resistant than twigs (Fig. 1, A and B). However, differences in vulnerability may be small enough in this species to ensure that petioles would nevertheless cavitate prior to branches when there is a large Ψ gradient between leaves, twigs, and branches.

Although the intraspecific variation in xylem vulnerability to drought-induced cavitation corresponded to the pattern of vessel dimensions in *Populus* (compare Fig. 1 with Figs. 2 and 3), there was no such relationship in *Alnus*, possibly because different trees of one species were compared. It follows that among different individuals there is not necessarily a distinct correlation between vessel dimensions and vulnerability to water-stress-induced cavitation. This is probably because the cavitation threshold is determined by maximum pore diameter in conduit walls (Zimmermann, 1983; Sperry et al., 1996), and a significant correlation between pore diameter and vessel dimension can be expected only within one individual. This view is supported by the results of Sperry and Saliendra (1994), who could demonstrate a significant correlation only between vessel diameter and vulnerability within one individual.

As in *Betula occidentalis* (Sperry and Saliendra, 1994), there was a small safety margin between midday Ψ values and critical cavitation levels; this small safety margin requires a sensitive stomatal control of Ψ. It is likely that xylem vulnerability in *Alnus* and *Populus* is a limiting factor on stomatal conductance and therefore on growth when Ψ approaches low values on hot summer days. It is interesting that both trees showed a highly vulnerable xylem, i.e. cavitation started at moderate xylem tensions. This corresponds to the fact that both species typically grow in moist soils. Trees such as *A. grandidentatum* that show a more resistant xylem (Alder et al., 1996) can maintain a high stomatal conductance over a broad Ψ range without risking a critical loss of hydraulic conductance. It is not yet clear why some trees have a vulnerable xylem. Thus, we need more information about the factors determining xylem vulnerability, i.e. pore diameter in conduit walls.

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