Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation

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ABSTRACT

Trees of *Juglans regia* L. shed leaves when subjected to drought. Before shedding (when leaves are yellow), the petioles have lost 87% of their maximum hydraulic conductivity, but stems have lost only 14% of their conductivity. This is caused by the higher vulnerability of petioles than stems to water-stress induced cavitation. These data are discussed in the context of the plant segmentation hypothesis.

Key-words: Juglans regia; walnut; leaf shedding; drought; plant segmentation.

Abbreviations: E, evaporative flux density $(kg\, m^{-2} s^{-1})$; F, water flow rate $(kg\, s^{-1})$; P, pressure (MPa); PLC, per cent loss of hydraulic conductivity; R, resistance (MPa $m^2 s\, kg^{-1}$); VC, vulnerability curve; Ψ, water potential (MPa); Ψ_{xp}, xylem pressure potential.

INTRODUCTION

Over the past 20 years, our knowledge of the hydraulic architecture of trees has increased and some hypotheses have been raised to explain how trees might be designed hydraulically to help them cope with periods of drought. Hypotheses have generally invoked a mechanism of plant segmentation, i.e. a mechanism that permits plants to shed expendable distal components of its shoots while preserving other parts that represent years of carbon investment. Leaf shedding is a potentially cost-effective way for plants to deal with drought stress by a plant segmentation mechanism. Walnut (*Juglans regia* L.) is known to shed leaves in response to drought. In this paper, we attempt to show that leaf shedding is an example of vulnerability segmentation.

Drought-induced water stress will cause xylem dysfunction by cavitation events in vessels and tracheids (Tyree & Sperry 1989), which ultimately leads to embolisms and loss of hydraulic conductivity in xylem. The vulnerability of xylem to cavitation is measured by the xylem pressure potential, Ψ_{xp}, that induces cavitation; a more vulnerable vessel or tracheid will cavitate at a less negative Ψ_{xp}.

Zimmermann (1983) was fascinated by plant segmentation, which can be defined as any structural feature of a plant that confines cavitation to small, distal, expendable organs in favour of larger organs representing years of growth and carbohydrate investment. Among these features, Zimmermann recognized the value of the decline of leaf-specific conductivity, LSC, with lessening stem diameter and the presence of hydraulic constrictions at stems and leaf junctions. These features will induce more negative Ψ_{xp}s in distal organs than if LSC were constant within the crown or if stems were without junction constrictions. This pattern of Ψ_{xp} distribution could cause more cavitation in distal than in basal components of shoots provided (1) the vulnerability to cavitation is the same in distal and basal components and (2) there is a large evaporative flux density, E, to cause substantial gradients in Ψ_{xp}. More recently, Tyree et al. (1991) defined a new kind of plant segmentation, vulnerability segmentation, that must also be considered. Vulnerability segmentation means that the more distal and expendable structures are more vulnerable to cavitation, i.e. that distal xylem might be 'designed' to cavitate at less negative Ψ_{xp} than basal proximal xylem. Vulnerability segmentation has been reported in *Acer saccharum* stems (Tyree et al. 1991), but we know of no other reports.

MATERIALS AND METHODS

Walnut (*Juglans regia* L. cv. Lara scions on wild walnut root stocks) saplings (1 year old) were transplanted to $200\, dm^3$ pots in 1990 and were grown for 2 years in a soil consisting of 50% clay soil +50% peat moss. Some trees were subjected to non-lethal drought cycles in previous years prior to studies described here. In September 1991, irrigation was withheld from selected trees for 5 d which was sufficient to induce leaf yellowing and leaf shedding (10–20 d later). Control trees were irrigated daily.

Midday leaf water potential, Ψ, was measured with a Scholander-Hammel pressure chamber and stem Ψ was

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measured with a temperature corrected stem hygrometer (Dixon & Tyree 1984) on stems between 1·5 and 2·0 cm diameter. Stem Ψ was measured on current-year shoots by a bagged-leaf method. Leaf and stem Ψ's were measured on control and droughted trees at midday (1200–1500 h) at the first sign of leaf yellowing in trees put through a single drought-stress cycle in September. After leaves yellowed, the trees were returned to a daily irrigation schedule. Midday evaporative flux densities from leaves, E, were computed by measuring stem flow (kg s^{-1}) of a whole tree by a heat balance method (Valancogne & Nasr 1989a,b) and dividing by leaf area of the tree.

Per cent loss of hydraulic conductivities, PLC, were measured on stems and petioles by methods described elsewhere (Sperry, Donnelly & Tyree 1987; Cochard *et al.* 1992a). Briefly, petioles or stem segments were excised under water and connected to a conductivity apparatus. The initial conductivity, K_i, was measured with a pressure difference, P, of 5 kPa, using 1 mol m^{-3} HCl, degassed and filtered through a 0·1 μm filter. The segments were then flushed with a pressure of 100–150 kPa for 15 min to dissolve air bubbles. The hydraulic conductivity of the segments were determined again and the flushing repeated until a maximum conductivity, K_m, was obtained (usually after 1 or 2 flushes). PLC was then calculated from PLC = 100 $(K_m - K_i)/K_m$. These measurements were performed on droughted trees within 2 d of leaf yellowing.

Vulnerability curves, VC, were constructed by dehydrating branches excised from control trees. Excised branches were 1 to 2 m long and were dehydrated either in a large pressure chamber or by bench-top dehydration in the laboratory or greenhouse. Bench-top dehydrations proceeded until desired levels of leaf or stem Ψ were measured with the pressure chamber or stem hygrometer for petioles and stems, respectively. Other branches were dehydrated by pressurizing with compressed air in a pressure chamber for several hours until water stopped exuding from the stem bases that protruded from the pressure chamber. They were returned to atmospheric pressure by reducing the air pressure at a rate of <0·1 MPa min^{-1}. After removal from the pressure chamber, PLC was determined on petioles and stems (current-year and 1-year-old shoots).

Hydraulic resistances to water flow in whole shoots, leaves, petioles, and stems were measured using a high-pressure flow meter described elsewhere (Tyree *et al.* 1993). Briefly, branches 1·5–2 m long and 0·02 m in diameter were cut in the air and immediately immersed in water, and the basal 0·1 m of stem excised to remove some of the air bubbles sucked into the stem during the initial cut. The base of the branch was connected to a potometer system that permitted perfusion of water into the base of the stem at an applied pressure, P, up to 0·2 MPa. Water flow was measured by measuring the pressure drop across a glass capillary tube 0·7 mm in diameter and 0·12 m long. The capillary tube was in series between the source of water under pressure and the base of the stem. The capillary tube had been previously calibrated to give the linear relationship between flow rate and pressure drop

(5·2 × 10^{-6} kg s^{-1} per cm H$_2$O in manometric pressure head). Perfusion continued for about 2 h until leaf air spaces became visibly infiltrated with water and water began to drip out of stomates of some leaves. At this time, a constant flow rate was achieved and the resistance to water flow was calculated from R = A$_L$P/F, where A$_L$ (m^2) is the leaf area and F is the flow rate (kg s^{-1}). Since shoots were perfused under pressure for >2 h, we presume that embolisms were dissolved and that computed resistances are thus less than in the native state. The leaf blades were then excised with a razor blade causing an increase in F and reduction in R. Leaf blade resistance to water flow was then calculated from $R_{leaf} = R_{whole\ shoot} - R_{shoot\ with\ leaves\ removed}$. Petioles, current-year shoots and 1-year-old shoots were removed in succession, and the resistance of the remaining part of the branch was measured after each component was removed. From the difference in resistance before and after removal of each component, the resistances of petioles, current-year and 1-year-old shoots were calculated. Leaf areas were measured with a Delta T leaf area meter (Delta-T Devices Ltd, Cambridge, UK).

RESULTS AND DISCUSSION

When both leaves and stems reached a midday Ψ of −1·9 MPa after 5 d without irrigation, the leaves turned yellow. The predawn Ψs before and after day 5 were −1·6 and −1·9 MPa, respectively, from which we conclude that E must have been low and gradients in Ψ were small within the droughted trees. Irrigated trees had Ψs of −0·7 and −0·5 MPa for leaves and stems, respectively (Table 1). Midday values of evaporative flux density from leaves were 6 × 10^{-5} kg s^{-1} m^{-2} in irrigated trees and five to ten times less in droughted trees.

The PLC in the petioles of droughted trees was 87% compared to 12% in well-watered control trees. The PLC in the stems of droughted and control trees were not significantly different; but the PLC of stems was significanly lower than PLC of petioles in droughted trees (Table 1).

Table 2 shows that there was a substantial hydraulic segmentation. The leaf blades accounted for about 80% of the R of branches 1–1·5 m long and about 0·02 m diameter at the base. Petiole R was about double that of current-year and 1-year-old shoots. The high resistance to water flow in leaves is probably located in the nonvascular parts of the water-flow pathways (Boyer 1985, p. 489; Tyree et al.

Table 1. Ψ of leaves and stems and PLC of petioles and stems of irrigated and drought-stressed walnut trees. Number of samples in brackets; errors are standard errors of the mean for PLC and midday ranges for Ψ

Condition	Midday Ψ, MPa		PLC	
	Leaves	Stems	Petioles	Stems
Irrigated	−0·7 ± 0·1	−0·5 ± 0·1	12 ± 1 (15)	10 ± 6 (10)
Droughted	−1·9 ± 0·1	−1·9 ± 0·1	87 ± 4 (15)	14 ± 3 (10)

Table 2. Resistances (MPa s m^2 kg^{-1}) to water flow though components of walnut branches, and calculated drop in Ψ across each component based on a typical evaporative flux density of 6×10^{-5} kg s^{-1} m^{-2} (for irrigated trees) times the value of the component resistance; for droughted trees, E and thus the computed drop in Ψ would be less by a factor of five to ten. Values of resistance are given as means ± SEM with $n = 5$

Component	R (MPa s m^2 kg^{-1})	$\Delta\Psi$ (MPa)
Leaf blade	6142 ± 588	0·37
Petioles	605 ± 127	0·036
Current-year shoots	300 ± 27	0·018
One-year-old shoots	310 ± 55	0·019
Total	7457 ± 600	0·44

1981; Cruiziat et al. 1980; Tyree & Cheung 1977; Tyree, Caldwell & Dainty 1975).

Figure 1 shows that the VC of stems and petioles of walnut gave an order of vulnerabilities of components of the tree of petiole > current-year shoots > 1-year-old shoots. When petioles reached 90 PLC, the leaf Ψ was approximately −1·9 MPa; at the same Ψ, the stems had lost only about 15% of their maximum hydraulic conductivity. This explains the pattern of PLC between petioles and stems reported in Table 1. This is in contrast to several *Quercus* species where there is no difference in the VC of stems and petioles (Cochard *et al.* 1992a).
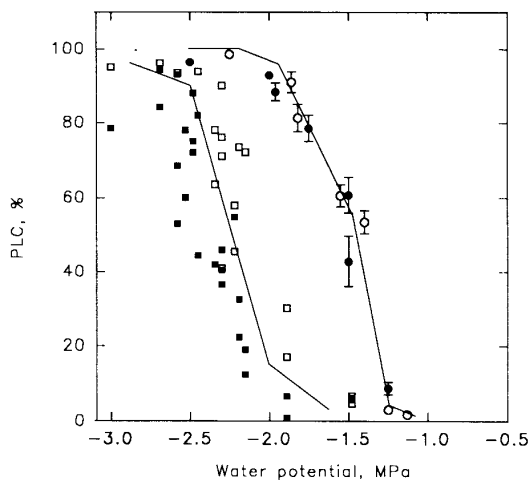
Figure 1. Vulnerability curves of walnut petioles and stems. PLC is the per cent loss of hydraulic conductivity. Open and closed circles are for petioles measured by bench-top and pressure-chamber dehydration, respectively. Error bars are standard errors of the mean ($n = 20$, error bars smaller than symbol size are not shown). Open and closed squares are stem segments of current-year and 1-year-old shoots, respectively. Each point represents an individual shoot. The x-axis gives the minimum Ψ reached based on pressure bomb determination of leaves for petioles and of stem hygrometer readings for stem segments.

Because of the high hydraulic resistance of the leaf blade, we were concerned about whether the Ψ of leaves measured in the pressure bomb (during bench top dehydration) accurately reflected the Ψ obtained in petioles. So we determined the VC for petioles by both bench-top dehydration and by dehydration in a pressure chamber. Figure 1 shows that the VCs obtained by the two techniques were similar. We are confident that the Ψ's will be similar in leaf blades, petioles and stems during pressure chamber dehydration. In previous studies (Tyree, Alexander & Machado 1992; Cochard, Cruiziat & Tyree 1992b), we have shown that very different methods of measuring VCs yield the same results.

Hydraulic segmentation (alone without vulnerability segmentation) will be an effective mechanism to confine embolisms to distal portions of a plant only if hydraulic segmentation causes a large gradient of Ψ_{xp} in shoots, so two conditions must be met simultaneously: There must be significant transpiration (E), and the product of E×R of the distal component (= the drop in Ψ within the component) must be enough to make the PLC in that component range from a low to a high value; for example, 20% at the base to 80% at the apex of the component. Only in that way can plant segmentation confine embolisms to expendable distal components. In most species, the change in Ψ needed to go from 20 to 80 PLC is 0·5–1·0 MPa; in instances it can be several MPa (Tyree & Ewers 1991; Fig. 1). It is unlikely that the hydraulic segmentation accounted for the high PLC in petioles versus stems of walnut. The typical E of irrigated walnut leaves at midday was 6×10^{-5} kg s^{-1} m^{-2}. This value of E was used with the data in Table 2 to calculate average Ψ drops across the leaf blade, petiole and stem components. In irrigated trees, the difference in Ψ across petioles and stems is likely to be less than about 0·04 MPa, but in droughted trees, E and thus the computed drop in Ψ would be five to ten times less. The most perilous time for plants in drought is when soils are so dry that the stomates remain closed all day long. Under these conditions, E will be very low and hydraulic segmentation will be ineffective because Ψ will be nearly the same everywhere in the plant from the roots to the shoot tips. Thus, hydraulic segmentation may be important only rarely, contrary to previous thinking (Zimmermann 1983).

This study is the first documented case we know of showing that drought-induced leaf shedding is preceeded by cavitation in petioles before cavitation in stems due to vulnerability segmentation. However, we can not say at this time if cavitation causes leaf abscission or what the underlying physiological processes are.

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