

Gas Penetration of Pit Membranes in the Xylem of *Rhododendron* as the Cause of Acoustically Detectable Sap Cavitation

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Abstract

The gas pressure required to force sap from *Rhododendron* stems was investigated. Sap was expressed from stems, and stem permeability to gas increased, at pressures of 1.3-3.5 MPa. We interpret the changing of permeability as a removal of water films in the pores of the pit membranes which normally limit the length of xylem conduits. Similar pressure differences exist across the pit membranes separating gas and sap-filled conduits when cavitation occurs in *Rhododendron*. It is suggested that cavitation in detached leaves and shoots of *Rhododendron* occurs when gas penetrates the pit membranes. The increase in the gas permeability of xylem subjected to high gas pressures was reversed by a soaking in water. It could not therefore have been a consequence of mechanical damage, caused when xylem conduits are subjected to high gas pressures, because such structural damage would be irreversible.

Introduction

Cavitation of xylem sap occurs when a gas phase is initiated and subsequently expands under tension. It may be initiated through failure of either cohesion within the sap (Oertli 1971), or adhesion between xylem sap and the cell wall (Greenidge 1954). Substances predisposing the sap to cavitation may enter sap-filled conduits from neighbouring injured or moribund cells (Coutts 1977). Alternatively a gas phase may expand from a 'nucleus' (a small bubble or unwetted particle) already in the xylem vessels (Harvey *et al.* 1944). This seems unlikely as the walls of the xylem conduits are almost fully hydrated from the time of their formation. Extraneous nuclei are probably prevented from invading the xylem by filtration during the passage of water through the cell walls and endodermis of the root and the pit membranes of the xylem.

Gas entering a sap-filled conduit via the submicroscopic capillaries of the pit membranes or cell walls might also cause cavitation (see discussion in Zimmermann 1983, pp. 44-45). In this instance the pressure differential across the sap-filled pore would determine whether or not gas could displace sap from the capillaries. This differential may be established either by a high gas pressure or, alternatively, a low pressure, i.e. a tension, in the sap.

The experiments described below were designed to measure the pressure required to force gas through the pit membranes of the xylem of a hardwood evergreen shrub (*Rhododendron ponticum*), and to compare this with the pressure difference across the membranes when acoustically detectable cavitation occurs.

Materials and Methods

Unbranched stems were cut either from large *Rhododendron ponticum* L. bushes in the grounds of the Garscube Estate (University of Glasgow, U.K.) or from the College of Advanced Education, Armidale, in the evenings in spring and late summers of 1981–1983. The branches were recut under water and were raised to full turgor by standing them in a humid atmosphere with their cut ends in distilled water overnight. Hydration, determined by weighing the shoots, is normally complete in a few hours. When fully hydrated the stems were trimmed to an overall length of 200–250 mm by trimming away the shoot tips, leaves and bark. The exposed xylem cylinder was wrapped in damp paper towelling which prevented evaporation from the exposed surfaces.

Water Potentials

A pressure chamber (Scholander *et al.* 1964) was used to measure the pressure component (sap tension) of leaf xylem water potential. The point at which sap was first seen to exude is termed the 'balancing pressure'.

Flow of Gas through Stems

The prepared stem was mounted in the pressure chamber with a minimum length of stem inside the chamber. The pressure chamber was lined with moist absorbent paper and the gas-inlet port was covered with a pad of moist polyurethane foam to minimize evaporative water loss from the stem during experiments. The chamber was pressurized with nitrogen; gas emerging from the stem was collected over brine in an inverted 1 dm³ measuring cylinder. The volume of sap emerging from the stem was insignificant in comparison to volume of gas collected. The rate of gas flow through the stem was found by timing the fall of brine between two marks on the measuring cylinder.

Separate collections of gas emerging from stem xylem and pith were made by paring away the xylem from the pith for a short length and collecting the gas emerging from each via separate plastic tubes. Gas flow through the pith was negligible compared to that through the xylem in both fresh and air-dry stems.

Artificial Modification of the Surface Tension (σ) of the Xylem Sap

The surface tension of xylem sap was adjusted by standing the shoots in solutions of butan-1-ol (see Table 1). The surface tension of butanol in contact with nitrogen, the gas used in the pressure chamber, was assumed not to differ significantly from that of butanol contacting air or its own vapour (Washburn 1926).

Table 1. Surface tension and osmotic potentials of liquids supplied to transpiring *Rhododendron* shoots

Values for surface tension are from Washburn (1926), those for osmotic potential are from Lang (1967)

Solution	Surface tension (mN m ⁻¹)	Osmotic potential (MPa)
Water (distilled)	71	0
4% butanol-water	48	—
9.5% butanol-water	27	—
NaCl (1 M)	74	-4.55

Collection of Xylem Sap

Sap expressed from stems in the pressure chamber was absorbed into paper tissue within plastic phials. After each pressure increment, sap collections were made over successive 2-min intervals until the final collection was less than 5% of the running total. This seldom took more than 480 s. Gas flowing through the stems evaporated significant amounts of stem water. Thus sap collections, while being indicative of periods of rapid expression of liquid sap, could not be used to determine accurately the amount of sap lost from stems over long periods.

Acoustic Detection of Cavitation

Cavitation of xylem sap was detected using a modified version of the acoustic detector of Milburn and Johnson (1966). Modifications included the use of lightweight ceramic transducers and improved noise elimination circuits (Crombie 1983). The acoustic detector was coupled to the leaf by inserting a needle attached to the transducer into the xylem of the petiole. Profiles relating cavitation to sap tension were derived as described by Crombie *et al.* (1985). In other experiments the effect of supplying liquids to the petiole of a leaf on the acoustic detector was studied.

Experimental Replication

Our procedure to test the experimental significance of results has been to refine an experimental design until reasonably convincing results were obtained. Next the experiments were repeated in full a few more times followed by many further repeats in somewhat abbreviated form. Although results varied somewhat the overall patterns reported were quite consistent though not amenable to conventional statistical tests. In this way the results in Fig. 1 are representative of 11 detailed experiments and 24 in all. Similarly the sap expression experiments (Fig. 2) were each repeated at least three times in detail but further replicates were simplified and 23 experimental replications were completed.

Results

Transmission of Gas through Stems

Experiments with Indian ink and similar suspensions (Farmer 1918; Crombie 1983; Zimmermann 1983) have shown that practically all the xylem conduits in *Rhododendron* stems reach a length of 110 mm. The spread of ink particles is limited by pit membranes which effectively limit the length of each conduit (vessels and tracheids in this instance). Ink particles, measured by electron microscopy, fell within the range 20–50 nm diameter, showing that the pit-membrane pores were narrower than these dimensions.

Nevertheless a small amount of gas was found to pass through even the longest unbranched stems obtainable (about 500 mm) at pressures of less than 0.1 MPa. This suggested that a few xylem vessels in the stems were at least as long as the stem sections used. Because from experimental tests it seemed unlikely that very significant advantages would be gained from longer stems, the shorter stems (20–250 mm) were used as they were more convenient to use and more readily available.

Gas Permeability of Stems

Gas pressure was applied in increments of 0.17 or 0.34 MPa to the basal end of *Rhododendron* stems within the pressure chamber. After attaining a maximum pressure, usually 4.1 MPa, the pressure was reduced incrementally to zero. Sometimes the pressure was increased for a second cycle of increments. For media of uniform and unchanging porosity (Adzumi 1937), the quotient of the gas flux through the medium (Q), divided by the pressure driving flow (P), is linearly related to P . Q/P was calculated and plotted against P for each experiment. An increase in the size or number of pores in the flow path would cause a greater Q/P for a given P (Yao and Stamm 1967).

Results of a typical experiment (Fig. 1) show that during the first cycle of increasing pressure there was a progressive steepening of the graph of Q/P against P indicating that the porosity of the shoot was increasing. The progressive increase in porosity found when P was first increased was not found when P was increased for a second time. The considerable hysteresis between the graphs of Q/P against

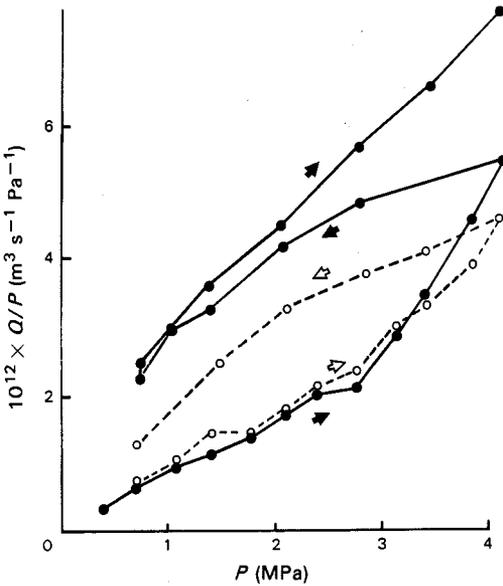


Fig. 1. Flow of gas (Q) through a *Rhododendron* stem with increasing applied pressure (P). The arrows indicate when pressure was increasing or decreasing.

- Pressure cycles with a fresh shoot.
- Pressure cycles after the shoot used above has been soaked overnight in distilled water.

P when P is increased for the first time and then decreased to zero and the nearly linear graph of Q/P against P on a second cycle of increasing pressure (Fig. 1) suggest that most of the change in porosity occurs soon after each pressure increment has been applied during the first cycle of increasing pressure. The slightly greater porosity measured when P was increased a second time (indicated by the steeper graph of Q/P against P) may have been due to evaporation of water remaining in the pit membranes by gas passing through the stems.

The flow of gas through stems from the above experiments was reduced by soaking them in water overnight (Fig. 1). As soaking would not have been expected to repair structurally damaged xylem we consider it unlikely that these increases in shoot porosity resulted from mechanical damage (e.g. burst pit membranes or splitting of the wood) caused by high gas pressures. Hysteresis between Q/P and P for a rehydrated stem was often not so marked as when the fresh stem was used. Clogging of pit membranes by bacteria (bacteriocides could not be included in the rehydrating water because of possible effects on surface tension) or changes in membrane structure following immersion in water of low ionic concentration (Zimmermann 1978) may have been responsible.

Q/P of air-dry stems at a pressure of 0.69 MPa was 7.5 (s.e. = ± 2.2 , $n=7$) times that of fully hydrated stems and 2.3 (s.e. = ± 0.6 , $n=7$) times that of stems subjected to a maximum pressure of 4.1 MPa in experiments similar to that shown in Fig. 1. This increase occurred even though there were no obvious signs of mechanical failure (e.g. cracking) in the xylem.

Expression of Sap from Stems

Typical collections of sap made during expression experiments are shown in Fig. 2*b*. Included for comparison also is a 'cavitation profile' (Fig. 2*a*; see Crombie *et al.* 1985) for *Rhododendron* leaves showing the relationship of cavitation detected using the acoustic technique to sap tension. Little cavitation occurred as sap ten-

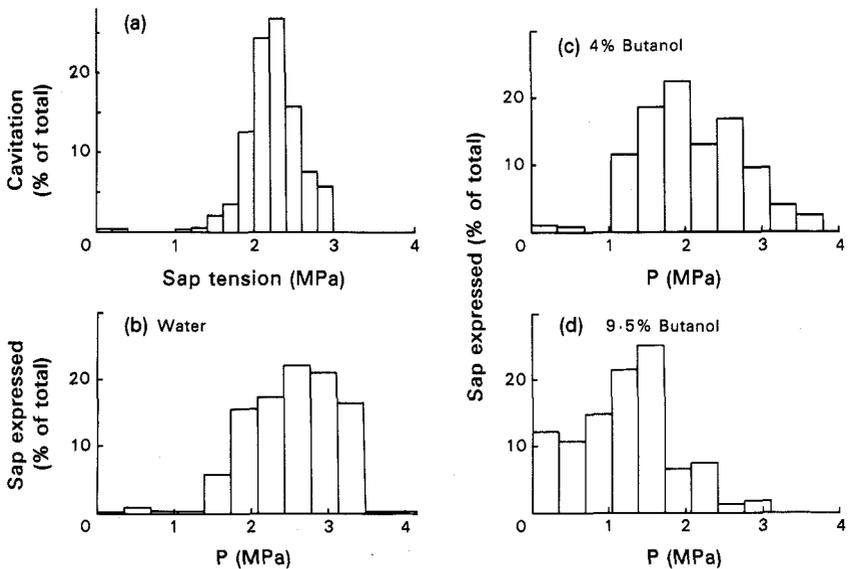


Fig. 2. Comparison of sap tensions causing cavitation and gas pressures (P) forcing sap from stems in *Rhododendron*. (A) Fraction of the total number of cavitation events occurring over successive 0.2-MPa increments in sap tension in a *Rhododendron* leaf. (b-d) Fraction of the total collection of sap expressed by successive 0.69-MPa increments of gas pressure applied to a *Rhododendron* stem that had been supplied with water, 4% butanol or 9.5% butanol.

sion (= balance pressure) increased from 0 to about 1.5 MPa (Fig. 2a). Cavitation frequency was maximal at sap tensions around 2 MPa and, after a rapid initial decline, there followed a prolonged fall at sap tensions greater than 3 MPa. Sap was expressed from the stems on the first cycle of increasing pressure but *not* on subsequent, equivalent, pressure cycles. A little sap was expressed from certain stems when pressure was first applied in each group of experiments. This sap probably originated from xylem conduits severed during preparation of the stems for experiments (Scholander *et al.* 1955). Ignoring this initial sap expression, the largest expressions of sap from stems previously supplied with water only began at chamber pressures of 1.3–1.5 MPa. Sap expression was essentially complete by a pressure of 3.5 MPa.

Sap collections from a stem infiltrated with 4% (v/v) butanol (surface tension 70% of that of water) are shown in Fig. 2c. Sap expression began at applied gas pressures of around 1 MPa. This is approximately 70% of the pressure at which expression began when stems contained nearly pure water after hydration (Fig. 2b). Major collections of sap from stems infiltrated with 9.5% butanol in water by volume (surface tension 40% that of water) began at applied gas pressures ranging from 0.5 to 1.0 MPa (30–70% of those at which sap was expressed when stems containing water were used) (Fig. 2d).

Acoustic Detection of Cavitation and the Effect of Low Surface Tension

When a drop of water was placed on the cut surface of the petiole of a cavitating leaf detectable cavitation events ('clicks') ceased immediately (Fig. 3b). After an

interval, during which the drop was absorbed while transpiration continued, cavitation began, slowly at first (Fig. 3*b*) but increasing over several minutes to a rate similar to that before the water was supplied. These results resemble those obtained when water was supplied to cavitating *Ricinus* leaves (Milburn and Johnson 1966).

In contrast if a drop of butanol-saturated water (surface tension approximately one-third that of water) was placed on the petiole of a leaf which had just begun to cavitate there followed immediately a burst of clicks (Fig. 3*b*). These clicks generally sounded less violent than those detected before the butanol was supplied. Many more clicks could be heard using the headphones attached to the detector than were detected by our rather less sensitive automatic recorder. The burst of clicks occurring when the butanol was supplied was short-lived and was followed by a long period in which few clicks were detected. Click rate was slow to return to the level established before the butanol was added. Sometimes the click rate failed to recover completely.

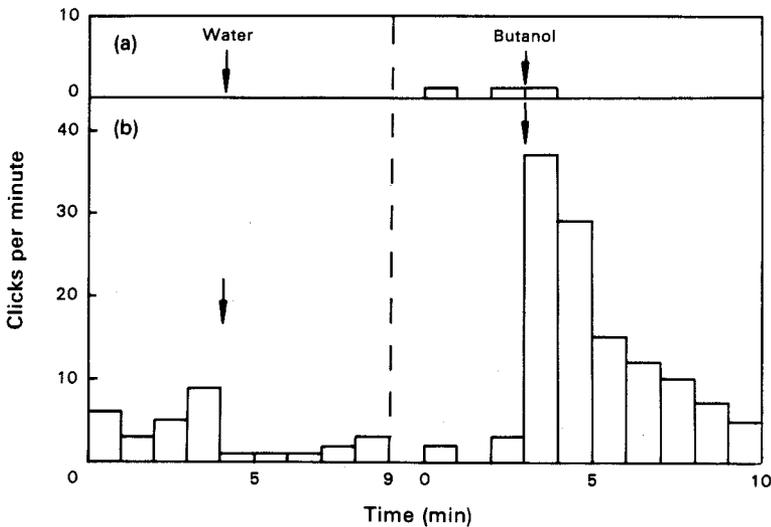


Fig. 3. Effect of supplying water or butanol-saturated water to the petioles of *Rhododendron* leaves on the acoustic detector. (a) Slightly stressed leaf (sap tension 0.3–0.5 MPa). (b) Severely stressed leaf (already cavitating — sap tension > 1.5 MPa).

No fixation noises (Milburn 1973) were detected when butanol was supplied to only slightly stressed leaves (balance pressure 0.3–0.5 MPa) on the acoustic detector (Fig. 3*a*). Nevertheless basic fuchsin supplied in the reduced form (Talboys 1955) to the petiolar stumps of slightly stressed leaves impaled on the detector penetrated the entire length of the midrib in seconds. We infer that the same applied to the butanol solutions. It appears that the clicks are not fixation noises because they are produced *only* when the tissue is suffering from fairly severe water stress.

Discussion

We suggest that expression of sap from stems and the cavitation of sap in detached leaves may occur when gas (nitrogen or air) displaces sap from the pores of pit membranes surrounding xylem conduits containing sap. Cavitation in intact plants may be produced in a similar way when gas invades sap-filled vessels from conduits (e.g. vessels and fibres) or interstitial spaces which contain gas as the result of prior cavitation or damage to the xylem (Zimmermann and Brown 1971). In living plants pressure differentials between gas and sap-filled conduits would be generated by reduced xylem sap pressure (sap tension) rather than by increased gas pressure as in these experiments. We regard the expression of sap from fresh stems, over a range of pressures similar in magnitude to the sap tensions at which cavitation occurs in *Rhododendron* (Figs 2a, 2b), as evidence supporting our hypothesis. The approximately proportional decrease in the pressure at which sap was forced from stems (Fig. 2) and the increase in the rate of cavitation of leaves (Fig. 3) brought about by adding butanol to the xylem sap provides additional evidence of the importance of surface tension in determining the sap tensions at which cavitation occurs. The reduced loudness of individual clicks in the presence of butanol may be explained also as a consequence of cavitation occurring at reduced sap tensions being less energetic than cavitation occurring at greater sap tensions if water only is present in the xylem.

We infer that the pressure at which gas first displaces sap from the pores in the intervening pit membranes can be determined from the sap expression data shown in Fig. 2. The sap collected at pressures between 1.4 and 3.5 MPa (Fig. 2) represents sap from conduits into which gas has gained entry, possibly by forcing sap away from the small pores in the pit membranes. If this is the case, and by assuming that the pores of the pit membrane retain liquid in the same way as does a capillary, a rough estimate of the size of the pores can be made using the formula for the retention of liquid in a capillary (Nobel 1970):

$$P_s = 2 \sigma \cos \theta / r.$$

P_s is the pressure required to force a liquid of surface tension σ from a capillary of radius r and with the walls of which the liquid has a contact angle θ . As a first approximation it is assumed that the surface tension of the sap equals that of water, that the contact angle is 0° (Nobel 1970) and that the capillaries have a circular cross-section. Taking 1.4 MPa and 3.5 MPa as the highest and lowest pressures at which gas penetrated wet pit membranes in *Rhododendron* stems (Fig. 2), the radii of the largest pores in the pit membranes fall within the range 41–100 nm.

These estimates have not included allowances for the tortuosity, the irregular cross section or the short length of the pit membrane pores. These factors, which arise from the 'felt like' construction of a pit membrane in contact with a gas (e.g. Petty and Puritch 1970) may have considerable effects on the pressure at which gas penetrates the pores. However, lack of precision in the data upon which the calculations are based, and the paucity of information regarding the factors themselves, probably does not warrant a more elaborate analysis.

The largest pores in this range are about the same size as those in the bordered pits of *Abies* (Petty and Puritch 1970) and *Picea* (Edwards and Jarvis 1982) but slightly smaller than those of other conifers (Stamm 1929). The smaller pores are less than half the radius of those in conifers, perhaps compensating for the absence of a valve-like torus in *Rhododendron* pits.

The large increase in xylem porosity found when the stems were air-dried may indicate that sap is displaced from a relatively small proportion of the pores in the pit membranes by gas at pressures of up to 4.1 MPa; i.e., although the largest pores in the pit membranes may be the major factor determining the sap tension at which cavitation occurs they may constitute a relatively small fraction of the total pore area of the pit membranes. Sizes of the smaller pores cannot be determined from sap expression studies, however, as sap exits from conduits immediately gas penetrates the largest available pores. Also, data obtained using air-dried stems must be interpreted with caution as drying in air can cause changes in the size of pores in the pit membranes (Petty and Puritch 1970). Fresh shoots were used in these experiments in part to avoid the possibility of structural changes which might follow drying or fixing of stems and also because substances released by dying cells might affect the surface tension of the xylem sap (Coultts 1977).

We have shown that when gas under pressure is applied to *Rhododendron* stems, xylem sap is expressed from the stem. Associated with this expression of sap there is a corresponding increase in the permeability of the stem to gas. The gas pressures inducing these changes correspond in magnitude, within experimental error, with the sap tensions at which sap cavitates in *Rhododendron* leaves and shoots (1.5–3 MPa, Crombie *et al.* 1985). Reduction of the surface tension of the xylem sap reduced the pressure at which sap could be expressed from stems and increased briefly the rate of cavitation in water-stressed leaves. In contrast, the incidence of cavitation decreased when water, which reduced sap tension without affecting surface tension, was supplied. These results in combination support the hypothesis that xylem sap, held in the pores of the pit membranes by surface tension, normally prevents the entry of gas into sap-filled xylem conduits. Cavitation occurs however when the surface tension is reduced and extraneous gas invades the conduits.

Differences in the tensions at which cavitation of xylem sap occurs in different species may be attributable therefore to differences in the structure of the xylem, especially the texture of the pit membranes. Solutes in the xylem sap, because of their effect on surface tension, may modify these effects significantly.

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