

Spring Filling of Xylem Vessels in Wild Grapevine¹

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ABSTRACT

Xylem vessels in grapevines *Vitis labrusca* L. and *Vitis riparia* Michx. growing in New England contained air over winter and yet filled with xylem sap and recovered their maximum hydraulic conductance during the month before leaf expansion in late May. During this period root pressures between 10 and 100 kilopascals were measured. Although some air in vessels apparently dissolved in ascending xylem sap, results indicated that some is pushed out of vessels and then out of the vine. Air in the vessel network distal to advancing xylem sap was compressed at about 3 kilopascals; independent measurements indicated this was sufficient to push air across vessel ends, and from vessels to the exterior through dead vine tips, inflorescence scars, and points on the bark. Once wetted, vessel ends previously air-permeable at 3 kilopascals remained sealed against air at pressures up to 2 and 3 megapascals. Permeability at 3 kilopascals was restored by dehydrating vines below -2.4 megapascals. We suggest that the decrease in permeability with hydration is due to formation of water films across pores in intervacular pit membranes; this water seal can maintain a pressure difference of roughly 2 megapascals, and prevents cavitation by aspirated air at xylem pressures less negative than -2.4 megapascals.

MATERIALS AND METHODS

Initial studies were made on *Vitis labrusca* L. growing at the Harvard Forest in Petersham, Massachusetts. Subsequent work was done on *Vitis riparia* Michx. in the vicinity of Burlington, Vermont. Repeated observations and measurements of root pressure were made at a single site on the banks of the Winooski river. Root pressure was measured using bubble manometers attached to severed vines; the manometers and methods of measurement have been described elsewhere (10). The vines used in laboratory experiments were obtained at the riverbank site, or from the Colchester bog near Lake Champlain. Vines in 'winter' condition were collected prior to onset of root pressure and were gas filled; vines in 'spring' condition exhibited root pressure at collection and were said to be 'bleeding.'

Documentation of Spring Filling of Vessels. The degree of refilling in spring vines was quantified by measuring the hydraulic conductivity of the xylem and expressing it as a percentage of the measured maximum value. A vine with 100% maximum conductivity was assumed to have all its vessels completely filled; lesser values would indicate gas emboli in vessels which were impeding flow. This method has been used successfully to indicate air embolism in palms (11), and other plants (12).

Intact tips measuring 1.5 to 2.0 m in length, and about 0.5 cm basal diameter, were cut from bleeding vines in the field and the basal cut end immediately placed in contact with the perfusing solution. The solution consisted of 10 mM NaCl and 0.05% formaldehyde in water that had been passed through carbon filters, deionizing columns, and particle filters of 5 μm and 0.2 μm retention. It was found from preliminary control experiments that, unless formaldehyde was present, hydraulic conductivity dropped steadily from its initial value. Vine sections were longer than the longest vessel, *i.e.* there were no vessels cut on both ends. Vessel lengths were measured according to the 'air' method detailed in Zimmermann and Jeje (14).

In the laboratory, the basal end of the vine segment was recut under water with a new razor blade and the extreme tip, which was commonly dead, was excised. A supply reservoir was connected to one end of the vine, and a drain reservoir connected to the other end with lengths of plastic tubing. The reservoirs and tubing were filled with perfusion solution that had been filtered through a 0.22 μm membrane filter (Millipore GS). Solution flowed from the supply to drain reservoirs through the vine at a known pressure gradient ($\Delta P L^{-1}$) dependent on the length of the vine (1), and the relative height of the reservoirs. The drain reservoir rested on an electronic balance (Sartorius 1702), and the mass flow rate (J) was calculated from the change in weight over specified time intervals (correcting for evaporation). Hydraulic conductivity was calculated as $J(\Delta P L^{-1})^{-1}$. The measurement of conductivity was automated by having a micro-computer in communication with the balance.

Following the first conductivity measurement, the vine was flushed with degassed solution at about 170 kPa for 10 to 20 min in order to dissolve air emboli in the vessels, and a second

Xylem vessels in the wild grapevines *Vitis labrusca* L. and *Vitis riparia* Michx. are gas-filled during winter. Prior to leaf expansion in spring, the vessels become filled with water by root pressures that can reach 500 kPa at the base of vines (7). Once leaves are expanded and transpiring the vessels must be able to withstand negative xylem pressure potentials; any remaining trace of gas would nucleate cavitation, thus disrupting water transport. Water conduction in these refilled vessels is critical since new vessels are not differentiated until well after leaf expansion (JS Sperry, personal observation). In fact, vessels remain functional for up to 7 years (9) and thus go through several cycles of emptying and filling. Vessels in grape are among the largest known, with maximum diameters of 0.5 mm, and lengths of over 8 m (14). How is all the gas so successfully removed from such large vessels?

We have documented the spring filling of grapevine vessels and have investigated three possible mechanisms for it: (a) condensation of water vapor—if the vessels contain water vapor, filling would occur readily when root pressures approached vapor pressure (about 0.23 kPa at 20°C); (b) dissolving of gas—sufficiently prolonged and elevated root pressure may be sufficient to dissolve the gas; and (c) expulsion of gas—root pressure may push gas out of the vessels, and eventually out of the vine.

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† Deceased.

measurement was made. The flush was repeated until conductivity reached a maximum value. To determine the degree and rate of vessel refilling, the initial conductivity expressed as a percent of the maximum was determined on 4 vine segments each at 1 week after onset of bleeding, bud break, and leaf expansion.

Composition of Gas in Vessels. Prior to the onset of root pressure, 1 to 2 m sections of vines were cut under water and transported to the laboratory being careful to keep both cut ends immersed. Still under water, one end of the segment was attached to a helium tank while the other end was placed in a water-filled chamber. Vessel lumina contents were flushed out with helium at a pressure of 35 kPa. The gas in the chamber was collected and transferred to helium-flushed syringes and analyzed using a gas chromatograph for percent composition of CO₂, O₂, and N₂.

Permeability of Vessel Ends to Air. To determine the feasibility of the gas-expulsion hypothesis, the pressure required to force air through vine segments lacking continuous vessels was measured. Preliminary results assured us that air penetrated vine segments only via xylem vessels; no air passed through the pith, probably because of the well-defined pith diaphragm at each node. The pressure required to force air through the vessels of the vine segments was termed the 'threshold pressure,' and represented the force required to push air from one vessel to the next. One end of the vine was inserted a few centimeters into a pressure bomb and the other immersed in water. The pressure was slowly increased (about 2.5 kPa s⁻¹) to the threshold value at which a steady stream of bubbles was observed from one or more vessels at the submersed end. The measurement was repeated several times for each vine by lowering the pressure to zero, waiting for all bubbling to subside, and again slowly increasing the pressure. Both winter (gas-filled) and spring (bleeding) vines were used.

Permeability of vessel ends to air was also studied as a function of vine water potential. In this experiment, we determined the water potential required to induce a threshold pressure of an arbitrarily low value (3 kPa) in a section of vine drying in the laboratory. Water potential was measured with a temperature-corrected stem hygrometer according to the methods detailed in Dixon and Tyree (3).

Filling of Xylem Vessels in the Laboratory. In an attempt to discriminate between the expulsion and dissolving hypotheses, we compared rates of refilling under conditions where gas could dissolve as well as escape *versus* conditions where escape was prevented and only dissolving was possible. Results indicated that dissolving and escape of gas could occur during refilling in winter vines; potential escape of gas from vessels was indicated by the high permeability of vessel ends to air (Fig. 2). In contrast, gas trapped in the vessels of spring vines could only dissolve because the vessel ends had become relatively impermeable to air (Fig. 2).

Rates of refilling were compared between winter vines, and spring vines in which some of the vessels were filled with air by one of the following methods: (a) vine sections were dried in air overnight or (b) sections were attached at one end to a compressed air source and 'air-injected' at high pressure (about 3.5 MPa). Air was the appropriate gas to use, because winter vines were found to have air-filled vessels (see Results). Refilling was followed by monitoring the hydraulic conductivity of vine sections for up to 13 h under conditions of continuous flow through the vine. The gravity pressure head in each experiment was near 25 kPa, a pressure typical of root pressures measured in the field. When conductivity appeared to reach its maximum value, the vine was flushed and the actual maximum conductivity determined as above. The time course of vessel filling was plotted as percent maximum conductivity *versus* time. In this experiment we were interested in relative rates between vines; the actual rate of refilling under field conditions was expected to be much slower

because intact tips would inhibit the flow of xylem sap, and root pressures would be subject to variation with factors such as temperature and soil moisture.

RESULTS

Field Observations and Documentation of Vessel Refilling. Root pressure, as evidenced from xylem sap flowing from severed vines, was first observed at the riverbank site on the week of April 8. At this time, and for 2 weeks thereafter, bleeding xylem was only observed at the base of vines; the distal portions remained gas filled. The boundary between bleeding and gas filled portions of the same vine was sharp; there was as little as a meter of vine separating the two regions. Observations indicated that gas in vessels of the distal dry portion of vines was compressed. Once cut, the dry part of the vine began bleeding within minutes, as if gas pressure released by severing the vine had been resisting the ascent of sap driven by root pressure. When dry portions of intact vines were cut and the rooted end immersed under water, gas bubbles streamed from the xylem vessels. A manometer attached to the distal dry portion of a vine registered a compression of 3 kPa; the basal portion was bleeding at a root pressure of 17 kPa. Root pressures in general ranged between 10 and 100 kPa. Bleeding continued through leaf expansion in late May during cloudy or wet weather.

The fact that a vine bled when cut did not necessarily mean that all of its vessels were sap-filled. Three of the four vine tips collected early in the bleeding season on April 22 (Fig. 1) had less than 1% maximum conductivity, indicating that many vessels were still air embolized. The rate at which vessels filled and conductivity recovered was highly variable between vine segments; one of the segments collected on April 22 was already at 100% conductivity, whereas two of the segments collected at bud break over 2 weeks later (Fig. 1, May 7) were still 55 to 70% of maximum. By the time the leaves were expanding, all vine segments measured were at 100% conductivity (Fig. 1, May 21), and were assumed to have completely sap filled vessels.

Composition of Gas in Vessels. Analysis of the gas in the vessels gave the following results: 5.7 ± 2.1% CO₂, 23.6 ± 0.8% O₂, 71 ± 0.7% N₂. Thus, the vessels are filled with CO₂ and O₂ enriched air. This result eliminated the vapor condensation hypothesis for vessel filling from consideration.

Permeability of Vessel Ends to Air. Vines in their air filled winter condition had vessel ends that were completely permeable to air (e.g. Fig. 2, reading 1, winter vine); as soon as pressure was applied to one end of the vine section bubbles streamed out of the other end from vessels around the entire circumference of the xylem. When winter vines were flushed with water, however, the threshold pressure increased dramatically to between 1.5 and

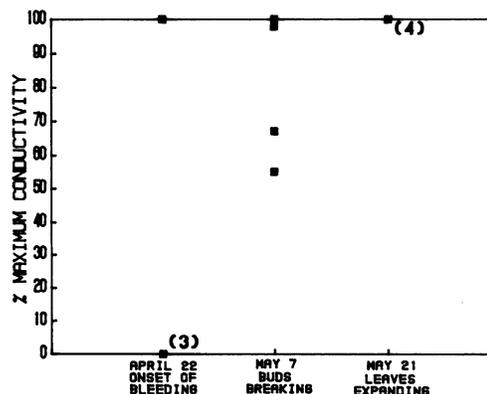


FIG. 1. Percent maximum hydraulic conductivity at three times during the bleeding season. Four vine segments were measured in each case, numbers in parentheses indicate superimposed data points.

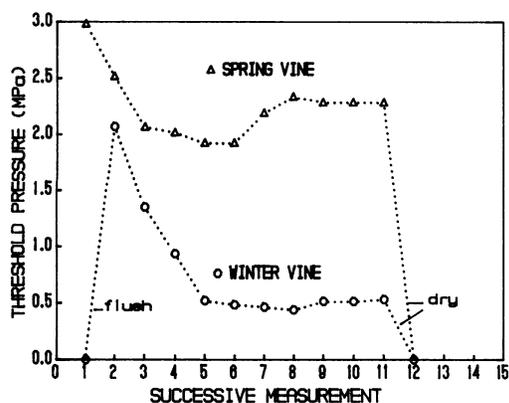


FIG. 2. Successively measured threshold pressures for the penetration of air through an originally air-filled winter vine, and an originally bleeding spring vine. The winter vine section was flushed with water between readings 1 and 2; both vines were air dried overnight between readings 11 and 12.

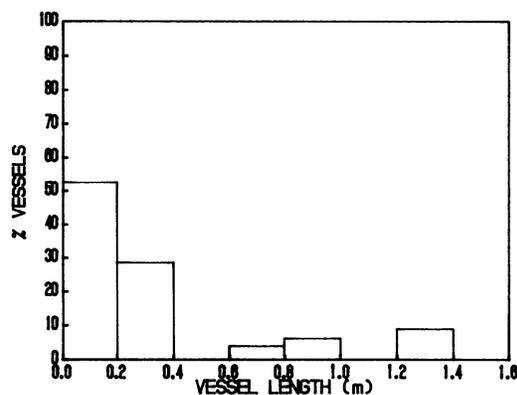


FIG. 3. Vessel length distribution in vine section typical of those used in experiments. All vine segments used were at least 1.5 m in length, and thus longer than the longest vessels.

2.3 MPa (e.g. 2.1 MPa in Fig. 1, reading 2, winter vine). In these wetted vines the threshold was marked by only 1 or 2 bubbling vessels. Repeated measurement of the threshold in a single vine caused a decrease to a relatively stable value typically between 0.3 and 0.6 MPa (e.g. 0.5 MPa in Fig. 2, readings 5–11, winter vine). If the vine was dried overnight in the laboratory, the threshold returned to its initial low value (Fig. 2, reading 12), and was again characterized by bubbling from nearly all of the vessels.

Although fewer measurements were made for spring (bleeding) vines, the initial threshold measurement tended to be higher than the highest obtainable value for winter vines (about 3.0 MPa, Fig. 2, spring vine), and the stable value obtained by successive measurements on the same vine was also higher than the corresponding value for winter vines (2.0–2.4 MPa, Fig. 2, spring vine). Air drying the spring vine produced the same result as for the wetted winter vine; the threshold dropped to near zero pressure. The vine segments used in these experiments were longer than the longest vessels; although most vessels (82%) were shorter than 0.5 m, some were as long as 1.4 m (Fig. 3).

In the experiment summarized in Figure 4 a spring vine was dehydrated to about -2.4 MPa before air at a pressure of 3 kPa could penetrate vessel ends in the segment. At this point between 5 and 10 vessels conducted air (about 7% of total). Further dehydration to -4.5 MPa increased the number of air-conducting vessels to more than 20 (the limit of reliable counting).

The fact that vessel ends in winter vines were highly permeable to air suggested that the expulsion of air could be a mechanism

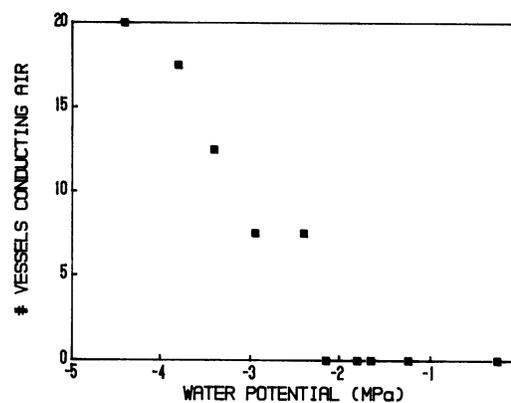


FIG. 4. Number of air conducting vessels at 3 kPa versus water potential of a dehydrating vine. Vessel counts had an uncertainty of ± 2.5 vessels.

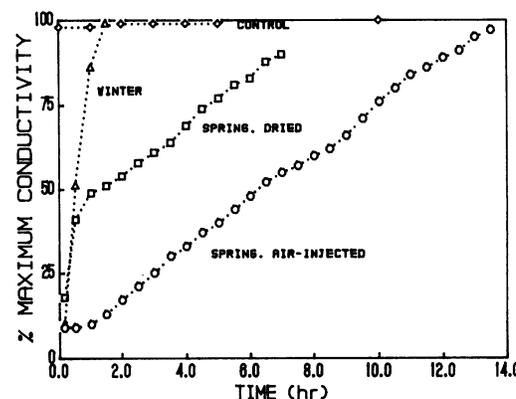


FIG. 5. Time course for recovery of maximum hydraulic conductivity in an initially air-filled vine in winter condition, an originally bleeding vine dried overnight, and a bleeding vine that had been injected with compressed air (3.5 MPa). The control was brought to maximum conductivity prior to the experiment.

for spring refilling. For this reason, we investigated pathways for air escape from intact vine tips. The basal cut end of an intact vine tip was inserted into a Scholander bomb pressurized at 5 kPa and rest of the vine was submerged in a tub of water. In every vine examined (over 6), bubbles were observed coming from one or more of the following places: extreme tips of vines (which were generally dead), inflorescence scars, and undefined points on the bark of the internode.

Refilling of Xylem Vessels in the Laboratory. Sections of vines collected in winter condition achieved maximum hydraulic conductance within 1.5 h (Fig. 5, winter) at a pressure representative of root pressures measured in the field (25 kPa). This rapid refilling was consistently observed in winter vines, and was accompanied initially by the expulsion of more than 1 ml of air from the downstream end of the vine. In contrast, spring vines in which at least some of the vessels were air filled by either partial dehydration (Fig. 5, spring, dried), or by injection of air at about 3.5 MPa in one end (Fig. 5, spring, air-injected), recovered full conductance at the same pressure only after 7 and 14 h, respectively; repeated trials with minor variations in the manner of air filling the vessels gave similar results. In these experiments, no air was observed to come from the downstream end during recovery of conductivity.

DISCUSSION

Of the three hypotheses considered, both air expulsion and dissolving appear to play important roles in the spring filling of

grapevine vessels. Vapor condensation does not occur because the vessels were found to contain air. The expulsion hypothesis is supported by the following results. Vessel ends in winter vines were highly permeable to air at pressures below 3 kPa. Refilling of vessels in these vines occurred readily in the laboratory at pressures characteristic of bleeding vines in the field and was accompanied by the expulsion of air from the xylem. In the field, the rise of sap under root pressure compressed the air in the vessel network ahead of it. This compression appeared to slow the upward progress of sap unless the vine was severed in the non-bleeding portion, in which case the sap ascended rapidly. Laboratory experiments showed that air under low pressure (5 kPa) leaked out of intact vines through dead vine tips and inflorescence scars; at these places the vessel network is in direct contact with the air outside the vine. Some air exited the vine through the bark of the internodes. The pathway in this case was probably through vessel walls (perhaps via pits) to the air spaces in the medullary rays, which Haberlandt (4, p. 668) reported to be in direct communication with the outer air. We think it is possible that the variation observed in the rate of recovery of maximum conductivity between vines (Fig. 1) may be due to variation in number of escape points for air; vines with dead, broken tips or other lesions would refill more readily.

Other results support the dissolving hypothesis. In the time course for refilling in the winter vine (Fig. 5, winter), air ceased to be expelled from the downstream end well before maximum conductivity was achieved. Apparently pockets of air remained trapped in vessels, continuing to impede flow until they dissolved. The fact that many bleeding vines in the field had hydraulic conductivities considerably below maximum (Fig. 1) also suggests that air remained in vessels after the sap had risen, and had to be dissolved before refilling was complete.

Dissolving is apparently necessary for complete filling of vessels, and may in fact be sufficient even in the absence of any escape of air. This is indicated by the eventual recovery of conductivity in the dried and air-injected spring vines in which the expulsion of air was not observed (Fig. 5). The presence of conductivity at the beginning of the experiments, however, means that some vessels (the smaller ones?) were water filled. Limited conductivity in a few vessels may have greatly enhanced dissolving rates by continually flushing air-saturated water away from the air emboli. Had we been able to fill *all* vessels in a vine section with air and yet not consequently cause vessel ends to be highly permeable to air, we would have had the definitive comparison between dissolving and expulsion.

Although the vessel ends in winter vines were highly permeable to air and had essentially no threshold pressure, the same vessel ends when wetted became quite impermeable requiring a pressure of 1.5 to 2.3 MPa before any air was forced through (*e.g.* Fig. 2, winter vine). We assume that air can readily escape the vessel as sap rises in spring but once the vessel is filled with sap and conducting the transpiration stream, air is prevented from entering the vessel under negative pressure and disrupting water flow. Our explanation for this is that in winter the pores in the pit membranes of vessel-to-vessel pits lack a water film, making possible the free movement of air between vessels. Once the sap has risen, however, formation of water films at the pores resists the movement of air between vessels.

According to this explanation, the threshold pressures in Figure 2 are a measure of the force required to break the surface tension and adhesion of water at these pores. From the capillarity equation, these pores would have to be between 0.10 and 0.15 μm in diameter to correspond with the peak threshold values of 2.0 and 3.0 MPa in Figure 2; these dimensions are within the range determined for several temperate hardwood tree species (8). We are not sure why the initial threshold pressure in the water filled vines was so much higher than values measured subsequently on the same vines. Rupture of microfibrils in the pit membrane during measurement can be ruled out because flushing the vine with water after the observed decline in the threshold restored

the initial high value. Similar results were obtained in a study of air permeability in *Rhododendron* xylem (2), and were attributed to local drying of the pit membrane during measurements which made the water film less stable once it reformed. We also wonder why the threshold pressures were higher in spring than winter vines; perhaps subtle changes in pore dimensions and/or adhesion between water and pore were brought about by prolonged hydration which increased resistance to air penetration. Prolonged hydration is reported to increase the wettability (defined by contact angle) of surfaces (6, p. 204), and may also cause fibrils in the pit membrane to swell (1, 5).

In Figure 2, air permeability of vessel ends was achieved by increasing air pressure relative to xylem pressure which remained near ambient. In Figure 4, however, the same result was obtained by the opposite circumstances: permeability was induced by lowering xylem pressure to negative values while air pressure was ambient. In both cases the pressure difference across the vessel ends at air penetration was between 2 and 3 MPa. We interpret this as the pressure difference required to rupture water films in intervascular pit membranes. From these results, we hypothesize that the water potential in grapevines can drop to -2.0 MPa before risking the rupture of water columns by aspirated air. In fact, water potentials seldom drop below -1.6 MPa (9). According to Figure 4, winter vines must be dehydrated below -4.0 MPa to possess their observed permeability, *i.e.* profuse bubbling from all xylem vessels at low air pressures.

The nucleation of cavitation in plants by air being sucked into the vessel lumen at critical negative xylem pressures has been most recently proposed by Zimmermann (13), and termed the 'air seeding' hypothesis. Evidence in favor of the hypothesis comes from recent work on *Rhododendron* in which a close correlation was found between the pressure difference required to force air through vessel ends and the negative xylem pressure required to induce cavitation as detected acoustically (2). Our investigation provides further support of this hypothesis. We have measured the pressure difference required to 'seed' air through vessel ends at both negative and positive pressures. This difference corresponds to reasonable pore dimensions and is large enough to support normal negative pressures recorded for grape. The continued investigation of the relationship between air permeability and the occurrence of cavitation in grape promises to be a further test of the air-seeding mechanism.

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