

The Effect of Reduced Hydraulic Conductance on Stomatal Conductance and Xylem Cavitation

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Received 10 November 1992; Accepted 19 March 1993

ABSTRACT

The vulnerability of xylem conduits to cavitation theoretically determines the maximum flow rate of water through plants, and hence maximum transpiration (E), stomatal conductance (g_s), and leaf area (A_l). Field-grown *Betula occidentalis* with a favourable water supply exhibit midday xylem pressures (ψ_{px}) approaching the cavitation-inducing range (-1.42 to -2.2 MPa). We studied the ability of the stomata to prevent cavitation-inducing pressures when whole-plant hydraulic conductance per leaf area (k_l) was reduced by making overlapping transverse cuts in the main stem. Controls were intact, or had only the phloem cut in the same pattern. Reducing k_l caused two responses: (1) variable g_s or E with ψ_{px} falling into the cavitation range causing up to 98% embolism and 100% leaf death, (2) decreased g_s and E with ψ_{px} remaining above the cavitation point and no leaf death or induction of cavitation. Shoots avoiding cavitation either produced new xylem and returned to control values of k_l , g_s and E (experiments in June and July), or they showed continued decline in g_s and E associated with increasing ψ_{px} and eventual premature senescence of leaves (experiments in August). Whether embolism occurred after reducing k_l probably depended on the response time of stomata, and the proximity of ψ_{px} to the cavitation range when the xylem was cut. Stomata probably responded indirectly to reduced k via small changes in leaf ψ ; root signalling was unlikely because of the constant rooting environment.

Key words: Stomatal conductance, hydraulic conductance, xylem embolism/cavitation, transpiration.

INTRODUCTION

Xylem cavitation represents an unambiguous limit to the drought tolerance of plants. It occurs when air enters functional conduits through pit membranes (see Sperry and Tyree, 1990, 1988; and related papers for details) and the result is a loss of hydraulic conductance due to vapour- and/or air-filled 'embolized' conduits. When xylem pressures (ψ_{px}) drop within and below the cavitation range, water conduction is reduced and eliminated and the plant ceases to function. Because cavitation pressure (ψ_{p-cav}) is determined by the permeability of interconduit pits to an air–water interface it is not expected to change over the course of a growing season, except during heartwood formation in the older xylem (Sperry *et al.*, 1991).

To a first approximation, ψ_{p-cav} is a constant that limits the maximum driving force for moving water from the soil to the xylem ($\psi_{soil} - \psi_{p-cav}$). The product of the maximum driving force and the hydraulic conductance between soil and xylem (k) gives the maximum possible steady-state flow rate through the soil–plant–atmosphere

system. Under steady-state conditions, flow rate will equal the transpiration rate. Expressing transpiration rate and hydraulic conductance on a leaf-area (A_l) basis, the maximum transpiration rate (E_{max} , per leaf area) will be:

$$E_{max} = (k/A_l)(\psi_{soil} - \psi_{p-cav}) \quad (1)$$

The term k/A_l is referred to as the leaf-specific hydraulic conductance (k_l ; Zimmermann, 1983; Tyree and Ewers, 1991), and E is the product of the leaf conductance to water vapour (g) and the difference in mole-fraction of water between leaf and air (Δw).

Modelling studies indicate that these theoretical limits on transpiration are approached under natural conditions in a variety of woody species (Tyree and Sperry, 1988). This suggests that in achieving maximum gas exchange, plants maintain a consistently small margin of safety from cavitation. The implication is that stomatal responses to drought stress are integrated with, and constrained by, the cavitation response. Our objective is to evaluate

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dawn leaf ψ_{px} . The average soil–trunk k_1 was 19.8 ± 6.66 mmol $s^{-1} m^{-2} MPa^{-1}$ ($n=8$ shoots). Soil–trunk k_1 for shoots showing leaf dieback (see Results) were approximated by dividing the average soil–trunk k_1 by the fraction of surviving leaves.

The k_1 from mid-trunk to the petioles of the leaves previously sampled for g_s and E was measured directly on harvested shoots in the laboratory using the technique developed for our earlier study (Sperry and Pockman, 1993). The shoot was harvested, recut at the base underwater to a standard length of 1.9 m, defoliated, and inserted in an elongated vacuum chamber with the cut end supplied with filtered (0.2 μm) solution (10 mmol oxalic acid) at atmospheric pressure. A partial vacuum (-35 to -55 kPa) pulled solution through the shoot and out of the cut petioles. Volume flow-rate was measured by collecting the solution in pre-weighed micro-centrifuge tubes fixed to the petioles and filled with cotton wool. Evaporation during the measurement caused less than 11% underestimate of k . Hydraulic conductance (volume flow-rate divided by pressure difference) to each petiole was expressed per unit leaf area (k_1 ; leaf-specific conductance). Sub-atmospheric pressures were used to minimize re-filling of any embolized vessels that might occur under positive pressures.

The k_1 from soil-to-petiole was calculated from the average soil–trunk k_1 in series with the individual trunk–petiole measurement.

Reduction of hydraulic conductance using overlapping cuts

To reduce k we made transverse cuts in the trunk with pruning shears. In general, 4–8 cuts were made across *c.* 50% of the main axis from opposite sides 1 cm apart; they were made *c.* 1.8 m proximal to the axis tip where the trunk diameter ranged between 1.13 and 1.19 cm. The trunk was secured with dowels to prevent breakage. After variable periods of time depending on the experiment, shoots were harvested and brought into the laboratory where trunk–petiole k_1 was measured inclusive of the notched region of the trunk. The notches were positioned outside the vacuum chamber to allow solution to be pulled around them without leaking. After this, k_1 was remeasured after removing the notched portion of trunk (*c.* 15 cm). Controls were measured in the same way, removing the same length of trunk, though with no notches included.

Controls showed no systematic change in k_1 before and after the trunk section was removed (Fig. 1b, 'controls'). In the absence of embolism induction above the notches, notched stems generally showed an increase in k_1 to control levels following notch removal (e.g. Fig. 3c). The extent of this increase was our measure of how much notching reduced k_1 . When embolism was induced, removing notches had less of an effect (Fig. 1c, d; shoots 1–5) and we could not estimate the original effect of the notches on k_1 .

Embolism measurements

Following the k measurements embolism was measured on 10 segments from the branch above the notched region using methods detailed in Sperry *et al.* (1988; and related publications). In this technique, embolism is expressed as the per cent decrease in hydraulic conductance resulting from embolism (= % embolism).

RESULTS

Notching the xylem 4 to 12 times reduced the soil–petiole k_1 by an average of 47% and caused two divergent responses: (1) stomatal closure and no cavitation above

control levels, (2) variable stomatal behaviour, cavitation, and leaf death.

Examples of these responses are shown in Fig. 1. In this experiment, six shoots from a clump were notched 6–7 times (all on August 10), one with just the phloem cut (Fig. 1, P-NOTCH), the others with both xylem and phloem cut (Fig. 1, XYLEM-NOTCHED). Controls had leaf ψ_{px} near but above the cavitation point (Fig. 1b, controls) and showed modest embolism levels (10%, Fig. 1c). The phloem-notched shoot was similar in all parameters to controls (Fig. 1, P-NOTCH).

Four of the five xylem-notched shoots showed embolism above control levels when harvested from one to 43 d following notching (Fig. 1c; 1d, 2d, 3d, 43d) and leaf ψ_{px} within the cavitation range (Fig. 1b). Embolism of 80% or more was associated with brittle, brown leaves that died from dehydration (Fig. 1c, dieback). The k_1 values (trunk–petiole) were below controls even after notch removal because of the embolism induced above the notch (Fig. 1d). Transpiration and g_s in the embolized shoots varied from zero in one completely dead shoot (Fig. 1, 2d) to control values in a shoot measured the day following notching (Fig. 1, 1d). This shoot had ψ_{px} in the cavitation range and 52% embolism; presumably this was increasing and would have led to leaf death if the shoot had not been harvested.

Some of the variability in g_s and E in these embolized shoots was probably time-dependent because shoots were measured at varying times following notching. However, we could not reconstruct the chronology of these changes from measurements on separate shoots because of variability in the rate and degree of response between shoots. For example, some shoots showed dieback on the same day they were notched (data not shown) whereas others did not (Fig. 1, 1d); and some shoots died completely (Fig. 1, 2d) where others sustained a few surviving leaves (Fig. 1, 3d, 43d).

One of the five xylem-notched shoots showed no embolism induction at all when measured 44 d after notching (Fig. 1c, 44d). In this shoot, g_s and E values were significantly below controls (Fig. 1A, 44d) and leaf ψ_{px} was higher than controls (Fig. 1b, 44d). Although there was no leaf dieback in the first few weeks after notching, after 44 d, 72% of the leaves had prematurely senesced (Fig. 1c) as evidenced by extensive yellowing.

The dichotomy in the notch response between embolism induction versus avoidance is more clearly seen in Fig. 2 where continuous measurements of xylem flow were made with the stem-flow gauge in two notched shoots. The shoot in Fig. 2A and B showed a rapid drop in flow rate immediately following notching the stem 8 times (Fig. 2A). Five days later the shoot was 65% embolized and had 45% leaf dieback due to dehydrated leaves (Fig. 2B). The dieback was evident as soon as the day after the treatment,

empirically the possibility of a link between stomatal behaviour and the carrying capacity of the vascular system.

In a preliminary study with *Betula occidentalis* (Sperry and Pockman, 1993) the stomatal response to reduced k was studied. This species cavitates at xylem pressures between -1.42 MPa to -2.2 MPa (Sperry and Pockman, 1993; Fig. 1). If it regularly uses its maximum driving force for flow (e.g. 1.42 MPa for ψ_{soil} of 0, and xylem sap of pure water) and avoids cavitating, then a reduction in hydraulic conductance should cause a proportional reduction in g due to reduced stomatal conductance (g_s) under conditions of constant ψ_{soil} and Δw . We reduced k by injecting air into the vascular system and inducing cavitation *in situ*. The treatment caused stomatal closure, but results suggested it was not sufficient to avoid cavitation and leaf dieback.

In this paper, we follow up on these preliminary studies with a more extensive investigation of the events following reduced hydraulic conductance. To improve the resolution of these events, we have used overlapping transverse cuts in the trunk to reduce hydraulic conductance rather than air-injection. These cuts locally restrict xylem transport because of embolization of the severed vessels, but there is no necessary induction of cavitation in undamaged vessels. The problem with air-injection was that it was difficult to control the distribution and amount of cavitation it induced, and it was impossible to separate the cavitation caused by the injection from cavitation caused by the plant's subsequent response.

MATERIALS AND METHODS

Plant material and experimental design

We studied *Betula occidentalis* because it favours riparian habitats resulting in high and constant ψ_{soil} throughout the growing season. It is small (to 15 m) enabling easy access to the crown, and has a multiple-shoot growth form allowing experiments to be replicated within a clump. Experiments were done in a riparian meadow in the Red Butte Canyon Research Area near Salt Lake City, UT. Shoots used were 5–7 m in height. The cavitation response was measured in the branch xylem of similar shoots in the same research area by Sperry and Sullivan (1992; see also Sperry and Pockman, 1993).

We used three treatments within a clump: non-manipulated control shoots, xylem-notched shoots where trunk xylem and phloem were interrupted by overlapping cuts, and phloem-notched shoots where only phloem was cut. Transpiration (E), g_s , and k_1 (k/A_1 ; leaf-specific hydraulic conductance) were measured for 10–20 leaves of the shoot distal to the notched region. Other measurements included ψ_{px} , leaf death, xylem flow rate, and embolism.

Xylem flow, transpiration (E), stomatal conductance, (g_s), and water potential (X) measurements

Xylem flow rate was measured with a stem-flow gauge (Baker and van Bavel, 1987) designed for diameters between 1.8 and 2.2 cm (Dynamax Inc., Houston TX); the gauge was interfaced with a datalogger (CR10, Campbell Instruments, Logan, UT).

Stem gauge readings and independent flow rate measurements were found to agree within 20% for flow rates exceeding $1 \times 10^{-5} \text{ kg s}^{-1}$.

Transpiration and g_s were measured with a null-balance porometer (Li-Corr 1600m, Li-Corr Inc., Lincoln, NE). 'Stomatal conductance' values were corrected for cuvette boundary layer conductance (g_b) to obtain true g_s (Li-Corr 1600 manual, section 3-1; D.K. McDermott, pers. comm.). Transpiration into the cuvette was calculated to exceed *in situ* rates by a maximum of 13% for estimated minimum g_b (wind speed, 0.5 m s^{-1} ; effective leaf size, 0.03 m ; equations in McDermott, 1990). Where possible, we measured E for control and treated shoots on the same day to minimize the effect of g_b on treatment versus control comparisons.

Leaf ψ_{px} was measured with a pressure bomb (P.M.S. Instruments, Corvallis, Oregon, USA) on four to 15 leaves per shoot to obtain a midday average. Balling and Zimmermann (1990) have questioned the reliability of the pressure bomb because it tended to give ψ_{px} readings lower (below -0.3 MPa) than those measured *in situ* with a pressure probe (above -0.3 MPa) on the same material. However, as Passioura has pointed out (1991) pressure bomb readings of ψ_{px} will always be lower than probe measurements under transpirational conditions because the bomb reading reflects the ψ of the mesophyll whereas the probe is a single-point measure of *in situ* ψ_{px} . Aside from this, the reliability of the pressure probe falls off dramatically for negative pressures below -0.2 MPa (absolute) because of cavitation induction during the measurement (Heydt and Steudle, 1991). Even carefully-constructed osmotic cells cannot sustain negative pressures below -0.3 MPa (absolute) without cavitation (Steudle and Heydt, 1988). This limitation creates obvious difficulties for using the probe to evaluate pressure bomb readings predicting ψ_{px} several MPa below the probe's measurement range.

Trunk ψ was measured with psychrometers (Plant Water Stress Instruments, Inc., Guelph, Ontario, Canada) on side-branches of the main trunk. The branch was cut distal to the psychrometer to eliminate flow-induced temperature gradients and to allow equilibration with trunk ψ (Sperry and Pockman, 1993). The instruments were usually installed the day before measurements began to ensure equilibration, and psychrometers were heavily insulated before being wrapped in plastic wrap and aluminium foil. The psychrometers measure the water potential corrected for the temperature gradient between the xylem and the psychrometer (Dixon and Tyree, 1984). Readings were not attempted when temperature gradients fluctuated over the course of the measurement; this usually resulted from inadequate shading of the instrument. Midday averages of trunk ψ were obtained from three readings spaced from 10.30 h to 13.00 h.

Hydraulic conductance (k) measurements

Hydraulic conductance (k) was defined as the volume flow rate divided by the water potential difference across the flow-path. It was estimated for the soil-to-petiole path for each leaf previously sampled for g_s and E . This was used to determine how close the measured transpiration rate approached the maximum allowable value without cavitation as predicted from equation 1.

Leaf-specific conductance (k_1) from soil to mid-trunk was estimated from average midday E and the soil-trunk water potential difference ($\psi_{\text{soil}} - \psi_{\text{trunk}}$):

$$k_1 = E / (\psi_{\text{soil}} - \psi_{\text{trunk}}). \quad (2)$$

Soil water potential in the rooting zone was estimated from pre-

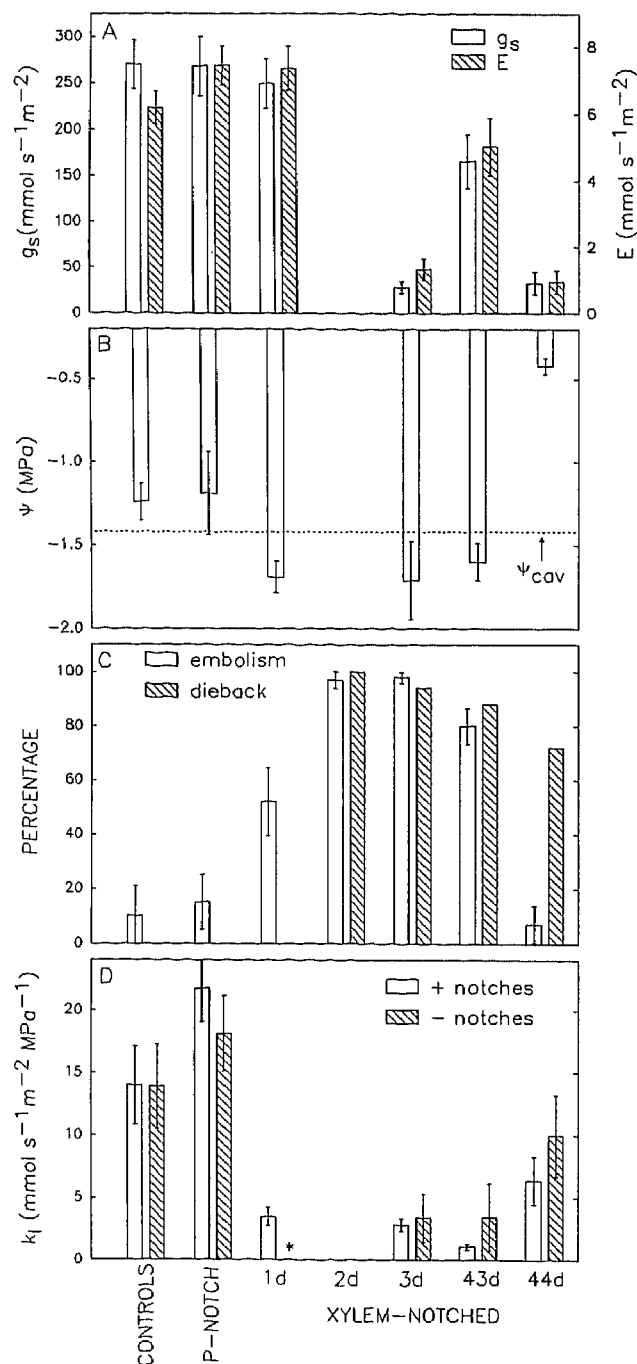


FIG. 1. (A–D) Response to 6–7 notches made in the phloem (P-NOTCH), and phloem plus xylem (XYLEM-NOTCHED). Notches were made on August 10 in shoots of one clump. The 5 xylem-notched shoots were measured 1 to 44d after notching as indicated; P-NOTCH was measured at 26d. CONTROLS are mean of 5 non-notched shoots (\pm s of shoot means); notched shoots are means for indicated sample sizes/shoot (\pm 95% confidence limits). (A) Midday g_s (open bars) and E (hatched bars; $n=15$). Data missing for 2d xylem-notched shoot because of 100% dieback. (B) Midday leaf ψ_{px} ($n=15$); dotted line is maximum cavitation-inducing ψ_{px} (ψ_{cav}). No data for 2d xylem-notched shoot (100% dieback). (C) Embolism (open bars; $n=10$) and leaf dieback % (hatched bars) above the notches. (D) Trunk-petiole k_i before (open bars) and after (hatched bars) removal of notches; controls had same stem length removed ($n=15$). Conductance was zero for 2d shoot. Data without notches missing for 1d shoot (*).

and leaf ψ_{px} at this time was within the cavitation range (data not shown).

The shoot in Fig. 2c and d also showed a decrease in flow following notching (5 cuts), but had only 8% embolism when it was measured after 20 d. No dieback was visible until c. 12 d following notching when leaves began turning yellow. At 20 d 33% of the leaves had senesced. Xylem pressures were higher than controls at the time of harvest as was observed in the non-embolizing shoot in Fig. 1 (44d).

The reduced transpiration, abnormally high leaf ψ_{px} , and eventual senescence in non-embolizing shoots was detected in more detail in the experiment summarized in Fig. 3. Four shoots on a single clump were measured for g_s , E , and ψ_{px} over a 2-week period beginning August 17; three were notched with 4 to 6 transverse cuts on the morning of August 18 (arrow). There was no difference in embolism between notched shoots and the control when shoots were harvested and measured in early September (Fig. 3d, open histograms). The reduction in k_i from the trunk to petiole caused by notching (Fig. 3c, + notches, – notches) was not related to the number of transverse cuts, and caused an estimated 20% reduction in soil–petiole k_i . During the first 3 d following notching, E declined to near $1 \text{ mmol s}^{-1} \text{m}^{-2}$ before stabilizing (Fig. 3a, solid circles) and ψ_{px} showed a corresponding rise (Fig. 3b, solid circles). Control ψ_{px} remained low between -1 MPa and the cavitation value throughout the period (Fig. 3b, open circles). Senescence was first observed 13 d after notching and had reached 20% at harvest-time (Fig. 3d, hatched histograms). A phloem-notched shoot on the same clump showed behaviour indistinguishable from controls (data not shown).

The senescence response in non-embolizing shoots (Fig. 1, 44d; Fig. 2c, d; Fig. 3) was only observed in August when the cambium was inactive. Non-embolizing shoots notched in June and early July when the cambium was still active showed a temporary decrease in g_s and E followed by a return to control values and no senescence (Fig. 4). Trunk–petiole k_i including the notch was in the control range (compare Fig. 1d, ‘control’ with Fig. 4b, k_i ‘+ notch’), and new xylem had been produced around the notches. Furthermore, k_i after notch removal was over twice that of the average control (36 versus $14 \text{ mmol s}^{-1} \text{m}^{-2} \text{MPa}^{-1}$, respectively; compare Figs 5b, k_i ‘– notch’ with 1d, ‘control’) indicating that greater than normal xylem production also occurred above the notch.

We examined the short-term (single-day) response to notching relative to controls in five experiments in August and early September. None of these resulted in cavitation, and all were qualitatively similar to the one summarized in Fig. 5. In this experiment, g and E declined within 20 min of making 12 transverse cuts and stabilized after 1 h at about 65% of the initial rate (Fig. 5a, compare control versus notched shoot). Xylem pressure remained

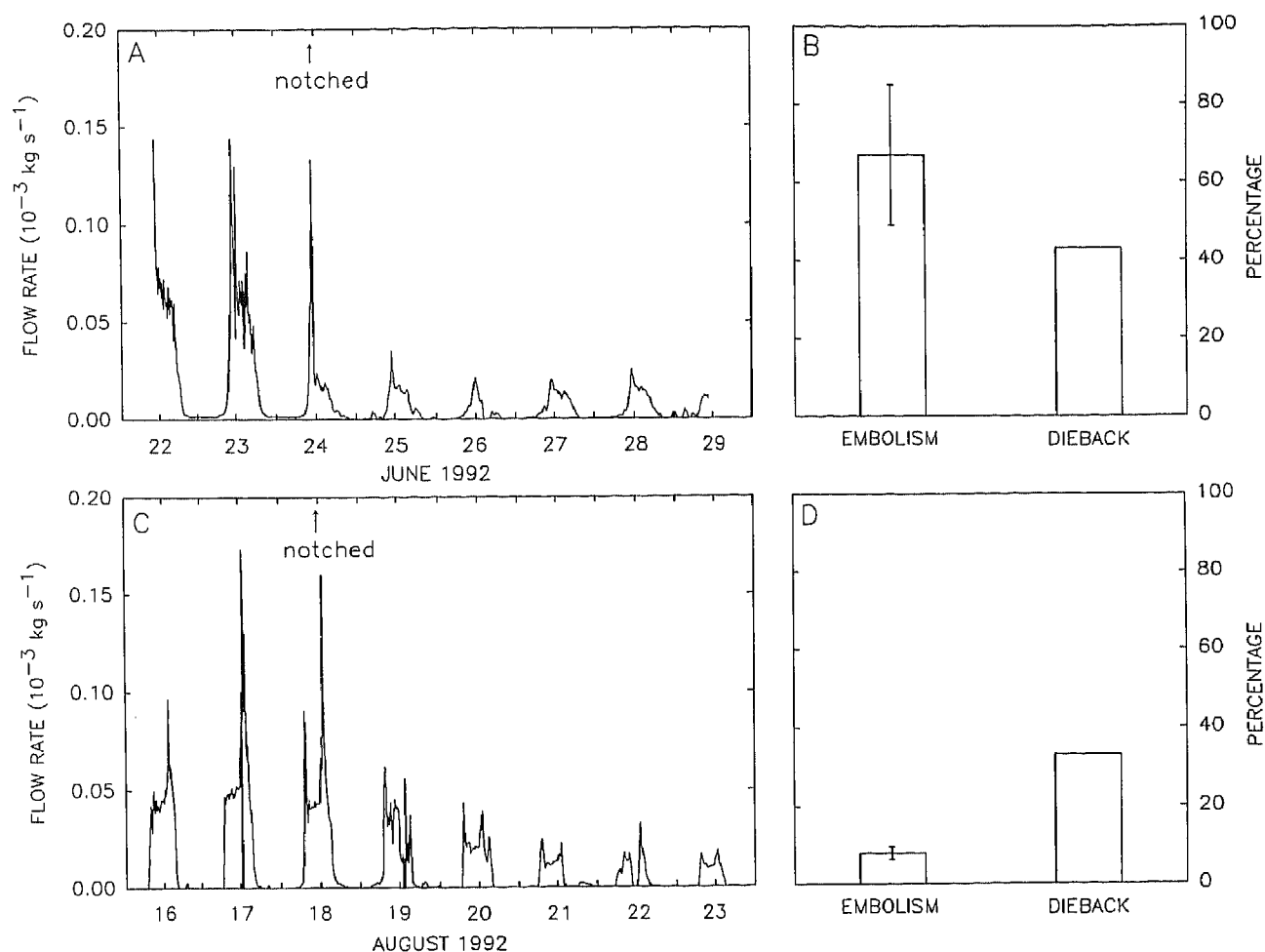


FIG. 2. (A) Flow gauge measurements of xylem flow rate before and after 4 notches were made above the gauge. (B) Embolism ($n=10$, $\pm 95\%$ c.i.) and dieback % 5 d after notching in (A). (C) Xylem flow rate before and after 5 cuts were made above the measuring point. (D) Embolism ($n=10$, $\pm 95\%$ c.i.) and dieback % 22 d after notching in (C).

approximately constant in both the control and the notched shoot throughout the experiment and remained just above the cavitation point (Fig. 5B); embolism in both shoots was below 10%. The 12 notches reduced soil–petiole k_1 by 65%.

The dashed line in Fig. 5C represents the maximum possible steady-state transpiration rate without cavitation as a function of soil–petiole k_1 . This was calculated from equation 1 using 1.14 MPa as the maximum available driving force ($\psi_{\text{soil}} - \psi_{\text{px}}$ at incipient cavitation). The ψ_{soil} estimated from pre-dawn ψ_{px} averaged -0.28 MPa and showed no trend over the summer, and cavitation for this species begins at -1.42 MPa (Sperry and Pockman, 1993). Prior to notching the measured leaves were entirely within the cavitation-free zone (Fig. 5C, circles). The notching reduced soil–petiole k_1 by 65% and was sufficient to bring all of the leaves into the cavitation zone in the absence of any reduction in E . However, stomatal closure following notching kept the shoot primarily within the safe zone (Fig. 5C, squares), and embolism was within the control

range ($< 10\%$). The control shoot was entirely within the safe zone throughout the experiment (data not shown).

When the same analysis was applied to the data in Figs 1, 3, and 4, the position of leaves relative to the expected cavitation zone was consistent with observed embolism levels in the branch xylem (Fig. 6). Controls fell for the most part beneath the line (Fig. 6, open circles) consistent with their low average embolism value of 10% (Fig. 1C). Shoots that avoided cavitation in response to notching and eventually senesced (Fig. 3, xylem-notched shoots) were well beneath the limiting line (Fig. 6, open triangles) when their transpiration rates had stabilized (> 3 d following notching, Fig. 3A). Shoots that avoided cavitation but recovered normal transpiration rates because of new xylem production (Fig. 4) were indistinguishable from controls (Fig. 6, open squares). Finally, shoots caviting in response to notching (Fig. 1, shoots 1–4) were for the most part above the line and within the cavitation range as expected given their elevated embolism levels (Fig. 6, solid triangles).

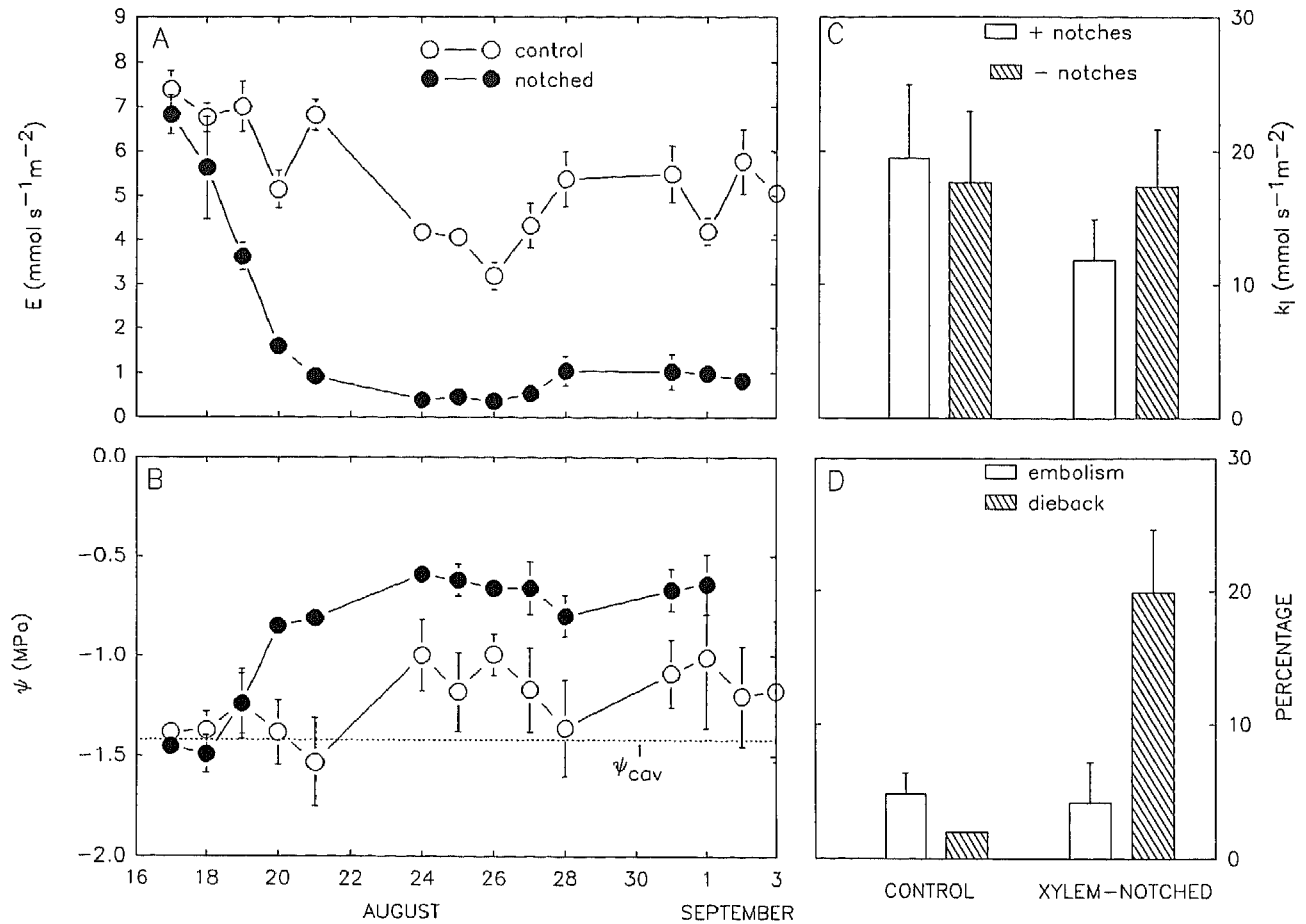


FIG. 3. (A–D) Response of three shoots notched 4–6 times on August 18 compared to control shoot on same clump. Notched shoots represented by the mean of three shoot means (\pm s of means); control is mean of single shoot for indicated sample size (\pm 95% confidence intervals). (A) Midday E ($n=15$) for control (open circles) and notched shoots (solid circles). (B) Midday leaf ψ_{mx} ($n \geq 4$) for control (open circles) and notched shoots (solid circles); dotted line is maximum cavitation pressure (ψ_{p-cav}). (C) Trunk-petiole k_1 before (open bars) and after (hatched bars) notch removal. Measurements made c. 16 d after notching; controls had same length of stem removed ($n=15$). (D) Embolism ($n=10$; open bars) and leaf dieback % (hatched bars) c. 16 d following notching.

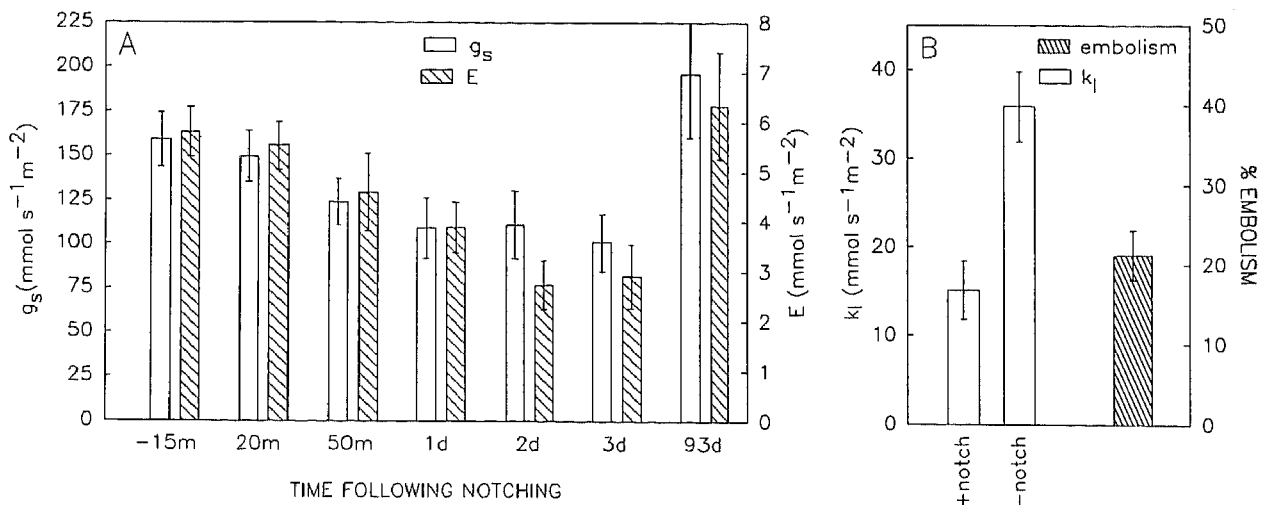


FIG. 4. (A, B) Response to four xylem notches made near midday on June 22 (mean \pm 95% confidence intervals for indicated sample size). (A) Midday g_s (open bars) and E (hatched bars) 15 m before notching, 20 and 50 m after notching, and on subsequent days as indicated ($n=15$). (B) Embolism (hatched bar), and trunk-petiole k_1 (open bars) 93d after notching before (+ notch) and after (- notch) notch removal ($n=10$).

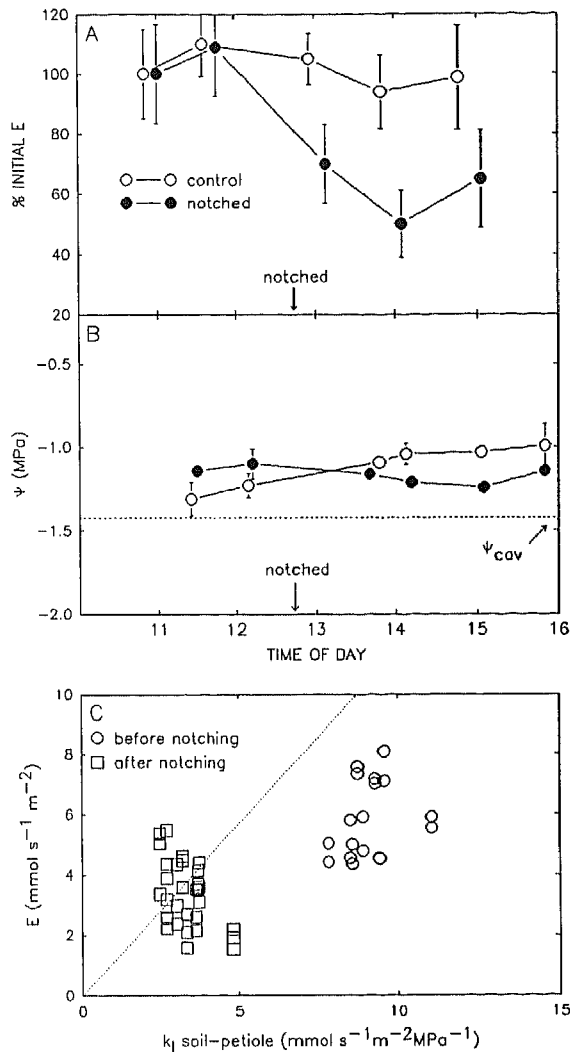


FIG. 5. (A–C) Response to 12 xylem notches made in a shoot at 12.50 h on September 17 compared to a control shoot on the same clump. (A) Mean E ($n=10$, $\pm 95\%$ c.i.) for control (open circles) and notched shoot (solid circles). (B) Mean leaf ψ_{px} ($n=3$, $\pm s$) for control (open circles) and notched shoot (solid circles). (C) Transpiration rate (E) versus estimated soil-petiole k_1 in the notched shoot before (open circles) and after notching (open squares). The dashed line is the minimum flow predicted to induce cavitation in the petiole for a given k_1 (equation 1).

DISCUSSION

There were two responses to reduced k_1 caused by transverse cuts: embolism induction and embolism avoidance. Avoidance resulted from stomatal closure which reduced E to values that kept leaf ψ_{px} from dropping into the cavitation range (Figs 5, 6). Induction resulted from leaf ψ_{px} dropping into the cavitation range (Fig. 1) due to E rates higher than the maximum allowable without cavitation (Fig. 6, solid triangles).

The factors determining whether or not embolism was induced were probably the response time of stomata to reduced k_1 and how close the xylem flow rate was to the cavitation inducing value. Although stomatal conduct-

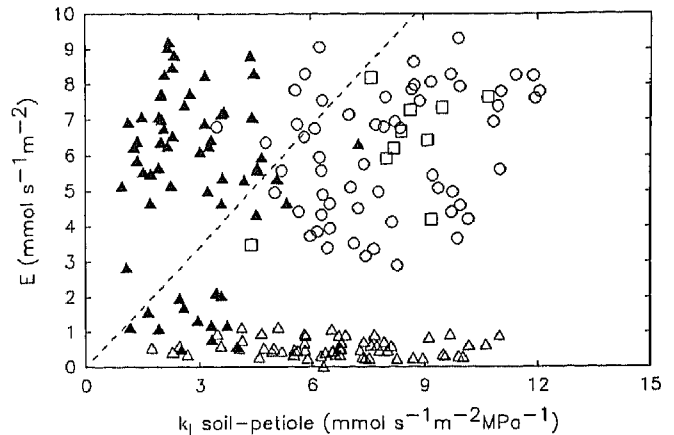


FIG. 6. Transpiration rate (E) versus estimated soil-petiole k_1 for control shoots (open circles), notched shoots that embolized (solid triangles), notched shoots that did not embolize and senesced (open triangles), and notched shoots that did not embolize and exhibited new xylem growth (open squares). The dashed line is the minimum flow predicted to induce cavitation in the petiole for a given k_1 (equation 1).

ance was reduced within 20 min after notching (Fig. 5), an almost instantaneous response would be required to avoid cavitation for flow rates at the maximum safe value; the actual required response time would depend on the capacitance of the leaf tissue. Shoots notched at midday when E is maximum would be more likely to embolize than shoots notched in the morning. This was consistent with the results shown in Figs 1 and 3; the five shoots in Fig. 1 were notched between 14.30 h and 15.30 h and four of them embolized, whereas the three shoots in Fig. 3 were notched before 10.30 h and none embolized.

How did stomata sense the reduction in k_1 and begin closing to prevent cavitation-inducing E values (Fig. 5)? Root signals can be ruled out because the rooting environment was constant during experiments. The simplest explanation is that stomata were responding to a threshold leaf water potential coincident with the upper end of the cavitation range. The reduction of k at constant E would reduce leaf water potential below the threshold bringing about stomatal closure probably via abscisic acid (ABA) release into the leaf apoplast (Hartung and Slovák, 1991). Modulation of g_s could maintain leaf ψ approximately constant near the threshold value.

It has been erroneously concluded from data similar to that in Fig. 4 that when leaf ψ remains constant while g_s drops, leaf ψ cannot be the signal inducing closure (Davies and Zhang, 1991). This is analogous to arguing that the temperature of a thermostatically regulated room is not the signal for its regulation because it remains approximately constant. In the same way that small changes in room temperature trigger its regulation through a thermostat, small changes in leaf ψ could trigger stomatal closure keeping leaf ψ within a limited range. Oscillations in leaf ψ near the threshold value need not be large enough and of low enough frequency to be detected by pressure bomb

measurements which only provide a volume-averaged leaf ψ . Pressure probe measurements of mesophyll turgor would be more appropriate for detecting the oscillations in ψ predicted to occur as the threshold was approached.

Although the stomatal closure observed within hours of notching was sometimes sufficient to avoid cavitation (Fig. 5), on successive days g_s continued to drop (Figs 3, 4) and E values became much lower than necessary to prevent cavitation (Fig. 6, open triangles). Similar 'after effects' of experimentally-imposed stress on stomatal closure have been reported previously and discussed in terms of leaf ABA concentrations (Hartung and Slovák, 1991). The over-compensation by stomata seen in our experiments may have resulted from an excessive release of ABA into the leaf apoplast triggered by the need for abrupt and sustained stomatal closure. Elevated leaf ABA levels would also be favoured by reduced export in the notched phloem.

Shoots notched in late June or early July (Fig. 4) showed a return to control E that was associated with control level k_1 . The implication is that the xylem produced after the notching increased k_1 to original levels and eliminated the need for sustained stomatal closure. This would not only reduce ABA release into the leaf apoplast, but speed its export by production of new phloem around the notches. Later in the summer vascular production after notching was not observed, and k_1 remained below controls (Fig. 3). The over-compensation of stomata was sustained and senescence set in within 2 weeks. In view of the well-known role of ABA in promoting senescence (Sacher, 1983), it seems likely that the premature senescence was due to prolonged elevation of ABA levels in the leaf.

Our results extend and clarify preliminary experiments reported on the same species using air-injection to lower k via induced cavitation (Sperry and Pockman, 1993). In these experiments, k was always reduced over the course of the afternoon when shoots were at maximum E , and all shoots showed dieback of the magnitude seen in the embolized shoots in Fig. 1. This study did not detect the long-term embolism avoidance seen here probably because k was always reduced at midday when E would have been closest to its limiting value.

Unfortunately, in all experiments where g_s and E were continuously monitored following notching, the shoots were successful in limiting E and avoiding embolism (Figs 4, 5). Thus, we could not unravel the reasons for the diverse response in g_s and E seen in the embolized and dying shoots in Fig. 1. Our earlier study suggested that initial stomatal closure was followed by loss of stomatal control and dieback (Sperry and Pockman, 1993). However, as shown by the experiment in Fig. 1, shoots apparently embolized and died back at different rates making reconstruction of a time-course based on separate shoots as was done in the earlier study difficult. The behaviour of stomata once critical E rates have been

exceeded is probably quite complex and requires more careful study.

ACKNOWLEDGEMENTS

This work was funded in part by NSF grant BSR-8806264 and USDA grant #9202487; N.N. Alder was supported by an NSF REU supplement.

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