



# The Major Veins of Mesomorphic Leaves Revisited: Tests for Conductive Overload in *Acer saccharum* (Aceraceae) and *Quercus rubra* (Fagaceae)

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# THE MAJOR VEINS OF MESOMORPHIC LEAVES REVISITED: TESTS FOR CONDUCTIVE OVERLOAD IN *ACER SACCHARUM* (ACERACEAE) AND *QUERCUS RUBRA* (FAGACEAE)<sup>1</sup>

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Many leaves survive the severing of their major veins in apparently excellent health. According to the classical explanation, the leaf minor veins provide “conductive overload,” an excess of parallel conductive paths, rendering the major veins hydraulically dispensable. Whether such an excess of conductive paths exists has important implications for vascular design and for leaf response to vascular damage. We subjected leaves of *Acer saccharum* and *Quercus rubra* to cutting treatments that disrupted the major vein system and determined leaf survival, stomatal conductance ( $g$ ), quantum yield of photosystem II ( $\Phi_{\text{PSII}}$ ), and leaf hydraulic conductance ( $K_{\text{leaf}}$ ). For *A. saccharum*, the cuts led to the death of distal lamina. For *Q. rubra*, however, the treated leaves typically remained apparently healthy. Despite their appearance, the treated *Q. rubra* leaves had a strongly reduced  $K_{\text{leaf}}$ , relative to control leaves, and  $g$  and  $\Phi_{\text{PSII}}$  were reduced distal to the cuts, respectively, by 75–97% and 48–76%. Gas exchange *proximal* to the cuts was unaffected, indicating the independence of lamina regions and their local stomata. Analogous results were obtained with excised *Q. rubra* leaves. These studies demonstrate an indispensable, vital role of the major veins in conducting water throughout the lamina.

**Key words:** biological networks; drought response; herbivory; hydraulic architecture; leaf hydraulic conductance; stomatal conductance; temperate forest; vascular architecture.

As shown in the classic studies of R. B. Wylie and co-workers, the leaves of many species survive the disruption of major vein system with little visible necrosis or desiccation, apparently in excellent health (see Figs. 1 to 9 in Plymale and Wylie, 1944). This survival was attributed both to the capacity of leaves to heal wounds (Wylie, 1927, 1930; Bloch, 1952; Davis, Miller, and Lineberger, 1991) and to “conductive overload”: the idea that the minor vein network in temperate deciduous leaves provides an excess of parallel vascular paths throughout the lamina, rendering the *major* veins hydraulically redundant and perhaps only essential for structural support (Wylie, 1938; Plymale and Wylie, 1944). However, the disruption of the major veins might have had large, though unperceived, effects on water supply within the leaf, with stomatal closure compensating for the loss in hydraulic capacity. A recent study of simulated herbivory in *Betula pendula* supports this interpretation. Two weeks after leaves had their midribs perforated, stomatal conductance and CO<sub>2</sub> assimilation had dropped to about 70% of values for untreated leaves (Oleksyn et al., 1998). This finding suggests a potentially indispensable role of major veins in leaf water transport. Whether leaves are dramatically oversupplied in vasculature has major implications for our understanding of the hydraulic design of leaves (Jeje, 1985; Canny, 1993; Roth-Nebelsick et al., 2001; Sack et al., 2002; Zwieniecki et al., 2002), as well as

the ways in which leaves cope with potential losses in transport capacity due to mechanical damage (e.g., Cholewa et al., 2001) or cavitation (Kikuta et al., 1997; Salleo et al., 2001).

In this study, we reexamine the role of the leaf major veins in *Acer saccharum* and *Quercus rubra*. Using a series of experimental treatments modeled after those of Wylie and co-workers, we tested for conductive overload by monitoring both leaf survival and rates of gas exchange. We also examined the effects of experimentally disrupting the major vein system on leaf hydraulic conductance and tested for short-term effects using excised leaves.

## MATERIALS AND METHODS

**In vivo experiments: plant material**—The in vivo experiments were performed at Harvard Forest, Petersham, Massachusetts, USA (42°54' N, 72°18' W) during summer 2001 on trees of *A. saccharum* and *Q. rubra* located along roads through the forest. The trees had diameters at breast height ranging from 2.2 to 4.3 cm for *A. saccharum* and from 1.8 to 3.8 cm for *Q. rubra*. Experimental leaves were those of highest exposure that could be accessed by hand from the ground. Experimental leaves for *A. saccharum* ranged in lamina area from 26 to 154 cm<sup>2</sup>, with mean  $\pm$  SE for all leaves of 75.4  $\pm$  2.1 cm<sup>2</sup> (leaf areas determined at the end of the study using a LI-COR leaf area meter; LI-COR, Lincoln, Nebraska, USA); those of *Q. rubra* ranged from 48 to 208 cm<sup>2</sup>, with mean  $\pm$  SE of 90.2  $\pm$  2.1 cm<sup>2</sup>. As an index of the light environment, the diffuse site factor (Anderson, 1964) was measured on an overcast day for five experimental leaves per tree, using matched quantum sensors (LI-COR LI-190). The *A. saccharum* leaves ranged from 8 to 15% daylight exposure and the *Q. rubra* leaves from 24% to 78%.

Leaf veins were classified according to branching architecture (Nelson and Dengler, 1997). Primary, secondary, and tertiary veins protrude as ridges visible on the abaxial face of the lamina. Primary veins are connected directly to the petiole. Thus, in *Q. rubra* there is only one primary vein, usually referred to as the midrib, while in *A. saccharum* there are typically seven. Secondary veins branch from the primary vein(s), and tertiary veins are smaller in diameter, branching from and sometimes linking the primary and sec-

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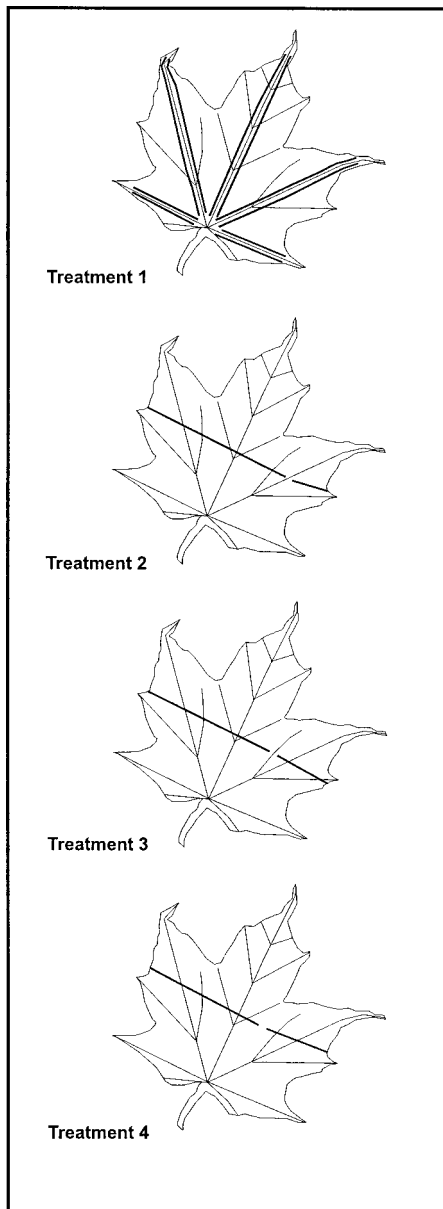


Fig. 1. Treatments applied to leaves of *Acer saccharum*.

ondary veins. The quarternary veins (henceforth “minor veins”) are fully embedded in the mesophyll and consist of several diameter classes, all smaller than the tertiary veins. The fine veins form a continuous mesh with the major vein system, dividing the lamina into regions referred to as areoles.

**In vivo experiments: experimental treatments**—Four treatments were applied, each of which involved partially severing different portions of attached leaves. All cuts were made using a scalpel while supporting the leaf on a styrofoam block. For *A. saccharum*, Treatment 1 consisted of parallel incisions on both sides of each primary vein, 5 mm from the primary veins, beginning 1 cm from the petiole-leaf junction, and for each vein finishing 1 cm from the margin (Fig. 1). The incisions were made on each side of the vein for the five central primary veins, but not for the first primaries on each side of the lamina, which were of notably smaller diameter. The other three treatments for *A. saccharum* consisted of cuts laterally across the lamina, approximately halfway along the middle primary length, perpendicular to the middle primary. The distal portion of the leaf remained attached to the proximal portion

by only a 5–6 mm section of lamina (the “bridge”). In Treatment 2, this bridge contained the second primary vein from the midrib, as well as minor veins; in Treatment 3, this bridge contained only a secondary vein and minor veins; for Treatment 4, the bridge contained only minor veins (Fig. 1).

The four treatments were modified for *Q. rubra* in accordance with its pinnate venation. Treatment 1 consisted of a 1-cm cut across the midrib, 1 cm from the petiole-lamina junction (Fig. 2). Treatment 2 consisted of parallel incisions on both sides of the midrib, 2–3 mm from the vein, beginning 1 cm from the petiole-leaf junction and finishing 1 cm from the margin (Fig. 2). Treatments 3 and 4 consisted of cuts laterally across the lamina, approximately halfway along the midrib length, perpendicular to the midrib, with a bridge connecting the distal portion of lamina to the proximal portion (Fig. 3). In Treatment 3, the bridge contained a secondary vein and minor veins; in Treatment 4, the bridge contained only minor veins.

Each cut leaf was splinted, or taped, as in the original Plymale and Wylie experiments (1944), to provide mechanical support. *Quercus rubra* leaves subjected to Treatment 1 were splinted with a 3 × 8 cm piece of cardboard, folded over the base of the lamina and petiole, and taped in place. All other *Q. rubra* leaves and all *A. saccharum* leaves were taped over the lengths of the cuts, such that the cuts were in the center of a strip of tape (3M Scotch transparent tape of 12.7-mm width), with the cut edges held apart under tape by about 1 mm. To indicate how rapidly sections of lamina would desiccate if completely severed, the distal halves of two leaves for each species were severed completely and then reattached with tape.

The first in vivo study was conducted using three trees each of *A. saccharum* and *Q. rubra*. Treatments 1 through 4 (see Figs. 1, 2, and 3) were applied to five leaves on each tree during 18–20 July for *Q. rubra* and 25–27 July for *A. saccharum*. Each treated leaf was paired with a control leaf that was nearby on the same branch (opposite leaves were used for *A. saccharum*) and matched in size and exposure, approximately, with its paired treated leaf. Control leaves were left uncut, but splinted and taped as for the treated leaves. Two weeks into the study, 12 experimental leaves from one of the *Q. rubra* trees were lost from the study due to destructive sampling (i.e., six treated leaves and their controls, two leaves each from Treatments 1, 2, and 3).

**In vivo experiments: gas exchange measurements**—Because the *A. saccharum* leaves soon browned, desiccated, and died (see RESULTS), only the *Q. rubra* leaves were available for physiological measurements. *Quercus rubra* leaves were measured three times—after 2, 3, and 5 wk, on 1–2, 6–7, and 22–23 August—for stomatal conductance ( $g$ ), transpiration, photosynthetically active radiation (PAR), and temperature using a steady-state porometer (LI-COR 1600; LI-COR, Lincoln, Nebraska, USA). All measurements were made between 0900 and 1330, alternating treated leaves with their matched controls for each of the experimental trees in sequence. For leaves subjected to Treatments 1 and 2, measurements were made with the cuvette applied to a location on the lamina between the midrib and margin, approximately half-way along the midrib length, avoiding secondary veins as far as possible. For leaves subjected to Treatments 3 and 4, and their uncut controls, measurements were made in the distal portion of the lamina, on the opposite side of the midrib from the cut (location A; Fig. 3). At the final measurement session, porometer measurements were also made at a second location distal to the cut—on the same side of the midrib as the cut (location B; Fig. 3)—and at a third location, in the section of lamina proximal to the cut (location C; Fig. 3).

A second, week-long study was made using the same treatments on *Q. rubra* leaves. On 24–26 August, eight leaves were selected for each cut type, and paired control leaves were taped but left uncut. The experimental leaves were chosen on six different trees (4–10 treatment–control pairs on each tree). One week later, on 1–2 September, between 0900 and 1530, leaves were measured for stomatal conductance, transpiration, PAR, and temperature, as above, and also for the quantum yield of photosystem II ( $\Phi_{PSII}$ , using a Walz MINI-PAM; Heinz Walz GmbH, Effeltrich, Germany), an index of overall photosynthetic capacity (Maxwell and Johnson, 2000). Measurements were made in the same lamina locations as in the first study (Figs. 2 and 3), alternating treated leaves and their matched controls.

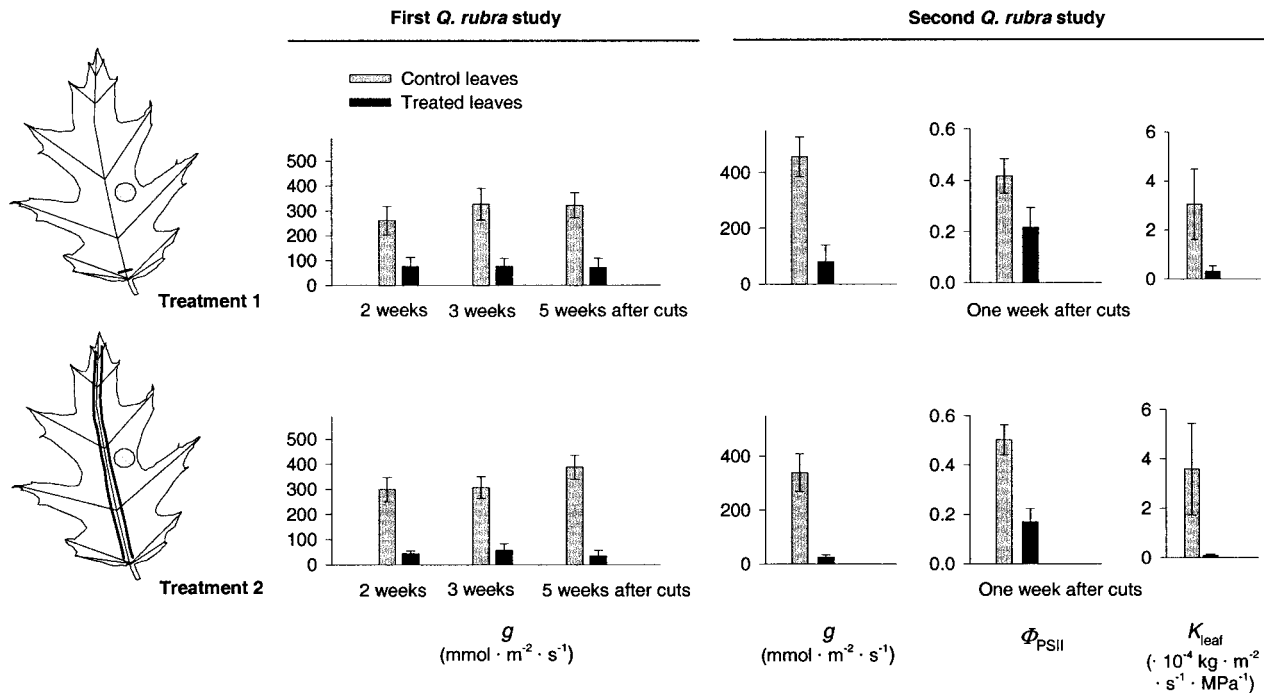


Fig. 2. Treatments 1 and 2 applied to leaves of *Quercus rubra* and their effects on gas exchange and leaf hydraulics relative to controls (means  $\pm$  1 SE). In the first study, measurements of stomatal conductance ( $g$ ) were made on three occasions following the cuts. In the second study, 1 wk after the cuts,  $g$  was measured, as well as the quantum yield of photosystem II ( $\Phi_{PSII}$ ) and leaf hydraulic conductance ( $K_{leaf}$ ). In the first study,  $N = 10-14$ . In the second study  $N = 6-8$  for  $g$  and  $\Phi_{PSII}$  and 3-6 for  $K_{leaf}$ .

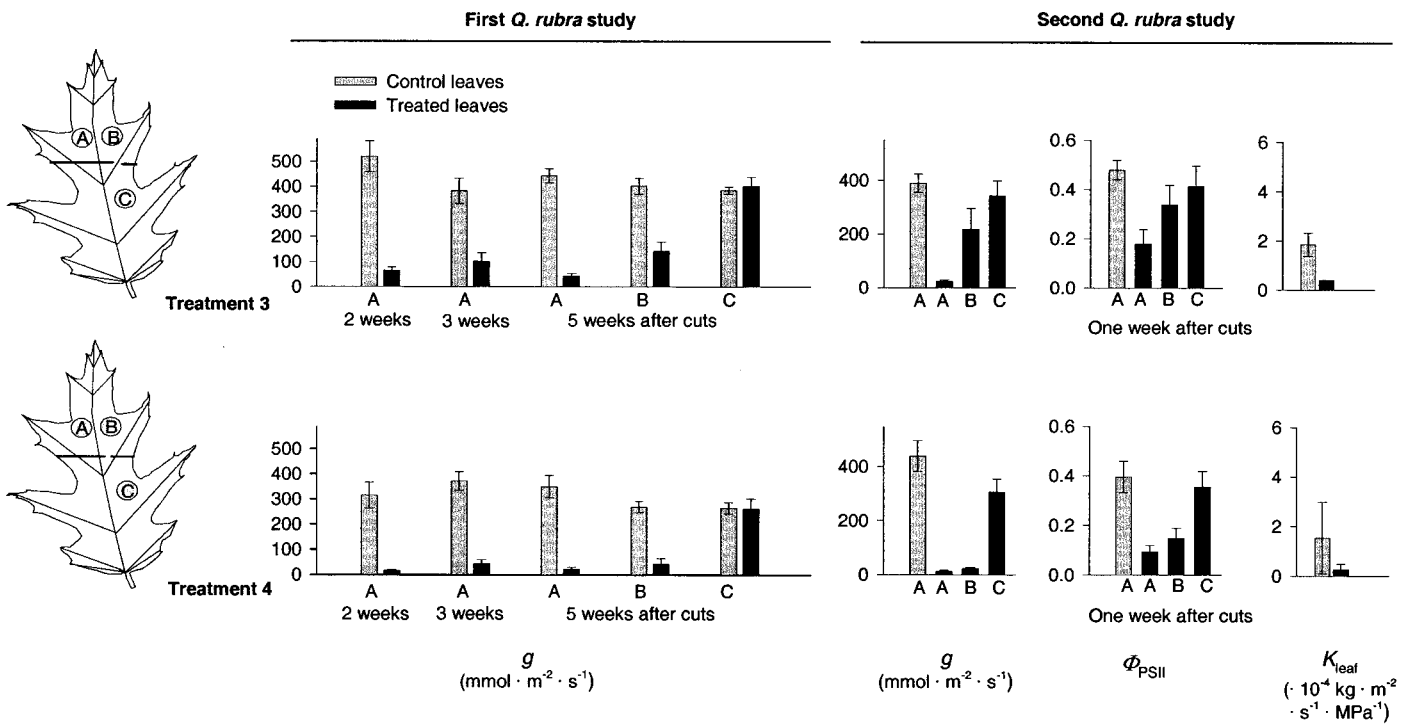


Fig. 3. Treatments 3 and 4 applied to leaves of *Quercus rubra* and their effects on gas exchange and leaf hydraulics relative to controls (means  $\pm$  1 SE). In the first study, measurements of stomatal conductance ( $g$ ) were made on three occasions following the cuts. In the second study, 1 wk after the cuts,  $g$  was measured, as well as the quantum yield of photosystem II ( $\Phi_{PSII}$ ) and leaf hydraulic conductance ( $K_{leaf}$ ). At the final measurement session of the first study, and in the second study, measurements were made at three lamina locations (A, B, and C). In the first study,  $N = 10-14$ . In the second study  $N = 6-8$  for  $g$  and  $\Phi_{PSII}$  and 3-6 for  $K_{leaf}$ .

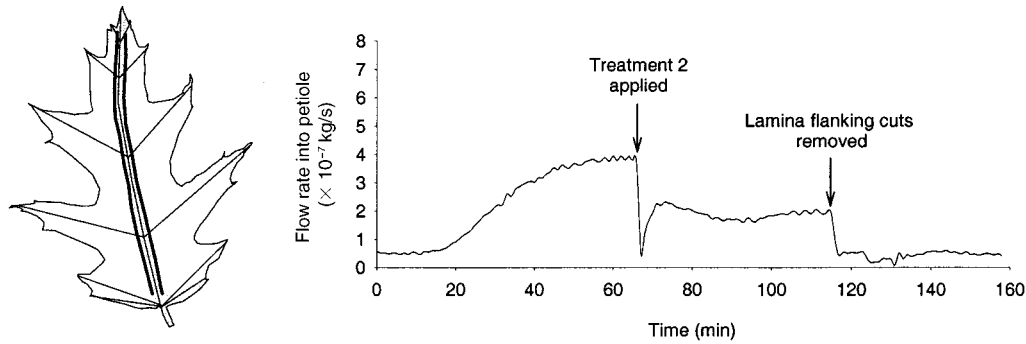


Fig. 4. The application of Treatment 2 to an excised leaf of *Quercus rubra*. In the schematic, the heavy line represents the cut (as in Fig. 2), the white section represents the “flanking” sections removed afterward, and the gray section represents the section remaining for the final reading.

**In vivo experiments: leaf hydraulic conductance measurements**—The leaf hydraulic conductance ( $K_{\text{leaf}}$ ) was estimated for *Q. rubra* leaves in the second study. The previous evening, leaves situated adjacent to the experimental leaves were covered with plastic bags and wrapped in aluminum foil. The following day, after measurement of stomatal conductance, transpiration, and quantum yield, each experimental leaf was covered with a quick-sealing plastic bag, and excised, along with one of the leaves covered with plastic bag and aluminum foil. Excised leaves were left for at least 1 h to equilibrate in their sealed plastic bags, inside a larger plastic bag with moist paper towel. The pressure drop across the experimental leaf ( $\Delta\Psi_{\text{leaf}}$ ) was estimated as the difference between its water potential (determined using a pressure chamber; Plant Moisture Systems, Corvallis, Oregon, USA) and that of its matched nontranspiring (bagged) leaf, which was presumed to have equilibrated with the xylem water potential of the branch proximal to the petiole. The leaf hydraulic conductance was estimated as the transpiration rate divided by  $\Delta\Psi_{\text{leaf}}$ ; values were standardized for the effects of temperature on the viscosity of water by correcting  $K_{\text{leaf}}$  to a value for 25°C (Weast, 1974; Yang and Tyree, 1993). For leaves subjected to Treatments 3 and 4, the transpiration rate used for  $K_{\text{leaf}}$  calculation was approximated using an area-weighted average:  $0.25 \times$  transpiration rate measured at location A +  $0.25 \times$  transpiration rate measured at location B +  $0.5 \times$  transpiration measured at location C. Leaf hydraulic conductance estimated in this way is an index of the whole-leaf integrated hydraulic conductance; the estimate contains some degree of uncertainty as porometers sometimes overestimate transpiration (Yang and Tyree, 1994). Sample sizes for estimating  $K_{\text{leaf}}$  were small ( $N = 3$  to 6 for each treatment), as the partially severed leaves were often fragile, and when broken could not be measured. Survival of all remaining experimental and control leaves was assessed on 2 September.

**In vivo experiments: analysis**—Paired-sample  $t$  tests were used to determine the significance of each treatment, by comparing measurements for treatment vs. control leaves (Zar, 1999; using Minitab Release 13.31 [MINITAB, State College, Pennsylvania, USA]). To compare the relative impacts of different treatments we tested impact indices, i.e.,  $\log[(\text{treated leaf value}/\text{control leaf value}) + 1]$ , using a repeated-measures ANOVA for the data in the first study (using GenStat 5 [NAG, Downer's Grove, Illinois, USA]; factors treatment  $\times$  measurement session), and using a one-way ANOVA for the data in the second study (using Minitab Release 13.31). Log transformation increased normality and heteroscedasticity (Sokal and Rohlf, 1995). To test the difference between measurements made at different lamina locations, repeated-measures ANOVAs were applied, blocking for leaf (using GenStat 5; Sokal and Rohlf, 1995).

**Excised leaf experiments**—Experiments were conducted on excised leaves of *Q. rubra* to assess the short-term effects of severing portions of the major vein system. Branches were detached from *Q. rubra* trees, covered loosely with plastic, and left overnight to saturate with cut ends in 10 mmol/L KCl aqueous solution. Each leaf to be measured was severed by cutting the petiole several times under solution (=10 mmol/L aqueous KCl) and then connected

by a compression fitting to low-resistance tubing (Bev-A-Line IV tubing, 3.2 mm internal diameter; Cole-Parmer, Vernon Hills, Illinois, USA) containing the KCl solution running to a graduated cylinder on an analytic balance ( $\pm 0.01$  mg; Sartorius 12 MP8, Sartorius AG, Goettingen, Germany;  $\pm 0.1$  mg; Mettler AG104, Mettler-Toledo GmbH, Greifensee, Switzerland). The balance logged data to a computer for calculation of flow rate into the petiole. The leaf was supported (abaxial surface down) using a wood frame strung with fishing line, which held the leaf horizontal and immobile above a large box fan. A light source was suspended above a plexiglass container full of water above the leaf, containing waxed paper as a diffuser, producing  $>1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  photosynthetically active radiation at the leaf surface while maintaining leaf temperature within 2°C of ambient. When the fan was turned on, flow into the leaf increased for typically 10–30 min before stabilizing with a coefficient of variation  $<5\%$  for 10 min ( $F_{\text{uncut}}$ ). At this time, leaves were prodded with the blunt end of a scalpel to test for the effects of mechanical shock. Next, leaves were subjected to the cuts of Treatments 1 through 4 (Figs. 2 and 3), using a scalpel, and the values recorded when the flow into the leaf again became stable ( $F_{\text{after cut}}$ );  $N$  for each cut type ranged from three to six leaves. We calculated the effect of the cut on the total water flow out of the lamina, as  $F_{\text{after cut}}/F_{\text{uncut}}$ .

We also estimated the effect of the cut on the water flow out of the lamina distal to the cuts for excised leaves subjected to Treatments 2, 3, and 4. Once  $F_{\text{after cut}}$  was determined, the regions of the leaf distal to the cut were excised. For leaves subjected to Treatment 2, the areas of lamina flanking the cuts were removed (Fig. 4); for leaves subjected to Treatments 3 and 4, the areas of lamina distal to the cuts were removed. The flow rate through the remaining lamina was recorded when it became stable again ( $F_{\text{distal section removed}}$ ). The impact of the cut on the lamina distal to the cut was estimated as  $(F_{\text{after cut}} - F_{\text{distal section removed}})/(F_{\text{before cut}} - F_{\text{distal section removed}}) \times 100\%$ . Paired  $t$  tests were used to test the differences in flow before and after application of the four treatments.

## RESULTS

**In vivo experiments: efficacy of treatments**—In both species, virtually all the control leaves remained green and apparently healthy throughout the duration of the experiment. Only one *A. saccharum* control leaf (i.e., 1/60) degreened and senesced.

A tape effect occurred for both treated and control leaves. Tissue directly beneath the tape often became partially discolored or desiccated, apparently due to the heating of lamina under the tape when exposed to direct sunlight. This effect was more pronounced in *A. saccharum* leaves, in which 116/120 leaves were affected, with the lamina underneath the tape often becoming completely brown. In *Q. rubra*, the tape led only to faint discoloration in 56/108 leaves. This effect took several weeks to develop and thus was not apparent in the

second study, in which *Q. rubra* leaves were sampled after only 1 wk. In 11/108 *Q. rubra* leaves the lamina between the veins under the tape was partially eaten by insects hatched from eggs preferentially laid under the tape. These tape effects occurred to the same degree, and in almost exactly the same number of control and cut leaves; thus, they are not expected to affect the findings of the study.

In *A. saccharum* and *Q. rubra*, the distal halves of leaves that had been completely severed from their proximal halves and reattached with tape desiccated within 3 d.

**In vivo experiments: effects on leaf survival**—The treatments had a visually striking effect on *A. saccharum* leaves: after 3 d, the majority of the lamina distal to the cuts had begun to turn brown. With a few exceptions, by 2 wk later, these regions had become completely brown, desiccated, and/or necrotic. For a few treated leaves, patches of lamina remained alive and green to the end of the study, for 4/15 *A. saccharum* leaves subjected to Treatment 1 (<10% of the lamina remained green for three leaves; 27% for the fourth) and for 3/45 leaves subjected to Treatments 2, 3, and 4 combined (30–80% of the lamina remained green distal to the cut). For *A. saccharum* leaves subjected to Treatments 2, 3, and 4, the lamina proximal to the cuts remained green and apparently healthy. In some of the *A. saccharum* leaves of Treatment 1, the strips of lamina containing primary veins between the cuts (2–3 mm in width) remained alive and green up to 3 wk, subject to patches of browning.

By contrast, in *Q. rubra* the treated leaves generally remained entirely green and, apparently, in full health. In the overwhelming majority of leaves, necrosis was largely limited to scarring at the edges of the incisions. In the first study, 1/13, 2/13, 1/13, and 1/15 of the leaves in Treatments 1 through 4 suffered some desiccation and tissue death (10% to all of the lamina area distal to the cuts was affected). In three other leaves partial desiccation of lamina regions distal to the cuts resulted from the severing of these regions by accident during gas exchange measurements or by insects. In the second study, partial desiccation or localized tissue death occurred in 2/8, 1/8, 0/8, and 3/8 of leaves subjected to Treatments 1 through 4, respectively, and in very small amounts (about 10% or less of the lamina area distal to the cut).

**In vivo experiments: effects on leaf gas exchange**—Despite the apparent health of the majority of the treated *Q. rubra* leaves, the four treatments had a strong impact on gas exchange. Each treatment significantly reduced  $g$  relative to that of matched control leaves in each study ( $P < 0.05$  in 5/16 tests;  $P < 0.001$  in 11/16 tests; Figs. 2 and 3). Stomatal conductance was reduced by 75% relative to control values in Treatment 1 in the first study (averaging across measurement sessions) and by 82% in the second study (Fig. 2). In Treatment 2  $g$  was reduced by 86 and 93% (Fig. 2), and (at lamina location A) by 84 and 94% in Treatment 3, and by 92 and 97% in Treatment 4 (Fig. 3). In the second study, the four treatments also strongly reduced  $\Phi_{\text{PSII}}$  ( $P \leq 0.003$ ; paired  $t$  tests; Figs. 2 and 3). In Treatment 1  $\Phi_{\text{PSII}}$  was reduced by 48% relative to control values, by 66% in Treatment 2, and (at lamina location A) by 62% in Treatment 3 and 76% in Treatment 4. The four treatments had different effects on gas exchange, and this pattern remained constant over time. In the first study, the impact of Treatments 1, 2, 3, and 4 on  $g$  differed significantly, considering location A for Treatments 3 and 4

(repeated-measures ANOVA, factors treatment and measurement session; for treatment,  $P < 0.05$ ; Figs. 2 and 3). In impact, the treatments ranked  $4 > 3 \sim 2 > 1$ . There was no difference between measurement sessions in this ranking, and there was no interaction between treatment and measurement session ( $P > 0.15$  in each case; Figs. 2 and 3). In the second study the treatments ranked empirically as in the first study, but no statistical differences could be resolved (one-way ANOVA;  $P > 0.3$ ).

The effect of Treatments 3 and 4 on gas exchange depended on where on the leaf lamina the  $g$  and  $\Phi_{\text{PSII}}$  were measured (one-way ANOVAs,  $P \leq 0.01$ ;  $P \leq 0.001$  in 4/6 tests; Fig. 3). In the control leaves,  $g$  did not vary among lamina locations A, B, or C (repeated-measures ANOVA, blocking by leaf;  $P > 0.05$ ; Fig. 3); similarly, when checked in the field,  $\Phi_{\text{PSII}}$  for control leaves was approximately the same across locations. In general, the cuts had a weaker impact at location B relative to location A. In Treatment 3,  $g$  measured at location B was reduced by 65% in the first study and by 44% in the second (cf. 84 and 94% at location A), while  $\Phi_{\text{PSII}}$  in the second study was reduced by 30% (cf. 62% at location A). In Treatment 4,  $g$  measured at location B was reduced by 85 and 95% (cf. 92 and 97% at location A), and  $\Phi_{\text{PSII}}$  by 63% (cf. 76% at location A). At location C (proximal to the cut),  $g$  and  $\Phi_{\text{PSII}}$  were statistically identical to control values in each study (paired  $t$  tests;  $P > 0.05$ ; Fig. 3).

**In vivo experiments: effects on leaf hydraulic conductance**—The treatments had a marked effect on  $K_{\text{leaf}}$ . The control leaves had  $\Delta\Psi_{\text{leaf}}$  of 0.7–0.8 MPa and  $K_{\text{leaf}}$  values (Figs. 2 and 3) similar to those of untaped exposed leaves of *Q. rubra* ( $2\text{--}3 \times 10^{-4} \text{ kg} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$ ; Sack et al., 2002). The treated leaves had higher mean  $\Delta\Psi_{\text{leaf}}$  values, 1.3–2 MPa, lower transpiration rates, and lower  $K_{\text{leaf}}$  values relative to control leaves. In Treatments 1 through 4,  $K_{\text{leaf}}$  was reduced respectively, by 90, 98, 78 and 83% relative to control leaves (Figs. 2 and 3). However, perhaps because of the small sample sizes, the effect was significant for only Treatment 4 (paired  $t$  tests; for Treatments 1, 2 and 3,  $P$  ranged from 0.064 to 0.086; for Treatment 4,  $P = 0.003$ ).

**Excised leaf experiments**—Excised leaves of *Q. rubra* also responded strongly to the partial severing treatments. Excised leaves achieved mean transpiration rates of  $2.5\text{--}4.5 \times 10^{-5} \text{ kg} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  (Table 1). Prodding the leaf with the blunt edge of the scalpel blade did not affect flow through the leaf. As shown in early studies of the stomatal conductance of attached leaves, once leaves have responded to mechanical shock (as inevitably occurs, in our case, during the connection of the leaf to the apparatus), they can be relatively resistant to further shock for hours (Williams, 1947, 1951). When Treatments 1–4 were applied, the rate of water uptake dropped abruptly and then partially recovered, typically reaching a stable flow rate within 15 min (Fig. 4). The removal of lamina distal to the original cuts again produced an abrupt drop, followed by a partial recovery of water uptake rates (Fig. 4 for Treatment 2). Treatments 1–4 significantly reduced rates of water uptake (paired  $t$  tests;  $P < 0.05$ ; Table 1), on average, by 22–52%. The treatments had significantly different impacts (one-way ANOVA;  $P < 0.05$ ). The effect of Treatments 2 through 4 was apparently stronger in the lamina distal to the cuts; estimated water loss was reduced in these regions by, on average, 48–74% (Table 1).

TABLE 1. Flow rates of excised leaves of *Quercus rubra* and the effects of treatments (means  $\pm$  1 SE). Total flow into petiole ( $F$ , in  $\times 10^{-5}$   $\text{kg} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), after 10 min of stable flow.

Treatment	$F$ before treatment ( $N$ )	Reduction of $F$ by treatment, relative to before cut (%)	Section of lamina distal to cut	$F$ after section distal to cut removed	Estimated reduction of $F$ by treatment, in lamina distal to cut (%)
1	2.47 $\pm$ 0.37 (6)	45 $\pm$ 5.6	Whole lamina	—	—
2	3.57 $\pm$ 0.39 (5)	52 $\pm$ 4.3	Lamina flanking the cuts	6.73 $\pm$ 0.38	65 $\pm$ 6.0
3	2.87 $\pm$ 0.61 (5)	22 $\pm$ 8.3	Lamina distal to cut	2.59 $\pm$ 0.48	48 $\pm$ 13
4	4.46 $\pm$ 0.38 (3)	48 $\pm$ 7.9	Lamina distal to cut	3.49 $\pm$ 0.77	74 $\pm$ 13

Some caution is advised in comparing the effects of the four treatments on the excised leaves with those of the in vivo studies. In the excised leaves, the short-term nature of the study means that the leaves did not have time to seal off the cut edges and thus some of the measured water uptake is likely to represent evaporation from cut edges of the lamina. Second, the water loss rates of the excised *Q. rubra* leaves were only 20–40% of midday transpiration rate for exposed leaves, as measured with a porometer (Sack et al., 2002). At the lower water loss rates of excised leaves, cuticular (non-stomatal) water loss accounts for a larger proportion of the total water loss rate, thus affecting calculations of percent change in water uptake rates. Finally, in estimating the impact of the treatments on the lamina distal to the cuts, our calculation neglects the possibility that there might be greater rates of water loss by regions of the lamina proximal to the incisions. All of these effects will lead to an underestimation of the impact of the four treatments on excised leaves, relative to that observed in the in vivo studies.

## DISCUSSION

Disruption of the major vein system in *A. saccharum* and *Q. rubra* had a profound effect on leaf function. In *Q. rubra*, stomatal conductance and photosynthetic capacity were strongly reduced in regions distal to the site(s) at which major veins were severed, while in *A. saccharum* disruption of major veins resulted in the desiccation and death of distal regions of the leaf lamina. We believe that these effects are due to reductions in the hydraulic capacity of the major vein network, rather than to a direct effect of wounding or to reductions in phloem capacity. Wounding should affect tissue on both sides of the cuts and we found no effect of the treatments on regions of the leaf proximal to the cuts. The rapid (2–3 d) desiccation of treated leaves of *A. saccharum*, the immediate reduction in water uptake in excised *Q. rubra* leaves subject to disruption of the major veins (Fig. 4, Table 1), and the marked reduction in  $K_{\text{leaf}}$  are all consistent with a hydraulic basis for the observed responses. In addition, the more pronounced effect on tissue survival and stomatal conductance in the second in vivo experiment (Fig. 2) may be attributable to the hotter and drier conditions during later summer, when evaporative demand would have been higher. Differences in the impact of the severing treatments on the two species are also consistent with a hydraulic interpretation. *Acer saccharum* leaves are known to desiccate faster than leaves of *Q. rubra* (Auge et al., 1998), contributing, along with their shallower root systems (Pallardy and Rhoads, 1993), to the greater sensitivity of *A. saccharum* to drought (Caspersen and Kobe, 2001). *Quercus rubra* leaves also have higher hydraulic conductance than leaves of *A. saccharum* (Sack et al., 2002). The fact that the experimental leaves of *A. saccharum* developed in deeper shade than those

of *Q. rubra* might have led to leaf properties that favored faster desiccation (e.g., a more permeable cuticle)—though the treated *A. saccharum* leaves would also have faced a lower evaporative demand.

The severing treatments reduced stomatal conductance differently across the leaf. In *Q. rubra* leaves subjected to Treatments 3 and 4,  $g$  was reduced only downstream of the cuts, with a greater effect on more distal regions (Fig. 3). Also, in the excised leaves the estimated impacts of the treatments on the lamina distal to the cuts was stronger than for the lamina as a whole (Table 1). These findings indicate a hydraulic independence of lamina regions, with localized stomatal responses. Such localized stomatal responses have been previously reported in intact leaves, but at much smaller spatial scales, especially during water stress—i.e., “patchy stomatal closure” (reviewed in Terashima, 1992). Patchy stomatal closure is particularly common in heterobaric leaves (Terashima, 1992), such as in our two study species, which have prominent bundle sheath extensions (Wylie, 1951). Whether bundle sheath extensions facilitate stomatal heterogeneity at the larger scale, as found in this study, remains to be investigated.

The results of this study do not support Wylie’s view of “conductive overload.” The marked effect of the treatments on  $K_{\text{leaf}}$  and lamina gas exchange indicates that the minor vein network does not provide sufficient conducting pathways in parallel with the major veins. Instead, the data presented here are consistent with the view of the leaf venation as an “irrigation system” composed of one or more highly conductive main lines and laterals that traverse the major dimensions of the leaf and that supply a network of low-conductance, leaky minor veins. A strong pressure drop between the major veins and the leaky minor veins will allow the leaf venation to function as a high-pressure manifold (see Cuenca, 1989), such that water is supplied to the mesophyll relatively evenly across the leaf (Jeje, 1985; Zwieniecki et al., 2002). According to this view, the major vein system and the minor vein network function primarily in series. Thus, severe disruption of the major vein system will lead to marked effects on the ability of the leaf to deliver water to regions “downstream” of the damaged site.

The experimental manipulations used here are probably extreme, when compared to the damage leaves face in nature. However, complete severing of individual veins is known to occur, for example, due to the activities of tent-making bats (Cholewa et al., 2001), certain insects, or mechanical damage. Total loss of conductive capacity in a vein might also arise from cavitation during drought. A more common occurrence is the partial loss of conductive capacity of major veins through cavitation (Kikuta et al., 1997). The data presented here do not bear on the milder effects of partial loss of conduction through major veins. In one study of desiccating *Prun-*

*nus laurocerasus* leaves, even after the midrib had cavitated by 70% of maximum,  $K_{\text{leaf}}$  was only reduced by 10%. In a study of two other Mediterranean species, however, the leaves did not appear so tolerant: stomatal closure began once the midrib had cavitated by <10% and 40% of maximum, respectively (Salleo et al., 2001). Thus, partial redundancy in the form of multiple xylem conduits within individual veins (i.e., in parallel) appears to provide leaves with some degree of protection against damage.

Leaves may be buffered against loss of vein transport capacity—partial or total—in a second way: through the reticulation of the vasculature (Wagner, 1979; Roth-Nebelsick et al., 2001). Although the data presented here do not support Wylie's ideas of conductive overload, *Q. rubra* leaves with disrupted major veins do survive, apparently due to the existence of alternative, albeit low-conductance, pathways. Leaves without reticulate venation lack this buffer against damage: in *Ginkgo biloba*, which has no minor veins, the interruption of primaries led to death of supplied regions of lamina (Shull, 1934). The interruption of the midrib in *Q. rubra* leaves (Treatment 1) reduced  $g$  by about 80% (Fig. 2). In previous studies, interrupting the midrib reduced  $K_{\text{leaf}}$  by 33% in *Prunus laurocerasus* (Nardini, Tyree, and Salleo, 2001) and reduced gas exchange by 30% in *Betula pendula* (Oleksyn et al., 1998). In these two studies, the less severe effect may be due to the interruptions of the midrib being made farther along its length, after more secondary veins had branched off.

We note that partial hydraulic redundancy is not always sufficient to allow survival of leaves with major veins disrupted. In *A. saccharum* areas of lamina died in this study, as was found for two of eight species that Plymale and Wylie examined (1944). We suggest that whether major vein disruption leads to tissue death or not depends at least as much on the evaporative demand and on the leaf's ability to reduce water loss (i.e., with an impermeable cuticle) as on the leaf's vascular architecture. However, the degree of buffering against damage is likely to depend on the architecture of the major vein system (Roth-Nebelsick et al., 2001). For instance, the disruption of a primary vein in a palmately veined leaf might have less impact than the disruption of the midrib of a pinnately veined leaf. One early study reported that severing of a primary vein in palmately veined *Pelargonium zonale* did not alter rates of water loss (Mer, 1940). However, that study was conducted on excised leaves and thus might have underestimated the impact of severing the vein.

The data presented here demonstrate that the major vein system plays a vital role in the hydraulic supply to the lamina. Our results are consistent with those of studies that indicate that the vascular system constrains  $K_{\text{leaf}}$  to an important degree (Tyree et al., 1981; Zweinieccki et al., 2002). Further, the hydraulic importance of the major veins is consistent with the expectation from Murray's Law (Murray, 1926; Sherman, 1981) that vein construction cost is correlated with hydraulic capacity (Canny, 1993). The major vein system is apparently very costly in mass, density, and volume (Plymale and Wylie, 1944) and, thus, should be expected to provide an indispensable high conductance path. However, we note that the cost of the major veins might be balanced against additional factors: in many leaves the major veins play an important mechanical role (Niklas, 1999). Further, tough veins apparently insure against damage, which (as seen here) severely reduces leaf function. Major vein structure is diverse in nature, and major vein architectures range widely, e.g., from the radial, parallel

system of *Ginkgo*, to the grid-like systems of grasses, to the palmate and pinnate systems of dicotyledonous trees. Further studies that examine the role of the major vein network in leaves with contrasting venation patterns will contribute to our understanding of the functional consequences of this diversity.

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