

## An analysis of resistance to water flow through wheat and tall fescue leaves during pressure chamber efflux experiments

R. L. STROSHINE\*, R. H. RAND†, J. R. COOKE‡, J. M. CUTLER§ & JEAN F. CHABOT¶ Department of Agricultural Engineering, Purdue University, West Lafayette, IN 47907, and †Theoretical and Applied Mechanics Department, ‡Agricultural Engineering Department, §formerly Research Associate in Agronomy Department, and the ¶Section of Ecology and Systematics, Cornell University, Ithaca, New York 14853, U.S.A.

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**Abstract.** This is a physical analysis of water movement in wheat (*Triticum*) and tall fescue (*Festuca arundinacea*) leaves placed in the Scholander pressure chamber. It takes into account the efflux resistances of water movement through the xylem and water flow across the cell membranes. Xylem resistance was estimated using Poiseuille's law.

Leaves which had been pressurized in the chamber were embedded, sectioned, examined under a light microscope and photographed. Cells were intact but distorted and xylem vessels were intact. Cells in portions of the blade squeezed by the chamber sealing grommet were crushed, but xylem vessels remained intact.

By applying pressure several tenths of a megapascal in excess of the balance pressure, water was forced from each leaf through the severed end which protruded from the chamber. Efflux curves were drawn by plotting the total water expressed as a function of time after the pressure increase. Water efflux from the shortest wheat leaf lasted only 10 min while efflux from the longest continued for up to 40 min. The efflux from a tall fescue leaf which was rehydrated and cut to a shorter length was much more rapid than efflux from the original leaf.

Experiments combined with mathematical analysis suggested that the effect of leaf length on efflux is related to a high resistance to water flow through vascular bundles. Xylem resistance would be sufficient to produce this effect if it were 10 times greater than that predicted by Poiseuille's law. Both the observations of water flow from the cut end of the leaf and the mathematical model suggested very little water flows from bundles with vessels of diameter less than 12  $\mu\text{m}$ . The apparent explanation is high resistance to water flow through these small diameter vessels.

*Key-words:* pressure chamber; xylem resistance; leaf water potential measurement; plant water movement; mathematical analysis; wheat leaves; tall fescue leaves.

\* Research partially supported by College of Agriculture and Life Sciences, Cornell University, Ithaca, New York. Address correspondence to R. L. Stroshine at Purdue University.

### Introduction

This paper summarizes a study of the rate at which water is forced from wheat and tall fescue leaves of varying lengths placed in a Scholander pressure chamber (Scholander *et al.*, 1964, 1965). A mathematical model of the water flow through leaves is presented as a tool for gaining insight into water movement within leaves placed in the chamber. Values for the resistances, permeabilities, volumes, dimensions, etc. used in the model were determined from data reported in the literature or from actual measurements on leaves. The model realistically predicts experimental results and also gives a possible explanation of effects of leaf length on water efflux.

Models have been developed which describe the pressure-volume curves derived from pressure chamber experiments (Tyree & Hammel, 1972; Cheung *et al.*, 1975, 1976) and the rate of water efflux from leaves in the pressure chamber (Tyree & Cheung, 1977; Tyree *et al.*, 1978). These models were useful in interpreting experimental results and gave insight into water movement in tissue during pressure chamber experiments. Our model builds upon these basic models, incorporates the effect of xylem resistance on efflux and describes the variation of water potential along the length of the leaf.

A mature wheat leaf (of the type on which we ran experiments) is typically 250–300 mm long and 10–12 mm wide and has primary vascular bundles running parallel to the length of the leaf. In their studies of wheat leaves, Kuo, O'Brien & Canny (1974) classified vascular bundles into laterals and intermediates. The larger bundles (laterals) develop as the leaf is elongating and have vessel elements with relatively large radii. The smaller bundles (intermediates) are not fully developed until the leaf has nearly stopped elongating. They are smaller in overall diameter and their vessels have smaller radii. Tall fescue leaves have a similar vascular pattern, but are longer and not as wide as wheat leaves.

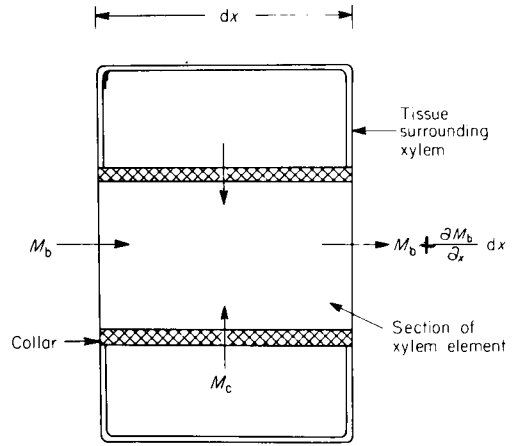
Two studies have presented evidence that under some conditions xylem elements offer significant

resistance to water movement. Rawlins (1963) reported gradients of several tenths of a megapascal (several bars) along the length of a rapidly transpiring tobacco leaf. These gradients would be maintained if xylem resistance to water movement were sufficiently high. Denmead & Millar (1976) measured resistances per unit length of  $0.44 \times 10^4$  Pa-s-mm<sup>-4</sup> for sections of wheat leaf and calculated resistance to flow of  $0.33 \times 10^4$  Pa-s-mm<sup>-4</sup> using Poiseuille's equation.

### Theory

Our objectives were to investigate and mathematically model the efflux of water from wheat and tall fescue leaves in the pressure chamber. Conditions chosen for the model were a 0.3 MPa (3 bar) overpressure applied near the zero turgor balance pressure. In the development of our model we assumed, as did Tyree & Cheung (1977), that water forced from the mesophyll cells travels to the nearest longitudinal vascular bundle and then flows out of the leaf through the vascular elements. This means that water from a given cell located between two adjacent bundles will flow to the nearest bundle. We assumed the water exuding from the ends of the xylem vessels is at zero potential. We also assumed that pressurizing the chamber compresses individual cells, causing cell water potential to increase by an amount equal to the chamber pressure. If the bundle sheath cells prevent gas penetration into the xylem vessels there will be a water potential gradient between the cells, the bundles and the outside atmosphere, and water will flow down that gradient. The middle lamella of the mesophyll sheath is suberized and completely envelopes the bundle (O'Brien & Carr, 1970). This could effectively prevent gas penetration into the xylem.

The detailed development of our model is described in the Appendix. Following is a brief description. A section of bundle of length  $dx$  is shown in Fig. 1. The cells adjacent to the bundle have been treated as a tissue and their characteristics described in terms of volume-averaged tissue parameters. (Tyree & Hammel, 1972, also used volume-averaged parameters in their model.) Justification for treating the cells as a tissue came from results of a preliminary model (Stroshine, 1980). In one of their three models, Tyree & Cheung (1977) assumed that water flows in leaf mesophyll cells by passing through several cell membranes. We developed a similar model and discovered that efflux curves for one cell with a low membrane permeability (high resistance) were similar to those in which water passed through two or three membranes which had a higher permeability (low resistance). This gives justification to the technique of using a single cell with lower membrane permeability to model a series of adjacent cells. For modeling flow through xylem vessels, we used the approach of Dimond (1966). He



**Figure 1.** Model of a section of a vascular bundle of a wheat leaf showing  $M_b$ , the volumetric flow of water through the xylem, and the  $M_c$ , volumetric flow of water into the xylem from the surrounding cells. The bundle consists of many vessel elements but has been represented as one large 'tube'. There are many cells surrounding the bundle but they have been represented as one large cell forming a 'collar' around the bundle.

was apparently the first to model vascular bundle resistance by using a single resistance equivalent to the resistance of parallel flow through several vessels of differing size.

The model gave us the following two equations which describe  $P(x, t)$  and  $V_r(x, t)$  at a given time  $t$  after application of an overpressure on the leaf:

$$\frac{\partial^2 P}{\partial x^2} = k_1 \frac{\partial V_r}{\partial t} \quad (1)$$

$$\frac{\partial V_r}{\partial t} = k_2 P - k_3 V_r - k_4 \quad (2)$$

**Table 1.** Expressions for the coefficients  $k_i$  used in equations (1) and (2). See Table 2 for definition of terms

$$k_0 = \frac{\pi \sum_{i=1}^j n_i r_i^4}{8v}$$

$$k_1 = \frac{NV_0}{k_0}$$

$$k_2 = \frac{L_{pl} A}{V_0}$$

$$k_3 = (\epsilon + \Psi_{op}^0) k_2$$

$$k_4 = (P_c - \Psi_{op}^0) k_2$$

$$a_1 = V_r' + \frac{k_4}{k_3}$$

$$\mu_m = \frac{-k_3}{k_2 k_1 \left(1 + \frac{\lambda_m^2}{\lambda_m^2}\right)}$$

$$\lambda_m = \frac{m\pi}{2l}$$

$$m = 1, 3, 5$$

For a given time,  $t$ ,  $P(x, t)$  is the pressure at a distance  $x$  from the protruding end of the leaf and  $V_r(x, t)$  is the value of the relative volume of the cells a distance  $x$  from the end of the leaf (see Appendix for mathematical definition). Relative volume is the ratio of the cell volume to its volume when turgor first reaches zero. Expressions for the variable coefficients  $k_1$ ,  $k_2$ ,  $k_3$  and  $k_4$  are given in Table 1. Although we show solutions only for cases of zero turgor ( $\varepsilon = 0$ ), for the sake of generality, we have included  $\varepsilon$  in our definition of  $k_2$ .

Equations (1) and (2) can be solved subject to one initial condition and two boundary conditions. We assumed the cells surrounding the vascular bundle were of uniform volume and had identical water potentials. The relative volume of the cells,  $V_r$ , is a function of the chamber pressure. Before an efflux experiment is begun, the chamber is at some initial balance pressure,  $P_c^i$ . Using  $P_c^i$ , we could determine  $V_r^i$ , the value of  $V_r = V_r(x, t)$  at  $t \leq 0$ , by using equation (2). For  $t \leq 0$ , the gauge pressure in the xylem vessels, was taken as zero. Setting  $\partial V_r / \partial t$  equal to zero and solving for  $V_r$  gave  $V_r^i$  equal to  $-k_4/k_3$ . The water potential at zero turgor,  $\Psi^0 = \Psi_{op}^0$ , will be a constant for each leaf. Using this fact and substituting the expressions for  $k_4$  and  $k_3$  from Table 1, we determined  $V_r^i$  for a given  $P_c^i$ . At  $t = 0$ , the chamber pressure is increased 'instantaneously', and, as can be seen from Table 1,  $k_4$  assumes a new value.

Boundary conditions for  $P(x, t)$ , the pressure in the xylem vessels, can also be determined. We assumed that the pressure at the end of the bundle open to the atmosphere ( $x = 0$ ) was zero. By assuming water flow into the bundle at the tip of the leaf ( $x = l$ ), to be negligible we could assume  $\partial P / \partial x|_{x=l}$  to be zero.

Solving equations (1) and (2) using the method of separation of variables gave us:

$$V_r(x, t) = -\frac{k_4}{k_3} + \sum_{m=1,3,5,\dots}^{\infty} \frac{4a_1}{m\pi} \exp(\mu_m t) \sin(\lambda_m x) \quad (3)$$

$$P(x, t) = \frac{-16a_1 k_1 l^2}{\pi^3} \sum_{m=1,3,5,\dots}^{\infty} \mu_m \frac{\exp(\mu_m t)}{m^3} \sin(\lambda_m x) \quad (4)$$

Definitions of  $a$ ,  $\mu_m$  and  $\lambda_m$  are given in Table 1.

The following derivation gave us an expression for  $Q$ , the total water expressed from the end of the stem between time zero and time  $t$ . We found the flow rate from the stem by substituting  $\partial P / \partial x$  into Poiseuille's equation:

$$\frac{dQ}{dt} = k_0 \left. \frac{\partial P}{\partial x} \right|_{x=0} \quad (5)$$

Integration of equation (5) with respect to time and substitution of the initial condition  $Q = 0$  when  $t = 0$  gave us the expression:

$$Q = \frac{8a_1 k_1 k_0 l}{\pi^2} \sum_{m=1,3,5,\dots}^{\infty} \frac{1 - \exp(\mu_m t)}{m^2} \quad (6)$$

Equation (6) is the equation we used to predict the water flow from the vascular bundles in the wheat leaf.

## Materials and methods

### Pressure chamber experiments

In 1979 we grew one variety of durum winter wheat (*Triticum durum* L. cv. 'Yorkstar') and one variety of spring wheat (*Triticum aestivum* L. em Thell cv. 'Super X') in a greenhouse. Cutting a leaf from the plant using a razor blade, we placed it in the pressure chamber with its base protruding through an airtight rubber seal. As we slowly increased the chamber pressure (c.  $0.01 \text{ MPa s}^{-1}$ ), water began to appear at the cut end of the stem. We assumed the chamber pressure at which this occurred (called the initial balance pressure) to be equal to the leaf water potential.

Tyree & Dainty (1973), Tyree, Caldwell & Dainty (1975), and Tyree *et al.* (1978) determined the rate at which water was expressed from leaves subjected to several tenths of a megapascal (several bars) over-pressure. We applied a similar procedure to wheat and tall fescue leaves. After determining the initial balance pressure, we used the method described by Cutler, Shahan & Steponkus (1979) to express water until the balance pressure was approximately 1.2–1.5 MPa. At this point the  $PV^*$  curve became linear and we assumed water loss from individual cells had reduced their volume-averaged turgor pressure to zero. After equilibrating the leaves, we rapidly (in less than 5 s) increased the pressure by 0.3 MPa (3 bars) and collected the expressed sap in 2-dram ( $7.4 \text{ cm}^3$ ) vials partially filled with cotton. These vials were changed at one or two minute intervals for the first 6–8 min of the test and at 4- or 5-min intervals thereafter. Sap expressed was determined from changes in vial weight. We graphed the cumulative volume of liquid expressed as a function of time after application of the over-pressure and gave these graphs the title 'efflux curves'.

After completion of the wheat leaf experiments, several unresolved questions about the effect of leaf length remained. In order to answer these questions we conducted efflux experiments on tall fescue (*Festuca arundinacea* Shreb). In the summer of 1980 we used plants grown in the field and repotted, and in the spring of 1984 we used plants growing in a greenhouse. We also measured interveinal distances on free hand cross sections from the leaves. Measurements were made at  $\times 100$  or  $\times 250$  magnification using a Carl Zeiss model 18 microscope with an ocular micrometer. During several of the efflux experiments we observed water coming from the protruding end of the leaf using a Bausch

\* The  $PV$  curve is a plot of the reciprocal of chamber balance pressure versus the cumulative volume of water expressed from the leaf.

and Lomb telescopic microscope capable of magnifications from  $\times 15$  to  $\times 45$ .

In several experiments we evaporated the water coming from the end of the leaf using a hair dryer. This enabled us to more easily observe efflux from individual bundles. Upon completion of the efflux experiments we examined free hand sections of the leaves at the protruding ends and at two or three other locations along their lengths. We also determined the diameters of the two largest vessels in each bundle.

In order to better establish the effect of leaf length on efflux, we rehydrated four of the fescue leaves used in the efflux experiments. Rehydration is necessary because the shape of the efflux curve is affected by the initial balance pressure. For our rehydration procedure we first placed water in the funnel-shaped sealing knob of the pressure chamber specimen holder. (This is the mechanism which seals around the portion of the leaf protruding from the chamber.) We covered the end of the leaves with water to a depth of 1–2 cm and then reduced the pressure to approximately 0.2 MPa below the balance pressure observed before the start of the previous efflux experiment. This pressure was maintained for 0.5 h so that the leaf could reabsorb water. At the conclusion of the rewetting, the balance pressure of this leaf was 0.22 MPa above the balance pressure for the efflux experiment conducted prior to rehydration. For the remaining leaves, we allowed 2–3 h for the water to be reabsorbed. The balance pressures of the second and third leaves were within 0.02 MPa of the balance pressure for the preceding efflux experiments. The fourth leaf was 0.16 MPa below the original balance pressure, but was brought within 0.05 MPa by applying a 0.3 MPa over-pressure for 3 min and thereby forcing water from the leaf.

In order to test the effect of leaf length, the first and second leaves were shortened by cutting. After rehydration they were removed from the chamber, cut to approximately 60% of their original length, and returned to the chamber. After we checked the balance pressure, we ran the second efflux experiment. In order to ensure that we could successfully rehydrate leaves, we performed the efflux experiments for the third and fourth leaves without cutting them to a shorter length or removing them from the chamber.

#### *Parameters used in the model*

In order to evaluate the coefficients used in our model (see Table 1) we had to determine values of the parameters shown in Table 2. We used the tissue hydraulic conductivity,  $L_{pt}$ , determined by Tyree & Cheung (1977) from pressure chamber experiments. Although changing the value of  $L_{pt}$  altered the shape of the curves predicted by the model, the curves still suggested the same conclusions. Therefore, even though our value for  $L_{pt}$  in the model may have been inaccurate, the choice would not affect our conclu-

sions. It must be emphasized that the tissue hydraulic conductivity  $L_{pt}$  defined in this study incorporates path length and is not the  $L_p$  used in irreversible thermodynamics.

Other values listed in Table 2 were measured from leaf cross-sections. Microscopic observation revealed that the mesophyll cells were irregular in shape with each cell having many 'fingers' or projections. Measurements of dimensions of the 'fingers' gave radii and lengths of approximately  $8.5 \times 10^{-3}$  mm and  $3.0 \times 10^{-2}$  mm, respectively. We assumed that each 'finger' acted like an individual cell and calculated the cell volume by assuming that each was a cylinder. We assumed that the area,  $A$ , across which water passed from the cell was equal to the area of one end of the cylinder. Essentially we were using a minimum value for area. If all of the surface of the cylinder were considered, the value could be as high as  $2.1 \times 10^{-3}$  mm<sup>2</sup> which is 9 times greater than the above.

In order to calculate  $k_1$  we needed the value of  $NV_0$ . Since  $N$  is the number of cells per mm of vascular bundle and  $V_0$  is the volume of a cell at zero turgor,  $NV_0$  is the zero turgor volume of mesophyll cells in the leaf per mm of vascular bundle. We estimated that 47% of the leaf volume was mesophyll cell using the method of Wiebel (1969) which Chabot & Chabot (1977) and Chabot, Jurick & Chabot (1979) adapted for use on cross-sections of plant leaves. After measuring the leaf thickness and width we calculated leaf volume using the prismoidal formula. We multiplied leaf volume by 0.47 and divided by total vessel length to find  $NV_0$ . Microscopic examination of leaf 3 showed that it had 10 lateral bundles and 25 intermediate bundles. We assumed four of the laterals were small laterals and 10 of the intermediates were small intermediates. For flow through all 35 bundles we assumed vessel length was 35 times 238 or 8330 mm. The value for  $NV_0$  given in Table 1 for flow through all bundles is the average value determined from measurements on four leaves. For flow through only the 10 laterals we assumed vessel length to be 10 times 238 or 2380 mm.

To evaluate the xylem resistance we needed to determine a value for  $\Sigma n_i r_i^4$  (See equation A2 in the Appendix). We took measurements of vessel radii from photographs of wheat leaf cross-sections and used them to calculate the values shown in Table 3. Also shown in the table are values reported by Kuo *et al.* (1974). Kuo classified the vascular bundles into five types: the midrib, large and small laterals, and large and small intermediates. We used his classification scheme but assumed the midrib behaved like a large lateral. Although Kuo reported values for a different variety of wheat, his values of  $\Sigma n_i r_i^4$  for the large laterals agree quite well with our values. The values for small and large intermediates agree within a factor of 4. The small intermediates in our plants had larger radii.

**Table 2.** Numerical values assigned to the parameters which define the coefficients  $k_i$  listed in Table 1

| Parameter     | Meaning  | Value   | Source  |
|---------------|--|---|---|
| $\nu$         | Viscosity of water expressed from the leaf   | $10^{-3}$ Pa-s  | Value for water at 20°C   |
| $V_0$         | The volume of a mesophyll cell when the turgor potential first becomes zero  | $6.9 \times 10^{-6}$ mm <sup>3</sup>  | Calculated by assuming the cell was a cylinder with radius $8.5 \times 10^{-3}$ mm and length $3.0 \times 10^{-2}$ mm |
| $NV_0$        | The zero turgor mesophyll cell volume per mm length of xylem. $N$ is the number of mesophyll cells per mm of bundle length and $V_0$ is the zero turgor volume of a single mesophyll cell                | 0.0238 mm <sup>3</sup> mm <sup>-1</sup> for flow through all bundles,<br>0.0802 mm <sup>3</sup> mm <sup>-1</sup> for flow through laterals only | Determined by dividing the estimated volume of mesophyll by the total length of xylem bundles                         |
| $L_{pt}$      | The hydraulic conductivity of the tissue adjacent to the xylem bundle  | $1.5 \times 10^{-10}$ mm Pa <sup>-1</sup> s <sup>-1</sup>   | Tyree & Cheung, (1977)  |
| $A$           | Area of the portion of the cell surface through which water flows out of the cell  | $2.3 \times 10^{-4}$ mm <sup>2</sup>  | The area of the end of a cylinder with a diameter equal to the diameter of the mesophyll cells                        |
| $\epsilon$    | Modulus of elasticity of the cell  | 0.0   | Only the case where the turgor potential was zero was modelled  |
| $\Psi_{op}^0$ | The osmotic pressure of the cell when the turgor potential first reaches zero, (which then becomes equal to the water potential, $\Psi$ )  | $+12.7 \times 10^5$ Pa  | Determined from a pressure <sup>-1</sup> -volume curve  |
| $P_c$         | The chamber pressure during the test   | $17.0 \times 10^5$ Pa   | This depends on the experiment being modelled   |
| $V_r^i$       | The value of $V_r$ (where $V_r$ is the relative departure of the cell volume from its zero turgor volume) when an efflux experiment is begun. For a definition see Appendix, equation A10 and discussion | 0.24  | Calculated from the initial conditions  |
| $l$           | The length of the leaf   | 238 mm  | The measured length of leaf 3   |
| $n_i, r_i$    | $n_i$ is number of xylem with radius $r_i$   | see Tables 3 and 4  | Measurements from leaf cross-sections   |
| $\pi$         | Geometrical constant   | 3.1416  |   |
| $m$           | Index of infinite series   | 1, 3, 5, ...  |   |

The values of the coefficients  $k_i$  used in the model are shown in Table 4. We determined the constants for each bundle type using values for  $\Sigma n_i r_i^4$  given in Table 3 and the values of the parameters given in Table 2. We also used the model to estimate flow of water from the leaf by assuming that water flowed through only the laterals. We changed the value of  $\Sigma n_i r_i^4$  to a weighted average of the values for large and small laterals. The value of  $NV_0$  became  $0.0802$  mm<sup>3</sup> mm<sup>-1</sup> because, as described previously, the vessel length changed.

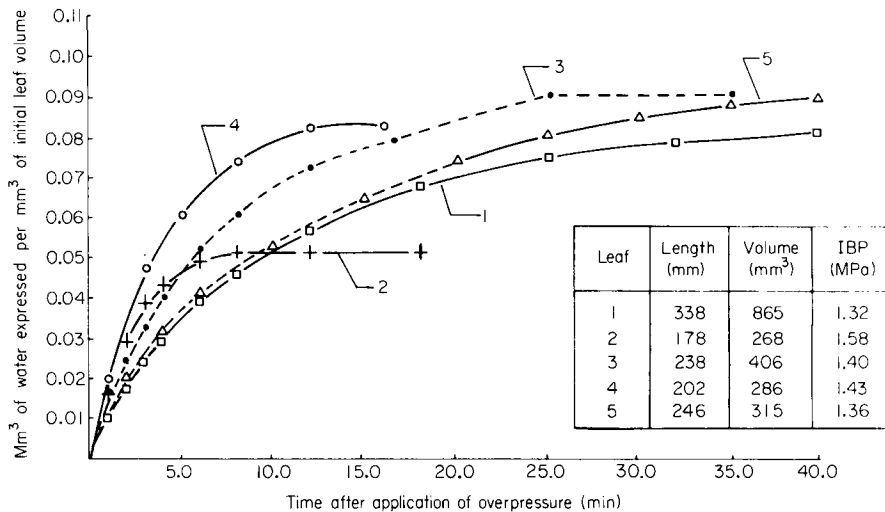
## Results

### Pressure chamber experiments

The efflux curves for several wheat leaves are shown in Fig. 2. Using Tyree & Hammel's (1972) method of analysis, we found  $\Psi_{vaop}$ , the volume averaged osmotic potential at zero turgor, for these leaves ranged between  $-1.22$  and  $-1.43$  MPa ( $-12.2$  to  $-14.3$  bars). The efflux experiments on individual leaves were run after bringing the leaves to a balance

**Table 3.** Values of the sums of the fourth power of vessel radii for various sized vascular bundles of wheat leaves. Standard deviations are included for measurements where they could be calculated. Radii were measured on photographs of wheat leaf cross-sections

| Bundle type        | Number of bundles on which measurements were taken |                          |                                    | Value of $\Sigma n_i r_i^4$ (mm <sup>4</sup> ) |                                 |                                    |
|--------------------|--|--------------------------|------------------------------------|--|---------------------------------|------------------------------------|
|                    | Samples collected 11/12/78                         | Samples collected 2/8/79 | Data from Kuo <i>et al.</i> (1974) | Samples collected 11/12/78                     | Samples collected 2/8/79        | Data from Kuo <i>et al.</i> (1974) |
| Large lateral      | 8  | 2                        | 6                                  | $3.85 \pm 1.5 \times 10^{-8}$                  | $4.94 \pm 1.62 \times 10^{-8}$  | $4.29 \times 10^{-8}$              |
| Small lateral      | 2  | 1                        | 4                                  | $9.17 \pm 1.06 \times 10^{-9}$                 | $3.19 \times 10^{-8}$           | $1.79 \times 10^{-8}$              |
| Large intermediate | 15   | 4                        | 13                                 | $2.56 \pm 2.20 \times 10^{-10}$                | $7.73 \pm 1.73 \times 10^{-10}$ | $1.89 \times 10^{-10}$             |
| Small intermediate | 11   | 5                        | 10                                 | $1.97 \pm 1.26 \times 10^{-10}$                | $3.35 \pm 2.54 \times 10^{-10}$ | $2.60 \times 10^{-11}$             |



**Figure 2.** Efflux curves for pressure chamber experiments on wheat leaves of varying length. IBP is the initial balance pressure before the overpressure of 0.3 MPa was applied. The overpressures forced water from the leaf.

pressure between 0.0 and 0.15 MPa above the zero turgor potential. Since the various leaves we studied varied in volume, we normalized the efflux curves by dividing efflux by the initial leaf volume. (Two other alternatives would have been to normalize the curves by dividing by either the volume of water in the leaf or the volume of osmotically active water.) All of the leaves were from plants grown in the greenhouse. Leaves designated 1, 2, 3 and 4 were from durum winter wheat (cv. 'Yorkstar') and were tested between 30 July 1979 and 2 August 1979. Leaf 5 was tested 20 November 1979 and was from spring wheat (cv. 'Super-X').

The preliminary results of our modeling of efflux from the intermediate bundles, which had a high xylem resistance, told us that if a given over-pressure were applied to both a short leaf and a long leaf, it would take less time to complete the efflux experiment on the shorter leaf. Acting on this clue, we experimented with leaves of varying lengths. We used the mature leaves directly below the flag leaf from plants which were in the early heading growth stage. Selecting leaves in this manner should have minimized differences in cell and vascular structure which might otherwise have affected results.

The effect of leaf length is apparent in the data shown in Fig. 2. All of the water was expressed from the shortest leaf (no. 2) in less than 10 min. However, all of the water had not been expressed from the longest leaf (no. 1) after 40 min. Time required for water efflux from the leaves of intermediate length increased with leaf length. Efflux rates (the slopes of the efflux curves) during the initial several minutes of the tests were relatively constant for both long and short leaves. With the exception of leaf 2, the asymptotic values of the normalized curves were approximately the same. Leaf 2 was 0.15 MPa above its zero turgor balance pressure when the over-

pressure was applied. The total efflux for a given over-pressure decreases as the initial balance pressure increases above the balance pressure at zero turgor. Therefore, it is reasonable that its efflux curve has a lower asymptote. In efflux experiments with tall fescue leaves during the summer of 1980 and the spring of 1984, leaf length had a similar effect on efflux. Therefore, for all three sets of experiments, leaf length affected efflux in the manner predicted by our model of efflux from bundles with a large xylem resistance.

The rehydration experiments gave further evidence that leaf length affects efflux. Results from the third and fourth leaves that were rehydrated demonstrated that efflux curves after rehydration were similar to the original curves. For both leaves, efflux from rehydrated leaves continued at similar rates to those observed during the initial experiments. At 20 and 35 min after the beginning of the efflux experiment on leaf four, cumulative effluxes from the rehydrated leaves were 94 and 91% of the original values respectively. For the first leaf, which was rehydrated and shortened, the efflux rates after 18 min were approximately  $0.78 \text{ mg min}^{-1}$  for the first efflux experiment and  $0.58 \text{ mg min}^{-1}$  for the shortened, rehydrated leaf. For the second leaf, efflux from the shortened leaf had stopped after 20 min, but the efflux rate for the leaf before shortening was  $0.30 \text{ mg min}^{-1}$  after 35 min. Eighteen minutes after the beginning of the efflux experiments, efflux rates before and after shortening were, respectively,  $0.32 \text{ mg min}^{-1}$  and  $0.08 \text{ mg min}^{-1}$ .

One observation made during the rehydration experiments confirmed that air does not penetrate the xylem vessels. When the tip of the leaf was covered with water, very small bubbles were escaping from three places at the end of the leaf. However, they were coming from the bulliform cells, not from

vessel elements. Had pressurized air in the plastic bag surrounding the leaf been forced into the vessel elements, rehydration of the leaf would have been impossible. As mentioned previously, the bundle sheath cells with their suberized layer may provide the sealing effect.

Our observation of water efflux from the ends of the bundles showed that the majority of water flows from the larger diameter vessels. Within seconds after chamber pressure was increased above balance pressure, water began to flow from bundles with vessels greater than *c.* 20  $\mu\text{m}$ . If efflux was allowed to continue and the water was evaporated from the end of the leaf, the decrease in efflux rate was evident. Efflux from the bundles with vessels of *c.* 20  $\mu\text{m}$  diameter continued but at a very low rate and there was a lesser amount of efflux from the bundles with vessels of *c.* 12  $\mu\text{m}$  diameter.

Interveinal distances and xylem vessel diameters were measured on both wheat and tall fescue leaves. At the base of the leaf the average interveinal spacing between the centerlines of the bundles in wheat leaves 1, 3 and 4 (Fig. 2) was 0.240 mm. The average spacing between lateral bundles was 1.08 mm. In the winter of 1984 we measured wheat leaves and found average spacings between all bundles and between laterals, respectively, of 0.245 and 0.924 mm for *Triticum durum* and 0.255 and 0.930 mm for *Triticum aestivum*. Spacings calculated from data in Table 1 of Altus & McCanney (1982) were 0.320 mm for all bundles and 0.727 mm for lateral bundles. The bundle spacing in tall fescue leaves from our 1984 experiments was 0.413 mm for all the bundles and 0.918 mm for lateral bundles. In our observations we found that the bundle spacing was slightly greater for wider leaves. The average diameters of the two largest vessels in bundles from wheat leaf number 3 (from 1979) were, respectively, 25, 23, 8 and 7  $\mu\text{m}$  for the large laterals, small laterals, large intermediates and small intermediates. Measurements on the two largest vessels in the bundles of the tall fescue examined in the spring of 1984 gave average diameters of 22  $\mu\text{m}$  (range 14–28  $\mu\text{m}$ ) for the laterals and 11  $\mu\text{m}$  (range 4–12  $\mu\text{m}$ ) for the intermediates. Measurements of vessel diameters near the tips of the leaves showed that the average diameter of the two largest vessels in the lateral bundles near the tip could decrease to 75% of the value at the base. This would decrease  $r_i^4$  to 32% of the value at the base.

Only if the xylem vessels and leaf cells are relatively undamaged can water flow through the leaf while it is in the chamber. Examination of a large number of cross-sections taken from portions of leaves that had been inside the chamber, such as those shown in Fig. 3A, showed that the xylem vessels were undamaged and that the mesophyll cells had not been ruptured. The portion of the leaf that is squeezed by the sealing grommet of the pressure chamber is subjected to large forces. In order to determine whether these forces damaged the xylem

vessels, we sectioned that portion of the leaf. A typical cross section is shown in Fig. 3B. Although the epidermal and mesophyll cells were either disrupted or crushed, the xylem vessels in both the large and small bundles were undamaged.

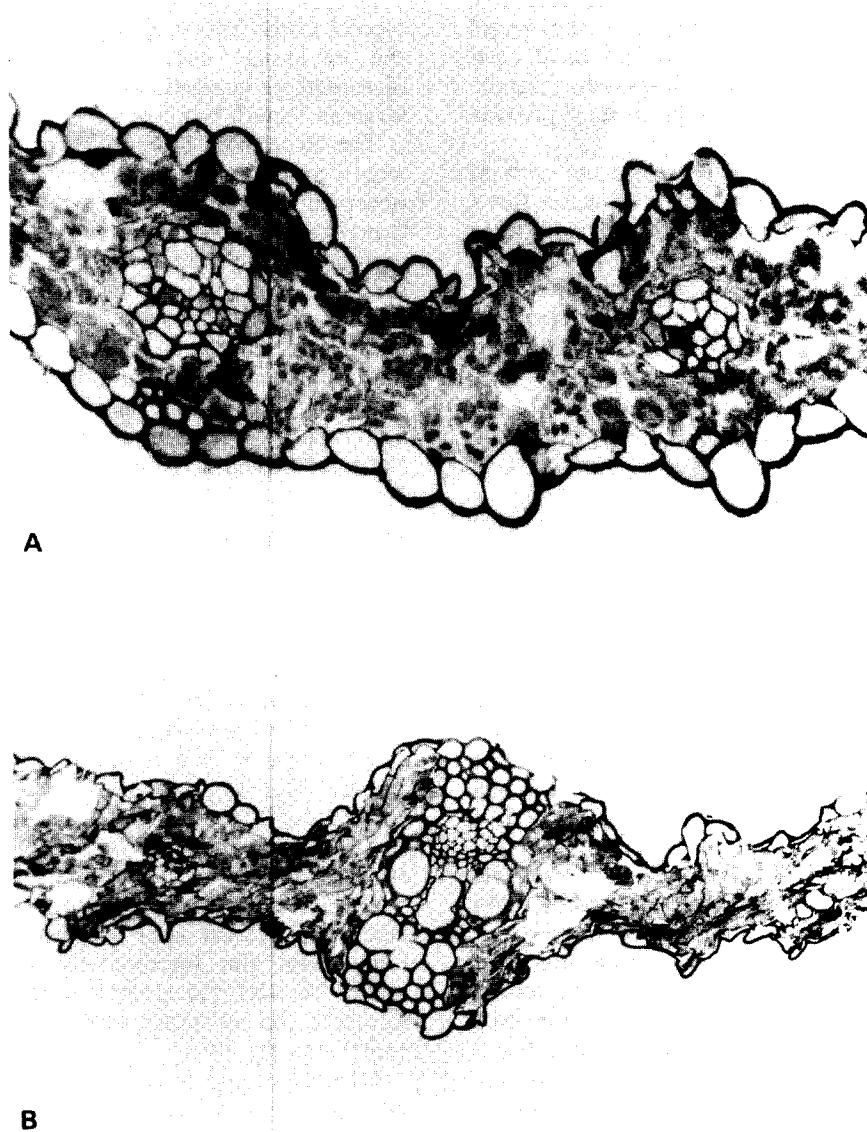
The experiments demonstrated the effect of leaf length on efflux. In addition, they suggested that the observed differences in time required to force water from the leaves were related to the length of the leaf rather than the spacing between bundles. Apparently the smaller diameter (<12  $\mu\text{m}$ ) vessels offer a high resistance to water flow. During the efflux experiments, the integrity of both the xylem vessels and the mesophyll cells is maintained. Furthermore, the movement of water through the vessels is not hindered by the compression of the leaf by the chamber sealing grommet.

#### Model predictions

Using the  $k$  values in the first four lines of Table 4 we calculated the values of the water efflux from each of the bundle types by multiplying the flow from a single bundle by the number of bundles of that type. This was equivalent to assuming that water from cells near intermediate bundles could not flow to the lateral bundles. We assumed there were six large laterals, four small laterals, 13 large intermediates and 10 small intermediates. The normalized efflux curves predicted by the model for each bundle type are shown in Fig. 4. Recall that the parameters of the model were determined in such a manner that the model leaf had the same length and volume as leaf 3. Flow from the intermediate bundles was much slower because the smaller diameter of the vessels increased the resistance to water flow by a factor of approximately 1000.

After observing the model prediction of low flow through the intermediates, we used the model to predict efflux when all of the water flows through the laterals and none flows through the intermediates. As previously mentioned, this changes the values of  $\Sigma nr_i^4$ ,  $k_0$  and  $k_1$  to those listed in the bottom line of Table 4. The resulting model predictions of efflux curves are shown in Fig. 5 as solid lines. The two curves are for a leaf with the same length as leaf 3 and for a leaf with half the length. Data points for actual efflux from the leaf are also shown. When the leaf length and volume were halved, efflux was changed very slightly.

Giordano *et al.* (1978) suggested that the resistance of the vascular bundles is greater than that predicted by Poiseuille's law. Therefore, we also investigated the effect of increased xylem resistance on the model predictions for efflux. We increased the model resistance by changing the values of  $k_0$  and  $k_1$ , to  $k'_0 = 0.1k_0$  and  $k'_1 = 10k_1$ . Since the reciprocal of  $k_0$  is proportional to xylem resistance, this was equivalent to increasing resistance by a factor of 10. The results for the two leaf lengths are shown as the

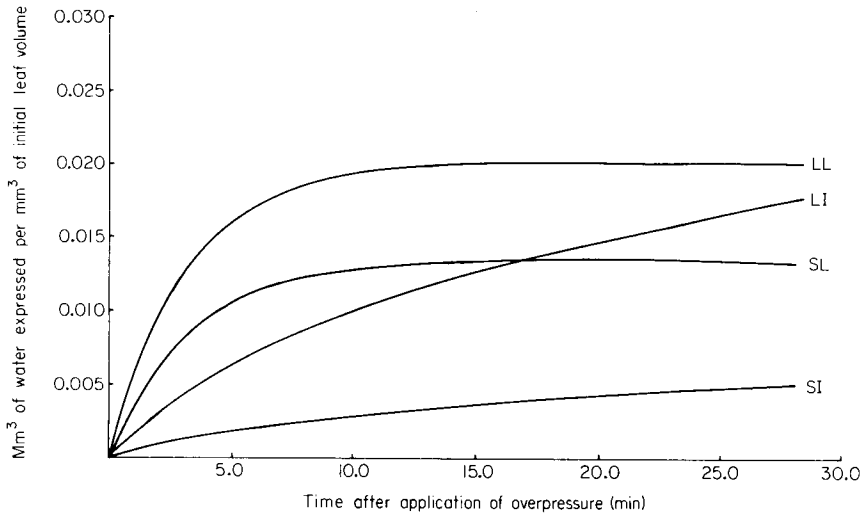


**Figure 3.** (A) Cross-section of a wheat leaf taken from a portion of the leaf inside the chamber after completion of an experiment. (Magnification  $\times 196$ .) (B) Cross-section of a leaf taken in the vicinity of the chamber seal after the leaf was removed from the chamber. Note that although the mesophyll and epidermal cells are deformed or crushed, the vessels are undamaged. (Magnification  $\times 178$ .)

**Table 4.** Numerical values for the coefficients  $k_i$  in Table 1 evaluated using the parameters from Tables 2 and 3

| Bundle type  | $\Sigma nr_i^4$ (mm <sup>4</sup> ) | Values of constants $k_i$                                  |                                |  |                          |                          | Numbers of bundles of this type in the leaf |
|--|------------------------------------|--|--------------------------------|--|--------------------------|--------------------------|---|
|  |                                    | $k_0$ (mm <sup>4</sup> Pa <sup>-1</sup> -s <sup>-1</sup> ) | $k_1$ (Pa-s mm <sup>-2</sup> ) | $k_2$ (Pa <sup>-1</sup> -s <sup>-1</sup> ) | $k_3$ (s <sup>-1</sup> ) | $k_4$ (s <sup>-1</sup> ) |   |
| Large lateral  | $4.94 \times 10^{-8}$              | $1.94 \times 10^{-5}$                                      | 1230                           | $5.0 \times 10^{-9}$                       | $6.35 \times 10^{-3}$    | $2.15 \times 10^{-3}$    | 6   |
| Small lateral  | $3.19 \times 10^{-8}$              | $1.25 \times 10^{-5}$                                      | 1900                           | $5.0 \times 10^{-9}$                       | $6.35 \times 10^{-3}$    | $2.15 \times 10^{-3}$    | 4   |
| Large intermediate   | $1.89 \times 10^{-10}$             | $7.42 \times 10^{-8}$                                      | $3.21 \times 10^5$             | $5.0 \times 10^{-9}$                       | $6.35 \times 10^{-3}$    | $2.15 \times 10^{-3}$    | 14  |
| Small intermediate   | $2.60 \times 10^{-11}$             | $1.02 \times 10^{-8}$                                      | $2.33 \times 10^6$             | $5.0 \times 10^{-9}$                       | $6.35 \times 10^{-3}$    | $2.15 \times 10^{-3}$    | 11  |
| Values assuming all flow is through the 10 lateral bundles | $4.25 \times 10^{-8}$              | $1.67 \times 10^{-5}$                                      | 4800                           | $5.0 \times 10^{-9}$                       | $6.35 \times 10^{-3}$    | $2.15 \times 10^{-3}$    | 10  |



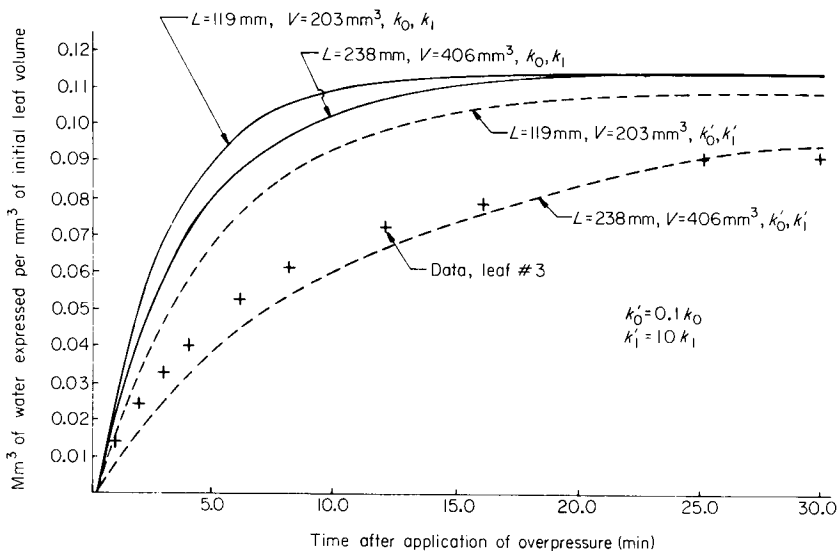


**Figure 4.** Mathematical model predictions of water efflux from various sized bundles. LL, SL, LI, and SI represent, respectively, the cumulative amounts of water expressed from the six large laterals, the four small laterals, the 14 large intermediates, and the 11 small intermediates.

dashed lines in Fig. 5. When  $k'_0$  and  $k'_1$  were used, the efflux time predicted by the model corresponded more closely to that experimentally observed and leaf length affected efflux in a manner similar to that observed in experiments.

The effect of changing tissue hydraulic conductivity was also investigated. This is important because wider leaves may have wider bundle spacings. A wider bundle spacing would mean a lower tissue hydraulic conductivity. Increasing  $L_{pt}$  to  $1.5 \times 10^{-9} \text{ mm Pa}^{-1} \text{ s}^{-1}$  (a 10-fold increase) increased efflux from the lateral bundles but had little effect on the efflux from intermediate bundles. Decreasing  $L_{pt}$  to  $5.0 \times 10^{-11} \text{ mm Pa}^{-1} \text{ s}^{-1}$  (one-

third of the original value), gave a cumulative efflux at  $t = 8 \text{ min}$  which was reduced to 67% of the previous value. Therefore, bundle spacing can affect efflux. However, with the decreased value of  $L_{pt}$ , halving the leaf length increased cumulative efflux at  $t = 8 \text{ min}$  by only 6%. This was much less than the experimentally observed effect of leaf length on efflux. As reported earlier, the distance between laterals is 3–5 times greater than the distance between adjacent bundles. Therefore, for the model of flow through only laterals, the values of  $L_{pt}$  could be changed to account for the longer flow path through mesophyll cells. Yet it would still not account for the effect of leaf length. Furthermore, changing  $L_{pt}$  may not be



**Figure 5.** Mathematical model predictions of efflux curves showing the effects of leaf length for two different xylem resistances. The solid lines are efflux curves for leaves with xylem resistance shown in Table 4, line five. The model is for flow through only the lateral bundles. The dashed lines are curves for leaves with xylem resistance ten times greater than the efflux curves shown in solid line.

justified. As mentioned earlier, the value for  $A$  was actually chosen to be a minimum. Decreases in  $L_{pt}$  are offset by increases in  $A$  because they both affect only the value of  $k_2$  (see Table 1). Therefore, the value of  $L_{pt}A$  used may already be appropriately small.

The mathematical model studies underscored the importance of the xylem resistance. Intermediate bundles with their small diameter ( $<12\ \mu\text{m}$ ) vessels have a very high resistance to water flow and apparently conduct relatively small amounts of water under conditions of rapid efflux. When the vessels of lateral bundles are assumed to have resistances calculated from Poiseuille's law, leaf length had very little effect on efflux. Changing the hydraulic conductivity, such as might occur if bundle spacing were wider, showed that bundle spacing can affect efflux. However, it did not explain the effect of leaf length on efflux. When xylem resistance was increased to 10 times the value calculated from Poiseuille's law, leaf length did have a significant effect on efflux.

### Discussion and conclusions

Although efflux experiments have been reported in the literature (Tyree & Dainty, 1973; Tyree & Cheung, 1977; Tyree *et al.*, 1978), to our knowledge, this is the first time that the effect of leaf length on efflux has been demonstrated. This leaf length effect was both demonstrated by the experiments and predicted by a mathematical model. Parameters used in the model were either taken from the literature or determined from physical measurements of the leaves tested. In view of this, we found the agreement between experiment and model to be quite encouraging.

Previous models of water efflux from the pressure chamber (Tyree, Benis & Dainty, 1973; Tyree & Cheung, 1977) have neglected the effect of xylem resistance. Dimond (1966) developed a simple approach to estimating the overall resistance of a vascular bundle based upon Poiseuille's law. When we incorporated his technique into a model for water efflux through a leaf placed in a pressure chamber, the model predicted very low flow rates through the intermediate bundles. Kuo, O'Brien & Zee (1972) noted the large differences in  $\Sigma n_i r_i^4$  values and suggested that the lateral bundles carry most of the water through the leaf.

The results of both experiments and the model suggest that resistance to water flow through the xylem is at least partially responsible for the effect of leaf length on efflux. Differences in bundle spacing alone cannot explain the effect of leaf length on efflux. Examination of the widths of the wheat leaves used in efflux experiments (Stroshine, 1980) showed that longer leaves were not always wider. Furthermore, in the experiments with rehydrated, shortened tall fescue leaves the bundle spacing did not change

yet the efflux time decreased substantially after shortening. Giordano *et al.* (1978) reported that in experiments with large diameter vessels ( $1.5 \times 10^{-2}$  cm diameter) of *Sechium edule* (Cucurbitaceae) measured resistance was 10–150 times greater than that predicted by Poiseuille's law. An increase in xylem resistance could also help to explain gradients in water potential along the lengths of leaves (Rawlins, 1963). The results of our model suggested that the xylem resistance could be 10 times greater than that predicted by Poiseuille's law. However, part of the greater resistance could be explained by the previously mentioned observation that vessel diameter decreases along the length of the leaf. Our model assumed vessel diameter did not decrease.

The average diameters of the two largest vessels of the lateral bundles of wheat leaves were similar to those of tall fescue. Average diameters of the intermediate bundles were slightly larger for the tall fescue. Therefore, the small vessel diameters suggest that intermediate bundles of both wheat and tall fescue transport very little water during forced, rapid efflux such as occurs in the pressure chamber. The spacing of the lateral bundles in the tall fescue leaves and wheat leaves was similar. Therefore, the effects of xylem resistance on efflux should be similar.

For the portion of this work involving mathematical studies, the simplifications imposed limitations. However, the simplified model was still a tool which gave insight. If transpiration rates could be accurately determined, the model in its present form (and more certainly in an extended form) could be useful for predicting water relations in rapidly transpiring leaves. The demonstrated high water flow resistance of the intermediate bundles suggests that much of the water being forced from the leaf cells must travel from the proximity of the intermediate bundles to xylem elements of the lateral bundles. Therefore, water movement across the width of parallel-veined leaves should be studied in more detail, and the effective resistance of the xylem vessels should be established. Crookston & Moss (1974) observed differences in vascular bundle spacing between  $C_3$  and  $C_4$  species of common crop and range Graminae. A study of efflux curves from several grass species with different vascular bundle spacings would be valuable.

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### Appendix

In this appendix we develop equations (1) and (2) from the model diagrammed in Fig. 1. By assuming water to be incompressible, equating flow into the section of bundle with flow out of the section gives:

$$\frac{\partial M_b}{\partial x} = M_c \quad (\text{A1})$$

where  $M_b$  = the volumetric flow rate through the vein ( $\text{mm}^3 \text{ s}^{-1}$ ), and  $M_c$  = the volumetric flow of water into a mm length of bundle from the surrounding cells ( $\text{mm}^2 \text{ s}^{-1}$ ).

If flow through the xylem is incompressible, laminar and steady, the value for  $M_b$  can be predicted from Poiseuille's law by assuming, as did Dimond (1966) that water can pass freely between xylem vessels. This means there will be parallel flow in the xylem vessels of a given bundle. When Dimond made this assumption he found that the equivalent resistance of a bundle of vessels is approximately equal to the sum of the reciprocals of the individual vessel resistances. Applying his technique:

$$M_b = \frac{-\pi \sum_{i=1}^j n_i r_i^4}{8v} \frac{\partial P}{\partial x} \quad (\text{A2})$$

where  $r_i$  = the radius of the  $i$ th element in the bundle (mm),  $n_i$  = the number of elements of radius  $r_i$  in the bundle,  $j$  = the number of different radii of the vessels in a bundle,  $v$  = the absolute viscosity of the xylem sap (Pa-s), and  $P = P(x, t)$  = the pressure a distance  $x$  from the end of the stem at time  $t$  after application of the overpressure (Pa).

Differentiating equation (A2) with respect to  $x$  and lumping all of the parameters into a single constant,  $k_0$ , gives:

$$\frac{\partial M_b}{\partial x} = -k_0 \frac{\partial^2 P}{\partial x^2} \quad (\text{A3})$$

If  $N$  is the number of cells per mm of the bundle and  $V$  is the average volume of the cells in the tissue, then

$$M_c = -N \frac{\partial V}{\partial t} \quad (\text{A4})$$

$V$  will depend on  $x$ , the distance of the cell from the end of the xylem open to the atmosphere and  $t$ , the time after application of the over-pressure. Substituting equations (A3) and (A4) into equation (A1) gives:

$$k_0 \frac{\partial^2 P}{\partial x^2} = N \frac{\partial V}{\partial t} \quad (\text{A5})$$

Slatyer (1967, equation 6.3) assumed the flow of water across the membrane of a single cell was the product of cell membrane permeability, the area across which flow occurs and the difference in potential on opposite sides of the membrane. We adapted Slatyer's equation to our model by assuming that water flows from the tissue across a membrane with area equal to a fraction of the total mesophyll cell wall area. Assuming that this membrane acts as though it separates the cells from the xylem, we assigned the water in the tissue the potential of the water inside the cell vacuoles and the water outside the tissue the potential of water in the xylem. This allowed us to write  $\partial V/\partial t$ , the change in cell volume with time, as:

$$\frac{\partial V}{\partial t} = -L_{pt} A (\Psi_{\text{tissue}} - \Psi_{\text{xyl}}) \quad (\text{A6})$$

where  $L_{pt}$  = the tissue hydraulic conductivity ( $\text{mm s}^{-1} \text{ Pa}^{-1}$ ),  $A$  = area across which water flows from cell to xylem (m<sup>2</sup>),  $\Psi_{\text{tissue}} = \Psi_{\text{tissue}}(x, t)$  = the water potential of the leaf mesophyll tissue (Pa), and  $\Psi_{\text{xyl}} = \Psi_{\text{xyl}}(x, t)$  = the potential of the water in the xylem at distance  $x$  from the cut end of the stem at time  $t$  after application of an overpressure, (Pa).

We assumed the potential of water in the xylem,  $\Psi_{\text{xyl}}$  to be equal to its pressure. Since the components of  $\Psi_{\text{tissue}}$  are the volume-averaged turgor and osmotic potentials, we used the equation developed by Tyree & Hammel (1972):

$$\Psi_{\text{tissue}} = \Psi_{\text{vat}} + \Psi_{\text{vaop}} + P_c \quad (\text{A7})$$

where  $\Psi_{\text{vat}} = \Psi_{\text{vat}}(x, t)$  = the volume averaged turgor potential of the cells (Pa),  $\Psi_{\text{vaop}} = \Psi_{\text{vaop}}(x, t)$  = the volume averaged osmotic potential of the cells (Pa), and  $P_c$  = the pressure of the cell contents (Pa) due to the forces exerted on the cell by the chamber (assumed equal to chamber pressure).

The values of the bulk (i.e., volume-averaged) parameters are described by Tyree & Hammel (1972) as follows:

$$\Psi_{\text{vat}} = \varepsilon \left( \frac{V - V_0}{V_0} \right) \quad (\text{A8})$$

$$\Psi_{\text{vaop}} = \frac{\Psi_{\text{op}}^0 V_0}{V} \quad (\text{A9})$$

where  $V$  = the average volume of the cells ( $\text{mm}^3$ ),  $V_0$  = the average volume of the cells at zero turgor ( $\text{mm}^3$ ),  $\varepsilon$  = the bulk modulus of elasticity of the tissue (Pa), and  $\Psi_{\text{op}}^0$  = the average value of the osmotic potential of the cells at zero turgor (Pa).

Substituting equations (A7), (A8) and (A9) into equation (A6) and assuming  $\Psi_{\text{xyl}}$  was equal to  $P$  gave us:

$$\frac{\partial V}{\partial t} = -L_{pt} A \left( P_c + \varepsilon \left( \frac{V - V_0}{V_0} \right) - \frac{\Psi_{\text{op}}^0 V_0}{V} - P \right) \quad (\text{A10})$$

By making the change of variable  $V_r = (V - V_0)/V_0$  we

wrote equations (A5) and (A10) in terms of  $V_r$  and  $P$ . We linearized the equations by assuming small values of  $V_r$  and using the binomial expansion  $1/(1+V_r) \cong 1-V_r$ . Thus, equation (A5) became equation (1) and equation (A10) became equation (2).

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