Water entry into detached root systems saturates with increasing externally applied pressure; a result inconsistent with models of simple passive diffusion

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Received 14 August 2001; revised 20 August 2001; in final form 18 February 2002

The most widely accepted model of radial water entry from the soil into the xylem of roots is based on principles of ordinary passive diffusion. However, long-standing problems with this model remain unresolved, which concern variable intrinsic properties of conductivity, \( L_p \), changing reflection coefficients, \( \sigma \), and inaccurate resolution of osmotic differentials between the soil and xylem. Our study re-examined pressure flow relationships in isolated roots of tomato (Lycopersicon esculentum Mill. cv. Montfavet), pea (Pisum sativum cv. Baccara) and soybean (Glycine max L. Merr cv. Essor) manipulated in a pressure chamber. In addition to problems previously recognized with the simple passive diffusion model, a new conflict, flow saturation, was observed at high pressures. Experiments revealed that the plateau in flow, \( J_{\text{max}} \), seen at high pressures followed natural rhythms diurnally and developmentally, and was not due to root damage or unnatural flow restriction. Near the end of the photoperiod, \( J_{\text{max}} \) closely correlated with root dry mass. The above inconsistencies between observations in pressure-flow kinetics and ordinary passive diffusion indicate that either the current model should be adjusted or a new model should be proposed.

## Introduction

There has been substantial progress concerning the nature and location(s) of the greatest resistance(s) to the uptake and transport of water through roots (Steudle and Peterson 1998, Tyerman et al. 1999). However, some fundamental details remain uncharacterized. This may be due, at least in part, to problems with the application of current quantitative models to data of water flow through roots.

### The ordinary passive diffusion model

Quantitative data is often acquired from experiments describing the transport of water through whole root systems by analysing flow of xylem from root systems subjected to artificially raised pressure in chambers designed to accommodate roots still in potted soil (Fiscus 1977, Miller 1985, Passioura 1988, Markhart and Smit 1990, Maggio and Joly 1995, Jackson et al. 1996). The most widely accepted physical model applied to the flow data generated from these experiments is analogous to models of ordinary passive diffusion across a membrane (Nobel 1991).

\[
J_j = P_j(c_j^p - c_j^i) \quad [1]
\]

Where \( J_j \) is flux of species \( j \) across the membrane of a given area (quantity/area/time, i.e. \( m^3/m^2/s \)), \( P_j \) is the permeability coefficient of species \( j \) (length/time, i.e. \( m \cdot s^{-1} \)) and \( (c_j^p - c_j^i) \) expresses the driving force across the membrane (referring here to the concentration of \( j \) outside and inside).

The analogous root model is suitable for a system of membranes (Fiscus 1977, Passioura 1988, Jackson et al. 1996): \( J = L_p(\Delta P - \sigma \Delta \pi) \quad [2] \)

Where \( L_p \) is the collective membrane conductivity (l/ time, m \( \cdot s^{-1} \)) and \( (\Delta P - \sigma \Delta \pi) \) is the driving force summing up components of water potential. \( \Delta P \) and \( \Delta \pi \) are...
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relationships was investigated at different times of day and
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that have been used extensively before (tomato and
soybean) and one that was yet unstudied (pea), by using
(1) external pressure range which was increased to higher
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M aterials and methods

Plant material and growing conditions

All plants were grown in a glasshouse in Dijon under
conditions that were designed to optimise homogeneity
of both shoot and root growth as described below. The
soil mixture that was developed specifically to the study
of root systems and avoids artefactual problems associ-
ated with pressure application to more sandy soils (Pas-
sioura 1988): a bottom layer of expanded fireclay balls
(6-10 mm diameter) facilitated effective pot drainage,
while an unpacked mixture of high absorption American
aluminium oxide (\( \text{Al}_2\text{SiO}_2, \) 0.5-4.0 mm, OIL DRI®.
US-Special Typr III R, Lobbe, Germany) and clay par-
ticles (2-5 mm) (50 : 50 v/v) allowed easy and homogeneous
diffusion of air and water and efficient water reten-

R.2

Tuitions of Lp and an unexplained offset on the pressure
and xylem flow. However, this model cannot resolve cer-
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cut surface thoroughly with distilled water, short sleeves of supple silicon tubing were fitted over the cut plant stumps. The internal diameter of the silicon tubing varied according to the species and maturity of the plants and was selected to give a snug airtight seal. The other end of the sleeve was fitted around a longer length of Tygon tubing, which in turn was connected to a rigid tube inserted into the bottom end of chamber lid coupling. All tubing-tubing connections were made using short silicon sleeves like those connecting the hypocotyl stump to the system. On the outside of the lid rigid tubing fit into the other end of the coupling was joined to a short length of Tygon tubing which directed exudate into pre-weighed Eppendorf tubes. Thus the pressure sealing occurred between the tubing and coupler rather than against the hypocotyl stump. Four couplings allowed simultaneous collection of exudate from up to four plants in the same pot. Mounting and sealing of the plant in the pressure chamber took less than 10 min from when the stem was cut.

Chamber pressure was increased by using compressed air injected through an inlet in the lid connected to a valve. A manual valve allowed fine regulation for changing or holding pressures constant over long periods of time. The initial 20 min of exudate flow was discarded, since it was comprised of the time for mounting the root system into the pressure chamber and the commencement of flow at 0.05 MPa and could not be reliably quantified. Thereafter, at a given pressure, or series of pressures, exudate was collected at regular intervals (usually of 10 min), and weighed to determine its volume and, thus, xylem flow rate. Estimates of L_p and P_i were determined as the slope of the regression of J_v on applied pressure (SAS Proc REG), including only points subjectively considered as comprising the linear phase of the curve.

After pressure manipulations were finished the roots were carefully recovered by washing. They were subsequently dried in an oven at 70°C for 48 h, and weighed.

Once a plant was subjected to pressure-flow manipulation it was not used in further experiments. In experiments involving pressure-flow relationships at different hours throughout an entire day, separate homogeneously grown plants were used at each hour.

Results
Pressure-flow relationships
Using procedures similar to those of Jackson et al. (1996) an ascending pressure gradient was applied to root systems of tomato in an effort to recreate flows that matched transpiration rates in intact plants. Fig. 1A shows a curve typical of those obtained. Flows per entire...
root systems, \( F \), were 30–50% lower than those reported previously (Jackson et al. 1996), but this probably reflects different cultivar and culture conditions. At low pressures the curves were qualitatively similar since, as \( \Delta P \) was increased in small steps, \( F \) from de-topped roots increased curvilinearly at first, and then linearly at higher pressures. Rates of transpiration were reached using less than 0.40 M Pa and well within the linear range of flows (50–80%) of the maximum flow per the entire root system, \( F_{\text{max}} \) generated with the pressure gradient. However, at 0.45 M Pa exudate flow began to saturate, and further increase in pressure distinguished a plateau in the pressure-flow curve giving it an overall sigmoidal shape, a feature not reported previously in studies that have measured pressure induced xylem flow. Fig. 1A also indicates how the parameters of the curves were derived according to interpretations previously proposed from models of simple passive diffusion. A straight line was fitted by regression to the linear phase of the pressure-flow curve, which was extrapolated back to the pressure axis to give an intercept value, \( P_i \), that should theoretically equal the osmotic potential of the soil \( \pi_{\text{soil}} \). The slope of the fitted straight line, \( L_p \), is considered a measure of root water conductivity. Other parameters that are not derived from simple passive diffusion are also marked on the figure. The maximum flow per root system, \( F_{\text{max}} \), was estimated from the highest flow rates achieved. \( P_{0.5} \) was determined as the pressure that resulted in half the estimated \( F_{\text{max}} \). For comparisons involving transpiration per plant \( F_{\text{max}} \) was used, but in other cases \( J_{\text{max}} \) was calculated by dividing \( F_{\text{max}} \) by the root DW. Used this way, root DW was taken as an indicator of the layers of surface area water passes through when entering the xylem from the soil. Comparisons in flux could then be made among root systems of different sizes, states of development and treatments. The means to directly determine surface area were not available, but it was assumed that root DW varies linearly with surface area. This should be true since roots were relatively homogeneous with respect to diameter within species and among experiments.

Similar experiments were done with soybean, since it was used previously to study pressure induced xylem flow (Fiscus 1977, Markhart and Smit 1990), to check whether saturation of flow was common across species (Fig.1B). Like tomato, the curve was sigmoidal, and saturation occurred at about 0.40 M Pa. Unlike tomato, \( F_{\text{max}} \) appeared to be barely adequate or slightly under transpirational loss, since rates of transpiration of intact plants did not always occur within the linear portion of the curve, but instead usually happened as flow saturated.

Finally, pressure-flow curves were tested in pea (Fig. 1C), since availability of a detailed model of its phenological progression would allow precise comparisons among experiments (e.g. Ney and Turk 1993, Roche et al. 1998, Lhuiller-Soundé et al. 1999). Accordingly, it represents an excellent model species to study water flow through roots according to development. The general curve shape for pea was different from tomato and soybean since flow from pea roots was linear from the initial low pressure of 0.05 M Pa onwards. Once again saturation of flow was observed with increasing pressure gradients, but flow from pea saturated at lower pressures than either tomato or soybean. These differences resulted in a curve that was more hyperbolic than sigmoidal. With the exception of a few rare cases under unusually humid conditions, transpirational loss of water from intact peas in the glasshouse far exceeded exudate flow from roots under pressure. For a set of 17 experiments encompassing measurements across the photoperiod and covering reproductive development (from flowering to the end of seed filling) \( F_{\text{max}} \) accounted for only 3.9–40% of transpirational demands. The shortfall between isolated root supply and transpiration was considerable since measurements of potential water stocks in the shoots were very small (from about 1–3 ml during the reproductive cycle of peas, which would supply from 5 to 60 min of water if it alone supplied transpiration) and made little impact on water budgets.

Results in Fig. 1 are curves obtained from single plants chosen because they were qualitatively representative of their species as inferred from over 65 pressure-flow manipulations. It is important to note however, that parameters such as \( L_p \), \( P_i \), \( F_{\text{max}} \) (or \( J_{\text{max}} = F_{\text{max}}/\text{root DW} \) and \( P_{0.5} \) varied among different series of plants, among different aged plants within a series (developmentally) and among the same aged plants diurnally (discussed below). Interestingly, although the water potential of the nutrient solution used to water the plants was constant among experiments at –0.065 M Pa, \( P_i \) rarely occurred close to this value.

Tests of root system integrity

Since flow saturation has not been reported before, it was important to determine if it was a result of root damage caused by the application of high pressure. If the integrity of the root were not compromised, one would expect that cycling between saturating and subsaturating pressures would result in predictable and constant flows characteristic of each pressure. On the other hand, damaged roots might be expected to have more variable flow, especially if pressure cycling caused further degradation in root integrity.

In tomato, cycling of external pressure application from saturating (0.55 M Pa) back to subsaturating (0.20 M Pa) pressures gave flows, at low pressures, slightly greater than those obtained before saturation was initially achieved (Fig. 2). This effect has been observed previously when pressure drops were imposed on soybean roots, even when saturating pressures were never imposed on the root system (Fiscus 1977). Furthermore, during the pressure cycling, the maximal flows reached, \( J_{\text{max}} \), were the same each time a saturating pressure was applied. Likewise, each drop to 0.20 M Pa produced a constant flow for that pressure.
Despite utmost care, occasionally root systems were obviously injured, probably due to tissue rupture during either mounting in the pressure chamber or after increasing the application of external pressure. Two types of injuries were noted: the most common was likely a rupture of tissue that allowed easy passage of air through the system consisting of the root and connected tubing. This most likely occurred close to where the root stumps were sealed by silicon tubing due to mechanical manipulation causing a cracking in the outer epidermal layers and cortex and creating a longitudinal fissure through which air could travel and break the seal formed by the silicon tubing. It was characterized by decreased xylem flow and a strongly increasing flow of air; such replicates were obvious and were discarded. The other type of injury was also characterized by decreased flow, but was likely due to a constriction by pinching. Problems of this nature were also recognized by a deviation from the usual pressure-flow relationship (Fig. 3). Such deviations consisted of premature and unstable flow saturation and their occurrence could be confirmed by pressure cycling. Figure 3 shows flows from one healthy vs. three damaged, pinched roots from plants manipulated simultaneously in the pressure chamber. If pressure was dropped from saturating to subsaturating levels, the effect was the opposite to that of undamaged roots (described above for Fig. 2) since flow would increase at the lower pressure, presumably due to an alleviation of the pinching effect. Any replicates that showed such deviations were not used further in pressure flow analyses.

Generally, pea had a more fragile root system, as indicated by a higher frequency of root injury due to a smaller and more irregularly shaped stem or root cross-section as compared to the thicker, rounder more robust ones of tomato or soybean. Otherwise the integrity of the pressure-flow relationships could be impressively strong. One mature pea root system was observed to withstand a large amplitude in pressure cycling between 0.1 M Pa (16.4 ± 1.9 μl min⁻¹) to 0.7 M Pa (28.8 ± 1.9 μl min⁻¹).

**Exudate flow in descending pressure gradients**

It has been observed previously that a diminishing flow rate may not follow the same kinetics as an incrementing flow rate (Fiscus 1977, Passioura and Munns 1984). Furthermore, the analysis of any differences between increasing- and decreasing-pressure gradients could give more insight to the nature of the resistance towards water entering roots. Descending pressure gradients were imposed on tomato root systems to see if characteristics of the pressure-flow curves such as Jmax, P0.5, Lp and Pi were constant, or a consequence of the direction in pressure gradient application.

Pressure flow relationships were investigated in two regimes involving declining-pressure gradients (Fig. 4A, B). In one case, an initial increasing pressure gradient (using 0.05 M Pa increments) was applied up to flow saturation (0.05–0.55 M Pa) and was followed by a mirror-image declining pressure gradient (Fig. 4A). The curve of the increasing pressure gradient was typical of those ob-
duced previously (Fig. 1), but some features were different during the pressure decline. The curvilinear lag in flow that was observed at low pressure (between 0.05 and 0.15 MPa) during increasing pressure gradients was less pronounced during declining pressure gradients. At intermediate pressures declining-pressure gradients showed more flow per unit pressure, although the linear portion of the curve was parallel and had a slope ($L_p = 375$) similar to that of the increasing-pressure gradient ($L_p = 371$). The curve following the declining-pressure gradient became less sigmoidal as flows close to the $J_{max}$ persisted at lower pressures, compared to the ascending pressure gradient, and did not drop significantly until the pressure went below 0.40 MPa. Overall these differences resulted in a change in values of parameters used to describe the curves. Accordingly, the pressure which yielded a flow half of $J_{max}$ ($P_{0.5}$) was lower for the declining-pressure gradient (0.213 ± 0.003) than the increasing pressure gradient (0.306 ± 0.003), and extrapolation of the linear portion of the declining-pressure gradient indicated a large decrease in the pressure axis intercept ($P_i = 0.04$ MPa) from that the increasing-pressure gradient ($P_i = 0.12$ MPa).

Similar pressure-flow changes were observed when the order was reversed and the decreasing-pressure gradient was followed by an increasing-pressure gradient (Fig. 4B). Once again, the reverse pressure gradient yielded a curve that was less sigmoidal and had higher flows at intermediate pressures and a less pronounced curvilinear flow depression at low pressures. Although it was more difficult to judge where the linear portions of the curves lay, especially for increasing pressure gradients, the slopes between increasing- and decreasing-pressure gradients remained similar (101 between 0.20 and 0.35 MPa for increasing pressure gradient, and 95 between 0.15 and 0.30 MPa for the decreasing pressure gradient), the pressure-axis intercept, $P_i$, and $P_{0.5}$ were lower for the decreasing pressure gradient ($P_i = 0.02$ MPa, $P_{0.5} = 0.188 ± 0.008$) than for the increasing pressure gradient ($P_i = 0.04$ MPa, $P_{0.5} = 0.241 ± 0.011$). At high pressure, however, there appeared to be a flow depression effect that was not seen at similar pressures in experiments that started with low pressure. Flow was relatively low at the initial high pressures of 0.55 MPa and then increased, despite the following steps of falling pressure from 0.55 to 0.40 MPa. By contrast, during the subsequent increasing-pressure gradient, flow increased towards a $J_{max}$ in the same manner as all other experiments involving an increasing pressure gradient in tomato.

The higher flows per unit pressure when roots were subjected to declining pressure gradients are consistent with observations during pressure cycling experiments (Figs 2 and 3) and with those from a previous study (Fiscus 1977) that imposed pressure gradients similar to that of Fig. 4A (although flow saturation was never achieved). Indications are therefore that tomato roots become more conductive to water flow once they have experienced either at least 10 min of pressure, of 0.40 MPa or greater, or flows resulting from those high pressures. Interestingly, this increased conductivity would not be detected by measures of $L_p$, since the slope did not change between ascending and descending pressure gradients.

Diurnal and developmental variability in pressure-flow relationships

Diurnal rhythms in water flux or measures of conductivity have been reported rather frequently (Parsons and Kramer 1974, Fiscus 1986, Carvajal et al. 1996, Henzler et al. 1999), so this was investigated in tomato and pea. Substantial changes in water flow through pressurized roots occurred during the course of a day. In tomato, for example, although the pressure-flow curves retained the same sigmoidal shape, the maximum flow ($J_{max}$) changed by 59% (Fig. 5) being greatest in at about 1130 h and lowest around 1600 h. Following previous methodology (Passioura 1988), a regression was made using points of the linear phase of pressure-flow curves to attempt to measure root conductivity ($L_p$) and soil osmotic potential ($P_i$). Although the decision about which data points comprised the 'linear' portion of the curve was subjective, especially when $J_{max}$ was low, it was nonetheless evident that $P_i$ decreased substantially from 1100 to 1600 h from about 0.17–0.02 MPa (Fig. 5). Corresponding slopes, $L_p$, also decreased from 163 to 77.

Similar rhythms were observed in pea where there was a peak around 1100 h in the apparent maximum flow of water through roots, $F_{max}$ (Fig. 6). $F_{max}$ was lower and approximately equal at 900 h and 1600 h, near the start and end of the photoperiod. As already indicated, pressure induced flow of water through pea roots rarely occur...
Fig. 5. Changes in effect of ascending pressure gradients on exudate flow from tomato roots sampled from mid-day to late-afternoon. Three experiments were conducted, which started at 11:30 h (●), 14:00 h (▲) and 16:00 h (■). Comparisons are made on level of flow at saturation (Jmax) and the pressure at which half-maximal flow is observed (P0.5). In addition a straight line was fit to the linear portion of the curves and extrapolated backward to determine the pressure axis intercept (Pi) and apparent conductivity as indicated by the slope (Lp).

Fig. 6. Patterns in gravimetrically determined transpiration rates (■) of glasshouse grown peas compared to the pattern of Fmax (●), the level of maximum exudate flow from isolated root systems determined from pressure-flow curves.

at rates great enough to account for transpirational losses (Fig. 1). The two-component model of water entry into roots predicts that water flow should increase during the periods of greatest transpiration (Steudle and Peterson 1998), so the timing of rhythms in Fmax and water supply shortfalls were investigated according to changes in transpiration. As indicated by Fmax, the greatest potential water flow occurred in the mid-morning, whereas transpiration of peas in the glasshouse was usually low in the morning and gradually increased from the morning at 11:00 h until peaking in mid-afternoon (Fig. 6). As indicated by Fmax compared to transpirational loss, there would be large shortfalls in water supply and the most serious apparent deficits would occur during the maximum rates of transpiration in the mid-afternoon.

The rhythms in maximal water flow through pea roots per root biomass, Jmax, were analysed in detail at 6 different stages from early flowering to the end of seed filling (Fig. 7A). The amplitude of the diurnal rhythms progressed in a logical sequence with development. They steadily increased from early flowering and reached a maximum from the middle to end of flowering. From that point the amplitude of rhythm dropped considerably, coinciding with the middle to end of seed filling. Jmax declined from midday to late afternoon at each stage of development, except for the end of grain filling when Jmax was constant throughout the photoperiod. By 16:00 h Jmax converged to a similar rate among developmental stages. The constancy of Jmax measured at 16:00 h was analysed further by plotting maximal water flow per root system, Fmax, versus biomass using the series of plants shown in Fig. 7A and an additional developmental series of measurements that spanned the reproductive cycle of pea. The plot shows that Fmax in the late afternoon is strongly associated with root biomass (r² = 0.96; Fig. 7B). This association held across different series of
Discussion

Problems with ordinary passive diffusion models

Ordinary passive diffusion models (Equation 2) kinetically describe a J shaped curve (Newman 1976, Fiscus 1977, Passioura 1988, Jackson et al. 1996). The initial slow increase of flow with pressure is perceived to be a result of a total driving force during which the osmotic component plays a significant role. Afterwards, a linear phase commences once osmotic potential effects become negligible at faster flows. Problems with ordinary passive diffusion models of water entry and flow through roots have been known for quite some time and include variable intrinsic properties of conductivity, \( L_p \), changing reflection coefficients, \( \alpha \), and the inability to accurately resolve osmotic differentials between the soil and the xylem (Passioura 1988).

Our results reveal similar phenomena and problems with their interpretation. According to the model, the slope of the linear portion of the curve should be a measure of membrane conductivity, \( L_p \). Deciding which points comprised the linear portion of the curve for regressions was subjective, especially for plants with lower flows. In fact, current results indicated that the pressure flow curves in tomato and soybean are sigmoidal and, accordingly, have an inflection point about which the slope changes; therefore, the practice of deriving constants from intercepts and slopes of a regressed line is unstable. Even if one ignores this, systematic use of line-fitting showed that \( L_p \) was subject to large variations with changing external pressure, both diurnally and developmentally. These changes cannot be rationalized easily with simple passive diffusion.

The model predicts that the pressure axis intercept from an extrapolation of the linear portion of the pressure-flow curve should correspond directly with the osmotic potential of the soil (\( \pi_{soil} \)), but it rarely does (Passioura 1988). The model has been amended with the inclusion of an intercept-offset term, \( P_{off} \), for which there is no confirmed physiological explanation. The seriousness of this was highlighted in one study of chilled root systems of spinach for which the pressure axis intercept was as high as 0.20 MPa despite the fact that the root systems were in a nutrient solution with an osmotic potential of 0.05 MPa (Fennel and Markham 1977). Present data underline the same problem in tomato and pea. In our experiments with tomato, the osmotic potential of the nutrient solution was constant, yet large differences in pressure axis intercepts were observed both among experiments (Figs 2, 3, 4 and 5) and within experiments (Figs 4A, B and 5). Moreover, in peas, it was not uncommon to have a negative pressure axis intercept despite the fact that the root systems were surrounded by nutrient solution with an osmotic potential of -0.065 MPa, which therefore should have resulted in a positive pressure axis intercept at 0.065 MPa.

Present results also highlight a conflict previously not revealed or discussed by earlier studies. Once the linear phase of the pressure-flow curve begins, Equation 2 predicts that it should increase linearly in proportion to the applied pressure ad infinitum. In practice this is not the case. The three species tested each reached a saturating maximum flow (Fig. 1), that persisted even when all the indications were that root systems were undamaged by pressure application. As discussed below, the maximum flow appeared to be related to root biomass and integrally regulated according to developmental stage of the plant and time of day (Figs 5, 6 and 7A, B). Can saturation be explained by adapting the simple passive diffusion model to incorporate some sort of inherent membrane ‘resistance’ that changes at the arrival of high water potentials? This study does not completely disprove that water crosses the membrane by non-Stokesian diffusion from the random thermal motion of the lipid tails. Using the Newman’s tangent test (Newman 1976), our data reveal a rather large probable accumulation of solutes at the principle barrier (limiting resistance) in the root, which might be created from solvent drag. This could tend to decrease the driving force at the principle barrier and explain saturation. However, we did not observe changes in saturation between plants that received half or full-strength nutrient solution prior to pressure chamber manipulations. Furthermore, plants flushed for several hours and supplied with distilled water throughout experiments neither prevented saturation nor altered the diurnal characteristics in saturated flow rates (\( J_{\text{max}} \)) in tomato or pea roots. Finally, like current determinations of \( L_p \) and \( P_i \), Newman’s tangent test relies on the assignment of a straight line through the linear part of pressure-flow plots and is open to the same criticism outlined above.

Is water conductivity by isolated root systems representative of intact plants?

The composite transport model of roots (Steudle and Peterson 1998) has been used to provide explanations for some of the problems associated with pressure flow curves and the application of ordinary passive diffusion formalism. For example, the apoplastic entry of water is perceived to have a changing role in water absorption based on the demands of transpiration, which may explain why \( L_p \) changes with pressure (Steudle et al. 1993). Accordingly, parallel apoplastic and transcellular pathways would play an important role during the passage of water across the different root tissues. The apoplastic paths are envisaged to lessen hydraulic resistance by allowing increased flow during periods of high transpiration, whereas water channels in the transcellular path would finely adjust water uptake in older, suberized parts of roots lacking a substantial apoplastic component.

While this may be true, relationships observed for pea between transpiration and pressure-flow data occurred...
inversely to the predictions of the two component hypothesis. In the glasshouse, peak transpiration for pea occurred at about 5 h after the mid-day peak in maximum water flow through pressurized roots, $F_{\text{max}}$. In fact, peak transpiration occurred when $F_{\text{max}}$ was apparently at its lowest. Moreover, the work of Henzler et al. (1999) showed that transpiration rate can be manipulated independently of the diurnal rhythm of root conductivity, which would be inconsistent with the hypothesis that root conductivity was a limiting factor for transpiration. Considering these discrepancies between transpiration and conductivity, there is serious doubt whether water flow through pressurized root systems is representative of what occurs in an intact transpiring plant. There were large deficits between maximum potential supply of water from an individual root system ($F_{\text{max}}$) and transpirational losses. The greatest deficits occurred between mid- to late-seed filling, when plants were transpiring their greatest amounts of water, but not visibly suffering from drought.

Other studies that applied pressure to isolated root systems have not reported differences in between maximum rates of flow from isolated roots and intact plants. Early on, Kramer (1959) warned that vacuum extracted sap flowed well below rates of transpiration, but surprisingly few studies have tried to compare the delivery of water from decapitated pressurized roots with measured transpiration rates. An exception is the work of Else et al. (1995) that showed tomato roots pressurized above 0.30 M Pa could furnish an amount of water sufficient for transpiration. Present results with tomato agreed, since a pressure between 0.35 and 0.40 M Pa was satisfactory. Tomato in this respect could be an exceptional species. Our results with soybean indicate maximum flow is, at best, barely adequate and usually deficient for transpirational demands. Perhaps most researchers working with decapitated root systems have not concerned themselves with accounting for transpiration because they have never observed or reported flow saturation at high pressures. Since ordinary passive diffusion models predict an ever-increasing flow with pressure, one could merely extrapolate flow curves to calculate the kind of pressure gradient required for transpirational requirements. However, if pressure-flow saturation occurs below transpiration rates this possibility is precluded.

By contrast, in studies of intact plants of several different species (Hordeum vulgare, Lupinus albus, tomato and Zea mays) discrepancies between rates of transpiration and pressure induced xylem flow rates have not been reported (Passioura and Munns 1984, Else et al. 1995, Stirzaker and Passioura 1996, Tiekstra et al. 2000). Those studies used a split-top pressure chamber design (Passioura 1980) that allowed pressurization of the root system without de-topping the plant and xylem collection from a cut leaf or petiole tip.

There is a strong possibility that at least one path of water flow is lost when shoots are excised. If true, this appears to be the major route of water flow to the shoot in the case of pea. It is difficult to speculate among the several possible causes that might explain the loss of a water entry route brought about by the lack of a shoot or loss of continuity in the water column (Passioura 1988, M arkhart and Smit 1990). Our pressure gradients were generated by ‘pushing’ from outside the root by raising external pressure, but transpiration operates by tension or ‘pulling’ from the inside by lowering internal pressure through cohesion and flow. Although the $\Delta P$ would be the same, physically this could have different effects on the conformation of membranes, water channels or apoplasm (i.e. Shone and Clarkson 1988). However, conductance of pressurized intact plants changes linearly, even at pressures close to 2 M Pa (M unns and Passioura 1984), so this explanation seems unlikely.

There may be a lost apoplastic route. Parallel apoplastic and transcellular pathways are proposed to play different roles during the passage of water across root tissues (Steudle et al. 1993). Increased apoplastic flow has been implicated during periods of high flow required by midday transpiration. Perhaps what is left after removing the shoot is the water channel component in the transcellular path that is normally implicated in only fine adjustments of water uptake in older, suberized parts of roots lacking a substantial apoplastic component tissues (Steudle et al. 1993). Intact plants may not suffer the same fate if they act like a manifold distribution system releasing the pressure drop more gradually over a larger distance and area (i.e. the leaves of many nodes) rather than suddenly at the cut root stump as in a shootless root system. The latter situation could lead to an apoplastic plug if a sudden pressure drop caused a form of intercellular compression.

Why has flow saturation not been reported earlier?

Although many examples of studies using isolated root systems have not reported flow saturation, they have not presented pressures adequately high according to present results (i.e. greater than 0.40 M Pa for tomato). Perhaps there has been concern that the plateau was an artefact of damaged roots. However, the pressures used here were less than 0.70 M Pa, and far lower than those known to affect cell physiology in plants (Passioura 1988). Observations of the present study indicate that $J_{\text{max}}$ is a characteristic of de-topped but otherwise well-functioning roots. For example, once flow saturation had been reached and the pressure decreased, flow rates returned consistently to values equal to or greater than those measured at pre-saturation rates during the initial pressure increase. Subsequent return to saturating pressures resulted in the same value of $J_{\text{max}}$. This sort of pressure cycling could be repeated several times with the same root system without changing flow rates at specific subsaturating or saturating pressures, and could be done with pressure changes as large as 0.50 M Pa.

During pressure cycling experiments, undamaged or uninhibited root systems were easily discerned from damaged or flow-inhibited root systems, for which flow appeared to be pinched off at higher pressures. For
pinched roots, a drop in pressure actually increased flow, presumably by relieving the pinch, and subsequent return to the higher pressure decreased flow once again. Pressure-inhibited and well-functioning roots were occasionally observed in the same experiment revealing a departure of the former from the normally smooth sigmoidal kinetics (Fig. 3). Maximum flows were typically reached earlier than those of unobstructed roots and the transition from linear flow to plateau was sudden rather than smooth and gradual. Moreover, the maximum flow declined with further pressure increase in pinched roots.

Another commonly observed type of root damage likely resulted from lesions in the root during the fitting of the root system to the pressure chamber. This occurred most commonly with peas, which did not have the woody strength of tomato or soybean, especially in the region of tubing attachment. In those cases the flow of air increased and that of sap decreased as pressure was increased. Root damage caused by lesions or pinching might be expected to occur in a relatively random fashion, whereas our observations of \( J_{\text{max}} \) followed predictable diurnal patterns, similar to those determined for root conductivity observed in several other plant systems (as based on measurements of \( L_p \) or other analogous parameters; Parsons and Kramer 1974, Passiourea 1988, Henzler et al. 1999).

Finally, careful scrutiny of previous studies sometimes reveals the tendency for saturating flow at high pressures. For example, in studies of tomato, one can see a point of saturation between 0.32 and 0.38 M Pa (Maggio and Joly 1995) and a tendency for flow to level off at 0.40 M Pa (Jackson et al. 1996). In the case of Maggio and Joly (1995), despite the apparent flow saturation in some treatments, the curves were deemed ‘highly linear’ and a straight line was fit to the data.

**An hypothesis based on facilitated passive diffusion**

Present results and problems with the current mathematical modelling of water uptake in pressurized roots could be reconciled mechanistically with facilitated passive diffusion and the existence of water channels, aquaporins, for transmembrane water entry into roots. Considerable physiological and molecular studies indicate flow is largely symplastic and that aquaporins may provide the greatest resistance to water flow through root systems (M arkhart and Smit 1990, Maggio and Joly 1995, Carvajal et al. 1996, 2000, Henzler et al. 1999, Kjellbom et al. 1999, Quintero et al. 1999, Wan and Zwiazek 1999).

Facilitated passive diffusion differs from ordinary passive diffusion on criteria (Nobel 1991) that are strikingly similar to attributes of aquaporin-mediated water flow. Facilitated diffusion is mediated by passive, selective transporters that allow certain solutes to cross a membrane to the exclusion of other solutes and at fluxes larger than those expected for ordinary passive diffusion (except at very high changes in water potential).

Likewise, aquaporins are transporters comprised of proteins that form channels to allow the passage of water. The driving forces of these channels are hydraulic or osmotic in nature and are not active; while they permit or facilitate the movement of water through membranes they do not act as pumps (Chrispeels and M auel 1994). Aquaporins are highly selective and allow water to pass freely while excluding ions or other small solutes. Accordingly, they are functionally defined as having a higher level of permeability than would be expected based on a model of ordinary passive diffusion (M auel 1997, Tyerman et al. 1999). In fact, the usual tests for the presence of functional aquaporins are the same as those for the detection of facilitated passive diffusion. This includes membrane crossing at lowered activation energy (\( E_a \)) and ratio of hydraulic permeability to diffusional permeability (\( P_h/P_d \)) greater than 1 (Kleinmans 1998, Coury et al. 1999). A aquaporin \( E_a \) in structural models indicates a low value of 16 kJ mol\(^{-1}\) that is similar to movement in bulk water and the corresponding \( P_h/P_d \) ratio is greater than unity in systems where diffusion facilitated by aquaporins is known (Schäffner 1998).

Selectivity of facilitated passive diffusion is generally poorly understood but the presence of transporters is assumed. Flux would saturate as all transporters became occupied at higher solute concentrations.

We are unaware of any studies reporting a saturation of aquaporin mediated water flow. However, in principle, saturation should occur once the finite number of aquaporins in a given membrane are occupied at high outside water potential. It should be noted that binding is not needed to explain how the process saturates. Any transport system that has a finite capacity will become saturated as that capacity is approached. Protein-mediated processes saturate because the number of protein molecules per cell is finite. Aquaporins somehow catalyse water movement through membranes. Regardless of the mechanism, which is poorly understood, one can think of \( J_{\text{max}} \) as the product of protein abundance and turnover number (the number of ‘catalysed’ events per aquaporin molecule per unit time). Since aquaporin abundance is finite, saturation is certainly possible, since the number of water molecules that can pass through the aquaporin channel per second is certainly finite. Moreover, according to our observations, water entry into isolated roots seems to saturate at physiologically relevant water flows.

Further evidence that aquaporins may be involved includes our observations of short-term regulation (diurnally) and long-term regulation (with changes in growth phase). Such flow regulation has recently been extensively documented for water crossing membranes via aquaporins (M auel 1997, Johansson et al. 1998). Evidence already exists showing that water flow through isolated roots is limited by the conductivity of aquaporins in the transcellular pathway in tomato (Maggio and Joly 1995) and a wide range of other plants including monocots, dicots, trees and legumes (Carvajal et al. 1996, 2000, Henzler et al. 1999, Kjellbom et al. 1999, Quintero et al. 1999, Wan and Zwiazek 1999). Therefore,
the water flow kinetics we observed in isolated roots of tomato, pea, and soybean may be more amenable to facilitated passive diffusion and models based on enzyme kinetics (Emery and Salon 2001).

Acknowledgements – We thank M. J. Jackson and R. S. Cole for sharing the detailed plans of their pressure chamber (University of Bristol, Lancaster, U.K.). Technical expertise was provided by G. Sanchez, F. Marmont and C. Jeudy in the design, building and maintenance of the pressure chamber and by V. Durrey for plant cultivation (INRA, Dijon, France). We are grateful to F. Marty, John Passioura and four anonymous reviewers for their criticism.

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