Water rise kinetics in refilling xylem after desiccation in a resurrection plant

H. SCHNEIDER, N. WISTUBA, H.–J. WAGNER, F. THÜRMER and U. ZIMMERMANN†
Lehrstuhl für Biotechnologie, Biozentrum, Universität Würzburg, Am Hubland,
D-97074 Würzburg, Germany

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SUMMARY

The acropetal water refilling kinetics of the dry xylem of branches (up to 80 cm tall) of the resurrection plant Myrothamnus flabellifolia were determined with high temporal resolution by observation of light refraction at the advancing water front and the associated recurving of the folded leaves. To study the effect of gravity on water rise, data were acquired for cut upright, horizontal and inverted branches. Water rise kinetics were also determined with hydrostatic and osmotic pressure as well as at elevated temperatures (up to 100°C) under laboratory conditions and compared with those obtained with intact (rooted) and cut branches under field conditions. Experiments in which water climbed under its capillary pressure alone, showed that the axial flow occurred only in a very few conducting elements at a much higher rate than in many of the other ones. The onset of transpiration of the unfolded and green leaves did not affect the rise kinetics in the ‘prominent’ conducting elements. Application of pressure apparently increased the number of elements making a major contribution to axial xylem flow. Analysis of these data in terms of capillary-pressure-driven water ascent in leaky capillaries demonstrated that root pressure, not capillary pressure, is the dominant force for rehydration of rooted, dry plants. The main reasons for the failure of capillary forces in xylem refilling were the small, rate-limiting effective radii of the conducting elements for axial water ascent (c. 1 μm compared with radii of the vessels and tracheids of c. 18 μm and 3 μm, respectively) and the very poor wetting of the dry walls. The contact (wetting) angles were of the order of 80° and decreased on root or externally applied hydrostatic pressure. This supported our previous assumption that the inner walls of the dry conducting elements are covered with a lipid layer that is removed or disintegrates upon wetting. Consistent with this, potassium chloride and, particularly, sugars exerted an osmotic pressure effect on axial water climbing (reflection coefficients > zero, but small). Although the osmotically active solutes apparently suppressed radial water spread through the tissue to the leaf cells, they reduced the axial water ascent rather than accelerating it as predicted by the theory of capillary-driven water rise in leaky capillaries. Killing cells by heat treatment and removal of the bark, phelloderm, cortex and phloem also resulted in a reduction of the axial rise rate and final height. These observations demonstrated that radial water movement driven by the developing osmotic and turgor pressure in the living cells was important for the removal of the lipid layer from the walls of those conducting elements that were primarily not involved in water rise. There is some evidence from field measurements of the axial temperature gradients along rooted branches that interfacial (Marangoni) streaming facilitated lipid removal (under formation of vesicle-like structures and lipid bodies) upon wetting.

Key words: Myrothamnus flabellifolia, xylem refilling, root pressure, lipid lining, capillary forces, resurrection plants.

INTRODUCTION

The resurrection plant Myrothamnus flabellifolia is a woody, multi-stemmed shrub growing in shallow soils on rocky outcrops to a height of 1–2 m. On dehydration, the decussately arranged leaves close like tiny inverted sunshades to become small brown rolls which furl very closely to the branches. The plant can survive desiccation during very long periods of drought. However, after watering, the leaves unfold immediately and display their green adaxial surfaces within c. 1 d.

Most research on desiccation tolerance of M. flabellifolia has focused on the structural, biochemical and molecular levels (Mauve, 1966; Gündel, 1968; Hoffmann, 1968; Vieweg & Ziegler, 1969; Carlquist, 1976; Gaff, 1977, 1989; Bewley & Pacey, 1981; Bewley & Krochko, 1982; Bartels et al., 1990;
Suau et al., 1991; Goldsworthy, 1992; Wilson & Drennan, 1992; Bianchi et al., 1993; Drennan et al., 1993; Sherwin & Farrant, 1996; Oliver & Bewley, 1997; Hartung et al., 1998; Farrant et al., 1999; Scott, 2000. There is little work on the mechanism of xylem refilling in ‘dry’ plants. Child (1960) placed dry branches of similar size in water, some upright and some inverted, so that the water covered a number of leaves. He found that the leaves of the upright branches rehydrated more rapidly and that on the inverted branches only the leaves in the water and those immediately above it unfolded. He concluded that rehydration occurred through the root system rather than via the leaf surfaces. Gaff (1977), working with intact ‘dry’ plants in the field, found that both root pressure and capillary forces were very important for refilling the xylem conduit and, therefore, for the entire rehydration process. Sherwin et al. (1998) measured hydraulic characteristics of the xylem, concluding that capillary forces could account well for the refilling of the xylem conduit, but that root pressure is not an adequate driving force because of its very low value (2.4 kPa).

The classical equations of Lucas (1918) and Washburn (1921) (which describe the rate of penetration of a liquid under its own capillary pressure into cylindrical capillaries with water-impermeable walls) are necessary for drawing conclusions about the involvement of capillary forces from water rise kinetic data. A theoretical analysis must also consider radial water transport into the tissue induced by osmotic/hydrostatic pressure gradients, transpiration and other (e.g. interfacial) forces. In addition, the inner walls of the empty conducting elements of the xylem of M. flabellifolia are presumably covered with a lipid film (Schneider et al., 1999) influencing wetting and thus water ascent.

Unfortunately, most of the above experimental observations are only qualitative, not allowing for analysis in terms of the Lucas and Washburn equations or safe conclusions about the involvement of capillary forces, root pressure or other forces during refilling of the empty xylem conduit of M. flabellifolia.

Here, we present high temporal resolution experimental data on the acropetal water rise kinetics in the dry xylem of cut and intact branches of M. flabellifolia, together with an adequate theory for water rise in leaky capillaries. We minimized methodological disadvantages and inaccuracies by using three different techniques for data acquisition. Water rise was studied under its own capillary pressure and in the presence of additionally applied forces. Kinetics were determined for cut upright, inverted and horizontal branches as well as for intact plants. This yielded comparative values for the unknown parameters, reducing the uncertainties involved in the theory (see later).

**Theoretical Considerations**

A liquid rises in a capillary because of attractive forces between its molecules and the capillary’s inner surface; these are distinct from the forces of cohesion that bind the molecules of a liquid together to form a bulk phase. The meniscus of water in a capillary assumes a concave shape because the adhesive forces are stronger than the forces of cohesion. The liquid tends to cover the greatest possible area of the inner surface of the capillary.

**Impervious capillaries**

For horizontal capillaries, or in general where gravity can be neglected, Washburn (1921) and Rideal (1922) gave the following equation for the rise of a liquid (neglecting air resistance) driven by the pressure difference across the curved surface of the meniscus:

\[ h^2 = \frac{2\sigma \cos \theta}{\eta g} \cdot r \cdot t \]  

(Eqn 1)

\(h\), height; \(\sigma\), surface tension between the liquid and the vapour; \(\eta\), dynamic viscosity of the liquid; \(\theta\), contact angle; \(t\), time; \(r\), radius of the capillary.

In the case of a nonuniform capillary, \(r\) represents the radius of the smallest cross-section. It is obvious from Eqn 1 that the rise height, \(h\), is proportional to \(\sqrt{t}\).

The corresponding equation for the rate of liquid rise is accordingly:

\[ \frac{dh}{dt} = \frac{\sigma r \cos \theta}{4\eta h} \]  

(Eqn 2)

Eqn 2 states that the (linear) rate at which a liquid penetrates any horizontal capillary under its own capillary pressure is directly proportional to the radius of the capillary, to the cosine of the contact angle, to the ratio of the surface tension to the viscosity of the liquid and inversely proportional to the height (i.e. the length) already filled by the liquid.

Eqns 1 and 2 were derived from the Hagen-Poiseuille equation, which relates the volumetric flow rate of a liquid through a straight pipe to the pressure drop imposed. The equation, which applies to laminar flow and to steady-state conditions, may be written as:

\[ \frac{dh}{dt} = \frac{\Delta P \cdot r^2}{8\eta h} \]  

(Eqn 3)

For horizontal capillaries (i.e. in the absence of gravity) the pressure drop is \(\Delta P = 2/r \sigma \cos \theta\) and integration of Eqn 3 leads to Eqns 1 and 2. For the motion of the advancing liquid front in a vertical capillary against gravity, the pressure drop is given by \(\Delta P = 2/r \sigma \cos \theta - \rho gh\) (where \(\rho\) = density) and accordingly in a vertical capillary with gravity by
\[ \Delta P = 2/r \sigma \cos \Theta + pgh. \] In the case of the application of an external pressure the total driving pressure against gravity is given by
\[ \Delta P = 2/r \sigma \cos \Theta - pgh + P_{rad}. \]

Integration of Eqn 3 for water rise in the direction of gravity leads to Eqn 4 for the rate of liquid movement (Lucas, 1918):
\[ t = \frac{h + c}{b + c} \ln \left( \frac{c}{bh + c} \right) \]
with \( b = \frac{\rho \gamma^2}{8\eta}; c = \frac{\sigma \cdot \cos \Theta}{4\eta} \) Eqn 4

In the case of water ascent against gravity, a final height is reached where the capillary force balances the gravity term:
\[ t = \frac{h + c}{b + c} \ln \left( \frac{c}{bh + c} \right) \]
with \( b = -\frac{\rho \gamma^2}{8\eta}; c = \frac{\sigma \cdot \cos \Theta}{4\eta} \) Eqn 5

**Leaky capillaries**

Xylem elements are not the ideal ‘pipes’ with impervious walls for which the Hagen-Poiseuille law (Eqn 3) was formulated. They are leaky capillaries, such that water extraction through the walls (pits) occurs (Altus et al., 1985; Canny, 1991, 1993). Radial water flow during xylem refilling is expected to interfere with longitudinal (axial) water flow in *M. flabellifolia* because ‘recurving’ of the folded leaves was observed close to the ascending water front (at least in the cases of water rise in the absence of and against gravity).

Taking into account the extraction of water from the xylem by lateral movement during longitudinal flow, Eqn 3 must be modified as follows according to the line of arguments given by Altus et al. (1985) and Canny (1991):
\[ \frac{dh}{dt} = \frac{\Delta P \cdot r^2}{8\eta h} \cdot \frac{V}{2\pi r^2} h \] Eqn 6

\( V \) is the extraction rate per unit length [m\(^3\) s\(^{-1}\) m\(^{-1}\)] assumed to be constant along the entire length of the branch (for justification of this assumption, see Altus et al., 1985). \( V \) has the dimension of a diffusion coefficient and can easily be related with the rate of radial water movement, \( v_{rad} \), by (Zimmermann & Steudle, 1978; Altus et al., 1985):
\[ V = 2\pi R \cdot v_{rad} \] with \( v_{rad} = L_{p, rad} \Delta P_{rad} \) Eqn 7

\( \Delta P_{rad} \) the radial driving pressure gradient; \( L_{p, rad} \), the hydraulic resistance of the radial pathway; \( R \), the radius of the lumen of the conducting element which might not be necessarily identical to the rate-limiting radius of axial water rise (see Eqn 4 and later in text).

Eqn 7 is useful if \( L_{p, rad} \) or \( \Delta P_{rad} \) are known and if \( \Delta P_{rad} \) is constant over the whole length of the leaky capillary.

Integration of Eqn 6 for capillary-driven water movement in a horizontal, leaky capillary yields for the rise height of the liquid as a function of time:
\[ h = \sqrt{\frac{c}{a}} \left( e^{2at} - 1 \right) \]
with \( a = -\frac{V}{2\pi r^2}; c = \frac{\sigma \cdot \cos \Theta}{4\eta} \) Eqn 8

Eqn 8 reduces to the Washburn Eqn 1 when the extraction rate per unit length, \( V \), is set to zero. This can be readily shown by expansion of the exponential function into a polynomial and by neglecting the higher-order terms.

Accordingly, integration of Eqn 6 for the motion of the advancing fluid front in a leaky vertical capillary against and with gravity is given by Eqns 9 and 10, respectively:
\[ t = \frac{1}{2a} \ln \left( \frac{ah^2 + bh + c}{c} \right) + \frac{b}{a} \sqrt{-\Delta} \]
with \( a = \frac{-V}{2\pi r^2}; b = -\frac{\rho \gamma^2}{8\eta}; c = \frac{\sigma \cdot \cos \Theta}{4\eta}; \Delta = 4ac - b^2 \); and
\[ t = \frac{1}{2a} \ln \left( \frac{ah^2 + bh + c}{c} \right) + \frac{b}{a} \sqrt{-\Delta} \]
with \( a = \frac{-V}{2\pi r^2}; b = \frac{\rho \gamma^2}{8\eta}; c = \frac{\sigma \cdot \cos \Theta}{4\eta}; \Delta = 4ac - b^2 \)

Note that it can readily be shown that Eqn 9 goes over into Eqn 5 when \( V = 0 \). When the gravitational term is neglected (i.e. \( b = 0 \)) Eqn 9 reduces to the Washburn equation for horizontal capillaries (Eqn 1).

Eqns 9 and 10 show that it is not possible to get an analytical expression for \( h \) as a function of \( t \). The experimental data must be fitted numerically by iterative solution of these equations using appropriate values for the various parameters.

**Assumptions and limitations**

A critical assessment of the theory is required when the fundamental equations for capillary transport are applied to the (poorly wettable) capillary network of
M. flabellifolia. The equations for impervious and leaky capillaries only hold provided (1) that the Hagen-Poiseuille equation is valid, (2) that the capillaries are of uniform radius over the whole length, deviating from circularity in cross-section by not more than a few per cent, and (3) that the surface tension and the contact angle do not vary along the individual capillary during water ascent, and that the wetting process can be accurately described by the term \( \sigma \cos \Theta \).

Condition (1) is usually not valid for short times because the steady-state approximation implicit in the derivation of the equations is inappropriate for the two ends of the liquid column in the capillary, that is, in the regions of entry into the capillary from the reservoir and in the vicinity of the advancing meniscus (Szekely et al., 1971; Adamson & Gast, 1997). However, such effects can presumably be neglected for water rise in dry branches of M. flabellifolia, since liquid movement is rather slow.

For longer times, the Washburn equation has been tested and found adequate for capillaries of radius 0.3–400 \( \mu \)m (Fisher & Lark, 1979). For narrower capillaries these authors found bubbles in the flowing liquid and an abrupt decrease in flow rates. The bubbles accounted for the decreased flow rates via the Jamin effect caused by the difference between advancing and receding contact angles.

Condition (2) is only roughly fulfilled for the branches of M. flabellifolia as the xylem vessels are not completely circular and contain reticulate/modified scalariform perforation plates at an average spacing of 500–600 \( \mu \)m (see Carlquist, 1976; Wagner et al., 2000). The average ‘pore radius’ of the perforation plates is \( \approx 1 \) \( \mu \)m and, therefore, much less than the radius of the vessels (10–18 \( \mu \)m).

The rhomboidal tracheids are a second type of ‘conducting element’ (Wagner et al., 2000). The corners of the tracheids cause a capillary under-pressure which is larger than predicted by the radius of the lumen. Rise times at the corners might be significantly faster than those in the capillary lumen (Finn, 1989), which can impose difficulties in the interpretation of the water rise kinetics. The use of a constant \( \sigma \cos \Theta \) term is only a rough approximation for water ascent in the xylem of M. flabellifolia.

The contact angle formed by a moving meniscus can differ from that formed by the static meniscus (Elliott & Riddiford, 1962; Fisher & Lark, 1979). Secondly, contact angle hysteresis can occur. This phenomenon has been long recognized and studied (Dettre & Johnson, 1964; Oliver et al., 1980). The general observation is (Adamson & Gast, 1997) that the contact angle measured for a liquid advancing across a surface exceeds that of one receding from the surface. This contact angle hysteresis can be as much as 50° for water on mineral surfaces, and velocity-(Elliott & Riddiford, 1962) and local-humidity-dependent (Zhu et al., 1994). An example is the appearance of a raindrop moving down a windowpane or an inclined surface (Lomas, 1970, 1971; Princen, 1971; Brown et al., 1980; Dussan, 1987).

Although not fully understood, contact angle hysteresis is generally attributed to surface roughness, surface heterogeneity, solution impurities adsorbing on the surface, or swelling, rearrangement or alteration of the surface by the solvent. Local tilting of a rough surface or local variations in interfacial energies on a heterogeneous surface can cause contact angle and surface tension variations.

Surface heterogeneity must certainly be taken into account in dry branches of M. flabellifolia because a lipid film apparently covers the inner walls of the xylem vessels and tracheids (Schneider et al., 1999; Wagner et al., 2000). The average value of the contact angle, \( \Theta \), may be given by the Cassie equation (1948):

\[
\cos \Theta = f_1 \cos \Theta_1 + f_2 \cos \Theta_2
\]

Eqn 11 (\( f_1 \) and \( f_2 \), fractions of the surface occupied by surface types having contact angles \( \Theta_1 \) (wettable) and \( \Theta_2 \) (nonwettable).)

Small-scale surface roughness influences the contact angle, as studied in model experiments by many authors (Shuttleworth & Bailey, 1948; Good, 1952; Eick et al., 1975; Adamson & Gast, 1997). For angles \(< 90^\circ \) (usually assumed for wetting of the inner walls of the xylem with water) the contact angle decreases with roughness.

The wetting of a hydrophobic surface depends on: the surface tension between the liquid and vapour (\( \sigma_{lv} \)); the interfacial tension between the solid and the liquid (\( \sigma_{sl} \)) and the surface tension between the solid and the vapour (\( \sigma_{sv} \)) (Adamson & Gast, 1997). Additionally, in M. flabellifolia the surface tension \( \sigma_{lv} \) reduces during wetting because the lipid film starts to disintegrate on contact with water. Thus, the assumption of \( \sigma = \sigma_{lv} \) being constant in the derivation of Eqns 8–10 is a very rough approximation.

Despite the complex behaviour of \( \sigma \) and \( \cos \Theta \), we demonstrate here and in Wagner et al. (2000) that the term \( \sigma \cos \Theta \) is very meaningful for the elucidation of the water rise mechanisms in M. flabellifolia. By measuring the refilling of the empty xylem of the resurrection plant under various driving forces and by using different methods and liquids it is possible to obtain values for the unknown quantities (i.e. surface tension, contact angle, radius and radial water extraction rate), thus reducing the theoretical ambiguities.

**Materials and methods**

**Plant material and environmental conditions**

Branches of various lengths (up to 80 cm) of Myrothamnus flabellifolia Welw. were collected from
intact dry plants at the Hofmann farm c. 10 km from Outjo, Namibia (Schneider et al., 1999). Branches were usually cut close to the root. Refilling experiments were performed in the laboratory in Würzburg and under field conditions in Namibia.

Field and laboratory experiments were carried out under various environmental conditions (Schneider et al., 1999). The ambient irradiance, temperature and rh were measured with a light meter and a hygro-thermometer (LI 189 SA, LI-COR Inc., Lincoln, NE, USA, and testo 610, Testo GmbH, Lenzkirch, Germany, respectively). Diurnal changes in the vertical (ambient) temperature gradients along the branches of intact plants at the collection site were measured by thermistors (Tiny Talks, RS Components, Mörfelden-Walldorf, Germany) fixed to the stem at different heights.

Water uptake and transpiration of cut branches were determined by weighing. In some experiments, the total water uptake through the cut base was measured additionally by using a potometer apparatus which consisted of a transparent, water-filled silicone rubber tube (diameter 3 mm) sealed onto the cut end of a dry branch. The tube was mounted horizontally, allowing calculation of water uptake rate from changes in the length of the water column.

**Determination of water ascent**

We used three experimental approaches to examine the rate of acropetal water ascent. For the ‘dye rise’ method, upright cut branches were placed in small flasks filled with ink (Quink, Parker, Newhaven, UK) or with aqueous solutions containing 0.1% fluorescein, toluidine blue O or aniline blue. The branches were dipped into the solutions to a depth of c. 3 cm. To determine the height the dye reached in a given time, we examined transverse sections of the stem at increasing distances from the apex by eye and/or with a microscope and epifluorescence device (Axiophot, Zeiss, Oberkochen, Germany). This method gives the ‘peak value’ of the flow velocity averaged over the conducting elements which contribute predominantly to water rise. As flow velocity can only be determined in cut branches, each data point needs a new branch of similar size and morphology.

To exclude dye-related artefacts (Canny, 1990, 1993), we studied short- and long-term kinetics of water rise without staining. Water-filled xylem regions of cross-sections are visible under appropriate illumination because of a change in the refraction of light. This method (‘light refraction’ method) required as many branches as the ‘dye rise’ technique. Because of its greater accuracy, this approach was applied to upright, inverted and horizontal branches. The branches were placed into small containers filled with tap water up to a height of c. 3 cm. For measurements on horizontal or inverted branches, we used stoppered culture flasks with tubes to ambient air to avoid subatmospheric pressures.

Water rise was coupled with an easily and accurately detectable enlargement of the angle between the branch and the initials of the leafy rolls at the advancing water front (‘leaf recurving’), presumably as a result of swelling of the dry material in the presence of water. Because this took a few minutes, the method lost accuracy at rates $>3–5$ cm h$^{-1}$, for example, during the initial phase of water ascent and particularly when refilling of the xylem was performed under pressure (i.e. root pressure, external pressure and filling in the direction of gravity). However, the ‘leaf recurving’ technique allowed complete kinetics to be measured on the same cut branch (thus consuming less time and plant material) or in intact plants under field conditions. The ‘leaf recurving’ method yielded very precise values of those xylem elements in which flow assumed a maximum value.

**Water rise under pressure**

We studied water rise dynamics in cut branches under field and laboratory conditions at applied pressures of up to 100 kPa. A transparent silicone rubber tube of 3 mm inner diameter, filled with tap water, was sealed to the cut end of a branch and the external pressure was varied by changing the height of the water column in the erect tube or by applying air pressure via a membrane to the tube in a horizontal position. The rate of filling of the empty xylem was determined by the ‘light refraction’ and ‘leaf recurving’ methods.

To determine effects of root pressure, we sealed an empty tube pressure-tight to the stub (c. 4 mm in diameter) of a branch cut from an intact dry plant in the field. Then the plant was watered and the rate of filling the tube with exudate determined by measuring the rise height as a function of time. Simultaneously, water rise kinetics in nearby intact branches were recorded by the ‘leaf recurving’ method.

**Results**

We measured the acropetal water rise kinetics in upright, inverted and horizontal dry branches under various field and laboratory conditions. In upright branches water rise occurred against, and in inverted branches in the direction of, gravity. The case of horizontal branches is equivalent to the refilling of the xylem conduit in a gravity-free environment.

Screening experiments with ink and fluorescein suggested that water rose much more rapidly in a few (directly neighbouring) xylem elements than in the other xylem region. Therefore we collected and analysed only the data of those conducting elements with the fastest axial advance of water.
Fig. 1. Acropetal water rise kinetics in the xylem (a) and water uptake kinetics (b) of cut (dry) 60- to 80-cm-tall leafy branches of *Myrothamnus flabellifolia* against gravity. Measurements were performed under laboratory conditions at an ambient temperature of 20–24°C. (a) ‘Leaf recurving’ (open squares), ‘light refraction’ (closed squares) and ‘ink rise’ (closed triangles) methods. Inset: comparison of water rise in leafy (open circles) and leafless (closed circles) branches (‘light refraction’ method). The data points represent mean values (± SD) calculated from at least three independent experiments. The solid line marks the best curve fit obtained for water rise by using Eqn 9. (b) Transpiration (curve a), weight increase (curve b), total water uptake (curve c) calculated from superposition of curves a and b, total water uptake measured by the potometer apparatus (curve d). The data show mean values of at least three independent experiments.

If not stated otherwise, 60–80-cm-tall cut leafy branches of similar growth geometry and with an intact apex were used. Screening experiments with cut branches of different morphology showed that it was particularly important to use branches without significant ramification in the lower part. Ramification resulted in a slower water rise in the main branch, with a simultaneously accelerated axial water movement in the side branch. Bifurcation of the stem into two branches of similar diameter significantly retarded the overall rise kinetics. These effects seriously complicated analysis of the data in terms of the theoretical framework given above. Therefore, such branches were discarded.

**Xylem water rise in upright branches**

When the cut ends of air-dry branches were dipped, water (and also dye) ascent was seen within 5 min in the bark up to a height of c. 15 cm. This ‘chromatographic effect’ was completely independent of the liquid (dye) rise in the xylem conduit presented in Fig. 1a, indicating that radical water uptake from the outside apparently did not occur. This was confirmed by experiments in which the outer bark was removed and a water droplet was placed carefully onto the outermost suberized tissue, remaining there for at least 30 min.

Fig 1a shows the average rise height of water in upright dry branches as a function of time. The measurements were performed in the laboratory at 20–24°C (20–50% rh, light intensity c. 6 μmol m<sup>–2</sup> s<sup>–1</sup>) by using the ‘dye rise’, the ‘light refraction’ and the ‘leaf recurving’ methods. Clearly, the ‘light refraction’ and ‘leaf recurving’ methods yielded similar rise kinetics and final rise heights. (The results of the ‘dye rise’ method are discussed later in this section.) As indicated by the standard deviation bars, the ‘leaf recurving’ method was usually much more precise than the ‘light refraction’ method, as
expected with nonuniform branch material. The standard deviation of the data did increase occasionally for heights \( > \approx 40 \text{ cm} \) where most branches exhibited more or less pronounced ramification which was associated with faster water rise in the (thinner) side branches than in the main branch.

Consistent with the findings of Gaff (1977) we found that water rise occurred most rapidly in the basal 10 cm of the plant (within \( \approx 30 \text{ min} \)) compared with the time taken for refilling the remaining upper part of the xylem. The final rise heights, which were reached after \( \approx 32 \text{ h} \), ranged between 40 and 55 cm (Fig. 1a) (i.e. the uppermost part of the xylem of cut branches was never refilled with water under these conditions).

**Xylem water rise in upright branches without an apex**

The interpretation of the data in Fig. 1a in terms of a capillary-driven water movement requires that branch apices do not offer high resistance to longitudinal water flow. A priori, an apex might be thought to block the efflux of the air (Child, 1960) displaced by the rising liquid in the xylem conduit. If so, a slight increase in pressure would retard (or, ultimately, prevent) movement of the liquid front. However, measurements performed on branches without apices yielded results similar to those on branches with apices (data not shown), ruling out (significant) effects of air resistance on water rise dynamics for upright branches.

**Water uptake and transpiration of upright branches during water ascent**

The basal leaves of the branches expanded and flourished \( \approx 3 \text{ h} \) after contact of the cut end with water. With time, recovery (i.e. rotation of the leaves from a vertical to a nearly horizontal plane, expansion and unfolding of the plicately folded leaves and resumption of a healthy green colour) progressed from the branch base to the apex. Transpiration started after \( \approx 4 \text{ h} \), increasing continuously with time (curve a in Fig. 1b). Measurements of the branch weight showed an almost linear increase with time up to 5–6 h (curve b in Fig. 1b). With the onset of transpiration, the weight increase slowed down and reached a plateau value after \( \approx 32 \text{ h} \). At this time, leaves below a height of 40–50 cm had expanded. Superposition of curves a and b yields the total water uptake by the branches (curve c) which is almost linear with time. This could be confirmed by measuring the total water uptake by means of the potometer apparatus (curve d).

Might the water needed for leaf expansion and transpiration affect the water rise, especially the final rise height of refilling (Fig. 1a)? Measurements of water ascent in the xylem of branches after complete removal of the leaves (Fig. 1a; inset) or in leafy branches at \( 100\% \text{ rh} \) (data not shown) yielded similar kinetics and final rise heights to those of leafy branches. Covering leafless branches with silicone rubber did not alter the water rise kinetics significantly, thus excluding possible artefacts due to evaporation through lesions induced by leaf removal. These findings indicate that the development of tensions due to leaf expansion and to transpirational water loss from the green leaves does not interfere with the rise of the water column in the xylem conduit and, therefore, does not need to be included in the theory of capillary-driven transport.

**Dye rise in the xylem of upright branches**

Despite some very large scatter of the data, the initial average acropetal rise rates measured using ink, fluorescein and other dyes were comparable with those measured by the other two methods (data not shown). However, significant differences in the kinetics of water and dye ascent occurred after 3–4 h of contact of the cut end of the dry branches with the solutions. Fluorescein, toluidine blue O and aniline blue closely mimicked water flow in the xylem elements and did not prevent recovery of the leaves (data not shown). However, they were not perfect markers for measurements over long rise periods, presumably because of dye adsorption to the walls and/or effects of osmosis and viscosity.

By contrast with these dyes, the forward movement of ink (frequently used by plant physiologists in field studies) almost ceased after \( \approx 3 \text{ h} \) (Fig. 1a). Correspondingly, the final height risen by the ink solution was a factor of 2–6 less (8–20 cm) than the maximum by tap water. This was also found under field conditions (data not shown). In addition, recovery of the leaves was not observed when ink was used as a marker for water ascent.

The apparent inadequacy of ink as a tracer for long-term studies of water rise could be due to viscosity effects, to the presence of cationic detergents in the ink (Adamson & Gast, 1997) and/or to the osmolality (\( c. 290 \text{ mOsmol} \); measured cryoscopically), which is much higher than that of the other dye solutions. The measured dynamic viscosity of the ink solution was comparable with that of pure water. However, the dye concentration might increase owing to radial water extraction from the xylem conduit (Canny, 1990, 1993). Ink enrichment is certainly associated with an increase in viscosity. Given its colloidal nature, there might be a height at which the dye is trapped in the perforation plates and/or in the corners of the tracheids.

Interference by the detergents with the lipid film on the inner walls of the vessels and tracheids (Schneider et al., 1999; Wagner et al., 2000) was suggested by studies in which branches were dipped into water containing various cationic or anionic detergents (e.g. 1% SDS, CASS, CTAB+...
solutions). With these detergents, final rise heights were on average c. 10 cm less than those of tap water (data not shown). This reduction was significantly less than that observed with ink, indicating that the osmotic pressure of the latter was mainly (or at least equally) responsible for its poor tracing performance. This could be confirmed by experiments measuring rise kinetics of solutions of various osmotically active solutes and osmolality.

**Osmotic-pressure effects on the xylem water rise in upright branches**

The specific conductivity of ink was c. 3 mS cm⁻¹. Neglecting charges on the colloidal particles, this is equivalent to the specific conductivity of a solution containing 23 mM of KCl or NaCl which corresponds to c. 40 mOsmol. Thus, the ink solution must contain c. 250 mOsmol of a nonelectrolyte.

Addition of KCl to the water showed (Fig. 2) that increasing concentrations (> c. 35 mM KCl) reduced the final rise height. About 1 M KCl (1.8 Osmol) was needed to reduce it to 20–25 cm (Fig. 2). Concentrations of mannitol, trehalose or raffinose ranging from 20 to 200 mM also reduced the final rise height (data partly shown in Fig. 2). The efficiency of the sugars increased with their molecular weight. About 200 mM mannitol or 50 mM raffinose were needed to decrease the final rise height to c. 13 cm (Fig. 2). Concomitant with the reduction in axial water rise by the sugars (which apparently contrasts with theoretical expectations) we found that rehydration and recovery of the leaves were reduced or even completely prevented. However, when the remaining dry parts of the branches were cut and placed in tap water, the usual refilling kinetics (Fig. 1a) associated with leaf recovery were again observed.

Measurements of the dynamic viscosity of the electrolyte and sugar solutions compared with those of tap water gave no indications of significant differences. Even though effects of accumulation of sugars in the xylem associated with changes in the viscosity cannot be completely excluded in the case of highly concentrated sugar solutions, it seems more likely that the retarding effects of KCl and sugars are mainly of osmotic nature. This implies that the xylem conduit must represent a compartment surrounded longitudinally and radially by solute-reflecting barriers which allow the generation of an osmotic pressure. In this case, the reflection coefficients for the sugars and, particularly, for KCl, must be rather low.

**Xylem water rise in upright, peeled branches**

Information about the tissue regions involved in radial water loss was needed to interpret the effect of osmotically active solutes and the finding of similar water rise kinetics for leafy and leaf-depleted branches. The outer living tissues (i.e. the bark, phellem, cortex and phloem) were completely removed using a razor blade. The peeled branches were protected against water loss with a thick, water-impermeable film of silicone rubber, and enveloped in parafilm. Entirely peeled and sealed branches in
Individual branches. The solid lines mark the best curve
symbols), 'leaf recurving' method, complete kinetics of
the aqueous phase via a membrane: 100 kPa (closed
independent experiments. Application of air pressure to
$q$
At final rise height, the gravitational term,
upright branches
water in the xylem.
promotional effect on the longitudinal rise height of
water refilling of the outer living cells has a
evaporation.
This again rules out hampering of water rise by
branches peeled only up to a height of 20 cm,
the xylem was as rapid as in unpeeled branches, the total
water uptake of the peeled and sealed branches was
rather slow, reaching a plateau value only after 32 h
(Fig. 3b).
By contrast, the total water uptake was very rapid
when peeled branches without protection against
water loss were used (Fig. 3b), demonstrating the
evaporation-protective features of the outer living
tissue. Despite this, the water rise kinetics and the
water volume finally taken up by the branches
(determined by weighing) were very similar to those
recorded for peeled and sealed branches (Fig. 3a,b).
This again rules out hampering of water rise by
evaporation.
The results can be interpreted as evidence that
water refilling of the outer living cells has a
promotional effect on the longitudinal rise height of
water in the xylem.

**Hydrostatic-pressure effects on xylem water rise in
upright branches**

At final rise height, the gravitational term, $\rho g h$, balances the capillary forces and water loss by radial
extraction (Eqn 9). The final rise height of 40–55 cm
(Fig. 1a) corresponds to a pressure of 4.0–5.5 kPa.
External pressures of 4.5 kPa, 8 kPa and 100 kPa
were applied to the water phase under laboratory
conditions to facilitate water ascent. Fig. 4 shows
that increasing pressure accelerated the refilling of
the empty xylem. A height of 50 cm was reached
within c. 4 h at 8 kPa, and within 30 min at 100 kPa.
Water rose to the apex of 60- to 80-cm branches and
leaves were recovered when pressures of at least
8 kPa were applied.

Under field conditions, root pressure developed
c. 3.5–4.5 h after watering of the intact dry plants for
30 min. The delay might have been due to root cell
reactivation after dormancy and/or to rate-limiting
properties of the soil as suggested by Gaff (1977).
Water rose to the apex of 60- to 70-cm-high plants
(Fig. 5a).
As in laboratory experiments, we found no effects
of transpiration on water rise kinetics under field
conditions. In Fig. 5a, normal shrubs and shrubs
completely enveloped in plastic bags are compared.
Rise kinetics and rise height for open and wrapped
intact plants were similar to those in cut branches at
an applied external pressure of c. 13.5 kPa. The
initial root pressure must be significantly larger than
the value of 2.4 kPa given by Sherwin et al. (1998).
Consistent with this conclusion, rise heights of up to
1.3 m were found (Fig. 5b) when a tube of 3 mm
diameter (with a cross-sectional area roughly cor-
Temperature dependence of the xylem water rise in upright branches

Ambient temperature was quite variable in the field (variation of up to $c. 40^\circ C$ during the day) by contrast with the nearly constant temperature ($20$–$24^\circ C$) in the laboratory. There were axial temperature gradients of up to $20^\circ C$ from the root to the apex of the intact plants as the day progressed. Typical diurnal temperature gradients are shown in Fig. 6a. Gradients developed around 10:00 hours, when the granite/soil became exposed to bright sunshine and ambient temperature increased above $30^\circ C$. The temperature gradient was particularly large between the granite surface and a branch height of $c. 20$ cm. Gradients decreased in the early afternoon but could still be recorded towards late afternoon. In addition, average daytime temperature at a soil depth of 10 cm was $c. 15^\circ C$ less than at the surface (data not shown).

Quite apart from viscosity effects, temperature gradients can greatly affect water rise kinetics in a conduit lined with hydrophobic film (Wagner et al., 2000). Despite much effort, we could not model these complex temperature profiles with cut branches under laboratory conditions. Therefore, we studied water rise kinetics in cut branches only in response to an increase in ambient temperature or to different heating regimes.

An increase of ambient temperature to $40^\circ C$ increased the initial rise rate significantly but the final height was comparable with that at room temperature (Fig. 6b). This is expected from Eqn 9 as temperature affects only the viscosity of the aqueous phase (Wagner et al., 2000). Similar results were obtained with cut branches in the field at fairly constant ambient temperatures of $c. 40^\circ C$ (Fig. 6b).

Ambient temperatures never rose above $60$–$70^\circ C$ in the field (even at the base of the intact plants). We also studied xylem refilling at much higher (lethal) temperatures. In the laboratory, $3$ cm of the cut basal end of branches were submerged in hot water ($80$–$100^\circ C$) for up to $23$ h (Fig. 6b). This treatment did not affect the initial (relatively fast) rise kinetics, but greatly reduced the final rise height of the water, which did not exceed $c. 20$ cm (Fig. 6b). A large number of lipid bodies in the xylem (already described; Schneider et al., 1999) were seen on microscopic inspection of transverse sections stained with Sudan III. Staining of sections with fluorescein diacetate (Romeis, 1989) strongly suggested that the parenchyma cells did not survive the excessive heat treatment. Cell death (and absence of leaf recovery) was restricted to the part of the branch through which the hot water had risen. This could be shown by removing the water-exposed part of the branch and placing the remaining piece in water at $20^\circ C$. The water rise kinetics were similar to those in Fig. 1a and water rise was associated with recovery of the nearby leaves (data not shown).

Similar reductions of final rise height (but not of initial climbing rates) also occurred when dry branches were exposed to water at $20^\circ C$ for $6$ h and
and 1a show (in agreement with the theoretical surface tension, the viscosity and the radial water extraction rate (see also the Theoretical Consider-

The initial velocity was similar to that measured in upright, unpressurized branches because of the variability in the morphology of the branches already mentioned. However, water traveled with gravity much faster than against it or in its absence. The rate of xylem refilling was almost constant over the whole length of the branches and reached the apex of 80-cm-tall branches within 7 h. In agreement with this, the leaves along the entire length of the branches became green within 1 d.

**Theoretical evaluation of the experimental data**

A general problem with curve fitting is that the number of unknowns can be too large to allow unambiguous values to be assigned to any of them. The unknown quantities here are the rate-limiting radius of the conducting element in which water rise assumed a maximum value, the contact angle, the surface tension, the viscosity and the radial water extraction rate (see also the Theoretical Consider-

**Xylem water rise in horizontal branches**

Fig. 7a shows acropetal xylem refilling of leafy branches in the absence of gravity measured using the 'light refraction' and 'leaf recurving' methods, which yielded similar results. The data in Figs 7a and 1a show (in agreement with the theoretical

**Ambient-temperature effect on the acropetal water rise kinetics under laboratory conditions of Myrothamnus flabellifolia.** Symbols in (a): ambient temperature of 20–24°C (‘leaf recurving’ method, open squares; ‘light refraction’ method, closed squares); ambient temperature c. 40°C (‘leaf recurving’ method, open triangles). Symbols in (b): upright branches (open squares, against gravity, ‘leaf recurving’ method); horizontally oriented branches (open circles, in the absence of gravity, ‘leaf recurving’ method); inverted branches (closed squares, in the direction of gravity, ‘light refraction’ method). The solid lines mark the best curve fits using Eqns 8–10. For further explanation, see ‘Xylem water rise in horizontal branches’ and ‘Xylem water rise in inverted branches’ sections.

Materials and Methods section). The height of the water column in the upper reservoir was c. 20 cm. The initial rate decreased only slightly when the height of the water in the reservoir was reduced to c. 2 cm, suggesting that the weight of the water column in the reservoir did not contribute significantly to the refilling of the xylem conduit, at least during the first hours. The flow kinetics exhibited a large scatter, especially above a height of c. 40 cm, presumably because of the variability in the morphology of the branches already mentioned. However, water traveled with gravity much faster than against it or in its absence. The rate of xylem refilling was almost constant over the whole length of the branches and reached the apex of 80-cm-tall branches within 7 h. In agreement with this, the leaves along the entire length of the branches became green within 1 d.

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**Xylem water rise in inverted branches**

In Fig. 7b the average acropetal rise heights as a function of time for inverted, upright and horizontal branches are compared. The data are of laboratory conditions (ambient temperature c. 20–24°C, 20–50% rh, light intensity 6 µmol m⁻² s⁻¹). For inverted branches, the ‘light refraction’ method was used. The ‘leaf recurving’ method could not be applied because of the high refilling rate (see the Materials and Methods section). The height of the water column in the upper reservoir was c. 20 cm. The initial rate decreased only slightly when the height of the water in the reservoir was reduced to c. 2 cm, suggesting that the weight of the water column in the reservoir did not contribute significantly to the refilling of the xylem conduit, at least during the first hours. The flow kinetics exhibited a large scatter, especially above a height of c. 40 cm, presumably because of the variability in the morphology of the branches already mentioned. However, water traveled with gravity much faster than against it or in its absence. The rate of xylem refilling was almost constant over the whole length of the branches and reached the apex of 80-cm-tall branches within 7 h. In agreement with this, the leaves along the entire length of the branches became green within 1 d.

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A general problem with curve fitting is that the number of unknowns can be too large to allow unambiguous values to be assigned to any of them. The unknown quantities here are the rate-limiting radius of the conducting element in which water rise assumed a maximum value, the contact angle, the surface tension, the viscosity and the radial water extraction rate (see also the Theoretical Consider-

**Xylem water rise in horizontal branches**

Fig. 7a shows acropetal xylem refilling of leafy branches in the absence of gravity measured using the 'light refraction' and 'leaf recurving' methods, which yielded similar results. The data in Figs 7a and 1a show (in agreement with the theoretical
Table 1. Values of the radial water extraction rate, $V_l$, the contact angle, $\Theta$, and the surface tension, $\sigma$, obtained from fitting the curves in Figs 1a, 2, 3a, 6b and 7 (see solid lines in the respective figures) by using Eqns 8 and 9 (rate-limiting radius: $r = 1 \mu m$).

<table>
<thead>
<tr>
<th>$\Delta P$</th>
<th>$T$ ($^\circ$C)</th>
<th>$V_l$ ($10^{-16}$ m$^2$ s$^{-1}$)</th>
<th>$\sigma \cos \Theta$</th>
<th>$\sigma$ ($10^{-2}$ N m$^{-1}$)</th>
<th>$\Theta$ ($^\circ$)</th>
<th>Fig.</th>
</tr>
</thead>
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<tr>
<td>Against gravity</td>
<td>21</td>
<td>0.95</td>
<td>0.0137</td>
<td>7</td>
<td>79</td>
<td>1a, 2, 3a, 7b</td>
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<tr>
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<td></td>
<td>24</td>
<td>0.8</td>
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<td>7</td>
<td>81</td>
<td>6b</td>
</tr>
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<td>0.96</td>
<td>0.0125</td>
<td>7</td>
<td>80</td>
<td>6b</td>
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<td>3</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Without gravity</td>
<td>21</td>
<td>0.7</td>
<td>0.0175</td>
<td>7</td>
<td>76</td>
<td>7a, 7b</td>
</tr>
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<td>55</td>
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</tr>
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<td></td>
<td>40</td>
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<td>0.0264</td>
<td>7</td>
<td>68</td>
<td>7a</td>
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<td>3</td>
<td>28</td>
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</table>

Values of $\Theta$ were calculated for different surface tensions in order to take into account a possible reduction of $\sigma$ by dissolved lipids during refilling. See text for further details.

Table 2. Values of the rate-limiting radius, $r$, the radial water extraction rate, $V_l$, the contact angle, $\Theta$, and the surface tension, $\sigma$, obtained from fitting the water rise kinetics under pressure and in inverted branches (solid lines in Figs 4 and 7b) by using Eqns 9 and 10 (temperature: 21$^\circ$C).

<table>
<thead>
<tr>
<th>$\Delta P$</th>
<th>$r$ ($10^{-8}$ m)</th>
<th>$V_l$ ($10^{-16}$ m$^2$ s$^{-1}$)</th>
<th>$\sigma \cos \Theta$</th>
<th>$\sigma$ ($10^{-2}$ N m$^{-1}$)</th>
<th>$\Theta$ ($^\circ$)</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>With gravity</td>
<td>1.0</td>
<td>0–3.8</td>
<td>0.0375</td>
<td>7</td>
<td>57</td>
<td>7b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Against gravity</td>
<td>1.0</td>
<td>1.1</td>
<td>0.033</td>
<td>7</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>$P_{ext} = 4.5$ kPa</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Against gravity</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0468</td>
<td>7</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>$P_{ext} = 8$ kPa</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>20</td>
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<td></td>
<td>4.0</td>
<td>0–2.0</td>
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<tr>
<td>Against gravity</td>
<td>1.9</td>
<td>0–2.3</td>
<td>0.0696</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>$P_{ext} = 100$ kPa</td>
<td>2.8</td>
<td>0–5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values of $\Theta$ were calculated for different surface tensions in order to take into account a possible reduction of $\sigma$ by dissolved lipids during refilling. Note that in the cases of pressure application several fits were possible, yielding different values for the rate-limiting radius of acropetal water rise. See text for further details.

To reduce the number of unknowns, we assumed usual values for density and dynamic viscosity of water at 20$^\circ$C and 40$^\circ$C ($\rho_{20} = 998$ kg m$^{-3}$, $\eta_{20} = 1.002 \times 10^{-3}$ kg m$^{-1}$ s$^{-1}$ and $\rho_{40} = 992$ kg m$^{-3}$, $\eta_{40} = 0.653 \times 10^{-3}$ kg m$^{-1}$ s$^{-1}$, respectively).

The equations for leaky capillaries (Eqns 9 and 8) were necessary to obtain satisfactory fits for water rise against and in the absence of gravity (Table 1). As expected from theory, the assumption of radial water loss from the xylem significantly affected the final rise height but not the initial water rise kinetics (up to $c. 10$ cm height). However, rise kinetics measured under pressure (i.e. in the direction of gravity, under root pressure and pressurization of the water bath) could be fitted quite accurately using the equations for either impervious or leaky capillaries, provided that the driving force, $\Delta P$, in Eqns 9 and 10, was changed accordingly (Table 2). This is not surprising, since radial water loss is a much smaller fraction of the total flow under these conditions.

For water rise under its own capillary pressure, there was good agreement between theory and experiment only when the first 10 min of water rise (i.e. up to heights of 4–6 cm) were neglected and the radius of the capillary was set to very small values compared with the average radius of the large vessels (18 $\mu$m; Carlquist, 1976). Fitting of the very accurate ‘leaf recurving’ data in Fig. 1a yielded a radius of 1 $\mu$m and a radial water extraction rate of $c. 1 \times 10^{-16}$ m$^2$ s$^{-1}$ (Table 1). Selection of other values for the radius resulted in poorer fits. Thus, this radius was used for
Refilling mechanisms of Myrothamnus flabellifolia

Table 3. Values of the radial water extraction rate, \( V_r \), the contact angle, \( \Theta \), and the surface tension, \( \sigma \), obtained from fitting the water rise kinetics in osmotically treated, peeled and heated branches (solid lines in Figs 2, 3a and 6b) by using Eqn 9 (rate-limiting radius: \( r = 1 \, \mu m \); ambient temperature: 21°C)

<table>
<thead>
<tr>
<th>( \Delta P )</th>
<th>( V_r \left( 10^{-14} , m^2 \cdot s^{-1} \right) )</th>
<th>( \sigma \cos \Theta )</th>
<th>( \sigma \left( 10^{-2} , N \cdot m^{-1} \right) )</th>
<th>( \Theta \left( ^\circ \right) )</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Against gravity</td>
<td>0–0.02</td>
<td>0.0011</td>
<td>7</td>
<td>89</td>
<td>2</td>
</tr>
<tr>
<td>200 mM mannitol</td>
<td></td>
<td>5</td>
<td>89</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>3</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Against gravity</td>
<td>0–0.05</td>
<td>0.0013</td>
<td>7</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>1M KCl</td>
<td></td>
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<td>2</td>
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<td></td>
<td></td>
<td>3</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Against gravity</td>
<td>0.34</td>
<td>0.0029</td>
<td>7</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>100 mM KCl</td>
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<td>5</td>
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<td></td>
<td></td>
<td>3</td>
<td>84</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Against gravity</td>
<td>0–0.01</td>
<td>0.0018</td>
<td>7</td>
<td>89</td>
<td></td>
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<tr>
<td>peeled</td>
<td></td>
<td>5</td>
<td>88</td>
<td>3a</td>
<td></td>
</tr>
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<td>3</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Against gravity</td>
<td>0–0.2</td>
<td>0.0018</td>
<td>7</td>
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<tr>
<td>80–100°C</td>
<td></td>
<td>5</td>
<td>88</td>
<td>6b</td>
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<tr>
<td></td>
<td></td>
<td>3</td>
<td>87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values of \( \Theta \) were calculated for different surface tensions in order to take into account a possible reduction of \( \sigma \) by dissolved lipids during refilling. Note that the accuracy of the fits was much less than for the curves for extraction of the values shown in Tables 1 and 2. See text for further details.

fitting of the other curves measured under various environmental conditions (Figs 2, 3a, 6b, 7). However, notable exceptions were found for water rise under external pressure, particularly when the pressure exceeded 8 kPa. In these cases, very good fits were also obtained when a radius of 1.7–4.4 \( \mu m \) was assumed and, at least in some cases, the capillary force was neglected (Table 2).

The values for the radial extraction rate, \( V_r \), and for the term \( \sigma \cos \Theta \) extracted from the fits are also listed in Tables 1 and 2. Comparison of Table 1 with Table 2 shows that \( V_r \) was quite constant when water rise occurred under its own capillary pressure, but varied considerably when refilling occurred under pressure (gravity, root pressure and external pressure). The values of the contact angle, \( \Theta \), depended on the assumption made for the surface tension, \( \sigma \), and vice versa (see the Theoretical Considerations section). In Tables 1 and 2 the calculated contact angles are given for \( \sigma = 7 \times 10^{-2} \, N \cdot m^{-1} \) (corresponding to the surface tension of pure water to air), for \( \sigma = 5 \times 10^{-2} \, N \cdot m^{-1} \) (corresponding to an oil–water interface; Ohki, 1988) and for \( \sigma = 3 \times 10^{-2} \, N \cdot m^{-1} \) (simulating the case of lipids dissolved in water; Johansson et al., 1994). Inspection of the data in Tables 1 and 2 allows two complementary conclusions: either the surface tension is very low or the contact angle is extremely high; and increase in the driving force reduces the contact angle.

Fitting of the water rise kinetics of osmotically treated, heated or peeled branches was not very accurate because of the large scatter of the data and the low final rise heights (c. 20 cm). Reasonable fits could be obtained when \( V_r \) was set to nearly zero or to very low values (Table 3). In the case of the 100-mM-KCl kinetics only, the best fit was obtained by assuming \( V_r = 3–7 \times 10^{-17} \, m^2 \cdot s^{-1} \), indicating still a significant radial water extraction. The analysis showed further (Table 3) that the contact angles were extremely high (80–89°) for the manipulated branches and independent of the selected surface tension value.

**DISCUSSION**

The data presented here allow some conclusions about the mechanisms of xylem refilling in *Myrothamnus flabellifolia*.

The refilling of the xylem conduit can be described satisfactorily and uniformly by the leaky capillary equations although these are only very rough first-order approximations. However, the analysis demonstrates that under its own capillary pressure alone water rises in the empty xylem too slowly to enable the survival of this resurrection plant in its dry environment.

**Root pressure drives xylem refilling**

The climbing rate and the final rise heights could be increased considerably in cut branches when external pressures of \( \geq 8 \, kPa \) were applied or when the refilling of the xylem was performed in the direction of gravity. Field measurements of rooted plants showed that *M. flabellifolia* apparently uses hydrostatic-pressure-driven flow for xylem refilling.
(Table 2). By contrast with Sherwin et al. (1998), we found that root pressure initially developed after water supply was high enough to drive water in the empty xylem to a height of 1.3 m within c. 10 h. This means that the xylem and the cells of the tallest plants (c. 80–90 cm) could be rehydrated in a relatively short time under field conditions.

The reasons for the low root pressure values measured by Sherwin et al. (1998) are not completely understood, but might be related to their use of potted plants. In our own preliminary experiments with glasshouse-grown potted plants, exudate rise heights were always very low (20–40 cm).

Why do capillary forces fail?

Refilling of the xylem with water under its own capillary pressure alone apparently plays a less important role in intact plants. The main causes of this are the small, rate-limiting effective radii of the conducting elements for axial water ascent and the very poor wetting of the dry walls.

Fitting of the respective curves by using the equations derived for leaky capillaries showed that the data could only be approximated when the value of the capillary radius for axial water ascent was set to c. 1 μm, above the critical radius of 0.3 μm, below which the Washburn equation fails (see the ‘Theoretical Considerations’ section), but much smaller than the average radius of the vessels (10–18 μm) and also smaller than that of the tracheids (c. 3 μm). Thus, water rise under its own capillary pressure is much slower than expected, considering the vessel and tracheid radii, but the final heights of the water columns in both conducting elements should be identical in the hydraulically connected water-conducting network.

A radius of c. 1 μm is not only compatible with the mean radius of the ‘pores’ of the perforation plates of the vessels, but also with the dimensions of the opening angle of the corners of the rhomboidal tracheids (Schneider et al., 1999; Wagner et al., 2000). As already mentioned, corners of rhomboidal capillaries considerably increase the rate of water spreading (i.e. more than predicted for cylindrical capillaries with uniform diameter (Finn, 1989)).

Thus, in the light of the analysis of the data presented here, the two pathways seem to be equally likely routes for refilling of the xylem.

The values extracted for the contact angle by curve fitting are quite large, even assuming a considerable reduction of surface tension at the liquid–vapour interface (Table 1). The contact angle was apparently decreased by an increase in the driving force (i.e. hydrostatic pressure) (Table 2) but remained extremely high when either osmotically active solutes were added to the aqueous phase, or the outer living tissue was removed or the cells were killed by heat treatment (Table 3).

Although several surface properties can affect the contact angle (see the ‘Theoretical Considerations’ section), we assume that the high values result from the hydrophobic properties of the inner walls of the xylem conduit (Schneider et al., 1999; Wagner et al., 2000). Hydrophobic impregnation and covering of the walls considerably limit water rise under its own capillary pressure in comparison with hydrophilic inner capillary walls.

If the lipid film on the inner xylem walls disintegrates (with consequent formation of lipid bodies) as water fills the vessels and the tracheids of dry branches, the contact angle will dynamically change as water penetrates the lipid film and subsequently wets the inner walls. Such changes in the contact angle are described by the Cassie equation (Eqn 11), but incompletely because the ratio of the wettable to the nonwettable surface increases as water rises in the xylem. This infers that the contact angle cannot be constant during water ascent as assumed in the derivation of the equations for the water rise kinetics (i.e. \( \cos \Theta = f(x,t) \)).

We have to conclude that the contact angle derived from curve fittings is a time-averaged value for the entire wetting process of those xylem elements with the greatest water advance rate. The contact angle should decrease with an increase in the rate of water rise (i.e. when additional pressure forcibly ruptures the film or (to a lesser extent) when capillary pressure-driven water movement occurs in the absence of gravity). This agrees with our results (Tables 1 and 2) and is also supported by the bulk of published experimental and theoretical evidence (Huppert, 1982; Melo et al., 1989; Troian et al., 1989, 1990; Cazabat et al., 1990, 1992). These authors have shown that in forced spreading the liquid front undergoes a fingering instability. It is quite likely that such effects will accelerate the wetting process, the disintegration of the lipid film and the subsequent formation of lipid bodies in the branches of *M. flabellifolia*.

Lipid removal changes the surface tension of water. We found that the initial water rise phase in upright and horizontal branches was faster than predicted by the equations (4–6 cm in 10 min). This can be easily explained if the disintegration of the lipid film and formation of floating lipid bodies takes a considerable time. Water penetration in the basal 6 cm of the plant would be driven by a capillary pressure corresponding to that of pure water in a capillary with a radius of 1 μm (i.e. c. 12 kPa for \( \Theta = 85 \)). As removal of lipids reduces the surface tension, axial water flow becomes ‘sluggish’. This is consistent with the lack of effect of heat treatments, peeling or osmotically active solutes on the initial refilling rate. It is likely that further acropetal water rise towards the apex occurs with reduced surface tension and that the values of the contact angles in Table 1 calculated for surface tensions of 3–5 x
10^{-2} \text{ N m}^{-1} are more realistic (further evidence: Wagner et al., 2000).

Why are the reflection coefficients so small?

With lipid film on the wall, and air above, the conducting elements of the xylem should be, at least temporarily, a compartment with very efficient solute-reflecting barriers. Experimentally, the reflection coefficients of the conducting elements for sugars and KCl are larger than zero, but substantially lower than those of cell membranes. The reflection coefficients of cell membranes for sugars are usually unity (Zimmermann, 1989). It is likely that the cell membranes of *M. flabellifolia* also have high reflection coefficients for these solutes, because leaves did not rehydrate when refilling was performed with sugar solutions of appropriate concentrations. The small reflection coefficients of the conducting elements could arise from an incomplete lipid film. However, it seems more likely that the low values result from disintegration of the lipid film and the concomitant wetting process upon water contact. This means that the ‘true’ reflection coefficients cannot be measured and experimental values, including changes of surface properties at the advancing water front, are ‘time-averaged’.

The most surprising result of this study was that suppression of radial water flow to the leaves (by osmotically active solutes or heat treatment) did not accelerate axial movement as predicted (Wagner et al., 2000). This forces a detailed discussion of the concept of radial water extraction on the basis of the kinetics analysis.

How is axial water ascent affected by radial spreading of water?

We must carefully distinguish between radial water loss from the conducting element and subsequent spreading of water across the tissue. The water extraction rate, \( V_i \), was introduced to account for radial water movement from those conducting elements with maximum water ascent. The fitting procedure showed that \( V_i \) could be varied only within quite narrow limits. If we interpret \( V_i \) in terms of Eqn 7, the radial velocity, \( \dot{V}_r \), assumes a value of \( c. 10^{-12} \text{ m s}^{-1} \) and \( 2 \times 10^{-11} \text{ m s}^{-1} \) for radii of 18 \( \mu \text{m} \) and 1 \( \mu \text{m} \), respectively. These values are extraordinarily low and lead to completely unreasonable values for the time of cross-section refilling. In view of the very low magnitude of \( V_i \) we have to conclude that the radial water extraction rate on the level of the single conducting element is ‘amplified’ considerably during the refilling process, resulting in a radial spreading rate compatible with the experimental observations of leaf recurving and recovery. This ‘amplification process’ demanded by the kinetics analysis is explored in Wagner et al. (2000). However, conclusions about the nature of the corresponding radial ‘tissue driving force’, its origin and its interaction with axial water ascent in the conducting elements can be drawn from the experiments with leafless and peeled branches in the present study.

During water ascent increasing transpiration rates were measured after \( c. 4 \text{ h} \), associated with successive recovery of the leaves from the base towards the apex. Since the rise kinetics in the xylem conduit were comparable for leafy and leafless branches, it seems clear that the tensions developed by transpirational water loss were too small to contribute significantly to the radial ‘tissue driving force’.

The experiments with peeled branches gave clear-cut evidence that the phelloderm, the cortical cells and/or the phloem were needed for capillary-driven water rise up to the maximum height of 40–55 cm in upright cut branches. The contribution of these compartments to water rise could only arise from the turgor pressure generated by the osmotic pressure within the cells upon water uptake. Filling of the ray parenchyma cells that are located near the water-filled conducting elements must precede the refilling of the outer cells. In peeled branches, the turgor pressure increases relatively quickly in the ray parenchyma cells. When the equilibrium value is established, the osmotic radial driving force disappears. In unpeeled branches, the ray parenchyma cells become only partly turgid because the rising turgor in these cells immediately drives the water further into other ray cells, and finally into the dry phloem, cortical cells and phelloderm (by analogy with the double membrane model used for explanation of water flow through epithelia (Katchalsky & Curran, 1975; Zimmermann & Steudle, 1978)). When axial water rise continues, the cells below the advancing water front gradually become fully turgid, but those above remain dry and thus osmotically active. This is clear from the experiments with heat- and osmotically treated branches, which demonstrated that refilling of the tissue occurred mainly radially, not axially, through the tissue.

The beneficial effect of radial water spreading in the tissue on axial water rise can also explain why water rises much faster in the upper side branches than in the main branch. The conducting area of the side branches (diameter \( c. 1.5–2.5 \text{ mm} \)) was smaller by a factor of at least two than the conducting area of the main branch (diameter \( c. 2.5–4 \text{ mm} \)), but the layer of outer living cells was of similar thickness (\( c. 60–90 \mu \text{m} \)). Thus, the ratio of water-supplying conducting elements to water-consuming cells was apparently less for thinner than for thicker branches. Consequently, the hydrostatic driving force remained longer in operation, resulting in an acceleration of axial water ascent in the reduced conducting area.
is frequently exposed to Myrothamnus flabellifolia

How can temperature affect the water rise kinetics?

Myrothamnus flabellifolia is frequently exposed to relatively high ambient temperatures. The experiments on cut upright and horizontal branches (Figs 6b, 7a) showed that an increase in ambient temperature from 20°C to c. 40°C significantly increased the initial rise rate of water, but that the final heights of the water columns in the xylem were comparable with those measured at room temperature. These findings can be explained by Eqs 8 and 9, provided that the decrease of the viscosity with increasing temperature is taken into account. However, the occurrence of axial temperature gradients during the day can induce a force in addition to root pressure under natural conditions. Temperature gradients could facilitate water rise further even though it must be mentioned that rehydration mainly occurs after rainstorms towards the end of cloudy afternoons (i.e. at moderate temperatures).

As shown by Eqn 1, capillary-driven water rise in impervious vessels in the absence of gravity varies exactly with the square root of time. The data can be approximated qualitatively by a similar relationship. However, this time dependence is not unique to capillary forces. Such a functional relationship is always expected for the penetration of viscous liquids into a tube (under a constant force). Thus, a $\sqrt{t}$-dependence of the rise height also holds for the Marangoni effect, first described correctly by Thomson (1853). The effect refers to flow produced by variations in surface tension. Surface tension gradients cause a shear stress at an air–water or oil–water interface (in the direction of increasing surface tension) which induces motion in the interface and the adjoining liquid layer.

In M. flabellifolia, surface tension gradients along the lipid-coated xylem walls can be created by the large axial thermal gradients. During the day these were directed from the granite to the upper leaves of the plants, pulling the liquid towards the region of higher surface tension (i.e. towards the apex).

Hydrodynamic equations account well for the rate of ascent of wetting films driven by temperature-induced surface tension gradients. Rigorous mathematical treatments of Marangoni streaming exist (Langbein & Heide, 1986; Troian et al., 1989, 1990; Cazabat et al., 1992; Wilson, 1997). In practice, more complicated flows with re-circulation are observed (Zimmermann et al., 1993). Most importantly in the context of the phenomena discussed here, mixed effects of gravity or other forces and surface tension gradients very frequently cause fingering instabilities and convection rolls which drive the spreading fluid more quickly than capillary forces alone. Thus axial temperature gradients along intact branches might, like root pressure and radial water extraction, play an important role in the disintegration of the lipid film on the lumen walls of the xylem. This is discussed in more detail in Wagner et al. (2000).

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