Physical mechanisms of rehydration in Polypodium polypodioides, a resurrection plant

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Resurrection plants have an amazing ability to withstand water drought. Here we investigate experimentally the rapidity of such revivals using the resurrection fern (*Polypodium polypodioides*) as a model example. Upon drying, the leaves of the resurrection fern fold into a thin cylindrical shell, thus protecting the photosynthetic area from light. In the dry state the fern looks dead, but will quickly come back once exposed to water by unfolding the cylindrical shell into a nearly planar sheet. We investigate here the mass and radius of curvature of the cylindrical shell as a function of time after rehydration and develop a phenomenological model to describe the observed phenomena. In particular, we demonstrate that the mass of the rehydrating plant follows a simple kinetic relationship, whereas the unfolding is governed by a more complex nonlinear constitutive relationship between the water uptake and the induced strain.

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I. INTRODUCTION

Most plants cannot tolerate longer periods of water deficiency and therefore rely upon suitable habitats where water is supplied on a regular basis. However, there is a certain class of plants, so-called resurrection plants, which can withstand water drought for up to several years. These include many bryophytes (nonvascular plants—e.g., mosses), a few ferns, and even fewer flowering plants [1–7]. The study of resurrection plants is of considerable ecological importance, as it could help us increase the desiccation-tolerance of crops in dry parts of the world and also improve our understanding of plant growth and regulation. In the dry state the resurrection plant may appear dead, but will quickly resume normal photosynthetic activity after exposure to water. The reason for this interesting behavior has not yet been revealed, although some biochemical mechanisms have been proposed [1–7].

Many desiccation-tolerant plants possess mechanisms to prevent conformational changes of the macromolecules in the cell as water is removed. In particular, the resurrection plants accumulate sugar in order to protect the dehydrated cells, since sugar may help stabilize the membranes and proteins in the dry state, maintaining hydrogen bonds between macromolecules [2,4,8]. It is also important to prevent or repair oxidative damage due to the accumulation of free radicals. Free radicals are highly reactive and bind with other molecules, and may therefore disrupt normal cellular processes. Absorption of light is probably a major source of oxidative damage, and some resurrection plants are known to protect their active photosynthetic areas by curling or folding the leaves. The folding is often driven by competitive stress in several directions, following a process that in some ways resembles the crumpling of a piece of paper. One example of such a two-dimensional folding is that of *Craterostigma plantagineum*, which is a flowering plant that starts folding after about 18 days of drought [4]. In the final folded state the leaves have about 15% of their original area [4,9]. To our knowledge the underlying physical reason for such a folding has not been reported for any resurrection plants, despite the fact that this may be one of the keys to understand how such plants protect themselves from mechanical damage and inhibit photosynthetic activity. Another crucial biological question is how quickly desiccation-tolerant plants regain normal metabolism after rehydration, since this may be related to their survival under ambient conditions.

In order to answer some of these questions, we investigate the resurrection fern (*Polypodium polypodioides*), which is a fascinating epiphyte that can be found in, e.g., the southeastern USA and South America. The leaves of this plant perform a one-dimensional folding from a sheet into a thin cylindrical shell after dehydration and may therefore serve as a model system to understand why and how fast the plant unfolds.

II. EXPERIMENT

Figure 1 shows this fern in the dry (a) and wet (b) states. Each plant is typically 50–100 mm long and is connected to other plants by a rhizome (horizontal stem) creeping—e.g., in the cracks of the bark of live oak (*Quercus Virginiana*). We note that in Fig. 1(a) each leaf is folded into a thin cylindrical sheet, and only their underside can be spotted by eye. Also the stem is slightly folded in the dry state, but this will not be studied here. Once it is exposed to water, the leaf starts to unfold, and after a few hours the plant has resurrected [Fig. 1(b)]. A cross section of an unfolding leaf is shown in the schematical drawing of Fig. 2. Note that the width q, the radius of curvature R, the height h, and the average thickness w all change as the fern opens its shell. We here wish to monitor these quantities as a function of time t after exposure to water.

The resurrection ferns used here were collected on the campus of Florida State University (mostly located on fallen and broken branches of live oak). We selected fully grown specimens with no visible damage for further study. The stomata ("breathing holes") of the plant are located on the underside of the leaves and were observed with a Leica DMPL polarization microscope using an objective of numerical ap-

FIG. 1. (Color) The folded (a) and unfolded (b) states of resurrection fern growing on live oaks on the campus of Florida State University. In picture (a) one can also see spanish moss (*Tillandsia usneoides*) as silvery gray threadlike masses. Resurrection fern and spanish moss are often observed to grow in beautiful harmony on live oaks. The white scale bar is about 30 mm.

erture NA=0.5. Figure 3 shows pictures of the closed stoma in the dry state (a) as well as the open stoma in the wet state (b). As the pressure builds up in the two guard cells flanking each stoma, the thin outer walls bulge out and force the inner walls into a crescent shape. The inner wall of each guard cell

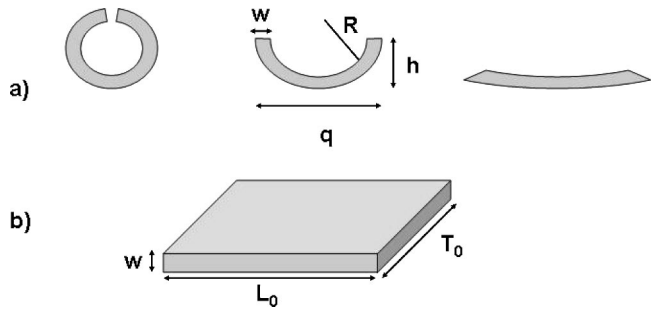

FIG. 2. Schematic drawing of a cross section of the leaf as it is unfolding after rehydration (a). Initially the upper side of the leaf containing the light harvesting complexes is folded to the inside, while the underside containing the the stomata folds to the outside. In the fully unfolded state (b) we have $q(t\to\infty)=L_0$, while the length of a piece of fern is T_0. In (b) the piece of fern is drawn as a flat rectangle, while in reality it is slightly curved.

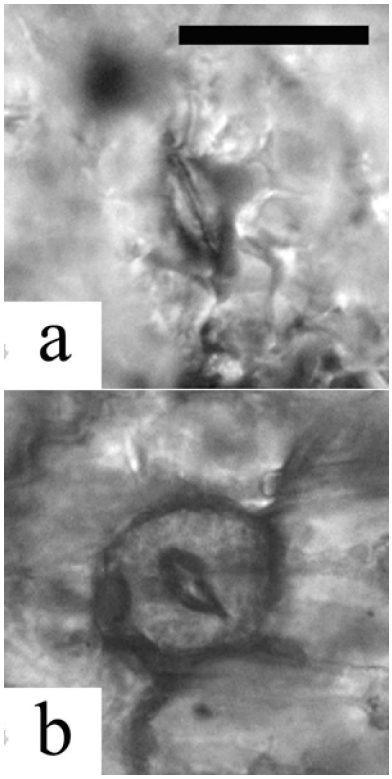

FIG. 3. Images of closed (a) and open (b) stomata in a resurrection fern. The scale bar is 40 μm.

is thick and elastic. Note the characteristic elliptical shape of the stoma (opening) formed by the two guard cells [10]. The long axis of the ellipse is $a\sim 10$ μm and does not change significantly as the guard cells grow. In order to probe the length b of the short axis as a function of time after rehydration, we deposited a droplet of water on the plant and monitored b with the microscope. We found that the short axis opens with an average rate of ~ 3 nm/s. It is nearly fully open after 20 min, thus typically resulting in $b\sim 3$–5 μm.

The ferns have a very porous cell structure that allow them to absorb 3–4 times their own weight in water. At the same time we observed that the volume of the fern grows substantially. This is also reflected in the area density of stomata, which decreases from typically 130 mm^{-2} in the dry state to about 70 mm^{-2} in the wet state.

In order to determine the mass of the fern as function of time, each plant was cut by a knife near the rhizome and then dried for about 2 weeks in an atmosphere of 60% relative humidity and temperature 20 °C . The mass was then determined with a Mettler-Toledo AB104-S balance. We simultaneously measured the height h and width q as function of time. Assuming that the radius of curvature of the fern is the same for any location on the leaf, we find

$$R(t) = \frac{q(t)^2 + 4h(t)^2}{8h(t)}. \tag{1}$$

Note that Eq. (1) is only an approximation here since the leaf is not perfectly cylindrical. We also emphasize that $q(t)$ can be measured to within an accuracy of 0.1 mm, whereas $R(t)$,

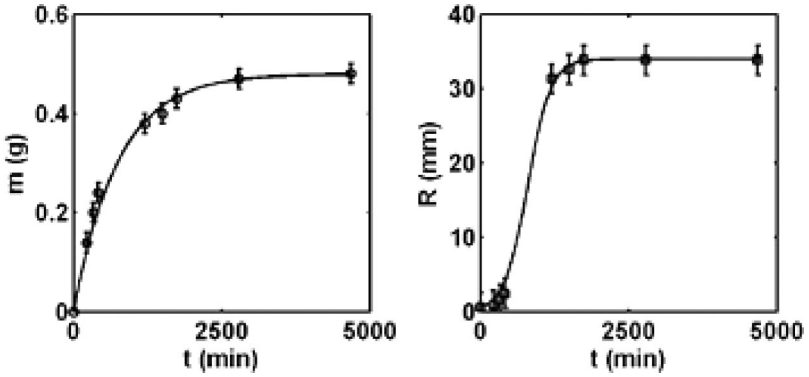

FIG. 4. The mass and radius of curvature of a whole fern. See the text for details.

which is a derived quantity, can only be measured with an accuracy of about 5 mm (due to the limited accuracy of h)

All further experiments were done in darkness in order to prevent, e.g., unwanted opening of stomata in presence of light. Figure 4(a) shows the mass $m(g)$ of the absorbed water and Fig. 4(b) the radius of curvature of the leaf of a fern. The leaf was placed in a clean petri dish, and ultrapure water was poured into it so that it almost covered the fern. Water exposure took place at $t=0$, and the leaf remained in contact with water during the experiments. We made sure that all parts of the fern were wet at all times, and water was refilled if necessary. Before hydration, the fern had a mass of 0.22 g, while after complete resurrection the mass was 0.70 g. Thus, the fern increased its mass by a factor of 3. The radius of curvature increased slowly in the beginning, until a solid increase started after about 7 h. We also note that this particular fern needed about 15 h to complete its resurrection. The characteristic time required to resurrect varied slightly from fern to fern, which will be discussed later in this study. However, we found that for one particular fern there is no change in the resurrection cycle over time periods of less than 3 months (approximately the time used to conduct these experiments). We verified this conclusion by putting the fern through 3–4 drying ($\sim$1 week) and wetting cycles, with the same result for $m(t)$ and $R(t)$ as seen in Fig. 4 (within the error margins). Repeating the wetting process while protecting the stem from water we found that the water uptake kinetics is unaltered to within the accuracy of our measurements, which suggests that the stem of the resurrection fern only plays a minor role in the revival. Moreover, we also measured $q(t)$ and $h(t)$ for ferns that were not separated from the rhizome by a knife (but otherwise subjected to the same conditions as above), without any change. This suggests that separating the fern from its rhizome by a knife does not alter its resurrection behavior over shorter periods of time (i.e., 2–3 months).

III. WATER UPTAKE

The uptake of water in the resurrection fern is a continuous process governed by three mechanisms. The first few minutes after the water hits the leaf surface are used to open the stomata. The cuticle—i.e., the waxy layer that covers the upper epidermis—keeps the water from entering any other way than through the stomata. As soon as a suitable opening is allowed, water starts flowing into the spongy layer of the leaf due to capillary pressure. See Fig. 5 for a schematic drawing of the interior structure of the cell. The spongy layer is very porous and can absorb a considerable amount of water by taking advantage of capillary pressure. Finally, the water is taken up by the cells in the spongy and palisade layers. The latter layer consist of a well-ordered array of nearly ellipsoidal cells, where most of the chlorophyll in the plant is located.

In order to understand the considerable water uptake of the resurrection fern, we require that the mass of water flowing into the fern must follow the law of mass conservation,

$$\frac{dm}{dt} = \phi A_0 \rho_0 v, \tag{2}$$

where ϕ is the fraction of air inside the leaf, A_0 is the surface area of the underside of the leaf, ρ_0 is the density of water in the fern, and v is the water flow velocity. Here ϕA_0 is assumed to remain constant. This is a reasonable assumption, due to the fact that before water is taken up by the cells in the spongy and palisade layers, there is no substantial increase in free area. After such an uptake, the cells increase by expanding outward and do not alter the inner available area significantly. We also assume that ρ_0 remains almost constant after the stomata have opened, while v is allowed to change. In a one-dimensional porous material we expect the flow velocity to follow Darcy's law (see, e.g., Ref. [11]),

$$v = -\frac{K_d}{\mu}\frac{dP}{dz}, \tag{3}$$

where K_d is the permeability of the porous medium, μ the viscosity of water, and P is the pressure. Moreover, it is clear

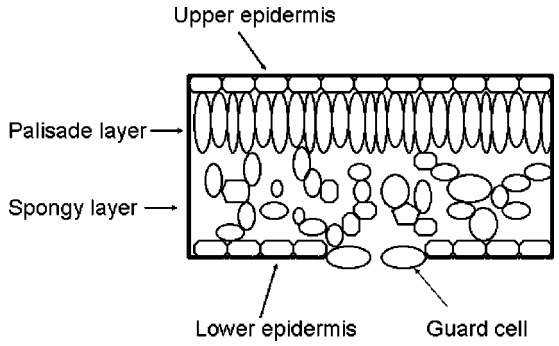

FIG. 5. Schematic drawing of a cross section of a leaf.

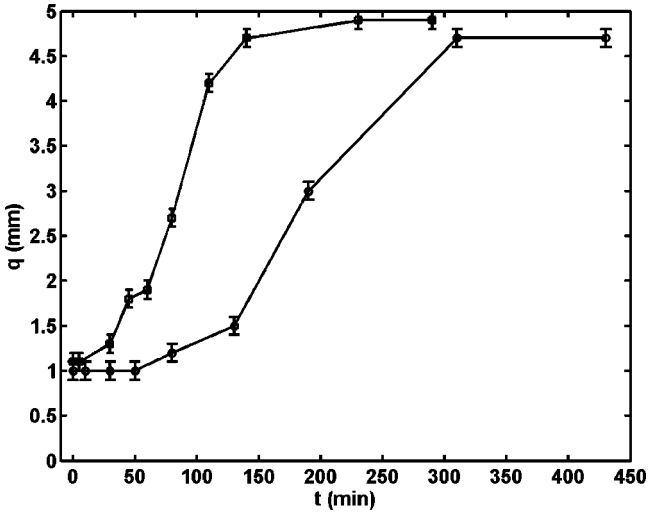

FIG. 6. The width as a function of time after rehydration for fern pieces of size $T_3=3.5$ mm (squares) and $T_6=9.0$ mm (circles). The two lines are guides for the eye.

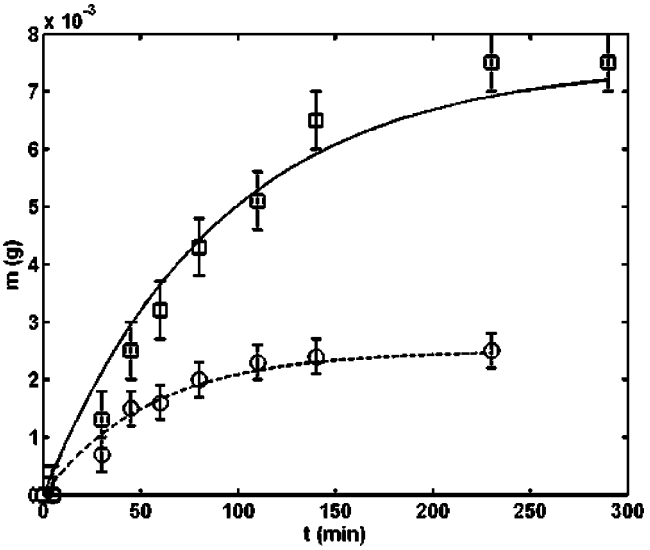

FIG. 7. The mass as a function of time after rehydration for fern pieces of size $T_1=1.4$ mm (circles) and $T_3=3.5$ mm (squares). The dashed and solid lines show the corresponding theoretical fit using Eq. (6).

that the pressure gradient decreases as the fern is filled up with water inside. Here we will assume a simple linear model with a driving pressure gradient in the z direction only, where

$$\frac{dP}{dz} = \frac{\gamma}{w_a}(m - m_\infty), \tag{4}$$

where γ is a constant and w_a is the average thickness of the fern. Then we have

$$\frac{dm}{dt} = -\frac{m - m_\infty}{\tau}, \quad \tau = \frac{w_a \mu}{\gamma K_d \phi A_0 \rho_0}. \tag{5}$$

Since $m(t \to \infty) = m_\infty$, we find

$$m(t) = m_\infty \left[1 - \exp\left(-\frac{t}{\tau} \right) \right]. \tag{6}$$

Equation (6) suggests that the time required to fill up the fern with water increases with the length of the path the water must flow as well as the viscosity of the water (or liquid), but decreases with increasing available area and permeability. We fitted Eq. (6) to the experimental data of Fig. 4(a) with $\tau=750$ min and $m_\infty=0.48$ g (which is the maximum amount of water the fern can take up). The fit is displayed as a solid line in Fig. 4(a) and suggests that our model is worth further study.

In order to understand the dependence of τ on size, we cut the leaves of a dry fern (dried for about 3 weeks) into six different sizes. In a single leaf two parallel cuts were made such that the water can access the open area of the two cut ends. The lengths T of the semicylindrical shells were $T_1=1.4$ mm, $T_2=1.7$ mm, $T_3=3.5$ mm, $T_4=4.1$ mm, $T_5=6.1$ mm, and $T_6=9.0$ mm. We then immersed these shells into water and monitored $m(t)$, $q(t)$, and $h(t)$. Figure 6 shows $q(t)$ for $T_3=3.5$ mm (squares) and $T_6=9.0$ mm (circles). Figure 7 shows $m(t)$ for $T_1=1.4$ mm (circles) and $T_3=3.5$ mm (squares), whereas the lines show the corresponding fits using Eq. (6) with $\tau_1=50$ min (dashed line) and $\tau_3=90$ min (solid line). It should be emphasized that water no longer flows only through the stomata, but is also allowed to flow through the cut areas. Thus, if the number of stomata is N, then the total area in which the water can enter is given by

$$A_0 = A_s + A_c \approx \frac{N\pi ab}{4} + 2w_a L_0. \tag{7}$$

For $T_6=9.0$ mm, we have $N \approx 4000$, and the stomatal area is found to be $A_s \approx 0.1$ mm^2. On the other hand, the cut area is $A_c \approx 0.7$ mm^2. Thus $A_s \ll A_c$, and it is clear that water flow through the cuts is important here. Since the water flow through the cut areas dominate over water entry through the surface of the leaf, the water uptake through stomata can be neglected and the effective width becomes $T/2$ for water flow through the area $w_a L_0$ of each cut end. This means that we have to substitute $w_a \to T/2$ in Eqs. (5) and (6). Figure 8 shows τ as function of T (squares) for the fern pieces discussed above, whereas the solid line is a corresponding fit using $k_m=22$ min/mm, where $\tau = Tk_m [k_m = \mu/(2\gamma K_d \phi A_0 \rho_0)]$. The graph suggests that our phenomenological model can describe the water uptake in the fern reasonably well.

IV. UNFOLDING

As noted from Fig. 4(b), the radius of curvature of the fern does not follow the same kinetics as the water mass. Instead, $R(t)$ is increasing slowly shortly after water exposure, then rapidly for intermediate times, before it finally saturates for larger times. In order to understand this phenomenon, we must remember that the palisade layer consists of a well-ordered array of cells. Upon drying, these cells shrink, thus exerting a compressive strain on the upper part of the leaf. The leaf length L decreases with a small amount ΔL, and the strain is found to be

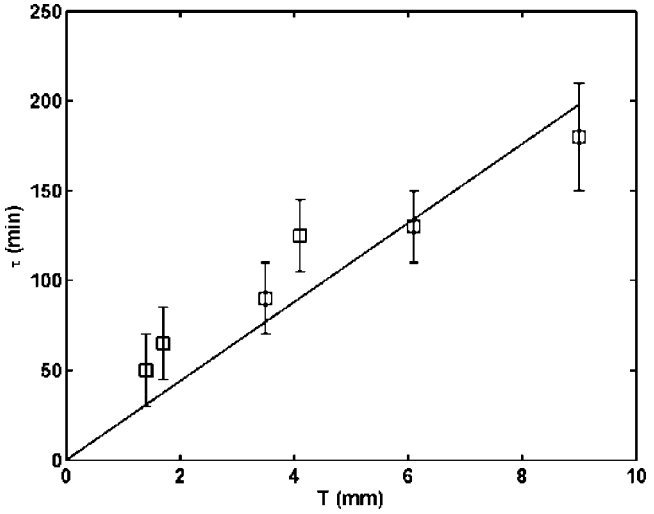

FIG. 8. The rate k_m as a function of leaf size. The solid line shows the corresponding theoretical fit $\tau = k_m T$ with $k_m = 22\ \mathrm{mm}^{-1}\ \mathrm{min}$.

$$\epsilon = -\frac{\Delta L}{L} = -\frac{w_{eff}}{R}. \tag{8}$$

The radius of curvature of the surface of the leaf segment is therefore given by

$$R(t) = \frac{w_{eff} L}{\Delta L}, \tag{9}$$

where w_{eff} is thickness of the layer involved in the folding (typically one may expect $w_{eff} \approx w/2$). Moreover, L is assumed to be constant since $\Delta L/L \ll 1$ during rehydration. It should be mentioned that the strain is most pronounced in the upper part of the leaf (near the palisade layer) and that the rest of the leaf does not contribute to the folding process. Moreover, we neglect the edge effects due to finite size, as well as any water-dependent changes in the elastic network structure.

The length change ΔL depends on the mass of the system. Moreover, we assume that ΔL only changes by a small fraction from its saturation value $\Delta L(t \to \infty) = \Delta L_\infty$, which allows us to expand ΔL in terms of the water mass—i.e.,

$$\Delta L = \Delta L_\infty + \alpha [m_\infty - m(t)]^\beta, \tag{10}$$

where α and β are constants. Upon using Eq. (6), we find

$$\Delta L = \Delta L_\infty + \alpha m_\infty \exp\left(-\frac{t}{\tau_R}\right), \quad \tau_R = \frac{\tau}{\beta}. \tag{11}$$

The radius of curvature can now be expressed as

$$R(t) = \frac{w_{eff} L}{\Delta L_\infty + \alpha m_\infty \exp\left(-\frac{t}{\tau_R}\right)}. \tag{12}$$

Here the boundary conditions require that $R(t \to \infty) = R_\infty = w_{eff} L / \Delta L_\infty$ and $R(t=0) = R_{min}$, such that

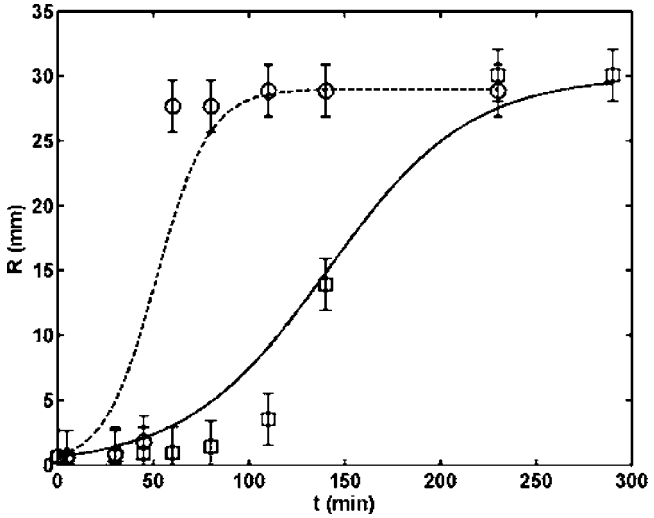

FIG. 9. The radius of curvature as a function of time after rehydration for fern pieces of size $T_1 = 1.4$ mm (circles) and $T_3 = 3.5$ mm (squares). The dashed and solid lines show the corresponding theoretical fit using Eq. (14).

$$\frac{R_\infty - R_{min}}{R_{min}} = \frac{\alpha m_\infty}{\Delta L_\infty}, \tag{13}$$

and Eq. (12) becomes

$$R(t) = \frac{R_\infty}{1 + \frac{R_\infty - R_{min}}{R_{min}} \exp\left(-\frac{t}{\tau_R}\right)}. \tag{14}$$

Interestingly, the behavior of $R(t)$ is similar to that of an autocatalytic chemical reaction, where the reaction rate at

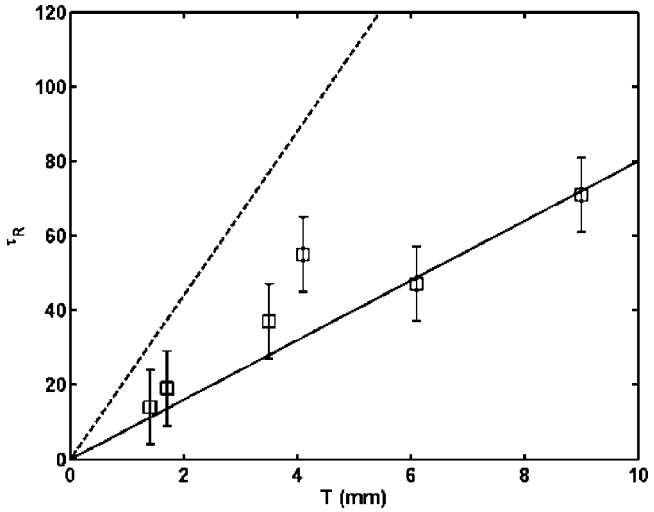

FIG. 10. The characteristic time τ_R as a function of leaf size. The solid line shows the corresponding theoretical fit using $\tau_R = k_R T$, where $k_R = 8\ \mathrm{mm}^{-1}\ \mathrm{min}$ ($\beta = 2.8$). The dashed line is the theoretical fit to the measured values of τ using $\tau = k_m T$ and $k_m = 22\ \mathrm{mm}^{-1}\ \mathrm{min}$, which would result from a linear relation between strain and mass ($\beta = 1$).

first increases slowly and is then followed by a steep increase before it finally goes to zero again.

We fitted Eq. (14) to the experimental data of Fig. 4(b) using $R_{min}=0.6$ mm, $R_\infty=34$ mm, and $\tau_R=196$ min. It is seen that our model reproduces the characteristic behavior of $R(t)$ and that $\beta\approx 3.8$. To find out whether this behavior is of a more general character, we also calculated $R(t)$ [using Eq. (14)] for the six samples discussed above. Once again we point out that $A_s \ll A_c$, so that water flow through the cut area is important, and we have to set $w_a \rightarrow T/2$ in Eq. (14). Figure 9 shows $R(t)$ for $T_1=1.4$ mm (circles) and $T_3=3.5$ mm (squares), whereas the lines show the corresponding fits using Eq. (14) with $\tau_R=14$ min (dashed line) and $\tau_R=37$ min (solid line). Figure 10 shows τ_R as function of T (squares) as well as a fit (solid line) using $\tau_R=\tau/\beta$ and $\beta=2.8$. Note that there is a good agreement between the model and our experimental data, which demonstrates that our model describes our samples reasonably well. Also for cut ferns of different sizes the constant $\beta\sim 3$, which suggests that the nonlinear behavior shown here is of a more general character, although the underlying reason is not yet known.

V. CONCLUSION

We have seen that water uptake in a resurrection plant is governed by the size of the plant and the area available for water flow. To this end, our experiments suggest that a small plant can resurrect faster than a big plant due to the fact that it takes longer time for the water to penetrate the porous network of a big plant. This observation can explain why smaller plants (e.g., bryophytes) can resurrect within an hour, whereas larger plants need up to several days [4–7]. Another interesting feature presented here is that the plant does not immediately react to the uptake of water. At first the water merely flows in to the elastic network of the fern (due to capillary action) without altering its elasticity significantly. However, after a while pressure starts building up in the cell network, thus changing the strain in the plant. When most of the network is filled up with water the fern finally stops unfolding. A prominent question then relates to the conductivity of water through the porous network. Upper bounds for the average conductivity in a homogeneous network were given by Durand and Weaire [12]. In a resurrection plant the porous structure expands and rearranges after wetting. It would therefore be interesting to obtain a fundamental understanding of liquid flow in a dynamic network.

Although our study has revealed some of the physical mechanisms involved in the resurrection of plants, several questions are left unanswered. For example, it would be interesting to understand in more detail the cellular mechanisms for water uptake and its correlation with the elastic properties of the cells. Moreover, the physical mechanisms that allow plants to survive severe water drought are not known yet.

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