PRESSURE PROBE STUDY OF THE WATER RELATIONS OF *PHYCOMYCES BLAKESLEEANUS* SPORANGIOPHORES

Daniel J. Cosgrove*, Joseph K. E. Ortega[‡], and W. Shropshire, Jr.[§]

*Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802;
[‡]Department of Mechanical Engineering, University of Colorado at Denver, Denver, Colorado 80202;
and [§]Smithsonian Environmental Research Center, Rockville, Maryland 20852

ABSTRACT The physical characteristics which govern the water relations of the giant-celled sporangiophore of *Phycomyces blakesleeanus* were measured with the pressure probe technique and with nanoliter osmometry. These properties are important because they govern water uptake associated with cell growth and because they may influence expansion of the sporangiophore wall. Turgor pressure ranged from 1.1 to 6.6 bars (mean = 4.1 bars), and was the same for stage I and stage IV sporangiophores. Sporangiophore osmotic pressure averaged 11.5 bars. From the difference between cell osmotic pressure and turgor pressure, the average water potential of the sporangiophore was calculated to be about −7.4 bars. When sporangiophores were submerged under water, turgor remained nearly constant. We propose that the low cell turgor pressure is due to solutes in the cell wall solution, i.e., between the cuticle and the plasma membrane. Membrane hydraulic conductivity averaged $4.6 \times 10^{-6} \mathrm{~cm~s^{-1}~bar^{-1}}$, and was significantly greater in stage I sporangiophores than in stage IV sporangiophores. Contrary to previous reports, the sporangiophore is separated from the supporting mycelium by septa which prevent bulk volume flow between the two regions.

The presence of a wall compartment between the cuticle and the plasma membrane results in anomalous osmosis during pressure clamp measurements. This behavior arises because of changes in solute concentration as water moves into or out of the wall compartment surrounding the sporangiophore. Theoretical analysis shows how the equations governing transient water flow are altered by the characteristics of the cell wall compartment.

INTRODUCTION

The sporangiophore of *Phycomyces blakesleeanus* is a single-celled aerial hypha which alters its growth rate and growth direction in response to light, gravity, wind, external barriers, and other stimuli (Bergman et al., 1969; Cohen et al., 1975). Because the sporangiophore is large (about 100 μm in diameter and many centimeters in length), grows rapidly (1–3 mm h^{-1}), and responds within minutes to a variety of stimuli, it has been favored as a model system for investigations of sensory transduction. Although much is known about its growth responses, the water relations of this unique cell still remain poorly characterized. For example, in the comprehensive review on *Phycomyces* by Bergman et al. (1969), no information is given about sporangiophore turgor pressure (P), osmotic pressure (π) or membrane hydraulic conductivity (Lp). Apparently the only attempt to measure sporangiophore turgor was by Roelofson (1950b) who used a pressure chamber technique to estimate turgor at about 2 bars. This dearth of basic information is remarkable because turgor pressure and water uptake are tied so closely to the growth

Address correspondence and offprint requests to Daniel J. Cosgrove, Department of Biology, Penn State University, University Park, PA 16802.

of these and other cells, and therefore to the output of sensory transduction (Cosgrove, 1986).

Water serves several important functions in the growth of this cell. Because water constitutes about 85% of the mass of the sporangiophore, growth depends critically on the ability of the cell to take up water. The chitinous wall, which physically constrains and shapes the cell, must irreversibly expand for growth to occur. In the stage IV sporangiophore, wall expansion is localized to a 2-mm region below the sporangium, and the rate of expansion is thought to depend in part on the mechanical stress borne by the wall. Since the magnitude of wall stress is a function of cell turgor pressure, values for cell turgor and for the factors which control turgor are needed for quantitative models of the mechanics of sporangiophore growth. Moreover, it has been argued that the avoidance and wind responses of *Phycomyces* are controlled by the concentration of water vapor surrounding the sporangiophore (Gyure et al., 1984).

Therefore, we carried out this study to measure some of the fundamental physical parameters that govern the water relations of *Phycomyces*, using the pressure probe technique (Cosgrove and Durachko, 1986; Cosgrove and Steudle, 1981; Hueskens et al., 1978). In this method, a cell is punctured with an oil-filled glass microcapillary which is connected to a pressure sensor and a moveable

plunger. With the appropriate manipulations, one can directly measure cell turgor pressure in individual cells, and what is more, one can induce volume flows across the cell membranes and thereby measure membrane hydraulic conductivity. In addition to providing needed data about *Phycomyces*, this study was of interest from a comparative standpoint. The cellular water relations of a number of algae and higher plants are well characterized (Dainty, 1976; Molz and Ferrier, 1982; Wendler and Zimmermann, 1985; Zimmerman and Steudle, 1978), but fungi remain relatively unstudied and uncharacterized. Our results show that the sporangiophore of *Phycomyces* has physical properties similar to those of other cells, but that it has a structural complexity which results in anomalous osmotic behaviors. We present a two-compartment model of the sporangiophore to explain these osmotic anomalies as due to dilution (or concentration) of solutes in the cell wall compartment which surrounds the aerial sporangiophore.

MATERIALS AND METHODS

Biological Material

Spores of wild-type (−) *Phycomyces blakesleeanus* were heat-shocked for 10 min at 45°C, inoculated on agar medium and grown as described by Dennison and Shropshire (1984). Cultures were grown either in glass vials (12 mm diameter) or Petri plates (9-cm diam) under cool-white fluorescent lights (10 μmol m^{-2} s^{-1}) at room temperature (20–24°C). In some cases cultures were inoculated in Denver, CO or in Rockville, MD and shipped by overnight mail to University Park. The medium for the Denver cultures consisted of 4% potatoe dextrose agar (Difco Laboratories, Inc., Detroit, MI) and 0.5% yeast extract, and differed slightly from the Rockville medium (Dennison and Shropshire, 1984). All cultures were enclosed in a plastic canopy to maintain high humidity. Straight, healthy-looking sporangiophores, typically 3–5 cm long, were selected from cultures 4–6 d old. Some experiments were carried out with an albino mutant, *car5*, grown as above (Dennison and Shropshire, 1984).

Pressure (P) Measurements

For some experiments, a small agar block (5–8 mm on a side) containing mycelia and one or more sporangiophores was carefully excised, separated from the culture plate and placed on a micromanipulator in front of a horizontal M5A stereomicroscope (Wild Heerbrugg Instruments Inc., Farmingdale, NY); in other cases, a vial with one or more sporangiophores extending 0.5–2 cm beyond the top of the vial was mounted on the micromanipulator. The region of the sporangiophore designated for pressure measurement was braced on one side with a stiff copper wire. A manual version of the pressure probe (Cosgrove and Steudle, 1981; Hueskens, Steudle, and Zimmermann, 1978) was mounted on a micromanipulator (E. Leitz, Inc., Rockleigh, NJ) which was used to guide the capillary tip of the pressure probe through the wall and into the vacuole of the sporangiophore (Fig. 1).

The pressure probe is like a miniature syringe, consisting of a glass oil-filled microcapillary, an adjustable rod which acts like a plunger, and a built-in pressure sensor. When the tip of the glass microcapillary penetrates the cell, the plasma membrane forms a tight seal around the capillary. The pressurized cell contents rapidly move into the capillary, forming a menicus at the boundary between the cell sap and the silicone oil (Fluid 200, 2 centistoke viscosity, Dow Corning Corp, Midland, MI). The meniscus is visible under the microscope, and its position must be carefully controlled. By advancing the adjustable rod, one can build up a back pressure in the probe and drive the oil/cell sap meniscus back to the tip of the capillary. When the meniscus is stationary, the pressures on either side of it are equal, and may be measured with the sensor installed in the body of the probe (for additional details, see Cosgrove and Durachko, 1986).

During our pressure probe measurements, we observed that the sporangiophores were growing, but we did not make quantitative measurements of their growth rate. All experiments were carried out under room lighting, with supplemental illumination from a fiber-optic illuminator. The illuminator filtered out nearly all of the infrared light and raised the temperature by less than 1°C, as measured with a thermocouple probe placed near the sporangiophore.

Pressure Relaxation Measurement

A small amount of silicone oil (about 0.2% of the sporangiophore volume) was rapidly injected into or removed from the sporangiophore, and the subsequent adjustment, or "relaxation", of pressure was monitored with
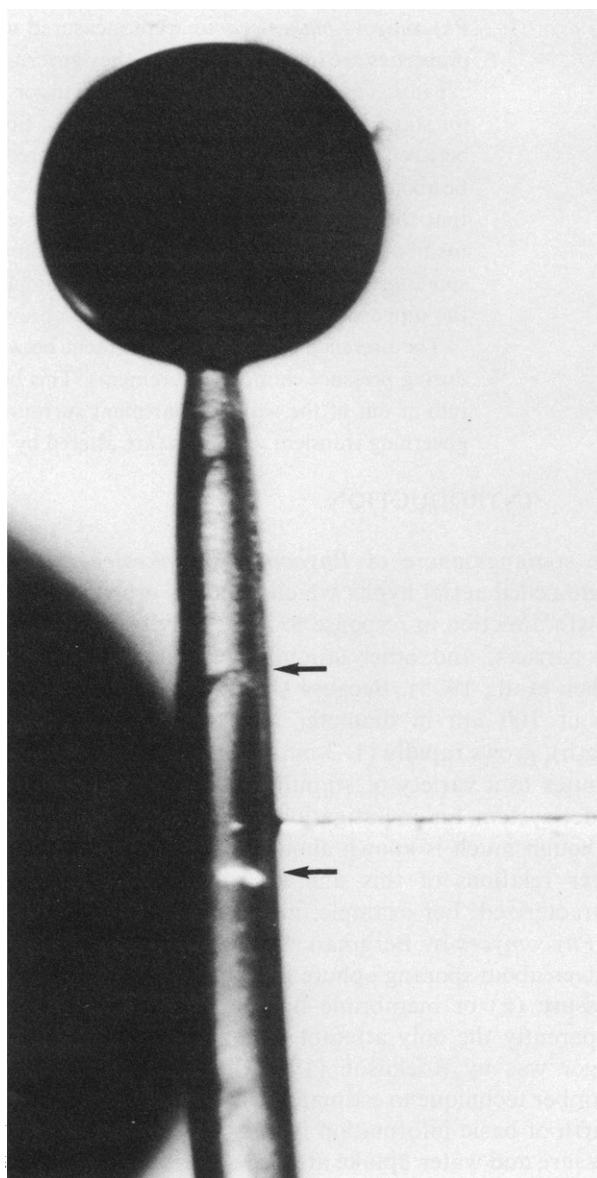

FIGURE 1   Photograph of stage IV sporangiophore, braced on the left side with a copper wire and impaled from the right with the glass capillary of the pressure probe. The vacuole is partially filled with silicone oil (boundaries marked with arrows).

the pressure probe. Ideally the time course is represented by an exponential decay to a new steady pressure (Dainty, 1976; Zimmerman and Steudle, 1978) with a halftime ($T'_{1/2}$) given by

$$T'_{1/2} = \frac{V \ln(2)}{A \, Lp \, (\epsilon + \pi)} \quad (1)$$

where V is cell volume, A is surface area, Lp is the membrane hydraulic conductivity, ϵ is the cell volumetric elastic modulus, and π is the osmotic pressure of the cell contents.

Pressure Clamp Measurement

We have modified the pressure clamp procedure developed by Wendler and Zimmerman (1982). We raised or lowered sporangiophore pressure to a new constant("clamped") value with the probe and measured the consequent flow of silicone oil into or out of the sporangiophore by visually following the movement of the oil boundary within the sporangiophore vacuole. After the start of a pressure clamp experiment, transport of water across the cell membranes is exactly balanced by the flow of oil through the capillary tip.

To begin a measurement, sporangiophore pressure was regulated to a constant value, then abruptly increased or decreased by about 1 bar and held constant. Movement of the oil boundary was monitored visually. To record the movement, an event marker was attached to a chart recorder and was triggered manually every time the boundary crossed one of the linear graduations of a reticle in the ocular of the microscope. From the chart record we calculated the velocity of movement of the oil boundary and, knowing the sporangiophore diameter, we could calculate the volume flow as a function of time from the start of pressure alteration. Ideally, the oil boundary moves quickly at first, but gradually slows, with the halftime ($T^c_{1/2}$) given by

$$T^c_{1/2} = \frac{V \ln(2)}{A \, Lp \, \pi} . \quad (2)$$

With the pressure clamp technique we estimated Lp without the necessity of knowing ϵ, using the relation

$$dV/dt = A \, Lp \, (\Delta\psi) \quad (3)$$

where dV/dt is the rate of volume flow, and $\Delta\psi$ is the water protential gradient across the cell membranes. At the start of the pressure clamp, $\Delta\psi$ equals the step change in pressure (ΔP) imposed on the sporangiophore and dV/dt is the initial rate of flow of oil into or out of the sporangiophore.

In most cases pressure was regulated manually by turning a micrometer screw to advance or retract the rod in the main body of the pressure probe. In a few experiments pressure was regulated by a computer-assisted version of the instrument. A microcomputer sensed the pressure via an analog-to-digital converter and regulated the pressure by controlling a digital stepper motor attached to the micrometer screw (for details see Cosgrove and Durachko, 1986). With the latter instrument it was possible to keep the pressure constant within ±0.02 bar.

The rate of water uptake because of sporangiophore growth was only ~0.02 that of the initial rate of water flux induced by our pressure clamp measurements, assuming a robust growth rate of 2 mm h^{-1}. Because the induced fluxes are comparatively large, water fluxes associated with growth were not considered in our calculations of membrane hydraulic conductivity.

Osmometry

To measure the osmolality of the sporangiophore contents, individual stage IV sporangiophores were decapitated and the cell sap was quickly collected using a fine glass microcapillary. For other experiments, droplets on the surface of intact sporangiophores were collected with the microcapillary. Samples of ~1 nl were loaded onto the temperature-controlled stage of a nanoliter osmometer (Clifton Technical Physics, Hartford, NY) and measured by the freezing point depression technique. Samples of the culture medium were measured with a model 5500 vapor pressure osmometer (Wescor, Inc., Logan, UT). Osmolality was converted to osmotic pressure using the formula $\pi = RTc$, where c is osmolality (in moles per kilogram) and RT has a value of 24.37 bar kg mol^{-1} at 25°C.

RESULTS

Pressure

Insertion of the microcapillary tip through the wall of the sporangiophore proved relatively straightforward, once the size of the capillary tip was chosen properly. Capillaries with very small tips became clogged, whereas large tips tended to cause cell leakage. We found that tips with outer diameters of 5–8 μm worked well. Once the tip penetrated the wall, generally into the vacuole, an exceptionally stable seal formed around the capillary.

We discovered that the fluid inside the sporangiophore was so viscous that the conventional pressure probe technique was not practical. Instead of regulating the position of a boundary between cell sap and the silicone oil *within* the capillary, as in the conventional method, we found it easier to regulate the size of an oil droplet injected into the vacuole of the sporangiophore (Fig. 1). This droplet typically displaced less than 1% of the volume of the cell and therefore did not represent a substantial osmotic disturbance. Except as noted below, we probed at the apical end of the sporangiophore, ~3 mm below the sporangium, i.e., at the base of the growing region.

The line in Fig. 2 labeled STEADY PRESSURE shows a trace from a pressure recording. The small, rapid changes in pressure are due to the physical manipulations involved in regulating the size of the oil drop. In measurements of 43 sporangiophores at stages I through IV, we found pressures ranging from 1.1 to 6.6 bars. As Table I shows, there was no significant difference between stage I
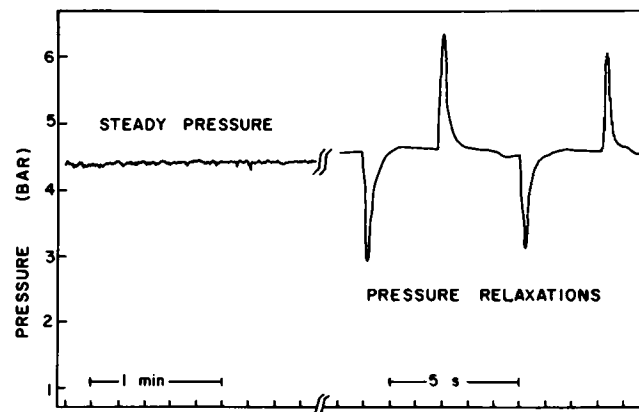

FIGURE 2 Recorder tracing from a turgor pressure measurement of a stage IV sporangiophore, using the pressure probe. At left is a tracing of pressure, at right volume was injected into or out of the sporangiophore. The time course of the pressure relaxation is determined in part by the membrane hydraulic conductivity.

Property	Material	Mean	SE	n	units
Lp	stage I	6.88	0.496	47	10^{-6} cm s^{-1} bar^{-1}
	stage IV	1.96	0.525	42	
	all	4.56			
$T'_{1/2}$	stage I	0.20	0.015	10	s
	stage IV	0.3	0.014	12	
P	MD*	3.83	0.163	33	bars
	CO‡	5.10	0.296	10	
	stage I	4.03	0.315	12	
	stage IV	4.21	0.203	29	
π_c	all	11.5	0.47	16	bars

*Cultures obtained from Rockville, MD (Dr. Shropshire).
‡Cultures obtained from Denver, CO (Dr. Ortega).

and stage IV sporangiophores, but the Denver cultures had a higher mean pressure than the Rockville cultures. This difference appeared to diminish after several days of culture in the lab at University Park. We have not established the reason for this difference, but it may be related to differences in culture medium, growing conditions, or treatment during shipment to the Penn State laboratory.

Pressure Relaxations

In these experiments, a small volume of silicone oil was rapidly injected into or withdrawn from the sporangiophore. The change in volume during a pressure relaxation measurement was judged from the position of the moveable oil/cell sap boundary.

Fig. 2 shows four pressure relaxations of a sporangiophore. In our experiments with 22 different sporangiophores, halftimes ranged from 0.15 to 0.39 s. Stage IV sporangiophores had significantly longer halftimes than stage I sporangiophores (see Table I). It is important to note that the mean halftime of stage I sporangiophores was 0.2 s, which was at the measuring limit of our pressure probe technique. This limit was determined by our ability to move and regulate the position of the oil/cell sap boundary. Hence, the mean halftimes shown in Table I probably overestimate the halftime of stage I sporangiophores, but are representative for stage IV sporangiophores.

Pressure Clamps

With the pressure probe we imposed a step change in pressure and measured the induced transport of water by monitoring the oil flow that was necessary to sustain the pressure at its new value. Fig. 3 shows the changes in pressure and oil volume during a typical pressure clamp measurement. Fig. 4 shows the distribution of Lp values calculated using Eq. 3. In 89 independent measurements made with 23 sporangiophores, Lp averaged 4.6×10^{-6} cm s^{-1} bar^{-1} and ranged from 0.78 to 20×10^{-6} cm s^{-1} bar^{-1}.
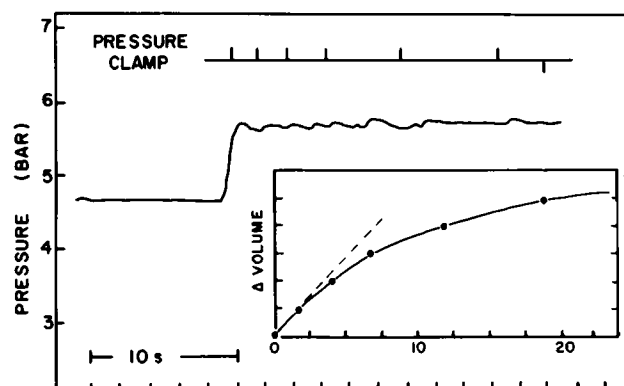

FIGURE 3 Pressure clamp experiment of a state IV sporangiophore. Turgor pressure was adjusted from about 4.7 bars to about 5.7 bars (middle trace) by injecting oil into the sporangiophore vacuole. Oil movement within the vacuole was observed visually, and each time the image of the moving oil boundary crossed a graduation of the ocular reticle, the pressure probe operation triggered an event marker. Top trace shows these tick marks. From these data and from the sporangiophore diameter, the change in volume was calculated (inset). The initial volume flux (broken line) was 4 ± 10^5 μm^3 s^{-1} and was used to calculate membrane hydraulic conductivity. Total oil influx at the end of the pressure clamp was about 0.72% of the sporangiophore volume.

Stage I sporangiophores had significantly greater values for Lp than did stage IV (Table I). This difference is consistent with the observed differences in halftimes for pressure relaxations. No difference in Lp between sporangiophores from Denver and Rockville cultures was found.

Basal Connection to the Mycelium

Gruen (1959) and others before him have reported that *Phycomyces* sporangiophores are not separated from the supporting mycelium by a septum. For our calculations of Lp, we assumed that water moved into or out of the sporangiophore by transport across the entire surface of the plasma membrane, and not via mass flow through a basal pore connecting the sporangiophore with the mycelium. Thus, we assumed that upon increase in cell pressure during a pressure clamp, the vast majority of the water would cross into the thin cell wall compartment which
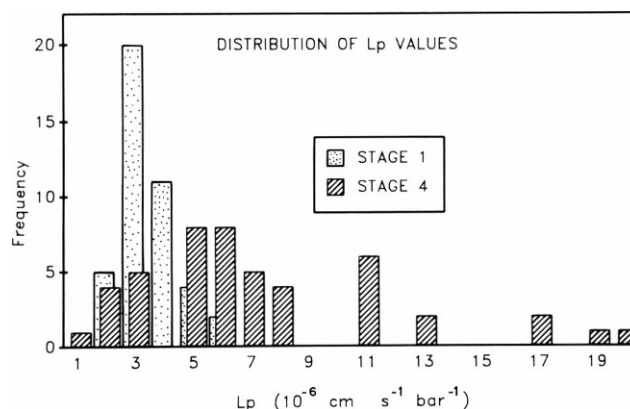

FIGURE 4 Disbribution of values of membrane hydraulic conductivity, calculated by pressure clamp measurements.

surrounds the sporangiophore. Therefore, it was important to ascertain the site of water transport in these experiments in order to validate the *Lp* calculations.

To determine the site of water transport into or out of the cell, we inserted the tip of the probe at various positions along the sporangiophore. We then carried out pressure clamp experiments and observed the oil movement as an indicator of the direction and velocity of volume flows. Because the base of the wild-type *Phycomyces* is heavily pigmented, we used the albino *car5* mutant for these experiments, to observe the oil movement more readily.

Our reasoning for these experiments was as follows. Consider a sporangiophore impaled at its base. If the main path for water flow is through a pore in the base of the sporangiophore, then the oil should move by bulk flow downward, out the base of the sporangiophore and into the mycelium. In contrast, if the base of the sporangiophore is sealed, then oil should move apically because the vast majority of the exchange surface, the plasma membrane, is apical to the oil. When we carried out this experiment with the capillary tip near the sporangiophore base, the oil moved apically. When the capillary tip was inserted in the middle of the sporangiophore, oil moved both upward and downward; and when the tip was inserted at the apical end of the sporangiophore, the oil moved mostly downward. From these observations we concluded that the basal end of the sporangiophore was effectively plugged and that the volume fluxes induced with the pressure probe occurred by water transport across the entire membrane surface of the sporangiophore.

We obtained additional evidence supporting this point from light microscopy of plucked sporangiophores. Under phase contrast illumination, fixed but unsectioned specimens showed well-defined septa separating the sporangiophore from the supporting hyphae (Fig. 5).

Osmotic Anomalies

In one respect the pressure clamp measurements, and to a lesser extent the pressure relaxation measurements, appeared anomalous. To raise turgor pressure by 1 bar to a new constant value, we found it necessary to inject a surprisingly small amount of oil into the sporangiophore— only about 1% of the sporangiophore volume (see Fig. 3). This value of 1% is the total value displaced at the end of a pressure clamp, i.e., when net transport of water has ceased, and is much less than that expected for an ideal osmometer. For example, in a simple osmometer with an osmotic pressure and turgor pressure of 4 bars, injecting oil to raise turgor to 5 bars will cause water to flow out of the cell, leaving behind the solutes and raising the osmotic pressure. At equilibrium, the osmotic pressure will have increased to 5 bars and oil will have diplaced 20% of the cell volume. In contrast to this ideal behavior, sporangiophores exhibited only about 1% oil displacement in such experiments.

FIGURE 5 Photomicrograph of the foot of a plucked stage I sporangiophore, under phase contrast illumination. Note the septa between the sporangiophore and the hyphae of the mycelium. Specimen fixed in OsO₄, dehydrated, and embedded in Spurr's medium.

In the appendix we present an analysis to explain this anomaly in terms of a two-compartment osmotic system: one compartment is the cell wall space, bounded on one surface by the cuticle and on the other surface by the plasma membrane; the second compartment is the combined volumes of the cytoplasm and vacuole and is bounded entirely by the plasma membrane (see Fig. 6). According to our analysis, when turgor pressure is stepped up during a pressure clamp, water moves from the sporangiophore into the wall space. Because the wall compartment is smaller than the cell compartment, the water transfer has a much greater effect on the external (wall) solute concentration than on the internal (cell sap) concentration. As a result, a new equilibrium is attained more rapidly and with less oil injection than for the simpler case of a cell surrounded by a large external solution which is unaffected by the water flux across the plasma membrane.

In addition to the osmotic anomaly noted above, two observations support the idea that the cell wall space acts like an outer osmotic compartment containing solutes. First, the cell wall solution of the sporangiophore appears to contain a high solute concentration. We noted that the surfaces of the lower half of sporangiophores are usually covered with tiny droplets (Fig. 7). Such exudation would be expected if the wall solution contained dissolved solutes and thereby attracted water from the sporangiophore. To test this idea directly, we measured the osmotic pressure of surface droplets with a nanoliter osmometer. Mean osmotic pressure of 12 samples was 10.2 bars (SE = 1.2 bars). This value may overestimate the osmotic pressure of the cell wall solution, however, because the exuded droplets

FIGURE 7 Photomicrograph showing small droplets exuding from the wall of a stage IV sporangiophore.
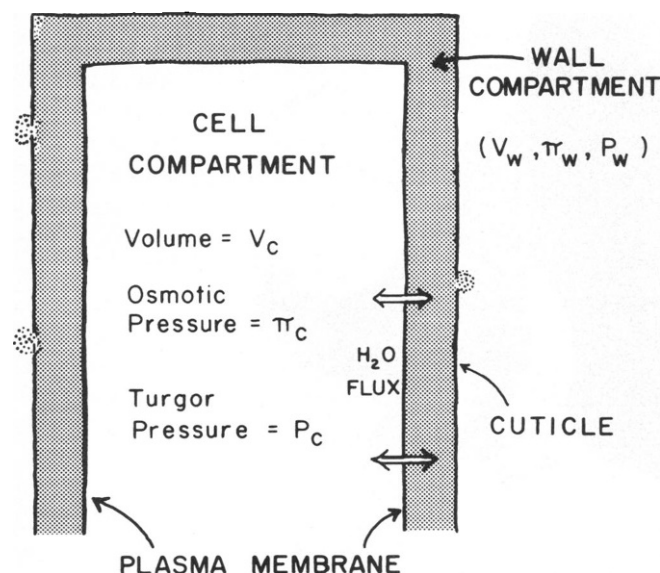

FIGURE 6 Idealized osmotic model of the sporangiophore as a cell compartment surrounded by a smaller wall compartment. Each compartment has distinct values for volume, osmotic pressure, and turgor pressure. We assume that exudation of solution through cracks in the cuticle prevents the wall compartment from becoming pressurized.

may be subject to evaporation. Nevertheless, this result does indicate that the wall solution contains a substantial concentration of solutes, as required by the two-compartment model.

As a second observation, we note that the water potential of intact sporangiophores was about −7 bars, and that this low water potential was not entirely due to transpirational water loss. Water potential was calculated as $(P - \pi)$, where turgor (P) was measured with the pressure probe and found to average 4.1 bars (Table I). Sap extruded from sporangiophores had a mean osmotic pressure (π) of 11.4 bars (Table I). Thus the water potential of the sporangiophores was about −7.4 bars. This value is substantially below the water potential of the culture medium, which was measured by vapor pressure osmometry to be about −4 bars. Thus there is a substantial water potential disequilibrium (about 3 bars) between the culture medium and the sporangiophore.

One possible reason for such disequilibrium might be that transpiration from the sporangiophore was great enough to reduce its water potential by about 3 bars. This would imply that the hydraulic resistance to water transport from the medium into the sporangiophore was great

enough to prevent water potential equilibration while the sporangiophore was transpiring. To test this hypothesis, hyphae and sporangiophores of explanted cultures were submerged under water; only the upper 3–5 mm of the sporangiophore was left in air for the pressure measurements. Submersion in this fashion should have reduced transpiration and increased the availability of water to the sporangiophore. If the cell equilibrated with the surrounding water, its turgor should have increased to about 11.5 bars. In fact, the turgor pressure of submerged sporangiophores remained low, in the range of 3–5 bars, even after 30 min of submersion. Because of reports that transpiraton might be localized to the growing region (Bergman et al., 1969), we also carried out experiments in which the sporangiophore was inverted and the apical 1/3 of the sporangiophore submerged in water. As before, turgor remained in the 3–5 bar range. Both types of experiments were carried out at least three times. In a third and better test, turgor was measured in sporangiophores mounted in air in a small chamber; then water was carefully added to the chamber to submerge the sporangiophore entirely, without disrupting the membrane seal around the microcapillary. In four replicates of this experiment, turgor stayed nearly constant in the first few minutes after submersion (increase of less than 0.1 bar), and during the ensuing 40–60 min gradually increased by less than 1 bar.

These results support the idea that the sporangiophore is isolated or buffered from high water availability in its surroundings by the impermeable cuticle covering the wall and by the osmotic compartment between the cuticle and the plasma membrane. As discussed below, the maximum water potential attainable by the sporangiophore is controlled by the concentration of solutes within the cell wall compartment.

DISCUSSION

Our study provides the first direct measurements of turgor pressure and other osmotic properties of the *Phycomyces* sporangiophore. The values for sporangiophore turgor pressure are similar to those found in giant-celled algae and many higher plant cells (Dainty, 1976; Zimmermann and Steudle, 1978) and they do not appear to change during sporangiophore development. Likewise, the values for membrane hydraulic conductivity (*Lp*) are similar to those found for giant-celled algae and for some cells from higher plant tissues (Cosgrove, 1986; Cosgrove and Steudle, 1981; Dainty, 1976; Zimmermann and Steudle, 1978). Interestingly, stage I sporangiophores had a significantly higher *Lp* than did stage IV cells. This appears to be the first report of a change in *Lp* associated with development. Although there are a number of developmental differences between stage I and stage IV cells, we do not know if the *Lp* difference has a functional or adaptive significance for the sporangiophore.

Previous workers have remarked on the lack of a septum between the sporangiophore and the supporting mycelium

(Bergman et al., 1969; Gruen, 1959). A number of studies (reviewed by Bergman et al., 1969) have used "plucked" sporangiophores, which appear to remain turgid and to grow when placed in water. To account for this behavior, several workers have suggested that the basal end of the plucked sporangiophore is clogged with a cytoplasmic plug. Our microscopic observations show that the connections between the hyphae and the sporangiophore are greatly constricted and are divided by septa. These findings lend additional credibility to earlier studies with plucked sporangiophores.

The oil-flow experiments with the pressure probe indicate that the sporangiophore base does not serve as a major pathway for water movement, either by bulk flow or by osmosis, under conditions where cell turgor pressure is altered with the probe. Instead, the major exchange of water in these experiments is within the cell wall, across the entire cell surface.

There has been much speculation about the normal path of water movement through *Phycomyces* (Bergman et al., 1969). Our results show that water is very readily exchanged between the wall compartment and the sporangiophore. In comparison, water transport at the basal end of the sporangiophore is slow, presumably because of the small exchange area. However, we cannot infer from these observations that the cell wall is the main path for normal transpiratory water flow through this system. To make this conclusion, it would be necessary to demonstrate first that the wall compartment makes good hydraulic contact with the culture medium, and second, the cell wall had a low hydraulic resistance in comparison with the parallel cell pathway. Use of an impermeable marker to follow extracellular water movement might help resolve this point.

Volumetric Elastic Modulus

The usual method for measuring ε with the pressure probe involves rapid volume injection and measurement of the corresponding pulse in pressure (Cosgrove and Durachko, 1986; Hueskens et al., 1978). This procedure proved unfeasible because of the high viscosity of the sporangiophore sap and because of the very short halftime for pressure relaxations. The time required to inject sufficient volume into the cell was so long that most of the change in pressure was dissipated by concomitant water efflux. It should be noted that with the size of our capillary tips, the halftime of the instrument itself is less than 25 ms (Cosgrove and Steudle, 1981); hence the limitation is not instrumental but in the meniscus-control technique.

For an ideal osmometer, we may calculate ε from the combination of pressure relaxation and pressure clamp measurements. By combining Eqs. 1 and 2, we may calculate ε as

$$\epsilon = \pi \left(\frac{T_{1/2}{}^{c}}{T_{1/2}{}^{r}} - 1 \right). \tag{4}$$

This procedure for estimating ε does not seem to have been tried in the past.

However, this approach was foiled because the sporangiophore did not behave as an ideal osmometer. Rather, it seemed to behave as a limited two-compartment system: the large volume of the sporangiophore compartment (vacuole and cytoplasm) surrounded by the small volume of the cell wall compartment. In such a system, the time course for both pressure relaxations and pressure clamps is strongly influenced by dilution or concentration of solutes in the wall compartment (see Appendix). Hence, Eq. 4 cannot be used to estimate ϵ in such a system, and we were not able to obtain a reliable measure of sporangiophore ϵ.

The Two-Compartment Model

The strongest evidence that the sporangiophore does not behave like an ideal osmometer comes from the pressure clamp experiments. At the completion of a typical pressure clamp, the oil injected into the cell was typically about 1% of the cell volume, yet turgor increased by about 1 bar, a 20–25% increase. For an ideal osmometer, a 25% turgor increase would require that oil displace about 20% of the cell water by the end of the pressure clamp. What accounts for the low oil displacements in *Phycomyces?* One possibility is that most of the water in the cell is "nonsolvent" water, i.e., water bound to proteins or other cell components and not osmotically active. To explain the anomalous oil displacement, the amount of nonsolvent water in the sproangiophore would need to be about 95% of the cellular water. This figure far exceeds the values of 10–20% found for cells containing high protein concentrations (reviewed in House, 1974). In cells as highly vacuolate as the sporangiophore, the nonosmotic water should be even less than 10%. Thus this explanation seems unlikely.

We propose that the osmotic anomaly noted above arises because water flow between the cell and the wall substantially modifies the solute concentration in the wall solution. As additional evidence for this hypothesis, we found that droplets on the sporangiophore surface contained a high solute concentration, and that cell turgor pressure did not increase when the sporangiophore was submerged under water. Apparently the cuticle covering the cell wall greatly retarded diffusional loss of solutes out of the wall compartment when the sporangiophore was submerged. Our analysis of water flow in a two-compartment system is presented in detail in the Appendix. Here we will briefly illustrate it.

Consider a sporangiophore with an osmotic pressure of 11 bars. It is surrounded by a wall compartment that is 5% of the volume within the plasma membrane and that has an osmotic pressure half that of the cell sap (see Fig. 6). At the start of the pressure clamp procedure, cell turgor pressure is raised by 1 bar. This upsets water potential equilibrium and induces an efflux of water from the cell to the wall. The dilution of solutes in the wall compartment is 10 times greater than the increase in solute concentration within the sporangiophore. This follows from the relative values for the volume and osmotic pressure of the two compartments. When the sporangiophore has lost 1% of its

water, a 20% dilution of the wall compartment will have occurred. Multiplying these factors by the osmotic pressures gives a 10-fold greater change in osmotic pressure in the wall compartment. Note also that the osmotic pressures are altered in opposite directions in the two compartments. By transferring water into the wall, the cell raises the water potential of the wall compartment, and thereby shortens the time required for the two compartments to reach water potential equilibrium. In an ideal osmometer, the equilibrium is attained exclusively by increase in cell osmotic pressure. As illustrated graphically in the Appendix, the new equilibrium will be reached 11 times faster than would occur in an ideal cell surrounded by a large water reservoir. Moreover, the total flow of oil into the sporangiophore will be 11 times less than that for an ideal osmometer. However, the initial water flux at the start of the pressure clamp procedure will not be affected, so it is still valid to use Eq. 3 to calculate Lp.

Cell Wall Solutes

The presence of droplets on the surface of sporangiophores (Fig. 7) seems to have drawn little comment in the *Phycomyces* literature. The droplets also occur in a number of other fungi (Colotelo, 1978). They clearly are not condensation drops and it would be remarkable if the sporangiophore could exude pure water, especially in an atmosphrere below 100% relative humidity. Roelofson (1950a) published a photograph showing small bubbles between the cell wall and the cuticle.

From our observations, we suggest that such surface droplets arise because the wall solution contains osmotically active solutes and tends to attract water. The tendency for the wall compartment to swell and become pressurized by water uptake would be defeated by escape of solution from the wall compartment via cracks or fissures in the cuticle. Such exudates ought to evaporate readily because they are not protected by the cuticle, and they may account for much of the transpiration of the sporangiophore. It should be possible to test this ideal by using the pressure probe to influence the flux of water from the sporangiophore into the wall compartment, while simultaneously measuring the rate of transpiration. We would expect that transfer of water in the wall compartment would transiently enhance exudation and transpiration.

Wall solutes have also been reported in tissues of higher plants. High apoplast solute concentrations seem most prevalent in tissues which act as strong sinks for phloem unloading and which are involved in high transport rates. Examples include developing grains (Wolswinkel, 1985), sugar beet storage tissue (Leigh and Tomos, 1983), and growing stem tissues (Cosgrove and Cleland, 1983). Because the wall compartment in many of these systems is open to the atmosphere, it cannot become highly pressurized. From this it follows that the maximum water poten-

tial attainable by these tissues is determined by the concentration of solutes in the wall space (Cosgrove and Cleland, 1983).

We conclude that the turgor pressure and water potential in *Phycomyces* sporangiophores are kept low by solutes in the cell wall. Furthermore, it is possible that the wall compartment is part of a turgor pressure control mechanism. By regulating the solute concentration of the wall solution, a sporangiophore could regulate its turgor pressure in an efficient manner. Because the wall compartment is small relative to the cell volume, it would require very little solute transport across the plasma membrane to raise or lower the osmotic pressure of the wall solution, and thereby to alter the osmotic pressure gradient across the plasma membrane. To test this hypothesis, one might use the pressure probe to raise or lower cell turgor pressure artificially, and measure the transport of solutes across the plasma membrane. For this experiment, it will be necessary to find some way to quantitate solutes within the wall, perhaps by permeabilizing the cuticle.

In our analysis of *Phycomyces* as a two-compartment system, solutes are contained in the wall and cannot diffuse away because they are bounded on one surface by the plasma membrane and on the other surface by the cuticle and air. We do not know whether diffusional efflux at the base of the sporangiophore is blocked, but in any case it would probably be slow because of the large distances (sporangiophores can be many centimeters in length).

The two-compartment model shows that in *Phycomyces* the halftimes for pressure clamp and pressure relaxation measurements will be faster than in an ideal osmometer. A pertinent question to raise is whether apoplastic solutes in multicellular tissues will have the same effect. If so, then some published values for membrane hydraulic conductivity (*Lp*), measured in situ with the pressure probe, may be erroneously high. Unlike the solutes in the sporangiophore wall which cannot diffuse away, solutes surrounding a cell in a tissue are free to diffuse through the apoplast. Because the probe induces water fluxes in a single cell, the vast majority of the cell wall solution in a tissue would be unaffected by pressure probe manipulations. However, in the wall space directly outside of a probed cell, water transport across the plasma membrane would tend to alter local solute concentrations next to the membrane. Diffusion would tend to equalize solute concentrations with neighboring regions of the cell wall. If diffusion were rapid enough, changes in wall osmotic pressure would be negligible and so the anomalous effects noted in *Phycomyces* would not occur. However, if diffusion were too slow to compensate for the water flows at the cell surface, then one would observe two exponential components during a pressure clamp or pressure relaxation measurement. The slower component would be due to re-equilibration of the local solute concentration by diffusion with the bulk of the wall space. Further studies will be needed to evaluate this effect of wall solutes in multicellular tissues.

CONCLUSIONS

The physical properties of the *Phycomyces* sporangiophore are similar to those of giant-celled alga and some higher plant cells. Values for turgor pressure, osmotic pressure and membrane *Lp* are in the ranges found for these cells. However, the behavior of the sporangiophore as a double compartment gives the cell anomalous osmotic properties. In particular, solutes in the wall compartment reduce the turgor pressure of the sporangiophore and also have a major influence on the time course of water flow during pressure relaxations and pressure clamps. Further studies will be needed to decide whether this double compartment system plays any role in the control of turgor pressure, growth, and sensory transduction in *Phycomyces*.

APPENDIX

Dynamics of Water Flow in an Osmometer Surrounded by a Small External Compartment

We wish to consider the osmotic behavior of an ideal two-compartment osmometer, illustrated in Fig. 6. It differs from the usual ideal osmometer in that the external compartment is of limited volume, contains solutes, and therefore becomes more dilute or more concentrated as water is transferred between the two compartments. The usual model of an osmometer assumes the external compartment is infinitely large and of constant solute concentration. As will be shown below, this difference substantially alters the time course of pressure clamp and pressure relaxation measurements and the volume flows associated with these measurements. Wendler and Zimmermann (1982) have published the relevant equations for the simpler model.

For this analysis, we assume that a single membrane separates the inner (cell) compartment from the outer (wall) compartment, that the membrane is permeable to water, and that it is completely impermeable to all solutes, e.g., its solute reflection coefficient is 1. Water flow from the wall compartment to the cell compartment is described by the phenomenological equation (Dainty, 1976; Zimmerman and Steudle, 1978).

$$\frac{dV}{dt} = A \, Lp \, [(\pi_c - \pi_w) - (P_c - P_w)] \qquad (A1)$$

where A is the membrane surface area, V is volume, Lp is membrane hydraulic conductivity, $P_c - P_w$ is the difference in hydrostatic (turgor) pressures between the cell and wall compartments, and $\pi_c - \pi_w$ is the difference in osmotic pressure between the cell and wall compartments.

In the case of the *Phycomyces* sporangiophore, we assume that P_w is constant and close to ambient pressure ($P_w = 0$). This is a reasonable assumption, considering the droplets which exude from the wall surface (Fig. 7), and the results of the osmotic experiments presented earlier.

Let $\pi_{c,0}$ be the osmotic pressure of the cell at some initial volume $V_{c,0}$, and let v be a volume exchange between the two compartments, such that

$$v = V_c - V_{c,0} = V_{w,0} - V_w \qquad (A2)$$

where $V_{w,0}$ is the initial volume of water in the wall, and V_w and V_c are the volumes of water in the wall and in the cell compartments after exchange of v. For v, it follows that

$$\pi_c = \pi_{c,0} \, [V_{c,0}/V_c] \approx \pi_{c,0} \, [1 - v/V_{c,0})] \qquad (A3)$$

and

$$\pi_w = \pi_{w,0} \left[V_{w,0}/V_w \right] \approx \pi_{w,0} \left[1 + v/V_{w,0} \right]. \qquad \text{(A4)}$$

Eq. A3 and A4 say that a gain of water by the cell dilutes cell osmotic pressure and concentrates wall osmotic pressure.

Making the appropriate substitutions into Eq. A1, we obtain

$$\frac{dV}{dt} = \frac{dv}{dt} = A \, Lp \left[\pi_{c,0}(1 - v/V_{c,0}) \right.$$
$$\left. - \pi_{w,0}(1 + v/V_{w,0}) - P_c \right] \qquad \text{(A5)}$$

which upon rearrangement becomes

$$\frac{dv}{dt} = -A \, Lp \left(\frac{\pi_{c,0}}{V_{c,0}} + \frac{\pi_{w,0}}{V_{w,0}} \right) v + A \, Lp \, (\pi_{c,0} - P_c - \pi_{w,0}). \qquad \text{(A6)}$$

Pressure Clamp Measurements

In a pressure clamp measurement, P_c is held constant, and $\pi_{w,0}$ and $\pi_{c,0}$ are constant by definition. Therefore, the last term, $(\pi_{c,0} - P_c - \pi_{w,0})$, is constant and is identical to the initial step change in turgor (ΔP) imposed on the cell. Eq. A6 is of the form $dv/dt = av + b$, and has the specific solution

$$v(t) = (\exp (at) - 1) \, b/a \qquad \text{(A7)}$$

where $v(t)$ is net volume exchange at time t. The rate constant of the exponential is

$$a = -A \, Lp \left(\frac{\pi_{c,0}}{V_{c,0}} + \frac{\pi_{w,0}}{V_{w,0}} \right) \qquad \text{(A8)}$$

and

$$b = A \, Lp \, (\pi_{c,0} - P_c - \pi_{w,0}). \qquad \text{(A9)}$$

Eq. A8 shows that the time course of a pressure clamp will speed up by the additive factor $\pi_{w,0}/V_{w,0}$. For a cell surrounded by a large external solution, this factor approaches zero.

Let us assume that the wall volume is 5% of the cell volume and that the wall osmotic pressure is half the cell osmotic pressure. In such a case, the quality $(\pi_{w,0}/V_{w,0})$ is 10 times greater than $(\pi_{c,0}/V_{c,0})$, and so a pressure clamp will go to completion 11 times faster (i.e., the terms are additive; see Eq. 8) than in the simpler case of the cell in contact with an external solution of large (~infinite) volume. The final volume exchange in a pressure clamp is given by $-b/a$, or

$$v(\infty) = \frac{(\pi_{c,0} - P_c - \pi_{w,0})}{\left(\dfrac{\pi_{c,0}}{V_{c,0}} + \dfrac{\pi_{w,0}}{V_{w,0}} \right)} = \frac{\Delta P}{\left(\dfrac{\pi_{c,0}}{V_{c,0}} + \dfrac{\pi_{w,0}}{V_{w,0}} \right)}. \qquad \text{(A10)}$$

In other words, the final volume exchange will also be smaller, by a factor of 11 in the above example. These effects are shown graphically in Fig. A-1.

Pressure Relaxation Measurement

In this method, cell turgor, P_c, does not stay constant, but changes as water moves across the membrane. We may express P_c as a function of v with the approximation (Dainty, 1976)

$$P_c \approx \epsilon \frac{v}{V_{c,0}} + P_{c,0} \qquad \text{(A11)}$$

where ϵ is the volumetric elastic modulus and $P_{c,0}$ is the turgor pressure
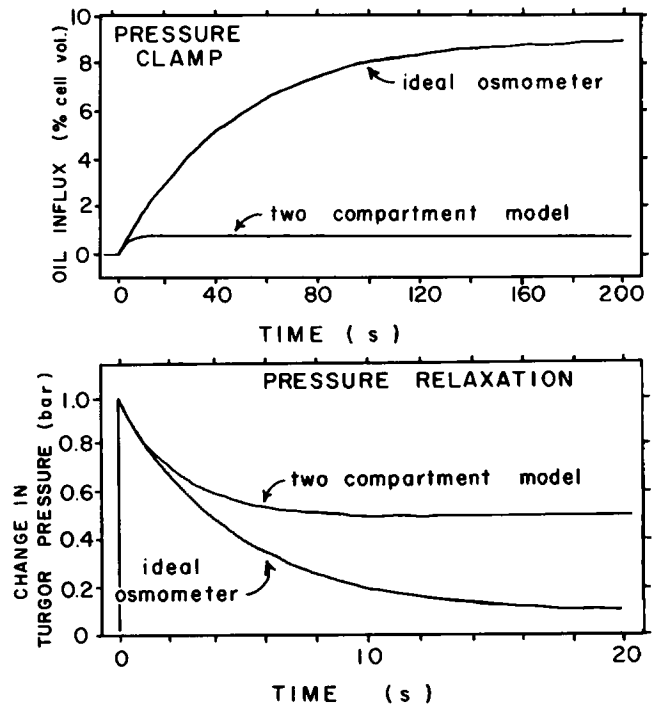

FIGURE A-1 Differences between an ideal osmometer and a two-compartment model during a pressure clamp experiment (top) and a pressure relaxation experiment (bottom). These are theoretical time courses calculated for a sporangiophore with the following characteristics: 30 mm length, 0.1 mm diameter, Lp of 5×10^{-6} cm s^{-1} bar^{-1}, cell osmotic pressure (π_c) of 11 bars, wall osmotic pressure (π_w) of 5.5 bars for the two-compartment model and 0 bars for the ideal osmometer, wall volume 5% of the cell volume, and ϵ of 100 bars. For the pressure clamp, a turgor pressure increase of 1 bar was imposed on the cell at time zero (i.e., from 11 bars to 12 bars for the ideal osmometer and from 5.5 to 6.5 bars from the two-compartment model). Then the oil influx required to balance the water efflux was calculated by equation (see Eq. A7). Actual measurements of *Phycomyces* sporangiophores resemble most closely the curve for the two-compartment model (compare with inset of Fig. 3). For the pressure relaxation calculation, the *change* in turgor is plotted. At time zero turgor pressure was raised by 1 bar by injecting a fixed volume into the cell. Note that the two-compartment model achieves equilibrium faster, but that turgor does not return as close to the pre-injection state as in the ideal osmometer.

when cell volume is $V_{c,0}$. Substituting Eq. A11 into Eq. A6 and solving as before, we obtain a similar solution, with a rate constant, a, given by

$$a = -A \, Lp \left(\frac{\pi_{c,0} + \epsilon}{V_{c,0}} + \frac{\pi_{w,0}}{v_{w,0}} \right). \qquad \text{(A12)}$$

This equation shows that the time course for a pressure relaxation speeds up by the additive factor $\pi_{w,0}/V_{w,0}$. The inclusion of the elastic modulus means that the influence of the wall compartment on the time course of a pressure relaxation is less than that for a pressure clamp. To illustrate this principle with the example given above, assume that the elastic modulus was 100 bars and cell osmotic pressure was 11 bars. Then the pressure relaxation would be speeded up by a factor of only about two, as compared with a factor of 11 for the pressure clamp. The same principle applies to net volume exchange during a pressure relaxation. Both effects are illustrated graphically in Fig. A-1.

Caveat

Eq. A3, A4, and A11 are obtained by truncations of Taylor Series expansions of π as a function of volume (Dainty, 1976). These approxima-

tions are suitable for small changes in volume. For our pressure clamp measurements, the imposed changes in sporangiophore volume were about 1%, and thus small enough for this approximation to be valid. For the wall compartment, on the other hand, volume changes were almost certainly larger, and the error associated with truncation of the Taylor Series may be significant.

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