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Phycomyces: TURGOR PRESSURE BEHAVIOR DURING THE LIGHT AND AVOIDANCE GROWTH RESPONSES

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Abstract—The turgor pressure of the stage 4b sporangiophore of *Phycomyces blakesleeanus* was continuously measured with a pressure probe before and during a period of increased elongational growth rate elicited by a step-up in blue light fluence rate (a positive light growth response) or by a double-barrier stimulus (avoidance growth response). In these and other experiments it was found that a step-up in turgor pressure between 0.02 and 0.05 MPa may elicit an increase in growth rate that is comparable to those of the light and avoidance growth responses. The results of the present work demonstrate that the turgor pressure does not increase during these growth responses, indicating that the increased growth rate is solely the result of altered cell wall mechanical properties. Furthermore, very small decreases in turgor pressure could be detected during the period of increased growth rate. This turgor pressure depression is predicted by the Growth Equations, and provides further support for the conclusion that the light and avoidance growth responses are solely the result of changes in cell wall mechanical properties.

INTRODUCTION

The sensory system of the sporangiophores of *Phycomyces blakesleeanus* has been studied by many investigators interested in the sensory transduction process. Importantly, most of the sporangiophore's sensory responses to environmental stimuli are observed as changes in the rate of cell enlargement (growth rate). Thus, it seems only natural that some investigators interested in the sensory system of *Phycomyces* have also become interested in the process by which the sporangiophore grows and regulates its growth rate. Most of the previous work in this area has been reviewed by Shropshire (1963), Bergman *et al.* (1969), and Cerde-Olmedo and Lipson (1987).

Work with higher plant cells, reviewed by Cleland (1971), Taiz (1984), Boyer (1985) and Cosgrove (1986), suggests that the rate of plant cell enlargement depends predominantly on the rates of two interdependent physical processes: water uptake and cell wall extension. Equations relating the rate of plant cell enlargement to both the rate of water uptake and the rate of irreversible cell wall extension were first published by Lockhart (1965). these equations were derived, and are valid, for plant cell growth with constant turgor pressure. These equations have been restated in a more general form by Ray *et al.* (1972) and termed the 'Growth Equations' by Taiz (1984). The Growth Equations, when the turgor pressure is constant, are:

$$\dot{v} = (dV/dt)/V = L(\Delta\pi - P) \quad (1)$$

and

$$\dot{v} = (dV/dt)/V = \phi\,(P - P_c) \quad (2)$$

where $\dot{v}$ is the relative rate of change in cell volume, V is the cell volume, L is the relative hydraulic conductance, $\Delta\pi$ is the osmotic pressure difference, P is the turgor pressure, ϕ is the irreversible cell wall extensibility, and P_c is the critical turgor pressure or yield threshold.

Subsequently, Eq. 2 was augmented by Ortega (1985) to extend the applicability of the Growth Equations to plant cell growth with changing turgor pressure, i.e. when both irreversible and reversible cell wall extension occur simultaneously. This augmented Growth Equation is:

$$\dot{v} = (dV/dt)/V = \phi\,(P - P_c) + (dP/dt)/\epsilon \quad (3)$$

where ϵ is the volumetric elastic modulus. Also, since the sporangiophores of *Phycomyces* are aerial hyphae which transpire, the Growth Equations must account for transpiration if they are to be used for these single plant cells. Recently, Ortega *et al.* (1988) augmented Eq. 1 with a 'transpiration' term:

$$\dot{v} = (dV/dt)/V = L\,(\Delta\pi - P) - \dot{T} \quad (4)$$

where $\dot{T}$ is the relative transpiration rate.

Referring to Eq. 3, it is apparent that the relative rate of change in cell volume, $\dot{v}$, or the growth rate, dV/dt, of the plant cell will change if the magnitude of the biomechanical parameters ϕ, ϵ, and P_c change, and/or the magnitude of the turgor pressure, P, changes. The magnitudes of the biome-

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chanical parameters ϕ, ϵ, and P_c determine the mechanical properties of the cell wall, and are considered to be regulated by the plant cell. The magnitude of the turgor pressure depends on the magnitudes of the biophysical parameters L and $\Delta\pi$, and on the magnitudes of $\dot{v}$ and $\dot{T}$ (see Eq. 4).

During the past two decades, Ortega *et al.* (1974), Ortega (1976), Ortega and Gamow (1976, 1977), Gamow (1980), and Chinn and Gamow (1984) working with the sporangiophores of *Phycomyces* have obtained substantial evidence that sensory stimulated changes in elongational growth rate (growth responses) are accompanied by simultaneous changes in cell wall mechanical properties. These growth responses may be elicited by several different environmental stimuli such as an increase or decrease in blue light fluence rate, an increase or decrease in the speed of a unilateral wind, or a 'double-barrier' stimulus.

Recently it has been demonstrated by Ortega and Keanini (paper in preparation) that step-up changes in turgor pressure (between 0.02 and 0.05 MPa) produced by a pressure probe, can elicit increases in growth rate of similar (but typically smaller) magnitude as those elicited by sensory stimuli. Smaller step-up changes in turgor pressure will elicit even smaller increases in growth rate. The smallest step-up change in turgor pressure which may produce a detectable increase in growth rate is approximately 0.006 MPa, however the magnitude of the increased growth rate is much smaller than those of the light growth and avoidance growth responses. This observed growth behavior to step-ups in turgor pressure is in agreement with the behavior predicted by the Growth Equations. Thus, these results together with those presented by Ortega *et al.* (1988) suggest that the Augmented Growth Equations (Eqs. 3 and 4) can be used to model the growth rate behavior of the sporangiophores of *Phycomyces blakesleeanus*.

An important question which has remained unanswered is whether these sensory-stimulated changes in elongational growth rate are only the result of changes in the mechanical properties of the cell wall? In other words, are the changes in growth rate also assisted by changes in the magnitude of the turgor pressure? This paper reports the results of the first measurements of the sporangiophore's turgor pressure before and during a sensory-stimulated change in elongational growth rate. The sensory-stimulated growth responses were elicited by a step-up in blue light fluence rate (positive light growth response), and by a 'double-barrier' stimulus (avoidance growth response).

MATERIALS AND METHODS

Biological system. Vegetative spores of the wild type strain of *Phycomyces blakesleeanus* NRRL 1555 (−) originally obtained from the California Institute of Technology, were inoculated on sterile growth medium in glass shell vials. The growth medium contained 4% (wt/vol) potato dextrose agar (Difco), 0.1% (vol/vol) Wesson oil, and 0.006% (wt/vol) thiamine and is similar to that used by Dennison and Shropshire (1984) and Cosgrove *et al.* (1987). Immediately after inoculation the vials were placed in an incubator which maintains an environment of high humidity and constant temperature (20°C), and which was illuminated from above with an incandescent light bulb. Mature stage 4b sporangiophores, 2 cm to 3 cm long, were selected for experiments from cultures that were 3 days to 7 days old.

Pressure measurements. The turgor pressure of the sporangiophore was continuously measured with a manual version of the pressure probe. The pressure probe is similar in design to that originally described by Zimmerman *et al.* (1969) and later by Husken *et al.*, (1978), except that it possesses a manually adjustable control rod which is used to adjust the pressure inside the chamber of the pressure probe. More recently, a pressure probe with a manually adjustable control rod was used by Cosgrove *et al.* (1987) to study the water relations of the sporangiophore of *Phycomyces*. In that work it was found that the cell sap of the sporangiophores of *Phycomyces* was very viscous, and sticky, especially in comparison to the cell sap of other plant cells. As a result, Cosgrove *et al.* (1987) injected an oil droplet into the vacuole and regulated its size. This method is accurate for measuring equilibrium turgor pressure, but it is relatively insensitive to small pressure changes due to the difficulty of measuring the droplet size and maintaining it at a constant volume. Subsequently it was found that by properly selecting both the size and shape of the microcapillary tip, it was possible to employ the usual technique of controlling a freely moving cell sap–oil interface within the capillary tip and maintaining it at a fixed location. In the present investigation, the cell sap–oil interface within the microcapillary tip is maintained at a fixed location with manual manipulation of the control rod. This change in technique improves the pressure probe's sensitivity to small pressure changes.

Two different pressure transducers were used in the pressure probe at different times. An 'absolute' pressure transducer was used for the initial experiments. In order to measure and record the turgor pressure directly a 'gage' pressure transducer was used for subsequent experiments. Both transducers were obtained from Kulite Semiconductor Products Inc., Ridgefield, NJ; Model XT-190-300A and XT-190-300G (the output was determined from calibration to be 0.307 mV/psi and 0.448 mV/psi, respectively). The pressure transducers were calibrated inside the pressure probe with a Heise Bourdon Tube Pressure Gauge Dresser Ind., Newton, CT (Model CMM, 0–200 PSIG Range). The output of the pressure transducers was determined to be linear over the range of turgor pressures measured in the experiments. Furthermore, the sensitivity of the pressure probe apparatus was determined to be such that pressure changes less than 0.00015 MPa could be detected. However, in operation we determined that only changes in turgor pressure of approximately 0.0015 MPa, and larger, could be readily detected. This reduction in sensitivity occurs because the manually-produced pressure changes which are required to insure that the cell sap–oil interface is free to move, introduce some background noise in the pressure readings. The transducer's electrical output was displayed on a digital multimeter (Keithly, 177 Microvolt DMM) so that small changes in pressure could be easily detected, and was recorded on a Houston Omniscribe Strip Chart Recorder (Model D5217-2).

Experimental protocol. The experimental procedure for most of the experiments was as follows. The experiment began by adapting a stage 4b sporangiophore to a broad band white light for 40 min. An overhead incandescent light bulb (40 W, located 45.7 cm from the sporangi-

ophore) was used as the light source in preliminary experiments. In subsequent experiments, a fiber-optic illuminator (Flexilux 90; HLU Light Source 90/W with a Two-Armed Swan Neck Light Guide), which provided bilateral illumination and filtered out nearly all of the infrared light, was used. The effective blue light fluence rates were different for different groups of experiments and are presented with the experimental results. After the 40 min adaptation period, the elongational growth rate was measured for the remainder of the experiment with a horizontally mounted microscope (Gaertner; 7011 K eyepiece and 32m/m EFL objective) and a stop watch. After a nearly constant elongational growth rate was obtained, the microcapillary tip of the pressure probe (approximately 3–5 μm in diameter) was inserted into the sporangiophore stalk approximately 5–7 mm below the sporangium using a micromanipulator. Generally, the sporangiophore was allowed to adapt to the inserted microcapillary for a period of 10 to 30 min, during which both the turgor pressure and the elongational growth rate were continuously measured. Then the sporangiophore was subjected to either a step-up in blue light fluence rate (to elicit a positive light growth response) or to a double-barrier stimulus (to elicit an avoidance growth response). The turgor pressure and elongational growth rate were continuously measured for the remainder of the experiment.

The step-up in blue light fluence rate was produced by increasing the power to the overhead incandescent light bulb in preliminary experiments, and in subsequent experiments by removing a neutral density filter (type NG4; Schott Optical Glass, Durea, PA) in the fiber-optic illuminator. The effective blue light fluence rate for both the adapting light and the light stimulus were measured using a photodiode (model PIN-10DP/SB; N.B.S. traceable calibration; United Detector Technology, Hawthorne, CA), a broad-band blue filter (type BG-28, Schott Optical Glass, Durea, PA) and a heat filter (type KG-5; Schott Optical Glass, Durea, PA).

The double-barrier stimulus was produced by sliding two parallel glass cover slips (5 mm apart) mounted on a rod, around the upper 1 cm of the sporangiophore stalk. It was previously demonstrated by Ortega and Gamow (1970) that several avoidance growth responses in succession may be elicited by placing the glass cover slips around the sporangiophore and removing them at regular intervals. In the present investigation and in some experiments involving the avoidance growth response, several double-barrier stimuli were given in succession to the sporangiophore by placing and removing the glass cover slips at regular intervals. At the end of the sequence, a step-up in turgor pressure was produced with the pressure probe by injecting inert silicon oil (Dow Corning Corp., fluid 200, 1–2 centistoke viscosity) into the vacuole. It has been demonstrated by Ortega and Keanini (paper in preparation) that the presence of the silicon oil in the sporangiophore's vacuole does not alter the elongational growth rate.

RESULTS

Figure 1 presents the results of a single experiment in which the turgor pressure was measured during a positive light growth response. The elongational growth rate (lower curve) is plotted against the time. The recorder tracing of the pressure from the pressure probe is presented above the elongational growth rate curve for corresponding times. The pressure transducer for this experiment was the absolute type (XT-190-300A), thus the atmospheric pressure (0.082 MPa in Denver) must be subtracted

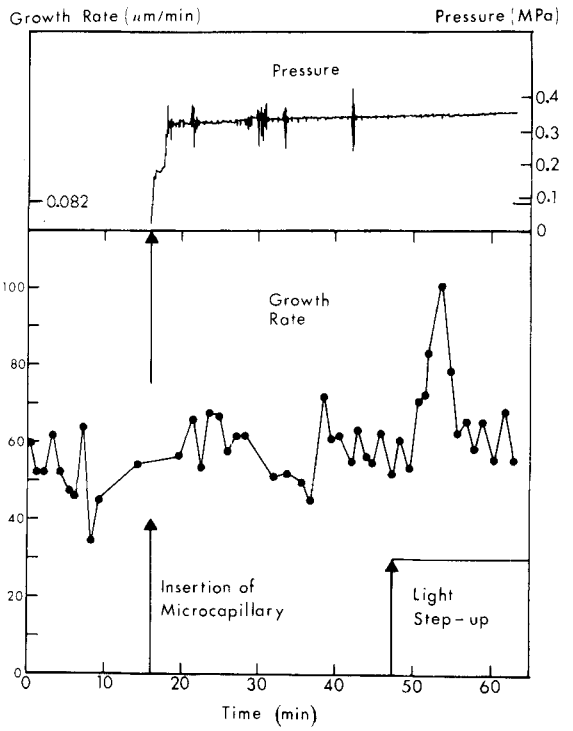

Figure 1. The results of a single experiment, in which the turgor pressure is measured during a positive light growth response, are presented. The lower curve is a plot of the elongational growth rate against time (●—●), and above it is a trace of the recorded pressure for the corresponding time period. The turgor pressure is obtained by subtracting the atmospheric pressure (0.082 MPa in Denver) from the recorded pressure. The first vertical arrow (left to right) indicates the time when the microcapillary tip of the pressure probe was inserted into the sporangiophore. The second vertical arrow indicates the time when the sporangiophore was subjected to a step-up in blue light intensity (0.0089 W/m^2 to 0.036 W/m^2).

from the recorded pressure (presented in Fig. 1) to obtain the turgor pressure. The rapid changes in pressure seen in Fig. 1 are due to manual movement of the control rod to produce pressure changes, which in turn move the cell sap–oil interface inside the capillary tip. These manually produced pressure changes are used to insure that the interface is free to move and thus accurately measure the pressure inside the sporangiophore. The magnitude of the rapid pressure changes needed to move the sap–oil interface is indicative of the viscous and sticky behavior of the sap–oil interface which varies with sporangiophores and capillary tips (size and shape). Major events during the experiment, such as insertion of the microcapillary tip of the pressure probe into the sporangiophore and the step-up in blue light fluence rate, are indicated on the time scale. For this experiment the adapting blue light fluence rate was 0.00889 W/m^2, and the stimulating blue light fluence rate was 0.03636 W/m^2. It can be seen that although the elongational growth rate fluctuates, there does not appear to be any significant

change in the average growth rate after the microcapillary tip of the pressure probe was inserted into the sporangiophore's vacuole. This was typical of most of the experiments, although in some experiments there did occur a decrease in elongational growth rate after insertion. The positive light growth response (the increase in the elongational growth rate) can be seen to begin approximately 3 min after the step-up in blue light fluence rate. Soon afterwards the elongational growth rate reaches a maximum of about 100 μm/min, then it returns to the prestimulus value of about 55 μm/min.

The magnitude of the growth response, R, may be determined by calculating the average growth rate for a 5-min interval after the beginning of the response, and dividing this value by the average growth rate for the 5-min interval before the beginning of the response: R = (average growth rate during response)/(average growth rate before response). The magnitude of the light growth response presented in Fig. 1 was calculated to be 1.46 (R = 1.46). The determination of R provides a method to compare the relative magnitude of responses elicited by different methods. It should be mentioned that the light growth response presented in Fig. 1 is very near the maximum response which may be elicited by sensory stimuli.

Inspection of the results presented in Fig. 1 demonstrates that the turgor pressure does not increase during the positive light growth response. This result is representative of more than 15 experiments conducted in which the turgor pressure was measured during the positive light growth response.

Figure 2 presents the results of an experiment in which the turgor pressure was measured during an avoidance growth response. As before, the elongational growth rate (lower curve) is plotted against time, and the trace of the turgor pressure for the corresponding times is presented above. The pressure probe's transducer for this experiment was a gage type (XT-190-300G), thus the pressure trace is the turgor pressure. In this experiment, the avoidance growth response begins approximately 3 min after the double-barrier stimulus is given. The magnitude of the avoidance growth response, R, is calculated to be 1.38. The magnitude of some of the rapid, manually-produced pressure changes recorded in the trace of the turgor pressure indicate that a sticky interface was encountered in this experiment. However, inspection of the results indicate that the turgor pressure does not increase during the avoidance growth response. These results are typical of more than 10 experiments conducted, and are similar to the results obtained with the positive light growth response.

Figures 3 and 4 present the results of more elaborate experiments. In these experiments the avoidance growth response was elicited several times consecutively, and then toward the end of each experiment, a step-up in turgor pressure was manually produced

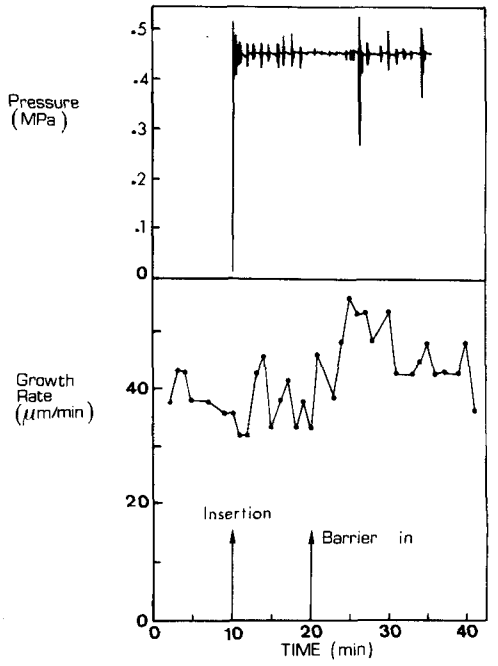

Figure 2. The results of a single experiment, in which the turgor pressure is measured during an avoidance growth response, are presented. The lower curve is a plot of the elongational growth rate against time (●—●), and above it is the trace of the recorded turgor pressure for the corresponding time period. The first vertical arrow (left to right) indicates the time when the microcapillary tip of the pressure probe was inserted into the sporangiophore. The second vertical arrow indicates the time when the sporangiophore was subjected to a double-barrier stimulus.

with the pressure probe. In both experiments, the placement and removal of the double barrier (indicated on the Figs. by arrows marked 'in' and 'out' respectively), and the pressure step-up are indicated on the time scale. As before, the upper curve is the recorder's trace of the turgor pressure (the gage-type pressure transducer was used for these experiments).

The experiment whose results are presented in Fig. 3 was conducted by two investigators. The second pressure-probe operator took over the experiment approximately 50 to 55 min after the turgor pressure measurements began (approximately 35 to 40 min on the time scale). A careful study of the pressure trace will reveal where the changeover occurred. The cell sap–oil interface was inadvertently relocated during changeover, which accounts for the slightly higher turgor pressure values between 35 and 40 min. Further inspection of the pressure trace will also reveal when the small step-up in turgor pressure was intentionally produced by the pressure-probe operator (also indicated by an arrow). The magnitude of the step-up in turgor pressure was small, 0.0062 MPa. The magnitude of the response, R, elicited by the step-up in turgor pressure (of 0.0062 MPa) is 1.18, and

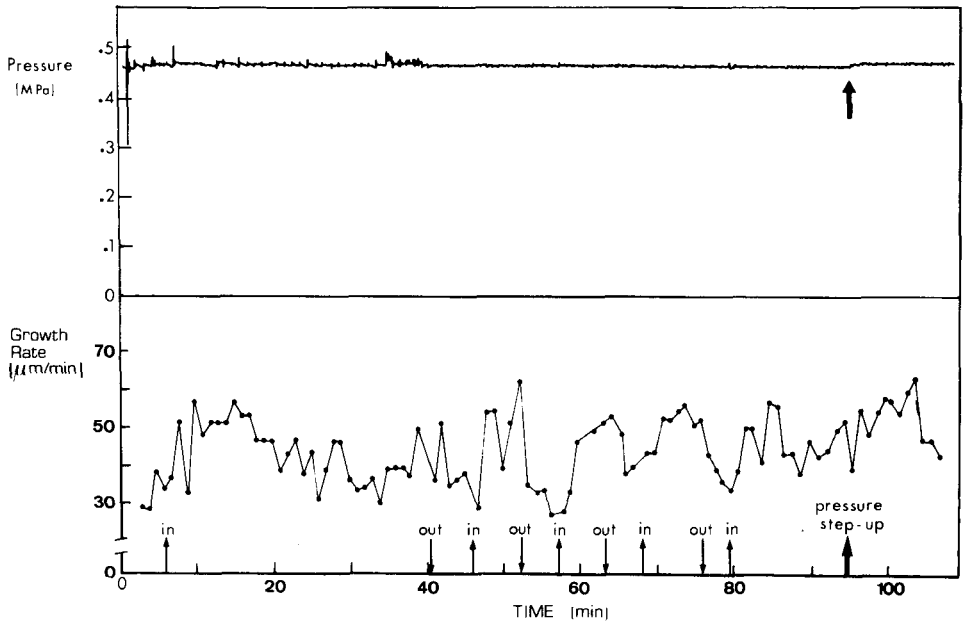

Figure 3. Presented are part of the results of a single, elaborate experiment in which the turgor pressure was measured during five consecutive avoidance growth responses, followed by a step-up in turgor pressure produced with the pressure probe. The lower curve is a plot of the elongational growth rate against time (●—●), and above it is the trace of the recorded turgor pressure for the corresponding time period. The vertical arrows labeled 'in' and 'out' indicate the time when the double barrier was placed around and removed from the sporangiophore, respectively. The last arrow on the time scale indicates the time when the 0.0062 MPa turgor pressure step-up was produced with the pressure probe.

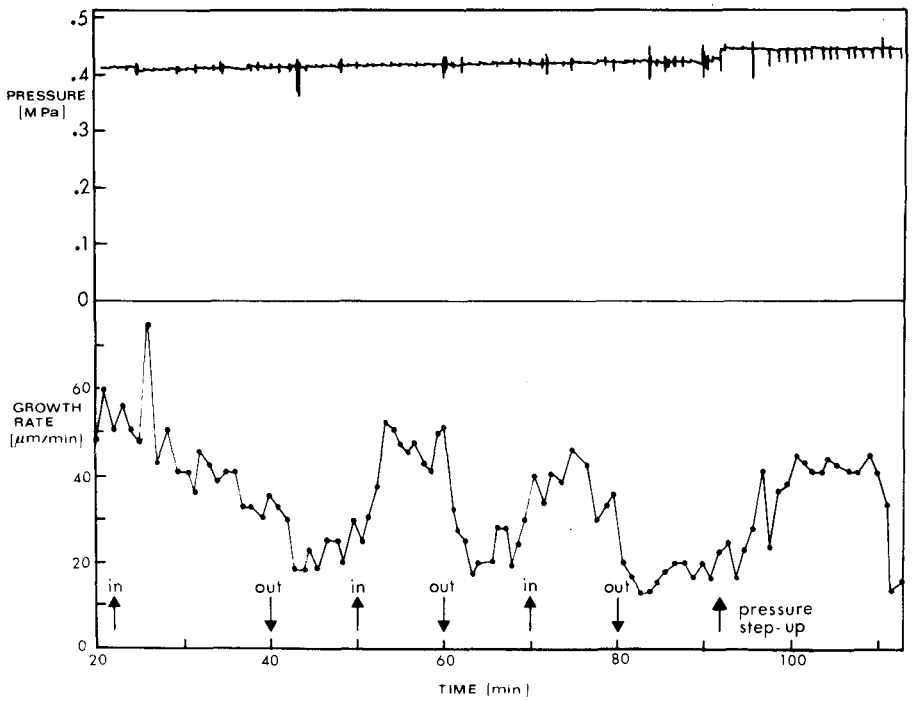

Figure 4. Presented are part of the results of another elaborate experiment in which the turgor pressure was measured during three consecutive avoidance growth responses, followed by a step-up in turgor pressure produced with the pressure probe. The lower curve is a plot of the elongational growth rate against time (●—●), and above it is the trace of the recorded turgor pressure for the corresponding time period. The vertical arrows labeled 'in' and 'out' indicate the time when the double barrier was placed around and removed from the sporangiophore, respectively. The last arrow on the time scale indicates the time when the 0.024 MPa turgor pressure step-up was produced with the pressure probe.

considerably smaller than the average R of 1.42 for the preceding avoidance growth responses ($1.62 \geq R \geq 1.28$).

Figure 4 presents the results of another experiment in which several double-barrier stimuli are given to the sporangiophore consecutively, and then a step-up in turgor pressure is produced with the pressure probe at the end of the experiment. In general the results are similar to those presented in Fig. 3, but there are a few noteworthy differences. One difference is that the elongational growth rate decreases after the tip of the microcapillary is inserted into the vacuole of the sporangiophore. In fact the first avoidance growth response (double-barrier stimulus is given at 22 min and removed at 40 min) is masked by the continual decrease in elongational growth rate which occurs for the first 40 min after insertion. Interestingly, the decreased steady-state growth rate does not prevent the sporangiophore from responding to subsequent double-barrier stimuli, or to a step-up in turgor pressure. Subsequently, two large avoidance growth responses are elicited by the double-barrier stimuli (R is 1.79 and 1.44). The subsequent step-up in turgor pressure (manually produced with the pressure probe) also elicits an increase in elongational growth rate of similar magnitude (R = 1.48) as that elicited by a double-barrier stimulus. It should be noted that the magnitude of the turgor pressure step-up (0.024 MPa) is considerably larger than the step-up presented in Fig. 3 (0.0062 MPa).

DISCUSSION

The results presented in this investigation demonstrate that an increase in turgor pressure greater than approximately 0.0015 MPa (the sensitivity of our pressure probe) does not occur during either the positive light growth response or during the avoidance growth response. Thus, it may be concluded that the transient increase in elongational growth rate, elicited by an increase in blue light fluence rate or by a double barrier-stimulus, is solely the result of changes in the mechanical properties of the cell wall.

This conclusion has some implications. In terms of the sensory system of *Phycomyces*, it may be deduced that the final step of the sensory transduction process need only result in altering the mechanical properties of the cell wall. In terms of the Growth Equations, the results suggest that the light growth response and the avoidance growth response are caused by a transient change in the magnitude of one, two or all three of the biomechanical parameters, ϕ, ϵ, and P_c. Ortega *et al.* (1988) have measured ϵ for the sporangiophores of *Phycomyces*, and have found that ϵ increases with the magnitude of the turgor pressure, as it does for higher plant cells. Ortega and Keanini (paper in preparation) have also determined the magnitudes of ϕ and P_c during steady-state growth for the sporangiophore of *Phycomyces*. Other experiments are needed to determine which of these parameters are altered during these two growth responses, although it is apparent from Eq. 3 that since there is no increase in turgor pressure ($dP/dt \simeq 0$) a change in the magnitude of ϵ cannot account for the observed change in growth rate.

Because the two physical processes of water uptake and cell wall extension are interdependent, subtle and somewhat complex turgor pressure behavior is expected during an increase in growth rate resulting solely from changes in cell wall mechanical properties. It is apparent that the Growth Equations are coupled by the relative rate of change in cell volume, $\dot{v}$. Thus an increase in growth rate due to changes in cell wall mechanical properties requires that there also occurs an increase in the rate of water uptake if the transpiration rate remains constant. An increase in the rate of water uptake may be achieved by increasing the magnitude of the relative hydraulic conductance, L, and/or the osmotic pressure difference, $\Delta\pi$; or by decreasing the magnitude of the turgor pressure. Although it cannot be determined whether the magnitude of L or $\Delta\pi$ change during the growth response, we can estimate the magnitude of the turgor pressure changes expected when L and $\Delta\pi$ remain constant. The following relationship can be derived from Eq. 4 (see Appendix):

$$P_r = P_s - (\dot{l}_r - \dot{l}_s)/L, \qquad (5)$$

where P is the turgor pressure, $\dot{l}$ is the relative rate of change in length, and L is the relative hydraulic conductance. The subscripts refer to values during the growth response (r) and during steady-state growth (s). It is apparent that during a positive growth response (when $\dot{l}_r > \dot{l}_s$) that there should be a decrease in turgor pressure ($P_r < P_s$).

Typical values for L, and $(\dot{l}_r - \dot{l}_s)$ may be obtained and used with Eq. 5 to estimate the magnitude of turgor pressure depression ($P_s - P_r$) that would be expected during a positive growth response (see Appendix). A value of 0.0021 MPa is obtained for the approximate magnitude of turgor pressure depression ($P_s - P_r$). This small change in pressure is very near the operating sensitivity of our pressure probe (0.0015 MPa). Naturally, any single sporangiophore will have values of L and $(\dot{l}_r - \dot{l}_s)$ that are slightly different from the typical values used in the estimation. Thus the turgor pressure depression may be slightly larger or smaller than 0.0021 MPa. It follows that the turgor pressure depression may be barely detected in some experiments and not at all in other experiments. A more critical study of the results presented in Figs. 1 and 2 (with the help of a rule, or by viewing the pressure traces from the side) may reveal a small decrease in turgor pressure during the time period corresponding to the positive growth responses. A critical

study of all the obtained results indicates that there is either no detectable change in turgor pressure or a slight decrease in turgor pressure during a positive growth response. This result provides additional support for the conclusion that only the mechanical properties of the cell wall are changed to elicit the light growth response and the avoidance growth response.

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APPENDIX

A relationship to describe the turgor pressure behavior during a growth response resulting from changes in cell wall mechanical properties can be derived from Eq. 4. Equation 4 is:

$$\dot{v} = L(\Delta\pi - P) - \dot{T} \tag{A1}$$

Rearranging Eq. 4 to solve for the turgor pressure, we get:

$$P = \Delta\pi - \dot{v}/L - \dot{T}/L \tag{A2}$$

Taking the time derivative of Eq. A2, and assuming that $\Delta\pi$, $\dot{T}$, and L are constant, we get:

$$dP/dt = -(d\dot{v}/dt)/L \tag{A3}$$

This equation describes the turgor pressure behavior when L and $\Delta\pi$ are constant. To determine the magnitude of the pressure change that results from a change in the growth rate, we multiply Eq. A3 by dt to obtain:

$$dP = -d\dot{v}/L \tag{A4}$$

Next Eq. A4 is integrated between values during the steady-state and during the response (subscripts s and r, respectively), and the following relationship is obtained:

$$P_r - P_s = -(\dot{v}_r - \dot{v}_s)/L \tag{A5}$$

For the special case of a cylindrical cell that is growing in length only, it may be assumed that the cross-section, A_0, is constant. Then it is apparent that:

$$\dot{v} = (dV/dt)/V = A_0(dl/dt)/(A_0 l) = (dl/dt)/l = \dot{l} \tag{A6}$$

where l is the length of the cell. For a cylindrical cell growing only in length, it follows that:

$$P_r - P_s = -(\dot{l}_r - \dot{l}_s)/L \tag{A7}$$

This is Eq.5 in the text.

A growing stage 4b sporangiophore of *Phycomyces* is a cylindrical cell that is essentially growing in length only. Thus Eq. A7, or Eq. 5 in the text, may be used together with typical values for L and $(\dot{l}_r - \dot{l}_s)$ to estimate the magnitude of the turgor pressure depression $(P_s - P_r)$ during a positive growth response.

A typical sporangiophore used in the experiments was approximately 3 cm in length and 100 μm in diameter. A typical growth response showed a maximum difference in elongational growth rate between the response and steady-state values of approximately 30 μm/min. Using these values, $(\dot{l}_r - \dot{l}_s)$ is calculated to be 0.001/min.

The relative hydraulic conductance, L, is calculated from the following relationship: $L = L_p A/V$, where L_p is the membrane hydraulic conductivity, A is the area of the cell membrane, and V is the volume of the cell. For a cylindrical cell, where the length, l, is much greater than the radius, r, then L may be approximated by the relation: $L = 2L_p/r$. Cosgrove *et al.* (1987) measured the L_p for stage 1 and stage 4 sporangiophore of *Phycomyces*. They obtained an average L_p of 1.96×10^{-6} cm/(bar s). Using this average L_p and $r = 50$ μm, L is calculated to be 7.84×10^{-4}/(bar s).

Substituting the values for $(\dot{l}_r - \dot{l}_s)$ and L into Eq. A7, or Eq. 5 in the text, we get: $P_r - P_s = -0.0021$ MPa, or $P_s - P_r = 0.0021$ MPa.