In vivo creep and stress relaxation experiments to determine the wall extensibility and yield threshold for the sporangiophores of *Phycomyces*

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ABSTRACT The pressure probe was used to conduct in vivo creep and in vivo stress relaxation experiments on the sporangiophores of *Phycomyces blakesleeanus*. The in vivo creep and in vivo stress relaxation methods are compared with respect to their utility for determining the irreversible wall extensibility and the yield threshold. The results of the in vivo stress relaxation experiments demonstrate that the growth usually does not cease when the external water supply is removed, and the turgor pressure does not decay for hours afterwards. A successful stress relaxation experiment requires that the cell enlargement rate (growth rate) be zero during the turgor pressure decay. In a few experiments, the growth rate was zero during the turgor pressure decay. However, in general only the yield threshold could be determined.

In vivo creep experiments proved to be easier to conduct and more useful in determining values for both the irreversible wall extensibility and the yield threshold. The results of the in vivo creep experiments demonstrate that small steps-up in turgor pressure, generally <0.02 MPa, elicit increases in growth rate as predicted by the growth equations and the augmented growth equations. The irreversible wall extensibility and the yield threshold were determined from these results. The results also demonstrate that steps-up in turgor pressure larger than ~0.02 MPa, produce a different response; a decrease in growth rate. The decreased growth rate behavior is related to the magnitude of the step-up, and in general, larger steps-up in turgor pressure produce larger decreases in growth rate and longer periods of decreased growth rate. Qualitatively, this growth behavior is very similar to the "stretch response" previously reported by Dennison and Roth (1967).

INTRODUCTION

In vivo stress relaxation experiments, which employ the pressure probe, have been used to determine the relative irreversible wall extensibility, ϕ, and the yield threshold, Y, of growing plant tissue (Cosgrove et al., 1984; Cosgrove, 1985 and 1987). Earlier work by Green (1968) and Green et al. (1971) employed another method to determine both the relative irreversible wall extensibility and the yield threshold of the growing *Nitella* cell. In their pioneering work, which was conducted before the introduction of the pressure probe, Green and his colleagues used a gas-filled capillary (micro-manometer) inserted into a *Nitella* cell to measure the turgor pressure. The turgor pressure was changed by adding an osmoticum to the solution in which the cell was immersed, and the subsequent growth rate behavior was measured. The relative irreversible wall extensibility and the yield threshold were determined from the resulting growth rate behavior. In fact, this method is equivalent to a creep test, where an instantaneous change in stress (turgor pressure) is imposed on the tested material (cell wall), and the subsequent strain rate behavior (growth rate behavior) is monitored and analyzed.

Here, the method of Green and his colleagues is modified to employ the pressure probe to continuously measure the turgor pressure and to produce instantaneous increases in turgor pressure (turgor pressure steps-up). Simultaneously, the growth rate is continuously measured. Because this method is similar to that of a creep test, we will refer to these experiments as "in vivo creep" experiments. Here, the results of both in vivo stress relaxation and in vivo creep experiments conducted on single-celled stage IVb sporangiophores of *Phycomyces blakesleeanus* are presented. Also, the in vivo creep and in vivo stress relaxation methods are compared with respect to their utility for determining the irreversible wall extensibility and the yield threshold. The long-term objective of this work, and other similar investigations, is to determine the magnitude and behavior of all the biophysical and biomechanical parameters in the growth equations, and augmented growth equations; thus establishing governing equations which describe and predict the growth rate behavior of the sporangiophore to physical and environmental stimuli.

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THEORY

Plant cell enlargement, or growth, is the result of two simultaneous and interrelated physical processes: the net rate of water uptake and the rate of cell wall extension. Lockhart (1965) was the first to derive rate equations for these two physical processes, which were later termed the "growth equations" by Taiz (1984). Subsequently, the growth equations were augmented with additional terms to extend their applicability to dynamic growth conditions, i.e., when the turgor pressure is changing (Ortega, 1985), and to plant cells which transpire (Ortega et al., 1988*a*). In general, two "augmented growth equations" describe the two physical processes involved in plant cell enlargement. The first augmented growth equation describes the relative rate of change in volume of the cell contents, ν, (Ortega et al., 1988*a*):

$$\nu = (dV/dt)/V = \mathcal{L}(\Delta\pi - P) - T, \quad (1)$$

where V is the volume of the cell contents, t is the time, $\mathcal{L}$ is the relative hydraulic conductance, $\Delta\pi$ is the osmotic pressure difference, P is the turgor pressure, and T is the relative transpiration rate. The second augmented growth equation describes the relative rate of change in volume of the cell wall chamber (Ortega, 1985):

$$\nu = (dV/dt)/V = \phi(P - Y) + (dP/dt)/\epsilon, \quad (2)$$

where V is the volume of the cell wall chamber, ϕ is the relative irreversible wall extensibility, Y is the yield threshold, and ϵ is the volumetric elastic modulus. It is apparent that the volume of the cell contents must be equal to the volume of the cell wall chamber, and that the respective rates of change must also be equal.

In vivo stress relaxation experiments

Mathematically and experimentally, the major criterion which must be fulfilled for an in vivo stress relaxation experiment, is that the relative rate of change in volume of the cell wall chamber must be zero ($\nu = 0$), or the volume of the cell wall chamber must remain constant (Cosgrove, 1985; and Ortega, 1985). Then Eq. 2 becomes:

$$dP/dt + \epsilon\phi(P - Y) = 0, \quad (3)$$

and the solution is:

$$P(t) = (P_i - Y)\exp(-\epsilon\phi t) + Y, \quad (4)$$

where the initial condition, $P = P_i$ at $t = 0$ is employed, and it is assumed that ϵ, ϕ, and Y are constant. It is noted that because the volume of the cell contents must be the same as the volume of the cell wall chamber, the net water uptake must be zero ($\nu = 0$, in Eq. 1). Experimentally, this condition may be achieved by eliminating the water uptake to the cell [$\mathcal{L}(\Delta\pi - P) = 0$], and eliminating transpiration from the cell (T = 0).

It is apparent that both ϕ and Y may be determined by measuring $P(t)$ after both water uptake and transpiration are eliminated, since P decays to Y, and the time constant of the decay is $1/\epsilon\phi$. A major requirement for using Eq. 4 to determine ϕ is that ϵ must be constant and known over the range of pressure change that occurs during the pressure decay (ideal stress relaxation).

In vivo creep experiments

Mathematically and experimentally, the only major criterion that must be fulfilled in an in vivo creep experiment, is that an instantaneous increase in turgor pressure (turgor pressure step-up) must be imposed on the plant cell. When a step-up in turgor pressure is given to the plant cell, the relative irreversible wall extensibility, ϕ, and the yield threshold, Y, may be determined from the subsequent growth rate behavior. These determinations are made using the following analyses, which are similar to those of Green et al. (1971). Noting that P is constant before and after the step-up ($dP/dt = 0$), and assuming that ϕ and Y are constant (as it was also assumed in the stress relaxation experiments), Eq. 5 is obtained by taking the differential of Eq. 2:

$$d\nu = \phi dP, \text{ or using finite differences; } \quad \Delta\nu = \phi\Delta P. \quad (5)$$

Solving for the relative irreversible wall extensibility, Eq. 6 is obtained:

$$\phi = \Delta\nu/\Delta P, \quad (6)$$

which is equivalent to the expression obtained by Green (1968) and Green et al. (1971). Thus, ϕ may be determined when ν and P are measured before and after a step-up in turgor pressure. Furthermore, once ϕ is determined, the yield threshold, Y, may be determined by using Eq. 2, and using the data before the step-up in turgor pressure (since ϕ, ν, and P are known and constant for this period of growth):

$$Y = P - \nu/\phi. \quad (7)$$

A theoretical advantage of the in vivo creep experiment over the in vivo stress relaxation experiment is that ϵ does not need to be determined in order to determine the magnitude of ϕ.

MATERIALS AND METHODS

Biological material

Vegetative spores of the wild type strain of *Phycomyces blakesleeanus* NRRL 1555 (−) were inoculated in glass shell vials on sterile growth

medium consisting of 4% (wt/vol) potato dextrose agar, 0.1% (vol/vol) Wesson oil, and 0.006% (wt/vol) thiamine. After inoculation the vials were incubated under continuous light from an incandescent light bulb (40 W) at high humidity and constant temperature (20 ± 1°C). Normally, sporangiophores appeared by the end of the third day. The sporangiophores were plucked daily so that a new crop was available the following day. Stage IVb sporangiophores (the stages of sporangiophore development are described by Castle, 1942) 3–5 cm in length were selected for experiments from the third to seventh crop.

Growth rate measurements

The stalk of the stage IVb sporangiophore is a long, cylindrical, single cell that enlarges (grows) predominantly in length, L. The elongational growth rate of the sporangiophore was determined by measuring the change in length at regular time intervals (usually 1–2 min; when the growth rate was small, longer time intervals were used) and calculating $\Delta L/\Delta t$. The change in length was measured using a long focal length horizontal microscope (Gaertner Scientific, Chicago, IL; 7011K eyepiece and 32 m/m EFL objective) mounted to a 3-D micromanipulator (model H-2; Line Tool Company, Allentown, PA, with large barrel micrometer heads or digital micrometer heads). A stop watch was used to measure the time intervals.

Pressure measurements

The turgor pressure of the sporangiophore was continuously measured with a manual version of the pressure probe (Ortega, et al., 1988*a*, *b*) which is similar in design to that originally described by Zimmermann et al. (1969). The pressure transducer used in the pressure probe was a gage type (that measures the difference between the absolute pressure and the local atmospheric pressure) for most of the experiments, and an absolute type for some experiments (before the gage transducer was obtained); both purchased from Kulite Semiconductor Products Inc., Ridgefield, NJ (model XT-190-300G and model XT-190-300A, 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 transducers' output (0.448 mV/psi and 0.307 mV/psi, respectively) were determined to be linear over the range of turgor pressures measured in the experiments. The transducer's output was recorded on a Houston omniscribe stripchart recorder (model D5217-2).

The pressure probe was mounted on a 3-D micromanipulator so that its microcapillary tip could be guided into the cell under visual observation using a horizontally mounted EMZ-2TR trinocular zoom stereomicroscope (Meiji Labax Optical Limited, Tokyo, Japan). The microcapillary of the pressure probe was filled with inert silicon oil (Dow Corning Corp., Midland, MI, fluid 200, 1 centistoke viscosity). After the cell was impaled, the usual technique of maintaining the cell sap-oil interface at a fixed location within the microcapillary tip was used to measure the turgor pressure of the sporangiophore (Ortega, et al., 1988*a*, *b*) instead of using the less accurate technique of maintaining the size of an oil droplet injected into the vacuole of the sporangiophore (Cosgrove et al., 1987).

Protocol for in vivo stress relaxation experiments

The following procedure was used to conduct the in vivo stress relaxation experiments. Initially, a large stage IVb sporangiophore was carefully removed from the mycelium with tweezers (plucked), and transferred to a small environmental chamber. A plucked stage IVb sporangiophore with its base immersed in water will continue to grow at a reduced rate for almost 20 h, exhibiting normal growth responses throughout (Bergman et al., 1969). A special environmental chamber was constructed for these experiments in order to suppress transpiration and to control the external water supply. The chamber was constructed of transparent acrylic plastic ~2-mm thick. The base of the chamber was connected to a syringe by a flexible plastic tube so that water could be added to, or removed from, the bottom of the chamber (see Fig. 1). The sporangiophore was attached to two thin horizontal supports inside the chamber. A thin layer of Vaseline was applied to the edge of the supports so that the sporangiophore would stick to the supports. The bottom of the chamber was filled with water so that the base of the sporangiophore was immersed in water. The sporangiophore's basal tip was generally immersed in <2 mm of water. Wet paper towels lined most of the inner surfaces of the chamber in order to saturate the chamber's air with water vapor, and suppress transpiration. The plucking and transfer procedure was usually completed in 5–20 s.

After the sporangiophore's basal tip was immersed in water, the pressure probe's microcapillary tip was inserted into the chamber in a position to impale the sporangiophore at a later time, and the chamber was sealed with Vaseline. The cell was then allowed to adapt in the chamber of ~100% relative humidity for at least 40 min. After the adaptation period, cell elongation measurements were initiated at ~1–2 min intervals to measure the growth rate. In the cases where the growth rate was very small, longer time intervals were used to determine the growth rate. When a 10–20 min period of steady growth rate was observed, the sporangiophore was impaled by the microcapillary tip of the pressure probe. Turgor pressure and cell elongation were then measured throughout the remainder of the experiment. Within 10–30 min after the sporangiophore was impaled, the water within the chamber was lowered below the sporangiophore's basal end to isolate the sporangiophore from its external water supply.

Protocol for in vivo creep experiments

The following procedure was used to conduct the in vivo creep experiments. A stage IVb sporangiophore in a glass shell vial was selected and adapted for 30 min to the room temperature of 21–22°C, to illumination from the overhead cool-white fluorescent lamps, and to bilateral illumination from a fiber-optic illuminator that filtered out nearly all of the infrared light (Schoelly Fiber Optics GMBH, Denelinger, West Germany Flexilux 90; HLU light source 90/W with a two-armed swan neck light guide). After this adaptation period, elongation measurements

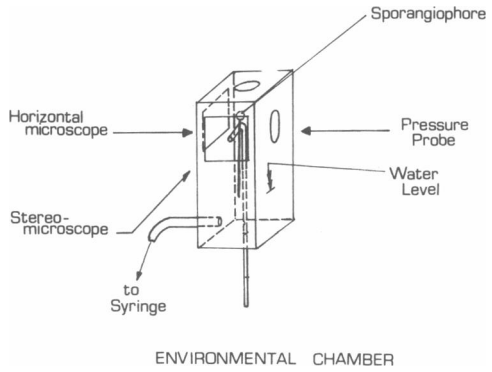

FIGURE 1 Schematic illustration of the environmental chamber used to conduct the in vivo stress relaxation experiments.

were initiated and continued at ~1–2 min intervals for the remainder of the experiment. When a 10–20 min period of steady growth rate was observed, the sporangiophore was impaled by the microcapillary tip of the pressure probe to measure the turgor pressure. Then the turgor pressure and elongational growth rate were simultaneously measured and monitored for another 10–30 min period. After this monitoring period, a step-up in turgor pressure was given to the sporangiophore by injecting inert silicon oil into the cell vacuole with the pressure probe. In general, the turgor pressure and the elongational growth rate were measured for another 20–50 min.

RESULTS

In vivo stress relaxation experiments

The results of the in vivo stress relaxation experiments varied significantly. In 50% of the stress relaxation experiments (four of the eight experiments conducted), the turgor pressure did not decay for an hour or more after the removal of the external water supply. In addition, the sporangiophores continued to grow at a rate of 1–8 μm/min during this period. Typically, a normal, intact, stage IVb sporangiophore grows at a rate of 35–60 μm/min. To ensure that an undetected external water source was not available for water uptake, the base of the sporangiophore was scanned visually with the stereomicroscope for water droplets. In addition, some experiments were conducted with the sporangiophores immersed in paraffin oil (within the environmental chamber). Oil immersion had no discernible affect; turgor pressure remained nearly constant, or decayed very slowly, while cell elongation continued for an hour or more at very small rates, as before. In one experiment, elongation continued and turgor pressure remained nearly constant for 5 h after the removal of the water supply and cell immersion in oil.

In the other four experiments the turgor pressure decayed to a constant value between 0.07 and 0.10 MPa. In two of these four experiments the turgor pressure decay was very slow, taking nearly 2 h to decay to a constant value. The results of one of these experiments is shown in Fig. 2, which is a plot of the elongational growth rate vs. time (*a*) and turgor pressure vs. time (*b*) for a single in vivo stress relaxation test conducted on a "plucked" stage IVb sporangiophore. At ~4 min on the time scale (indicated by the arrow), the water was removed. It is seen that the growth continued for ~135 min afterwards at a rate of 2–8 μm/min, during which time the turgor pressure slowly decayed. Finally, after 140 min, the growth stopped when the turgor pressure decayed to a constant value of ~0.07 MPa. These results are similar to those obtained from another experiment. Two other experiments exhibited a faster decrease in turgor pressure. Fig. 3 shows the results of one of these

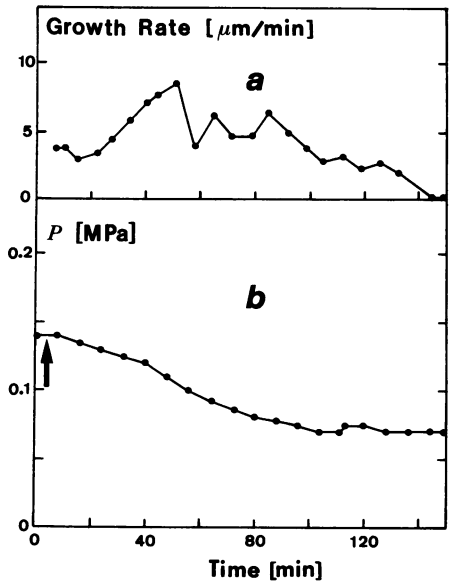

FIGURE 2 Plots of the elongational growth rate vs. time (*a*) and the turgor pressure, *P*, vs. time (*b*) for a single in vivo stress relaxation experiment.

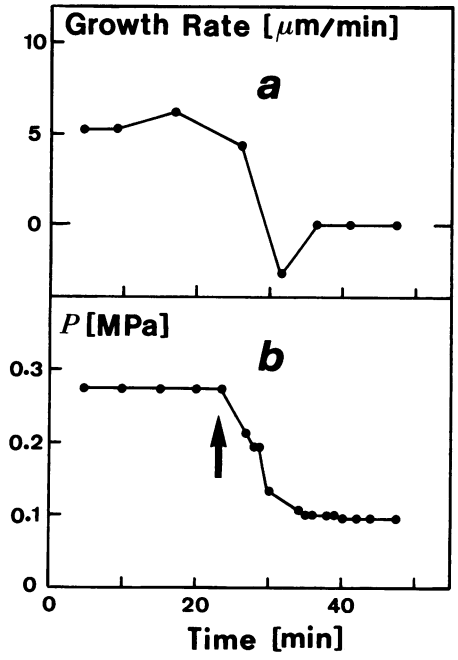

FIGURE 3 Plots of the elongational growth rate vs. time (*a*) and the turgor pressure, *P*, vs. time (*b*) for a single in vivo stress relaxation experiment.

experiments. Note that the initiation of the rapid turgor pressure decay occurs nearly simultaneously with the cessation of elongational growth, thus satisfying the condition for a stress relaxation experiment. In both experiments the turgor pressure decayed to a constant value within 10–30 min. The final turgor pressure values for all four experiments varied between 0.07 and 0.10 MPa.

In vivo creep experiments

The results of the in vivo creep experiments demonstrate that the growth rate behavior elicited by a step-up in turgor pressure depends on the magnitude of the step-up. A small step-up in turgor pressure, generally <0.02 MPa ($\Delta P \leq 0.02$ MPa), elicits an immediate increase in growth rate. Typically, this increased growth rate is sustained for a period of 15–30 min, after which it returns to its basal value (the value before the turgor pressure step-up). The increased growth rate elicited by the turgor pressure step-up is predicted by the growth equations and the augmented growth equations. A larger turgor pressure step-up ($\Delta P \geq 0.02$ MPa) elicits an initial increase in growth rate, followed by a period of decreased growth rate.

Small steps-up ($\Delta P \leq 0.02$ MPa)

The growth rate behavior of a single stage IVb sporangiophore to a small step-up in turgor pressure ($\Delta P = 0.0077$ MPa) is presented in Fig. 4. In this figure, both the turgor pressure (pressure trace from the chart recorder; *upper curve*) and the natural logarithm of the length (*ln L*) of the sporangiophore (*lower curve*) are plotted against the same time scale. The slope of "ln *L*" curve, or $d(ln\ L)/dt$, is equal to the relative rate of change in length (dL/Ldt) of the sporangiophore. The relative rate of change in length is equivalent to the relative rate of change in volume ($v = dV/Vdt$) because the cross-sectional area of the stage IVb sporangiophore remains constant for the period of growth during which the experiments are conducted. The results are plotted this way so that they can more easily be compared with those obtained by Green (1968) and Green et al. (1971), working with the large single internodal cell of *Nitella*. Major events in the experiment are indicated on the curves; such as the time when the sporangiophore was impaled (*short arrow on the lower curve*) and the time when the step-up in turgor pressure was given (*long arrows on upper and lower curves*). Note that after a turgor pressure step-up of 0.0077 MPa, the slope of the "*ln L*" curve increases for a period of 20–25 min. This result is representative of more than 20 experiments conducted.

Longer and more elaborate experiments were also conducted. Fig. 5 shows the results of a longer experiment, during which four small steps-up ($\Delta P = 0.0077$ MPa for each step-up) were given in succession (~10 min apart). It is noted that the slope of the "*ln L*" curve increases after each of the four steps-up, but the magnitude of the increases are not the same; the increase of the "*ln L*" curve is larger after the first and third steps-up, suggesting that some strain-hardening may be occurring. Qualitatively, the results presented in Figs. 4 and 5 are similar to those obtained by Green (1968) and Green et al. (1971) for *Nitella;* and identical to those obtained by Ortega et al. (1988*b*) for stage IVb sporangiophores of *Phycomyces blakesleeanus*.

It is noted that for the experimental results presented in Figs. 4 and 5, the elongational growth rate did not change after the sporangiophore was impaled. In general, this is representative of ~60–70% of the in vivo creep experiments conducted. In the other 30–40% of the experiments, the slope decreased soon after the sporangiophore

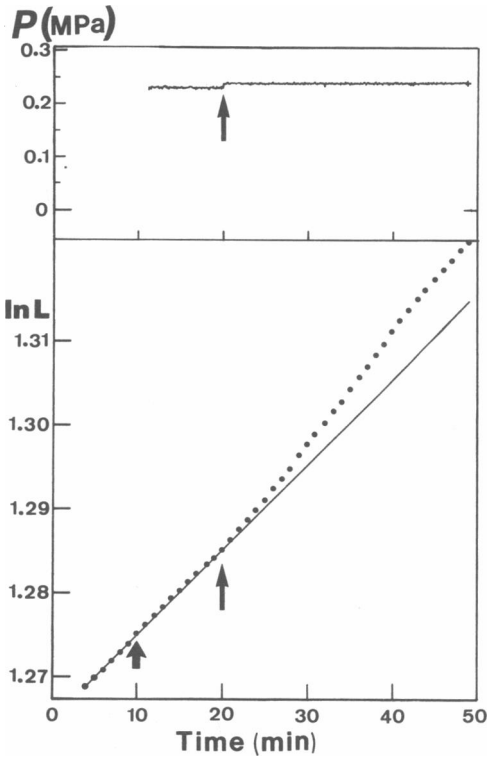

FIGURE 4 The turgor pressure, *P*, trace from the chart recorder (*upper curve*) and a plot of the natural logarithm of the length (ln L) of the sporangiophore (*lower curve*) v.s. the same time scale for a single in vivo creep experiment. The first short arrow on the time scale (*left to right on the lower curve*) marks the time when the sporangiophore was impaled by the pressure probe to measure the turgor pressure. The long arrows (*on both upper and lower curves*) mark the time when a step-up in turgor pressure was imposed on the sporangiophore. The magnitude of the turgor pressure step-up was 0.0077 MPa.

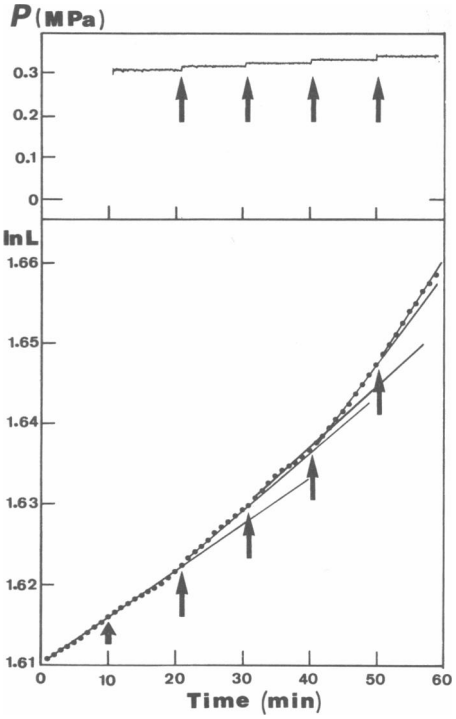

FIGURE 5 The turgor pressure, *P*, trace from the chart recorder (*upper curve*) and a plot of the natural logarithm of the length (ln L) of the sporangiophore (*lower curve*) vs. the same time scale for a single in vivo creep experiment. The first short arrow on the time scale (*left to right on the lower curve*) marks the time when the sporangiophore was impaled by the pressure probe to measure the turgor pressure. The long arrows (on both *upper* and *lower* curves) mark the time when a step-up in turgor pressure was imposed on the sporangiophore. The magnitude of each turgor pressure step-up (four steps-up were given) was 0.0077 MPa.

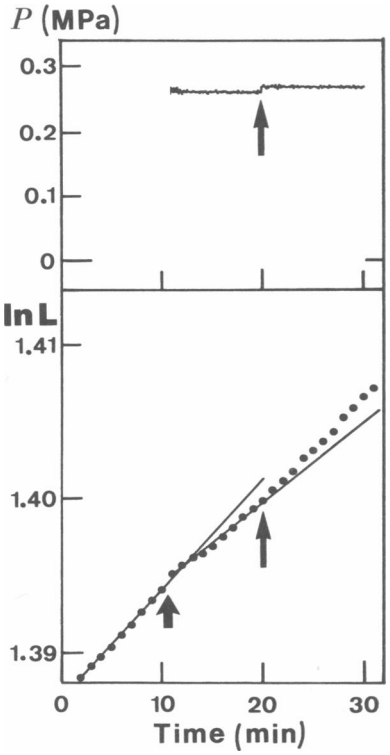

FIGURE 6 The turgor pressure, *P*, trace from the chart recorder (*upper curve*) and a plot of the natural logarithm of the length (ln L) of the sporangiophore (*lower curve*) vs. the same time scale for a single in vivo creep experiment. The first short arrow on the time scale (*left to right on the lower curve*) marks the time when the sporangiophore was impaled by the pressure probe to measure the turgor pressure. The long arrows (on both *upper* and *lower* curves) mark the time when a step-up in turgor pressure was imposed on the sporangiophore. The magnitude of the turgor pressure step-up was 0.0077 MPa.

was impaled. However, the growth rate behavior to small turgor pressure steps-up remained qualitatively the same. For example, Fig. 6 shows the results of an experiment in which the growth rate decreased after the sporangiophore was impaled, and subsequently a step-up in turgor pressure of 0.0077 MPa was given to the sporangiophore. As before (as shown in Figs. 4 and 5) the slope of the "*ln L*" curve increases after the step-up in turgor pressure.

To assure that the observed changes in growth behavior were not caused by the injection of the silicon oil into the vacuole, but only by the change in turgor pressure, control experiments were conducted. The protocol for the control experiments was the same as before, but instead of imposing a step-up in turgor pressure, the pressure was pulsed up, from and then back down to the equilibrium turgor pressure, to inject silicon oil without producing a net increase in turgor pressure. The pressure pulse was usually 2–10 s in duration and usually $< \sim 0.003$ MPa in magnitude. The results demonstrated the slope of the "*ln L*" curve, remained constant after the silicon oil was injected into the sporangiophore.

Large steps-up ($\Delta P \geq 0.02$ MPa)

In general, steps-up in turgor pressure larger than 0.02 MPa produced growth responses that are qualitatively different from those demonstrated in Figs. 4–6. Somewhat surprisingly, a step-up in turgor pressure $> \sim 0.02$ MPa results in an increase, followed by a decrease in growth rate. Fig. 7 presents the results of such an experiment, where the step-up in turgor pressure is 0.031 MPa. Subsequent to the step-up in turgor pressure, the growth rate increased slightly for a minute, then decreased to a smaller magnitude. Soon afterwards (~15 min after the step-up) the growth rate resumed its basal growth rate (growth rate before the turgor pressure step-up) and then increased to a value larger than the basal growth rate.

Larger steps-up in turgor pressure elicit growth behav-

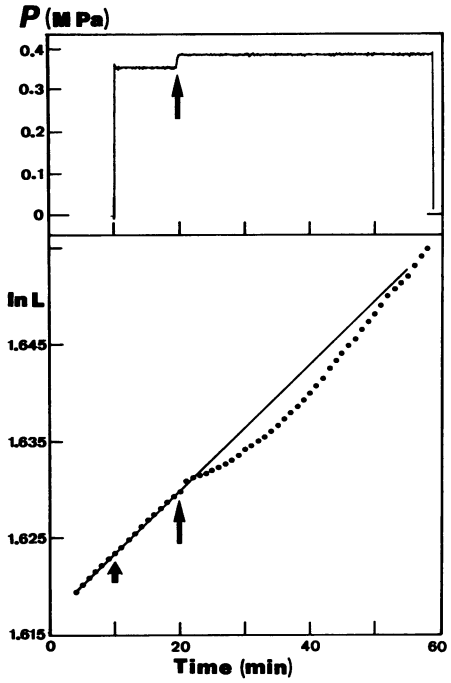

FIGURE 7 The turgor pressure, *P*, trace from the chart recorder (*upper curve*) and a plot of the natural logarithm of the length (ln L) of the sporangiophore (*lower curve*) vs. the same time scale for a single in vivo creep experiment. The first short arrow on the time scale (*left to right on the lower curve*) marks the time when the sporangiophore was impaled by the pressure probe to measure the turgor pressure. The long arrows (on both *upper* and *lower* curves) mark the time when a step-up in turgor pressure was imposed on the sporangiophore. The magnitude of the turgor pressure step-up was 0.031 MPa.

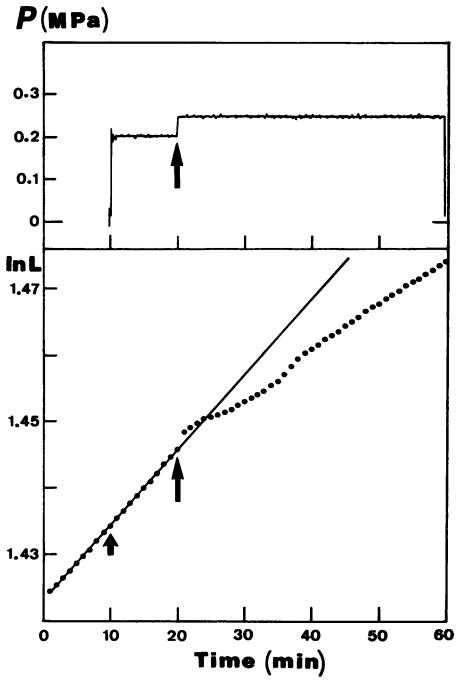

FIGURE 8 The turgor pressure, *P*, trace from the chart recorder (*upper curve*) and a plot of the natural logarithm of the length (ln L) of the sporangiophore (*lower curve*) vs. the same time scale for a single in vivo creep experiment. The first short arrow on the time scale (*left to right on the lower curve*) marks the time when the sporangiophore was impaled by the pressure probe to measure the turgor pressure. The long arrows (on both *upper* and *lower* curves) mark the time when a step-up in turgor pressure was imposed on the sporangiophore. The magnitude of the turgor pressure step-up was 0.046 MPa.

ior that is qualitatively similar, but different in detail. Fig. 8 presents the results where the step-up in turgor pressure is larger ($\Delta P = 0.046$ MPa). Similarly, after the step-up in turgor pressure, the growth rate increased for a minute, then decreased to a smaller magnitude. Soon afterwards (~15 min after the step-up) the growth rate resumed its basal growth rate for ~4–5 min, and then decreased to a smaller value. Fig. 9 presents the results where the step-up in turgor pressure is still larger ($\Delta P = 0.062$ MPa). Again, it is apparent that after the step-up in turgor pressure, the growth rate increased for a minute, then decreased to a smaller value. However, the growth rate remains lower than the basal value for at least 40 min, the remainder of the experiment.

The results presented in Figs. 7–9 suggest that the initial increase in growth rate is proportional to the magnitude of the turgor pressure step-up. This initial increase is thought to be partly elastic (reversible) and partly plastic (irreversible) extension of the cell wall, which results from the relatively large and instantaneous increase in turgor pressure. This relatively large increase in cell wall extension may cause strain-hardening of the cell wall, and may account for the subsequent decrease in growth rate. The subsequent decreased growth rate behavior, although somewhat complex, appears also to depend on the magnitude of the turgor pressure step-up: larger steps-up produce larger decreases in growth rate and longer periods of decreased growth rate.

ANALYSES

In vivo stress relaxation experiments

In general the in vivo stress relaxation experiments proved very difficult to conduct in comparison to the in vivo creep experiments. Importantly, the results were only occasionally similar to those of a normal stress relaxation test. In fact, only two of the eight experiments conducted satisfied the requirement for a stress relaxation experiment; that the growth rate is zero during the stress relaxation. In addition, it was previously shown that the volumetric elastic modulus is a nonlinear function of the turgor

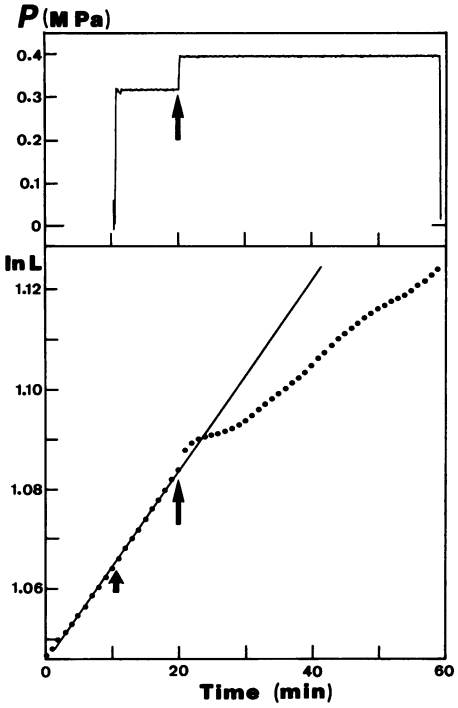

FIGURE 9 The turgor pressure, P, trace from the chart recorder (*upper curve*) and a plot of the natural logarithm of the length (ln L) of the sporangiophore (*lower curve*) vs. the same time scale for a single in vivo creep experiment. The first short arrow on the time scale (*left to right on the lower curve*) marks the time when the sporangiophore was impaled by the pressure probe to measure the turgor pressure. The long arrows (on both *upper* and *lower* curves) mark the time when a step-up in turgor pressure was imposed on the sporangiophore. The magnitude of the turgor pressure step-up was 0.062 MPa.

pressure over the range of turgor pressure decay measured in the stress relaxation experiments (Ortega et al., 1988*a*); thus it is not possible to estimate ϕ from the obtained results using the equation describing ideal stress relaxation (Eq. 4). Furthermore, the growth rates of plucked sporangiophores were generally smaller than those of normal sporangiophores, thus the estimated values of ϕ using in vivo stress relaxation experiments would probably not be representative of normal, growing sporangiophores.

In four of the eight experiments, the growth rate was zero after the turgor pressure decayed to a constant value. Theoretically then, it is possible to estimate the yield threshold. The yield threshold, Y, for plucked sporangiophores in these four experiments was between 0.07 MPa and 0.10 MPa (0.07 MPa $\leq$ Y $\leq$ 0.10 MPa).

In vivo creep experiments

In general, the increased growth rate exhibited by stage IVb sporangiophores to small turgor pressure steps-up ($\Delta P \leq 0.02$ MPa) is predicted by the growth equations and augmented growth equations. Thus, the relative irreversible cell wall extensibility, ϕ, and the yield threshold, Y, may be determined from the results presented in Figs. 4 and 5, and other similar experimental results when $\Delta P \leq 0.02$ MPa. Previous work by Green et al. (1971) demonstrate the use of Eq. 6 to calculate ϕ for *Nitella* cells. Applying their method, it is noted that the sporangiophore is a cylindrical cell that grows predominantly in length, L, and the cross-sectional area may be considered constant for the period of growth during which the experiments were conducted. Then, $v = (dV/dt)/V = (dL/dt)/L = d(ln\ L)/dt$, where $d(ln\ L)/dt$ is simply the slope of the curves of "$ln\ L$ vs. t" presented in Figs. 4 and 5. Then ϕ may be determined by measuring the change in slope of the "$ln\ L$ vs. t" curves and dividing by the change in turgor pressure (Eq. 6). However, an important difference exists between *Nitella* cells and the stage IVb sporangiophores which slightly alters the analysis; *Nitella* grows along its entire length, and the stage IVb sporangiophore does not. In fact, the stage IVb sporangiophore only grows in a region immediately below the sporangium termed the "growing zone," and the length of the growing zone, L_g, remains nearly constant during this stage of development: $L_g \simeq 3$ mm. This difference requires that Eqs. 6 and 7 be slightly modified for the sporangiophores of *Phycomyces*.

Modified forms of Eqs. 6 and 7 may be obtained from the basic rate equation for irreversible wall extension (Green et al., 1971):

$$dL/dt = m(P - Y), \tag{8}$$

where m has the dimensions of the reciprocal of viscosity. Now, taking the differential of Eq. 8, assuming m and Y are constant, Eq. 9 is obtained:

$d(dL/dt) = m dP$, or using finite differences;

$$\Delta(dL/dt) = m\Delta P. \tag{9}$$

Solving for m, Eq. 10 is obtained:

$$m = \Delta(dL/dt)/\Delta P. \tag{10}$$

Therefore m can be determined by measuring the difference in elongational growth rate, $\Delta(dL/dt)$, before and after the step-up in turgor pressure, and dividing by the magnitude of the step-up in turgor pressure, ΔP. The average growth rate for a 10 min period before and after the turgor pressure step-up was used to determine the difference in elongational growth rate. The average value for the irreversible wall extensibility, m, was determined to be 898 μm MPa^{-1} min^{-1} with a standard deviation of $\pm$660 μm MPa^{-1} min^{-1} for 20 experiments (m = 898 $\pm$ 660 μm MPa^{-1} min^{-1}, n = 20).

A relative rate equation for irreversible wall extension

in the growing zone may be obtained by dividing Eq. 8 by the length of the growing zone, L_g:

$$(dL/dt)/L_g = (m/L_g)(P - Y). \quad (11)$$

The coefficient, m/L_g, is defined as the "relative irreversible wall extensibility for the growing zone of the sporangiophore," ϕ_g, and may be determined by dividing m by the length of the growing zone: $\phi_g = m/L_g$. The average value for ϕ_g was determined to be 0.33 MPa^{-1} min^{-1} ($\phi_g = 0.33 \pm 0.23$ MPa^{-1} min^{-1}, $n = 20$).

The yield threshold, Y, may be determine from Eq. 8 and the data before the step-up in turgor pressure since m, P, and dL/dt are known and constant for these periods of growth. The average value for the yield threshold was determined to be 0.26 MPa ($Y = 0.26 \pm 0.049$ MPa, $n = 20$). Furthermore, the average difference in the magnitude between the turgor pressure and the yield threshold ($P - Y$) was determined to be 0.053 MPa [$(P - Y) = 0.053 \pm 0.043$ MPa, $n = 20$].

Because it is not known why the sporangiophore's growth rate was reduced in ~30–40% of the experiments and what quantitative effect this might have on the subsequent growth responses (even though, qualitatively, the responses appear identical), only the results in which the sporangiophore's growth rate remained constant before and after the cell was impaled, were used to determine m, ϕ_g, Y and $(P - Y)$.

Importantly, an average growth rate of 47.6 μm/min is calculated when the average values obtained for m and $(P - Y)$ are substituted into Eq. 8 ($m = 898$ μm min^{-1} MPa^{-1}, and $(P - Y) = 0.053$ MPa). The value of 47.6 μm/min for the average growth rate falls within the range of 30–78 μm/min reported by most investigators (Bergman et al., 1969; and Cerda-Olmedo and Lipson, 1987).

DISCUSSION

In the past few years, the pressure probe has been used to conduct in vivo stress relaxation experiments to determine the relative irreversible wall extensibility, ϕ, and the yield threshold, Y, for higher plant tissue (Cosgrove et al., 1984; Cosgrove, 1985 and 1987). Although the in vivo stress relaxation method represents a significant improvement over earlier methods, it also presents difficulties in experimental practice. First, the growing plant tissue must be excised from the rest of the plant so that it may be isolated from its water source Cosgrove (1985 and 1987); and in the case of the single-celled sporangiophore of *Phycomyces blakesleeanus,* the sporangiophore must be plucked from the mycelium. Typically, plucked sporangiophores grow at slower rates than do normal sporangiophores, and after hours of growth have smaller diameters. Thus the irreversible wall extensibility, and perhaps the yield threshold, determined from the in vivo stress relaxation experiments may not be representative of normal sporangiophore growth. The results of the in vivo stress relaxation experiments conducted on the stage IVb sporangiophore demonstrate that the growth usually does not cease when the water supply is removed, and the turgor pressure does not decay for hours afterwards. A requirement for a successful stress relaxation experiment is that the cell enlargement rate (growth rate) be zero during the turgor pressure decay (Cosgrove, 1985; and Ortega, 1985). Presumably, in addition to the reduced ϕ resulting from the reduced growth rate of plucked sporangiophores, there exists an unknown water supply, possibly in the cell wall chamber (Cosgrove et al., 1987), which continues to supply water for slow growth after the external water supply is removed. A second difficulty is that transpiration must be eliminated, or effectively suppressed, in order to conduct a successful stress relaxation test (Cosgrove, 1985). Thus, a special environmental chamber must be constructed to increase the relative humidity to 100%. The third difficulty revolves around the requirement that the volumetric elastic modulus, ϵ, must be constant and known over the range of turgor pressure decay in order to determine ϕ. In general, it is known that ϵ is a nonlinear function of P for higher plant cells (Zimmermann, 1978) and for the sporangiophores of *Phycomyces blakesleeanus* (Ortega, 1988*a*). This result demands that $\epsilon(P)$ be measured to insure that ϵ is constant over the range of turgor pressure decay. Furthermore, if ϵ is not constant over the range of turgor pressure decay, ϕ cannot be determined from the equation that describes the turgor pressure decay for an ideal stress relaxation experiment (Eq. 4). In the case of the sporangiophores, ϵ is not constant over the range of turgor pressure decay.

Because the in vivo stress relaxation experiments could not be used to determine the irreversible wall extensibility of the sporangiophore, we looked to the earlier method used by Green (1968) and Green et al. (1971) to determine both the relative irreversible wall extensibility and the yield threshold. Their work was conducted before the introduction of the pressure probe, thus we altered their method slightly to employ the pressure probe. The use of the pressure probe to conduct in vivo creep experiments offers two advantages over the earlier technique. First, it more accurately measures the turgor pressure, and does so continuously. Second, it is possible to change the turgor pressure in <1 min with the pressure probe, and thus more accurately approximate a step-up in turgor pressure. This, in fact, was one of the difficulties Green (1968) and Green et al. (1971) had identified with their method of changing the turgor pressure by adding an osmoticum to the solution in which the *Nitella* cell was

immersed, because a significant amount of time is require for the osmoticum to diffuse through the cell wall and impose a change in turgor pressure. A disadvantage to using the pressure probe to change the turgor pressure, is that a step-down in turgor pressure cannot be produced and maintained. In theory, a turgor pressure step-down can be achieved simply by lowering the pressure in the pressure probe chamber (by retracting the control rod) and drawing out cell sap and/or silicon oil into the microcapillary tip. In practice, the cell sap of the sporangiophore forms a plug in the microcapillary tip and the turgor pressure can no longer be monitored and/or controlled.

In general, the in vivo creep experiments proved to be easier to conduct when compared with the in vivo stress relaxation experiments, and the obtained results were much more reproducible. Furthermore, because the sporangiophore does not need to be plucked, removed from its water source, or isolated and maintained in an environment of 100% relative humidity, the results of the in vivo creep experiments are more representative of the sporangiophore in its normal condition and natural environment. The results of the in vivo creep experiments demonstrate that the growth rate increases after a small step-up in turgor pressure ($\Delta P < 0.02$ MPa). Using these results, the relative irreversible wall extensibility of the growing zone, ϕ_g, the yield threshold, Y, and the difference between the turgor pressure and yield threshold, $(P - Y)$, were determined ($\phi_g = 0.33 \pm 0.23$ MPa^{-1} min^{-1}, $n = 20$; $Y = 0.26 \pm 0.049$ MPa, $n = 20$; and $(P - Y) = 0.053 \pm 0.043$ MPa, $n = 20$, respectively).

Green et al. (1971) determined $\phi \simeq 0.0167$ MPa^{-1} min^{-1}, and $(P - Y) \simeq 0.02$ MPa for *Nitella*. Thus, ϕ_g for the stage IVb sporangiophore's growing zone is approximately an order of magnitude larger than ϕ for *Nitella*, while $(P - Y)$ for the stage IVb sporangiophore is comparable with that of *Nitella*. Also, Green et al. (1971) and Taiz (1984) determined that there exist two kinds of yield thresholds for *Nitella;* adjustable, Y_a, and nonadjustable, Y_{na}. They determined $Y_a \simeq 0.48$ MPa and $Y_{na} \simeq 0.2$ MPa for *Nitella*.

Results from the in vivo creep experiments conducted on the stage IVb sporangiophores of *Phycomyces* indicate that $Y \simeq 0.26$ MPa. On the other hand, the results from the in vivo stress relaxation experiments indicate that $Y \simeq 0.1$ MPa (although it is to be noted that the growth rates of the plucked sporangiophores were smaller than those of normal sporangiophores). These results may suggest that the stage IVb sporangiophore also has an adjustable, Y_a, and nonadjustable, Y_{na}, yield threshold. Because the turgor pressure decay in the in vivo stress relaxation experiments take at least tens-of-minutes to complete, the yield threshold may adjust to its lowest "nonadjustable" value during that time. Thus, the turgor pressure in the in vivo stress relaxation experiments may decay to Y_{na} ($Y_{na} \simeq 0.10$ MPa). On the other hand, because the yield threshold is obtained from a period of time when the turgor pressure and growth rate are constant in the in vivo creep experiments, this method may give Y_a ($Y_a \simeq 0.26$ MPa). If this is true, then the respective yield thresholds differ by a factor of approximately two, as they do in *Nitella*.

Interestingly, larger steps-up in turgor pressure ($\Delta P > 0.02$ MPa) produce an initial increase in growth rate for a short period of time (typically 1 min), followed by a decrease in growth rate which is less than the original rate before the step-up. The initial increase appears to be proportional to the magnitude of step-up and is thought to cause strain-hardening of the cell wall. This may account for the subsequent decrease in growth rate. The behavior of the subsequent decrease in growth rate also appears to be related to the magnitude of the step-up; where larger steps-up in turgor pressure produce larger decreases in growth rate and longer periods of decreased growth rate. This growth rate behavior may also be explained by strain-hardening of the cell wall after the turgor pressure step-up.

Dennison and Roth (1967) demonstrated that the growth rate decreased for a period of time (several minutes) after a constant longitudinal load was applied to the stage IVb sporangiophore (a weight was attached to an inverted stage IVb sporangiophore). Minutes afterwards, the growth rate resumed its basal rate. When the longitudinal load was removed (the weight was removed), the growth rate increased for another period of time (several minutes), after which it returned to its basal growth rate. They termed this growth rate behavior to a step-change in longitudinal load as the "stretch response." Qualitatively, the decreased growth rate behavior elicited by large steps-up in turgor pressure ($\Delta P > 0.02$ MPa), is very similar to the stretch response reported by Dennison and Roth (1967).

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