Cell Wall Extension Behavior of *Phycomyces* Sporangiophores during the Pressure Response

Joseph K. E. Ortega, Martin E. Smith, and Mark A. Espinosa
Department of Mechanical Engineering, Bioengineering Laboratory, University of Colorado at Denver, Denver, Colorado 80217-3364 USA

ABSTRACT The cylindrical, single-celled sporangiophore of *Phycomyces blakesleeanus* grows (enlarges) predominantly in the longitudinal direction during two stages of development; stage I and stage IVb. Cell enlargement (cell wall extension) occurs in a distinct region termed the "growing zone." It was previously reported that a large step-up or pulse-up in turgor pressure, greater than approximately 0.02 MPa, will elicit a transient decrease in longitudinal growth rate of the stage I and stage IVb sporangiophore. This transient decrease in longitudinal growth rate is termed the "pressure response." Both the magnitude and duration of the pressure response depend on the magnitude of the turgor pressure step-up or pulse-up. Qualitatively, the pressure response is similar to the stretch response, which is produced with the application of a longitudinal force (load) on the sporangiophore. In this investigation, the growth (extension) behavior of the cell wall in the growing zone is studied during the pressure response. It is found that both the extension rate of the cell wall in the growing zone and the length of the growing zone decrease during the pressure response, and that together they account for the observed decrease in longitudinal growth rate.

INTRODUCTION

One reason that single-celled sporangiophores of *Phycomyces blakesleeanus* have been extensively studied is because they demonstrate many sensory responses to environmental signals. Most of these responses are either growth responses (transient changes in growth rate) or tropic responses (Bergman et al., 1969; Cerda-Olmedo and Lipson, 1987). It is well documented that the cell wall of this cylindrical sporangiophore grows in a distinct region termed the "growing zone," and that during two stages of development (stage I and stage IVb) the sporangiophore enlarges predominantly in the longitudinal direction (Bergman et al., 1969; Cerda-Olmedo and Lipson, 1987). Typically, the stage I sporangiophore grows from its mycelium as a tapered tube to a length of 1–3 cm and has a diameter of 0.15–0.25 mm near the mycelium. The growing zone is located between the apical tip and a region 0.5–2.0 mm from the tip. Longitudinal growth rates of stage I sporangiophores range between 0.3 and 1.5 mm/h. The stage IVb sporangiophore consists of a cylindrical stalk with a spherical sporangium (0.5 mm in diameter) on top. The diameter of the single-celled stalk varies from near the mycelium (approximately 0.25 mm) to the base of the sporangium (where it attaches to the sporangium; approximately 0.10 mm). The approximate location of the growing zone is between 0.10 and 2.5 mm from the base of the sporangium. Longitudinal growth rates of stage IVb sporangiophores range between 1.5 and 3.6 mm/h. The stalk can grow to a length of 10–20 cm.

In addition to many other sensory responses, stage I and stage IVb sporangiophores also demonstrate growth responses to steps-up in turgor pressure that are produced with a pressure probe. Ortega et al. (1989, 1991) demonstrated that a step-up in turgor pressure will elicit either an increase or decrease in longitudinal growth rate, depending on the magnitude of the turgor pressure step-up. A turgor pressure step-up smaller than approximately 0.02 MPa will elicit an increase in longitudinal growth rate, whereas a larger turgor pressure step-up will elicit a transient decrease in longitudinal growth rate. The transient decrease in longitudinal growth rate, produced by a large turgor pressure step-up ($\Delta P > 0.02$ MPa), is termed the "pressure response." Both the magnitude and duration of the pressure response 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. A large turgor pressure "pulse-up" (produced with a pressure probe) will also elicit a pressure response; typically the magnitude of the pulse-up must be greater than 0.02 MPa. Again, both the magnitude and duration of the pressure response depend on the magnitude of the turgor pressure pulse-up. The pressure response appears to be qualitatively similar to the "stretch" response previously reported by Dennison and Roth (1967).

In the present investigation, the growth (extension) rate of two cell wall regions (originally in the growing zone during steady-state growth) are measured and compared, before and during the pressure response. The pressure response was produced by a large turgor pressure pulse-up, typically larger than 0.05 MPa.

MATERIALS AND METHODS

Biological material

Vegetative spores of the wild type strain of *P. blakesleeanus* NRRL1555 (−) were inoculated in glass shell vials on sterile growth medium consisting of

Received for publication 14 June 1993 and in final form 8 August 1994.

Address reprint requests to Joseph K. E. Ortega, Director of the Bioengineering Laboratory, Department of Mechanical Engineering, University of Colorado at Denver, Campus Box 112, P.O. Box 173364, Denver, CO 80217-3364. Tel.: 303-556-8489; Fax: 303-556-6371.

0006-3495/95/02/702/06 $2.00

4% (w/v) potato dextrose agar, 0.1% (v/v) Wesson oil, and 0.006% (w/v) thiamine. After inoculation, the vials were incubated under continuous light from four fluorescent light bulbs (cool-white, 40 W each, located 0.5 m above the vials) at high humidity and constant temperature (21 ± 1 °C). Typically, 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 I sporangiophores (1–2 cm in length) and stage IVb sporangiophores (2–4 cm in length) were selected for experiments from the third to seventh crop.

Turgor pressure

The turgor pressure of the sporangiophore was continuously measured with a manual version of the pressure probe (Ortega et al., 1989, 1991). The pressure transducer used in the pressure probe was the "gage" type that measures the difference between the absolute pressure and the local atmospheric pressure; it was purchased from Kulite Semiconductor Products Inc. (Ridgefield, NJ; model XT-190–300G). The pressure transducer was calibrated inside the pressure probe with a Heise Bourdon Tube Pressure Gauge from Dresser Industries (Newton, CT; model CMM, 0–200 PSIG Range). 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 (model H-2 from Line Tool Company, Allentown, PA, with large barrel micrometer heads) so that its microcapillary tip (typically ≈15 μm outer diameter) could be guided to impale the sporangiophore under visual observation using a horizontally mounted EZM-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., Corning, NY; fluid 200, 1–2 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 equilibrium turgor pressure of the sporangiophore (Ortega et al., 1989, 1991). A step-up in turgor pressure was produced in the sporangiophore by injecting inert silicon oil into the cell vacuole with the pressure probe, using the method previously described by Ortega et al. (1989, 1991). A pulse-up in turgor pressure, of any predetermined duration, was produced by stopping the injection of oil in a step-up and returning the turgor pressure to its initial equilibrium value.

Growth and cell wall extension measurements

Longitudinal growth of stage I and stage IVb sporangiophores was determined by measuring the longitudinal displacement of the apical tip (for stage I) or the longitudinal displacement of the base of the sporangium (for stage IVb) at regular time intervals (1 min). The cell wall growth (extension) behavior within the growing zone was studied by attaching small starch grain markers (10–60 μm in diameter) at different locations on the cell wall within the growing zone and measuring their longitudinal displacement as a function of time. A sharp needle was used to pick up and attach the starch grain markers to the sporangiophore; typically, the starch grains adhere to the cell wall surface as soon as they contact it. The longitudinal displacement of each starch grain marker was determined by measuring its location on the sporangiophore at regular time intervals (1 min) and plotting its displacement, X, from its initial location as a function of time. The longitudinal displacement of the apical tip (stage I), the base of the sporangium (stage IVb), and the individual markers were measured using a long focal length horizontal microscope (Gaertner; 7011K eyepiece and 32 m/m EFL objective) mounted to a 3-D micromanipulator (Line Tool Company, Allentown, PA; model H-2) with digital micrometer heads (resolution of 1.0 μm). A stop watch was used to measure the time intervals.

Protocol for the pressure response experiments

The following procedure was used to conduct the pressure response experiments. A stage I or stage IVb sporangiophore in a glass shell vial was selected and adapted for 30 min to the room temperature of 21–22°C, to room lights (cool-white fluorescent lamps hung from the ceiling), and to bilateral illumination from a fiber-optic illuminator (Flexilux 90; HLU Light Source 90/W from Schoelly Fiberoptic, Denzlingen, Germany, which filtered out nearly all of the infrared light) with two swan neck light guides (the end of each light guide was positioned ≈8–12 cm on either side of the sporangiophore at an angle of about 60° from the horizontal). The irradiance of the adapting light at the location of the sporangiophore (fluence rate) was measured with an irradiance probe (Tektronix, Beaverton, OR; model J6502) and typically ranged from 25 to 50 W/m^2. After the adaptation period, typically two small starch grain markers (10–60 μm in diameter) were attached at specific locations on the cell wall within the growing zone of either the stage I or stage IVb sporangiophore. Then the longitudinal displacement of starch grain markers and the longitudinal growth of the sporangiophore were measured every min for the remainder of the experiment.

When a 15 min period of steady growth rate was observed, the sporangiophore was impaled with the microcapillary tip of the pressure probe to measure the turgor pressure. Then the turgor pressure, longitudinal growth, and longitudinal displacement of the starch grain markers were measured and monitored for another 10 min period. After this monitoring period, a pulse-up in turgor pressure (1 min duration) was produced with the pressure probe by injecting inert silicon oil into the cell vacuole. Subsequent to the pulse-up, the turgor pressure was clamped at its previous equilibrium value, and the longitudinal growth and the longitudinal displacement of the starch grain markers were measured for another 20 min. Then a second turgor pressure pulse-up of equal magnitude and duration was produced, and again, subsequently the turgor pressure was clamped at its previous equilibrium value, during which time the longitudinal growth and the longitudinal displacement of starch grain markers were measured for another 20 min. In a couple of experiments, a third turgor pressure pulse-up of equal magnitude was produced.

Displacement curves and "RDR" values

Fig. 1 illustrates the longitudinal displacement of three markers (M1, M2, and M3) attached to the growing zone of a sporangiophore. The longitudinal displacement of each marker (as a function of time; T1 − T5) can be represented by a "displacement curve," which is produced by plotting the marker location at equal time intervals; in Fig. 1, this is done by simply connecting the marker location at each subsequent time interval.

In Fig. 1 *A*, it can be observed that when growth is occurring below the marker, the respective displacement curve has a positive slope, and if the marker displacement rate is constant, the curve is a straight line (M1 and M2). Also, the end of the growing zone can be determined with these displacement curves, because once the growing zone has moved above the cell wall region to which the marker is attached, the marker will no longer be displaced, and the slope of its displacement curve will be zero (M3, when $t >$ T3). It is also observed that displacement curves with different positive slopes are produced when growth is occurring between the markers.

A quantitative assessment of the relative growth rate (relative extension rate) occurring in the region between any two markers can be made using the following method. Consider the displacement of M1 and M2 during the time interval, Δt = T5 − T4 (Fig. 1 *A*). The rate at which M1 is displaced with respect to M2 is simply the difference between their displacement rates; $\Delta X(1)/\Delta t - \Delta X(2)/\Delta t$. A measure of the average rate of extension per unit length of cell wall (between M1 and M2) can be obtained by normalizing the difference in marker displacement rate to the distance between the

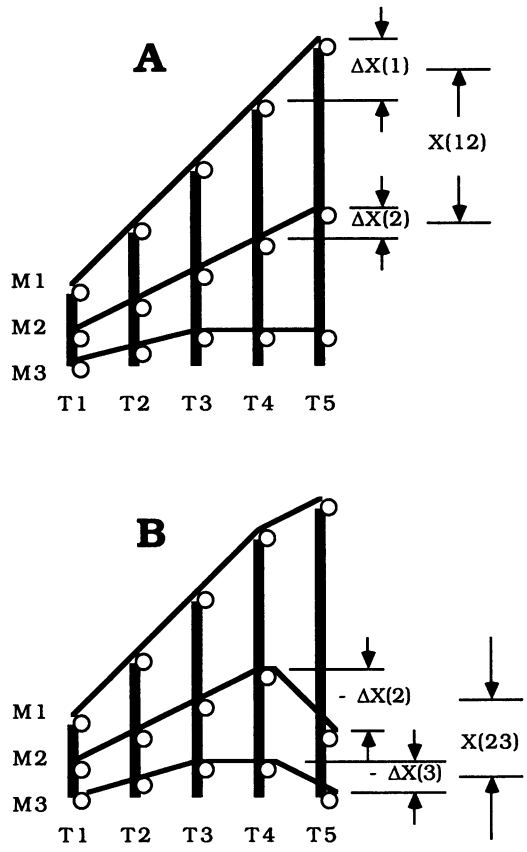

FIGURE 1 A schematic illustration of three markers (M1, M2, and M3) initially located in the growing zone of the sporangiophore and their displacement in time, for two different cases. In the first case (*A*), extension (growth) occurs between the markers in all subsequent time intervals. Lines connecting each marker at successive times produce three displacement curves that diverge. It is also noted that when net extension occurs below a marker, its corresponding displacement curve has a positive slope; a curve with zero slope indicates that the net extension in the regions below it is zero (M3 at T3, T4, and T5). In the second case (*B*), two characteristics of the displacement curves are illustrated when cell wall contraction occurs in the region between two markers and in the region below it. It can be seen that contraction between M2 and M3 during the time interval between T4 and T5 produces displacement curves that converge during that time interval. Furthermore, corresponding displacement curves for M2 and M3 have negative slopes during the time interval when contraction is occurring in the region below it.

markers, $X(12)$; then the *relative* displacement rate (RDR) between M1 and M2 is calculated using the following equation: $RDR = [\Delta X(1)/\Delta t - \Delta X(2)/\Delta t]/X(12)$. The advantage of determining the relative displacement rate (RDR) between two markers is that the RDR values obtained for different regions in the growing zone can be compared with one another to determine which regions are growing faster or slower per unit length, because the distances between markers are not always the same. In general, the "relative displacement rate" for any two markers, M1 and M2, is defined and calculated using the following equation

$$\mathrm{RDR} = \frac{dX(1)/dt - dX(2)/dt}{X(12)}, \qquad (1)$$

where $dX(1)/dt$ is the longitudinal displacement rate of M1, $dX(2)/dt$ is the longitudinal displacement rate of marker M2, and $X(12)$ is the average distance between the two markers during the time interval. The RDR value can provide qualitative as well as quantitative information. Consider the marker displacement behavior displayed in Fig. 1 *B*. The distance between M2 and M3 is shortened during the time interval, Δt = T5 − T4; this represents a "contraction" in the cell wall region between M2 and M3. When the RDR value is calculated for M2 and M3 during this time interval, the following expression is obtained: $RDR = [\{-\Delta X(2)/\Delta t\} - \{-\Delta X(3)/\Delta t\}]/X(23)$, and because the magnitude of $\{-\Delta X(2)/\Delta t\}$ is greater than that of $\{-\Delta X(3)/\Delta t\}$, the sign of the RDR value will be "minus." Therefore, a "minus" RDR value represents a contraction in the region of cell wall between respective markers. It should also be noted that the displacement curve has a "negative" slope when the cell wall in the region below the marker is undergoing contraction.

RESULTS

Fig. 2 presents the results of a pressure response experiment conducted on a stage IVb sporangiophore. The turgor pressure trace from the chart recorder is presented in Fig. 2 *A*. Three turgor pressure pulses-up (0.16 MPa in magnitude and 1 min in duration) were produced with the pressure probe at 20 min intervals. Fig. 2 *B* shows the displacement of the top of the growing zone (base of the sporangium) and the displacement of three starch grain markers (M1, M2, and M3) as a function of time (marker displacement curves; the same time scale is used for both *A* and *B*); for convenience, the marker displacement curves are plotted so that they begin at a common point. Typically, growing zones of stage IVb sporangiophores are located in the region between the base of the sporangium and a distance of approximately 2.5–3.0 mm from the base. The upper curve (Fig. 2 *B*) is the displacement of the base of the sporangium (labeled "base") as a function of time; the curve below the upper curve represents the displacement of M1 (initially M1 was located 1.02 mm from the base of the sporangium); the displacement of M2 is represented by the next curve (initially M2 was located 2.57 mm from the base); and the displacement of M3 is represented by the bottom curve (initially M3 was located 9.01 mm from the base, which is far below the growing zone). The first narrow arrow on the time scale (*left to right*; at 10 min) indicates the time when the sporangiophore was impaled to measure the turgor pressure. The second, third, and fourth broad arrows on the time scale (*left to right*) indicate the times when a turgor pressure pulse-up was produced with the pressure probe.

The RDR values (in brackets) for the regions between respective markers are presented in Fig. 2 *B*. The "average" marker displacement rate for the 10 min interval before and after the respective turgor pressure pulse-up was used to calculate the respective RDR value. The results demonstrate that RDR values for both the upper region (0–1.02 mm; between the base of the sporangium and M1) and lower region (1.02- 2.57 mm; between M1 and M2) of the growing zone

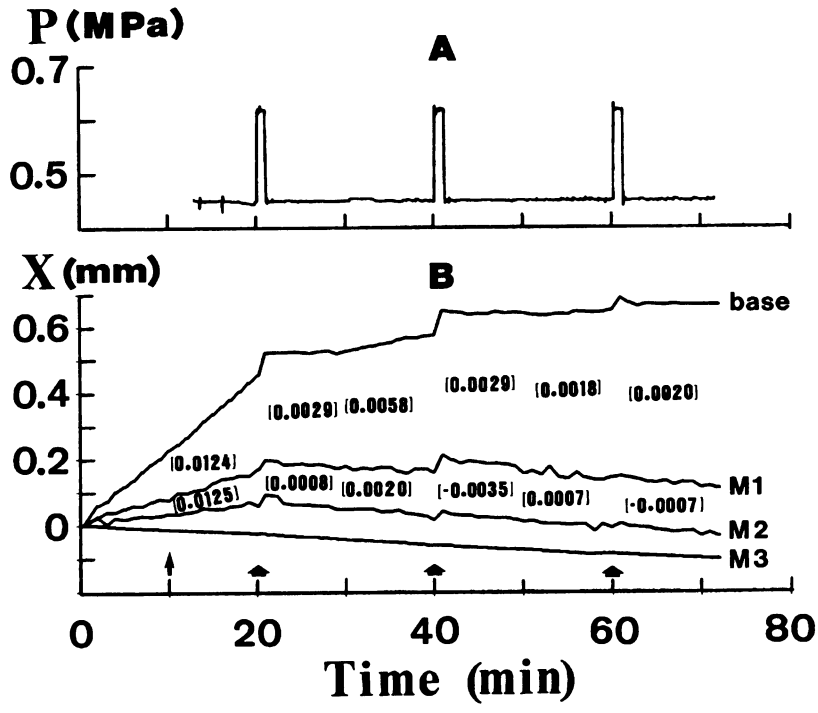

FIGURE 2 The turgor pressure, P, trace from the chart recorder versus time (A). The displacement, X, of the base of the sporangiophore (overall growth) and of three starch grain markers (M1, M2, and M3) versus the same time scale for a single pressure response experiment conducted on a stage IVb sporangiophore (B). The first narrow arrow on the time scale (*left to right*; at 10 min) marks the time when the sporangiophore was impaled by the pressure probe to measure the turgor pressure. Subsequent broader arrows on the time scale mark the time when turgor pressure pulses-up (1 min in duration and 0.16 MPa in magnitude) were produced in the sporangiophore with the pressure probe. The numbers in brackets are RDR values.

are nearly identical for the 10 min interval before the first turgor pressure pulse-up (RDR values of 0.0124 and 0.0125, respectively). This indicates that, in this experiment, the growth rate per unit length of cell wall is approximately the same in the upper and lower regions of the growing zone. The results also demonstrate that the magnitude of respective RDR values for both upper and lower regions of the growing zone are reduced after the first and second pulse-up. There is some recovery (magnitudes of the RDR values increase) in both upper and lower growing zone regions during the 10 min period before the second pulse-up. Interestingly and importantly, the sign of the RDR values for the lower region (1.02–2.57 mm; between M1 and M2) is "minus" after the second ($RDR = -0.0035$) and third ($RDR = -0.0007$) pulse-up, which suggests that a small amount of cell wall contraction is occurring in this lower region during these time periods. Another characteristic of the displacement curves, which would suggest cell wall contraction, is the "negative" slope of the bottom displacement curve (for M3, which is far below the growing zone) throughout the experiment, and the "negative" slopes of the two displacement curves above it (for M1 and M2) after the first, second, and third pulse-up. However, upon further investigation, it was found that this negative slope can be accounted for by "agar shrinkage"; because water is continually being removed from the agar by evaporation and by the sporangiophores (because the sporangiophores are growing and transpiring), the agar decreases in volume and continuously lowers the mycelium on which the sporangiophores grow. This "agar shrinkage" *cannot* account for the indicated contraction between markers.

Average RDR values obtained for upper and lower growing zone regions of stage IVb sporangiophores are summarized in Table 1. The mean values (±SE) for locations of M1 and M2, measured from the top of the growing zone (base of the sporangium), were as follows: $X_{M1} = 2.07 \pm 0.21$ mm ($n = 7$), and $X_{M2} = 3.19 \pm 0.19$ mm ($n = 5$). Therefore, the "average" upper growing zone region is between the base of the sporangium and the average location of M1 (0–2.07 mm), and the "average" lower growing zone region is located be-

TABLE 1 RDR values of upper and lower growing zone of stage IVb

Period (10 min)	Upper region (0–2.07 mm) RDR (min^{-1})	n	Lower region (2.07–3.19 mm) RDR (min^{-1})	n
Pulse-up #1				
before	0.0121 ± 0.0008	7	0.0042 ± 0.0010	7
after	0.0035 ± 0.0009	7	0.0014 ± 0.0008	7
Pulse-up #2				
before	0.0050 ± 0.0008	6	−0.0004 ± 0.0005	5
after	0.0017 ± 0.0011	6	−0.0006 ± 0.0004	5
Pulse-up #3				
before	0.0013 ± 0.0005*	2	−0.0023‡	1
after	0.0007 ± 0.0013*	2	−0.0010‡	1

Comparison of the average relative displacement rate (RDR) values (see Eq. 1 for definition), for the upper region (0–2.07 mm; 0 is the base of the sporangium) and lower region (2.07–3.19 mm) of the growing zone of stage IVb sporangiophores (typically the growing zone is located in the region between the base of the sporangium and 3 mm from it). The RDR values are determined using displacement rates that are averaged over a 10 min period, before and after the first, second, and third turgor pressure pulse-up. Each value presented is the mean ± SE; n = the number of experiments (values) used. Magnitudes of turgor pressure pulses-up were between 0.08 and 0.16 MPa ($\Delta P = 0.12 \pm 0.01$ MPa; $n = 7$), and magnitudes of equilibrium turgor pressure were between 0.38 and 0.48 MPa ($P = 0.43 \pm 0.02$ MPa; $n = 7$).

*These SE values are unreliable because of the small sample size.

‡For the cell wall region between 2.60 and 9.01 mm from the base of the sporangium.

tween average locations of M1 and M2 (2.07–3.19 mm). Three pressure response experiments were also conducted with stage I sporangiophores. Average RDR values obtained for upper and lower growing zone regions of stage I sporangiophores are summarized in Table 2. The mean values for the locations of M1 and M2, measured from the apical tip (top of the growing zone) were as follows: $X_{M1} = 0.67 \pm 0.11$ mm ($n = 3$), and $X_{M2} = 1.39 \pm 0.09$ mm ($n = 3$). Therefore, the "average" upper growing zone region is between the apical tip and the average location of M1 (0–0.67 mm), and the "average" lower growing zone region is located between the average location of M1 and M2 (0.67–1.39 mm).

DISCUSSION

The results presented in Fig. 1, Table 1, and Table 2 demonstrate that, in general, relative rates of cell wall extension (RDR values) in both upper and lower regions of the growing zone are reduced in magnitude after large turgor pressure pulses-up ($\Delta P > 0.05$ MPa). Smaller RDR values occur during the same time period as do the pressure responses of stage I and stage IVb sporangiophores (transient decreases in growth rate produced by large turgor pressure pulses-up; $\Delta P > 0.02$). In addition, some experimental results indicate that the growing zone length, l_{gz}, also decreases during the pressure response. Results that demonstrate this behavior can be seen in Fig. 2 *B*. Observe that the RDR value for the lower cell wall region (between M1 and M2) is positive before the second turgor pressure pulse-up ($RDR = 0.0020$) and changes to a negative value ($RDR = -0.0035$) after the pulse-up. This behavior indicates that this cell wall region (between M1 and M2) was in the growing zone before the second pulse-up (because the RDR value is positive), and then below the growing zone (because the RDR value is negative) during the second pressure response (after the second pulse-up). This result indicates that l_{gz} decreased during the pressure response. This cell wall extension behavior cannot be explained simply by the idea that the cell wall region moved out of the growing zone because of normal steady-state growth, because during the subsequent 10 min time period (the recovery period before the third pulse-up) the RDR value for the same cell wall region (between M1 and M2) becomes positive again ($RDR = 0.0007$), which indicates that this region is again in the growing zone. This result indicates that l_{gz} increased again during the recovery period after the second pressure response. In general, the RDR behavior between M1 and M2 indicates that the end of the growing zone moved above M1 (to the region between the base of the sporangium and M1) during the second pressure response, and then moved below M1 (to the region between M1 and M2) during the recovery period after the second pressure response (10 min period before the third pulse-up). This type of RDR behavior between markers was found in 3 of the 10 experiments conducted. In general, this behavior was not observed in all of the experiments, probably because it is necessary to have markers at just the right location to detect this change in l_{gz}. In theory, the probability of having markers in a location that can be used to detect this behavior will increase by using more markers, similar to the experiments conducted by Ortega et al. (1991), where six markers were used to determine l_{gz}. However, the protocol for the previous experiments (Ortega et al., 1991) did not require the simultaneous use of the pressure probe. In the present investigation, experiments with more markers were attempted. However, it proved to be too difficult to measure the displacement of the additional markers, and to operate the pressure probe at the same time.

TABLE 2 RDR values of upper and lower growing zone of stage I

Period (10 min)	Upper region (0–0.67 mm) RDR (min^{-1})	Lower region (0.67–1.39 mm) RDR (min^{-1})
Pulse-up #1		
before	0.0071 ± 0.0016	0.0026 ± 0.0002
after	0.0034 ± 0.0013	0.0004 ± 0.0005
Pulse-up #2		
before	0.0054 ± 0.0014	0.0015 ± 0.0005
after	0.0013 ± 0.0005	−0.0017 ± 0.0020

Comparison of the average relative displacement rate (RDR) values (see Eq. 1 for definition), for the upper region (0–0.67 mm; 0 is the apical tip) and lower region (0.67–1.39 mm) of the growing zone of stage I sporangiophores (typically the growing zone is located in the region between the apical tip and 1.5 mm from the tip). The RDR values are determined using displacement rates that are averaged over a 10 min period, before and after the first and second turgor pressure pulse-up. Each value presented is the mean ± SE of three experiments (values) used; note that the SE values are unreliable because of the small sample size. Magnitudes of the turgor pressure pulses-up were between 0.05 and 0.08 MPa ($\Delta P = 0.06 \pm 0.01$ MPa; $n = 3$), and magnitudes of equilibrium turgor pressure were between 0.40 and 0.62 MPa ($P = 0.53 \pm 0.06$ MPa; $n = 3$).

In general, the obtained results indicate that the transient decrease in growth rate of the pressure response is achieved by reducing the relative rate of cell wall extension in the growing zone and shortening the length of the growing zone.

The suggestion that the length of the growing zone, l_{gz}, is involved in growth rate regulation during the pressure response is supported by findings of Trinci and Halford (1975) and Ortega et al. (1991). Work with stage I sporangiophores (Ortega et al., 1991; Trinci and Halford, 1975) demonstrate that the magnitude of steady-state longitudinal growth rate correlates with l_{gz}; i.e., faster growing cells have longer growing zones. Ortega et al. (1991) obtained an equation that correlates the average growth rate, dl/dt, with the average value for l_{gz}

$$dl/dt = 0.007(\text{min}^{-1})l_{gz}(\mu\text{m}) + 3.7(\mu\text{m min}^{-1}). \quad (2)$$

Ortega et al. (1991) also obtained a relationship between the irreversible wall extensibility, m, and l_{gz}

$$m = 0.28(\text{min}^{-1}\,\text{MPa}^{-1})l_{gz}(\mu\text{m}) - 24.0(\mu\text{m min}^{-1}\,\text{MPa}^{-1}); \quad (3)$$

m is an important biomechanical parameter in the "Augmented Growth Equations" (Ortega, 1990, 1993, 1994;

Ortega et al., 1989, 1991) that participates in growth rate regulation of plant and fungal cells. Importantly, these equations (Eqs. 2 and 3) produce correct average values for steady-state growth rate and irreversible wall extensibility of stage I and stage IVb sporangiophores when respective average values of l_{gz} for stage I and stage IVb sporangiophores are used (Ortega et al., 1991).

Earlier work by Ortega (1976) indicates that the growing zone length may also be involved in growth rate regulation during the "light growth response" (a transient increase in longitudinal growth rate produced by a step-up or pulse-up in light intensity). These results demonstrate that l_{gz} increases during the period of increased growth rate of the light growth response (produced by a pulse-up in light intensity).

A decreasing l_{gz} after a turgor pressure step-up or pulse-up is also consistent with earlier findings of Ortega et al. (1975). When the growing stage IVb sporangiophore was repeatedly loaded and unloaded (longitudinal loads were applied with a tension-compression machine), strain hardening of the cell wall occurred (Ortega et al., 1975), resulting in less extension per unit load (demonstrating a decrease in mechanical extensibility) for each subsequent loading-unloading cycle (mechanical conditioning). This decrease in mechanical extensibility continued for approximately 13 cycles, after which there was no further decrease. It was demonstrated that the cell wall region with the largest mechanical extensibility (located within the growing zone) reduces in length after mechanical conditioning, thus contributing to the observed reduction in total mechanical extensibility. The strain hardening and mechanical conditioning processes can be explained with the "fibril reorientation" hypothesis proposed by Ortega and Gamow (1974), which was used to explain spiral (helical) growth behavior of sporangiophores in all growing stages of development. The fibril reorientation hypothesis predicts that chitin microfibrils initially oriented in the transverse direction (with respect to the longitudinal axis of the sporangiophore) are reoriented toward the longitudinal axis during longitudinal extension of the cell wall caused by turgor pressure during normal growth, or by longitudinal loads applied with a tension-compression machine. It is predicted that the mechanical extensibility of the cell wall will decrease as more microfibrils are reoriented toward the longitudinal axis.

The "negative" RDR values obtained in the pressure response experiments (Fig. 2, Tables 1, and Table 2) suggest that the lower cell wall region of the growing zone (which was initially extending during steady-state growth) not only stopped extending, but actually contracted during the pressure response. These results are consistent with those of Smith (1990), which also suggest that a small amount of cell wall contraction may be occurring in the cell wall region adjacent to the lower region of the growing zone. This cell wall contraction could be explained in terms of a wall-stiffening process that would result in a change in the elastic properties of the cell wall. Hohl and Schopfer (1992) have observed changes in cell-wall elasticity of plant tissue (*Zea mays* L.) that is associated with changes in growth. In terms of the Augmented Growth Equations, this process might be reflected by a change in the magnitude of the volumetric elastic modulus, ϵ. Future investigations will seek to confirm the suggested cell wall contraction and to determine whether it is accompanied by a change in magnitude of the volumetric elastic modulus.

This work was supported by National Science Foundation grant IBN-9103760 to J. K. E. Ortega.

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