Phycomyces: Irregular Growth Patterns in Stage IVb Sporangiophores

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ABSTRACT   Net rotation and net elongation of a stage IVb *Phycomyces* growing zone were simultaneously measured minute by minute with a photographic apparatus coupled with a rotating stage. A direct correlation between a growth response and a twist response after either a light stimulus or a house stimulus was found. There were significant irregularities in growth rate in both the elongation and rotation that were not a result of measurement error; these irregularities were poorly, if at all, correlated. We believe that these fluctuations reflect the underlying molecular mechanism of cell wall synthesis.

INTRODUCTION

More is known about the two-dimensional growth patterns generated by the giant sporangiophores of *Phycomyces* than any other living plant cell wall, with the possible exception of the work done by P. B. Green and his colleagues (Gertel and Green, 1977) on the internode cells of the aquatic algae of *Nitella axillaris*. This situation stems from the fact that, for the past 100 years, the *Phycomyces* sporangiophores have been extensively studied by sensory physiologists and biophysicists whose primary interest is to unravel the molecular mechanisms of sensory transduction; for them the *Phycomyces* is an elegant but simple model sensory system that may hold the key to understanding more complex sensory systems (Bergman et al., 1969). With two exceptions[1] (Bergman, 1972; Cerda-Olmedo, 1974), all the graded sensory responses described in *Phycomyces* involve a change in the growth patterns of the living cell wall. In recent years, work in our laboratory has shown that additional growth patterns can be created by mechanically deforming the living cell wall (Ortega et al., 1975; Gamow and Böttger, 1979). These new and old growth patterns must in some way reflect the underlying molecular structure of the growing cell wall (Ortega and Gamow, 1974). The discovery of spiral growth in mature *Phycomyces* sporangiophores was first made by A. J. Oort (1931). Operationally, spiral growth is said to occur if a marker on the growing zone (GZ) is displaced both vertically by the elongational growth occurring above and below the marker and horizontally because of the rotation of the GZ. The rotational displacement is measured either in respect to some fixed object on the sporangiophore, such as the sporangium, or in respect to a fixed observer, i.e., the ground. This growth is two dimensional in the sense that the two-vector

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[1] The initiation of new sporangiophores can be cycled as a function of a day and night cycle. Secondarily, the induction of the pigment of β-carotene is also triggered by a light stimulus.

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components of growth, twist, and stretch can be thought of as occurring on a plane that has been folded into a conical stalk. Although an early report by Castle (1937) suggested that rotation and elongation (twist and stretch) were proportional to one another, recent reports (Cohen and Delbrück, 1958; Ortega et al., 1974) have clearly shown that they are not. Cohen and Delbrück (1958) realized that a material section of the GZ is not equivalent to a miniature GZ since each section of the GZ is in a steady-state flux characteristic of just that one GZ section. To quote from their 1958 paper, "The whole GZ is a constant structure, in the absence of stimulation, although it is in a state of turnover, like the flame of a candle. Each material section of the GZ, however, is not constant: it stretches, twists and moves down within the GZ. Experimentally, the chief difficulty of defining growth responses of material sections lies in the establishment of standards of reference." In any case, the motion of a particle placed *above* the GZ directly reflects the summation of all the twisting and stretching occurring in the entire GZ.

In this report, we describe a technique that allows us to measure with high accuracy the minute-to-minute displacement of a particle above or in the GZ. We find that both the vertical and horizontal displacements are quite irregular, and in addition, that they appear to be uncorrelated. There are two papers claiming that the growth rate is not in fact smooth—the 1931 paper by Oort and the 1940 paper by Castle. Both authors discuss in detail these irregular growth patterns. Certainly, the comparatively recent advent of the tracking machine has made the task of measuring the average growth rate and the changes in growth rate after a stimulus quite easy (Foster and Lipson, 1973), but this technique has to date shed no light on the question of whether the mature *Phycomyces* sporangiophore grows smoothly or not, because only average growth rates have been reported.

The aim of this report is to substantiate that these growth irregularities are real and significant and are not a result of measurement errors.

MATERIALS AND METHODS

Wild-type *Phycomyces blakesleeanus* sporangiophores, NRRL1555(−), originally obtained from M. Delbrück, were grown in shell vials containing 5% potato dextrose agar (PDA) with 1.0% yeast extract. The shell vials were incubated under diffuse incandescent light in a high-humidity room with a temperature range between 22° and 27°C. Before each experiment the sporangiophores were dark adapted in red light for at least 20 min. During the experiment, the humidity was reasonably high as a result of open water dishes surrounding the experimental sporangiophore. Unless otherwise stated, all experiments were carried out with a water-filtered red light source.

The apparatus used to simultaneously measure minute by minute the net rotation and the net elongation of a stage IVb GZ was a modification of an apparatus first described by Cohen and Delbrück (1958). The mature stage IVb sporangiophore in a glass shell vial was firmly secured to a stage that rotated clockwise once every 60 s. To ensure that the GZ of the sporangiophore was vertical (parallel to the axis of rotation of the stage), a double knee was inserted between the stage and the vial. The rotating stage allowed us to measure the angular velocity of any particle situated above, below, or in the GZ. Because the net rotation of a mature stage IVb

sporangiophore is in the same direction as the rotating stage (clockwise), a particle either above or in the GZ takes <60 s to complete one revolution. By determining how much less, we calculated the angular velocity of the particle in respect to the observer. (For instance, if a particle completes one revolution in 58 s, we can readily calculate that the entire region below the particle must have a total angular velocity of 12°/58 s or 12.41°/min.) For our present experiments, we used a glass bead ~15 μm in diameter. The bead was placed ~50 μm below the sporangium in the stalk region, which does not stretch. The GZ, with the attached glass bead, was observed continuously through a motor-driven 35-mm camera attached to a low-powered (~X 10) microscope. Each time the particle "came around," it was photographed. The effectiveness of the method stems from the fact that, at the instant the shutter is clicked, an electronic timer records the time to within 10 ms. The electronic timer and the camera are coupled by way of a signal to the flash mechanism generated by the camera; i.e., this signal triggers the timer. The photograph was only used to verify that the marker particle has indeed returned to its original starting point. In principle, if a timed particle had been seen to be "off center," we could have back corrected to determine the correct time; but we never found the correction necessary.

Although the photographs were not necessary for calculating the angular velocities, they were needed for measuring elongational velocities. To ensure that our apparatus was working properly, an artificial sporangiophore ("artificial Phyco") was constructed. It consisted of a straight, 0.5-mm diameter wire attached to a motor-driven micrometer screw. A small spur gear on the motorshaft of a synchronous clock motor (1 rpm) drove a larger spur gear on a metric micrometer head with a gear ratio of 1:8. The micrometer screw was constructed to advance at 63 μm/min. Two timed photograph methods were used to measure the growth rate of either a known object (the artificial sporangiophore) or the real sporangiophore. First, a calibrated scale placed in the ocular of the microscope was used to determine the growth rate of a straight sporangiophore. To avoid parallax errors, this method requires the use of straight sporangiophores. Perfectly straight, growing sporangiophores are nearly impossible to obtain, because they show both fast, 5–7.5 min, and slow, 30–60 min, oscillations; a term used to describe these oscillations is "sporangiophore hunting" (Shropshire, 1963). To determine whether our measurements were significantly influenced by either a parallax problem or sporangiophore hunting, we placed an additional marker below the growing zone and calculated directly the change in length between the two markers. The data obtained in this manner were no different from data obtained using the calibrated scale with reasonably straight sporangiophores. From a large series of photographs small bends were seen to occur in the GZ, but, since the bend angle between any pair of photographs taken in any 1-min interval was <1°, the error caused by bending was considered to be negligible. We have determined that hunting results in a bending rate of ~0.5°/min, but in a continuous set of measurements lasting for 38 min, the bending angle did not vary more than 5° from the vertical. From a single pair of photographs we, of course, cannot eliminate the possibility that the GZ is bent directly toward or directly away from the camera, but, since we find no significant bends in a large random sample of photographs, we have neglected this possibility. To ensure that our elongation measurements were not influenced by the rotating stage itself, we repeated a set of measurements with a stationary stage; we found that the growth measurement was not influenced by the rotating stage.

To measure the light growth response, a 1-min saturating light stimulus was unilaterally given to the rotating sporangiophore. The light source was an incandescent bulb (GE44, G.3 V, 0.25 A, General Electric Co., Cleveland, Ohio), with the

light passing through both a water filter and a Corning 5-61 blue filter (Corning Glass Works, Corning, N. Y.).

To obtain the house response, a glass house 1 X 1 X 4.5 cm was placed over the entire rotating sporangiophore. In both the light growth response and the house response, a photograph was taken each time the marker above the GZ completed one revolution.

RESULTS

Control Experiments

As described in Materials and Methods, a particle above or in the GZ would be expected to gain on the table, i.e., it would take < 60 s to complete one revolution. On the other hand, a particle below the GZ should rotate with the table; therefore, a series of measurements with a particle placed below the GZ makes an excellent control. In one such experiment, we timed the rotation for 56 consecutive minutes and obtained a sample average of 60.10 ± 0.28 s. Converting the standard deviation from second units into degree per minute units, we obtained a standard deviation of ±1.7°/min. That our sample average was 60.10 s instead of 60.00 s does not change our error measurement but indicates only that some small constant bias is occurring in our measuring instrument. We could have further decreased our error by back correcting from our photographs, but our error is small enough that we did not feel this to be necessary. The beauty of this method of measuring angular velocities is that the measurements are virtually insensitive to parallax problems, to hunting problems, and to changes in growth rate. Determining elongation velocities, on the other hand, is beset with numerous difficulties. We have determined the growth velocities from enlarged 35-mm photographs of the GZ with the sporangium. Our control is an artificial Phyco attached to a motor-driven micrometer screw (see Material and Methods). To obtain the average "growth" rate, the artificial Phyco was photographed once every minute for nine consecutive minutes, each photograph was remeasured 10 times, and the sample average and the standard deviation of all 90 measurements were found to be 62.87 ± 1.4 μm/min.

Growth and Rotation Experiments

Fig. 1 A and B shows the irregular rotation rate (bottom curve) and the irregular elongation rate (upper curve) of the entire GZ for two sporangiophores. The error bars on the elongation and growth curve, as described above, represent only our uncertainty in making repeated measurements from identical photographs and do not include the error that can occur because of hunting. There also appears to be little or no correlation between the fluctuations seen in growth and in rotation.

Rotation and Elongation Rates after a Light Stimulus

We used a procedure identical to the one described above for measuring simultaneously the rotation and elongation rates, except we gave a 1-min saturating blue light stimulus during the seventh minute. Fig. 2 A and B (data from two sporangiophores) shows that the correlation between the stretch response and twist response is quite good.

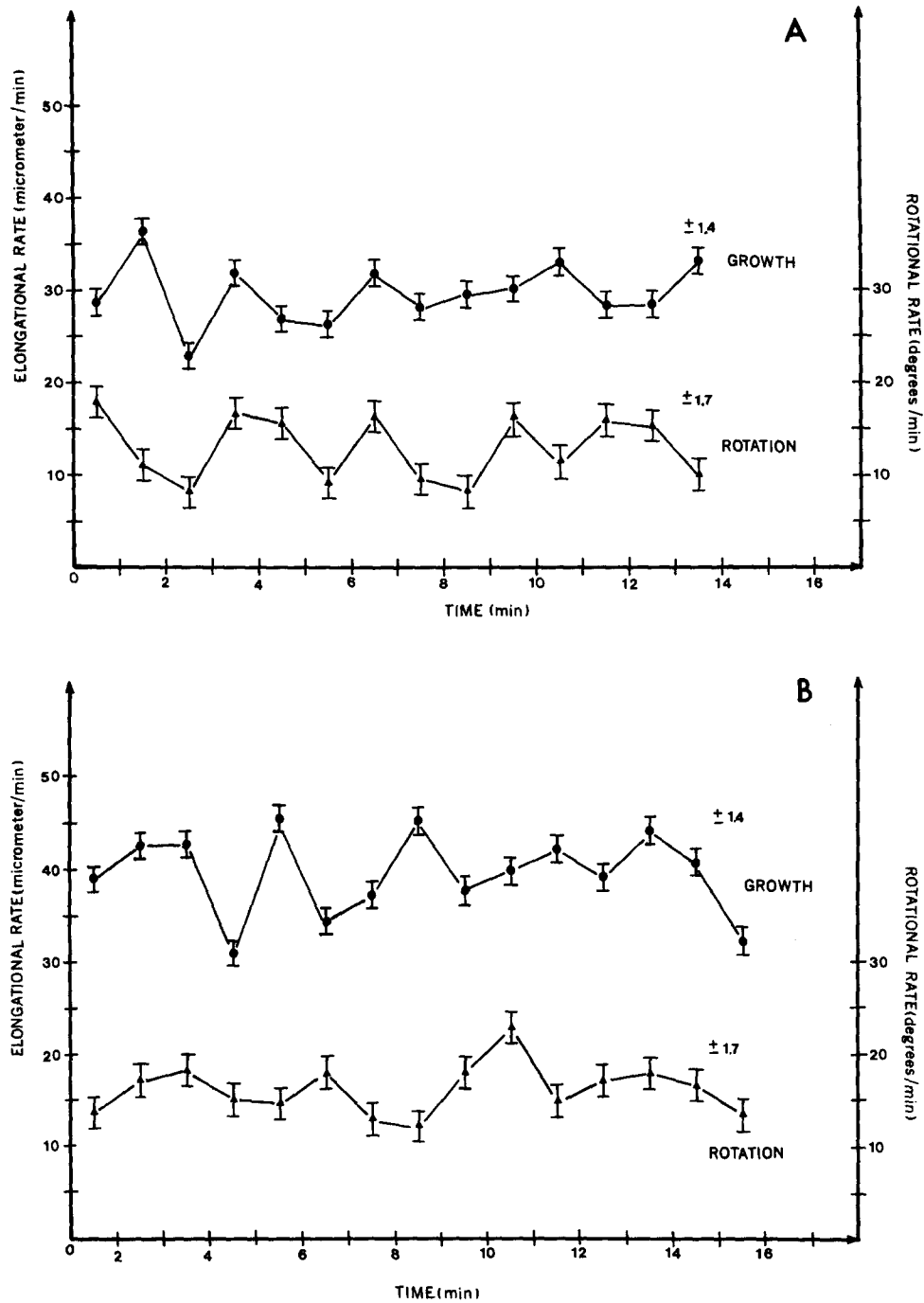FIGURE 1*A* and *B*.   The top curve represents the elongational growth rate of a stage IVb sporangiophore. The error bars were determined from the artificial Phyco. The bottom curve shows the rotation rate of the same sporangiophore. The error bars were calculated from our control experiment in which the glass bead marker was placed just below the GZ.

Rotation and Elongation Rates after a House Stimulus

Again, we used the identical procedure, except in this case a glass house 1 ×
1 × 4.5 cm was placed over the sporangiophore. Because the walls of the glass
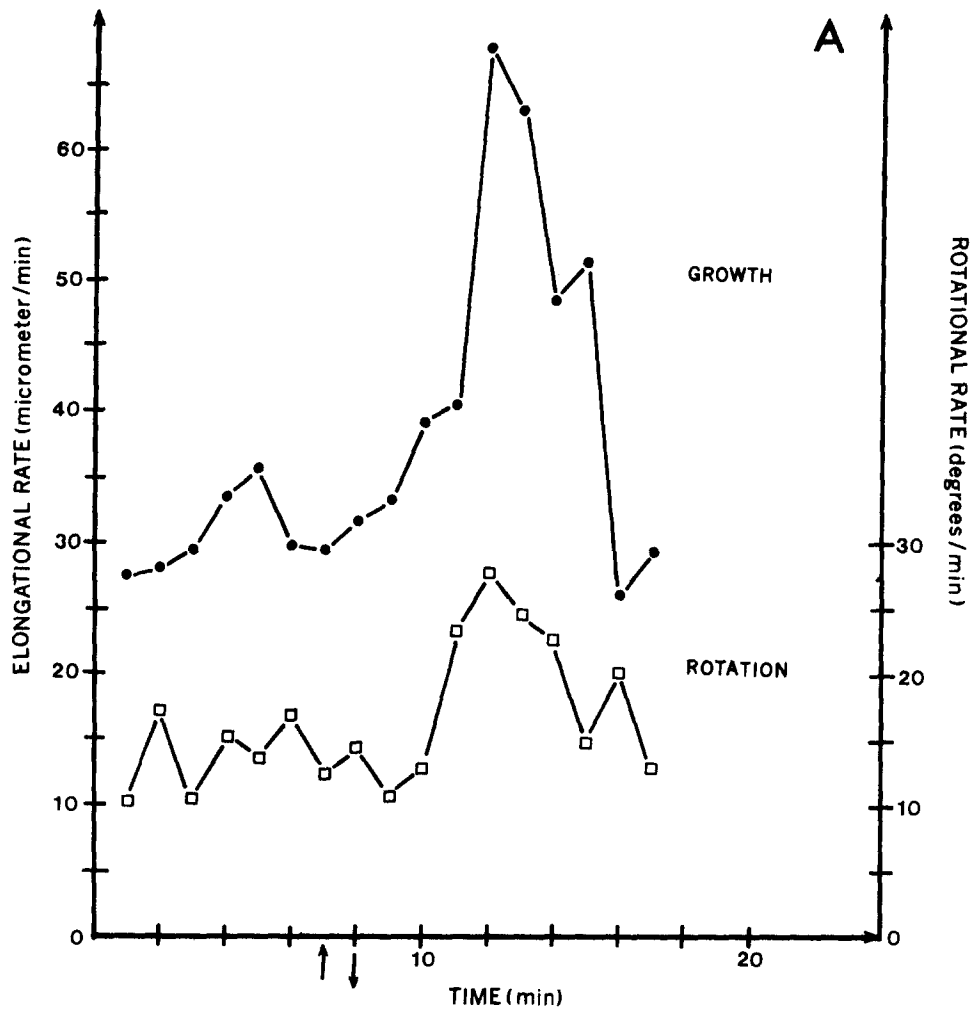house are flat, one can continue making rotation and elongation measurements

FIGURE 2A and B.   Rotation (□) and elongation (●) growth rates for red light–
adapted stage IVb sporangiophores were measured as described in the text. The
arrows indicate the time and duration of a saturating blue light stimulus.

after the house is in place. Fig. 3 A and B (data from two different sporan-
giophores) shows that the "house response" is similar to the light response just
described in that there is an increase in both the rotation and the elongation
growth rate.

DISCUSSION

Fungi cell wall growth is thought to be a result of an interplay between lytic and synthetic enzymes (Bartnicki-Garcia, 1970). The softening of the cell wall by a lytic enzyme such as chitinase results in cell wall extension; the driving
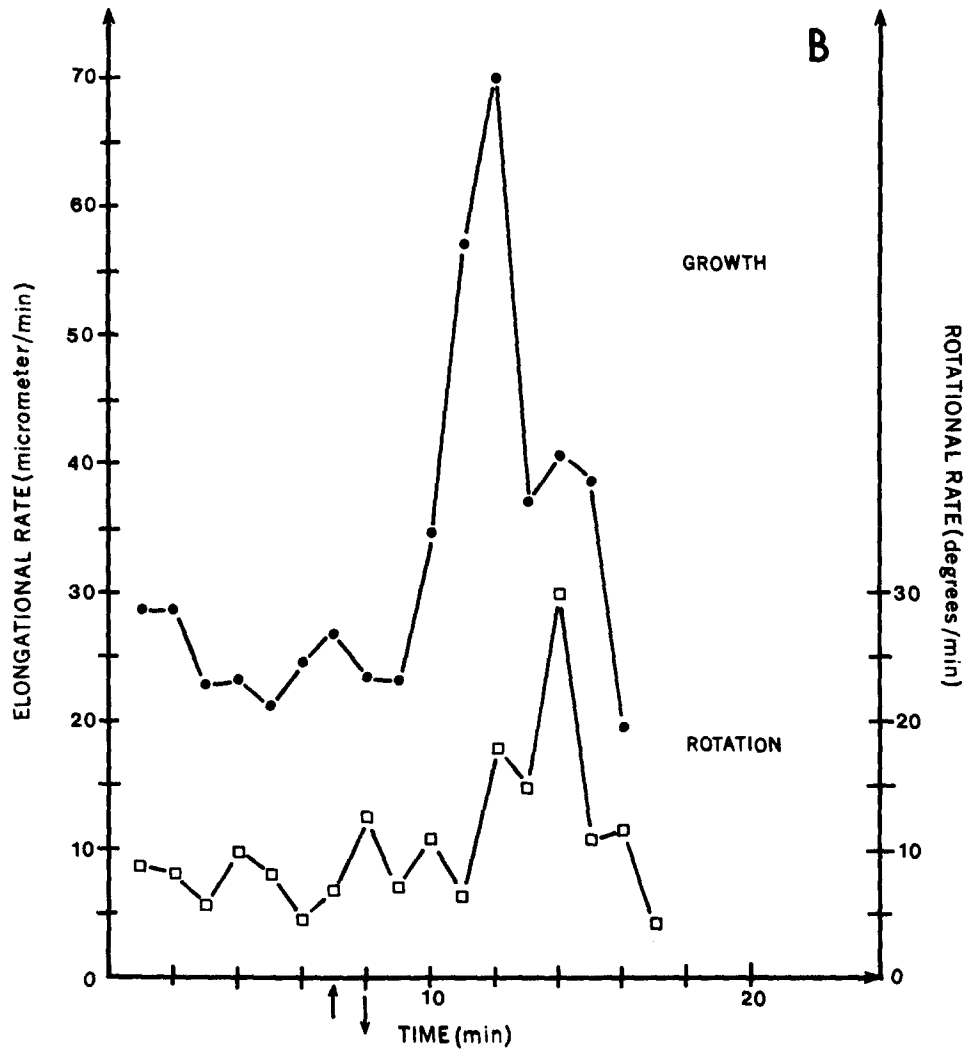
FIGURE 2.

force of this extension is the cell's internal turgor pressure. To avoid catastrophic rupture of the cell wall during extension, cell wall synthesis must be continuous. Matraux et al. (1980), using the mercury technique pioneered by Kamiya et al. (1963), have concluded that in *Nitella* both turgor pressure and some metabolic event are necessary for cell wall extension. It seems possible

that these fluctuations in growth rate are a result of the changing activity of the lysis-synthesis mechanism. Similarly, "random" changes in the cell's turgor pressure could account for these fluctuations. Green (1971) has made detailed measurements of turgor pressure during growth of *Nitella*, but measurements have not yet been made in *Phycomyces*. We favor the turgor pressure explanation, because it is hard to envision that the breaking of even a large number
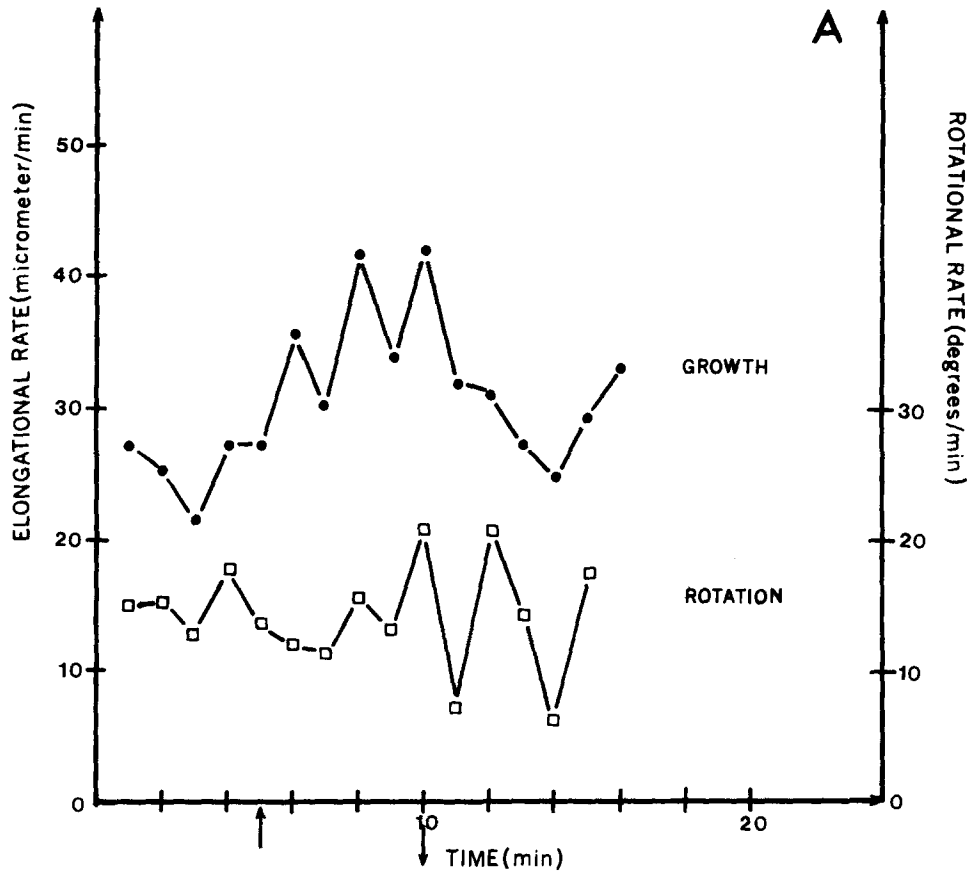

FIGURE 3A and B. The same procedure as described in Fig. 2, except a glass house was placed over the entire stage IVb sporangiophore for a house stimulus instead of the light stimulus. The arrows indicate the putting on and the taking off of the house.

of molecular bonds at the *micro* level would be so accurately reflected in the growth patterns at the *macro* level. In any case, Castle (1940) appears to have had a great deal of insight in attempting to explain the discontinuities in the growth rate when he wrote, "Whether the outer layers of such thickened wall could be supplied within the materials necessary for intussusceptive growth is questionable. If such outer layers are, on the contrary, passively stretched during growth, there must come moments when parts of these layers 'give

way,' resulting in extra increments of growth for a short period." It should be noted that, although Castle was measuring irregularities occurring during 200-ms intervals, his statement may also be true for irregularities measured during 1-min intervals.

These irregularities in cell wall growth can also play a role in understanding the nature of differential cell wall growth. For instance, in an avoidance response the sporangiophore grows away from a barrier at a bending rate of ~2°/min as a result of a bias favoring cell wall extension in the wall nearest the barrier. It now appears that this bias is a statistical one, with the only
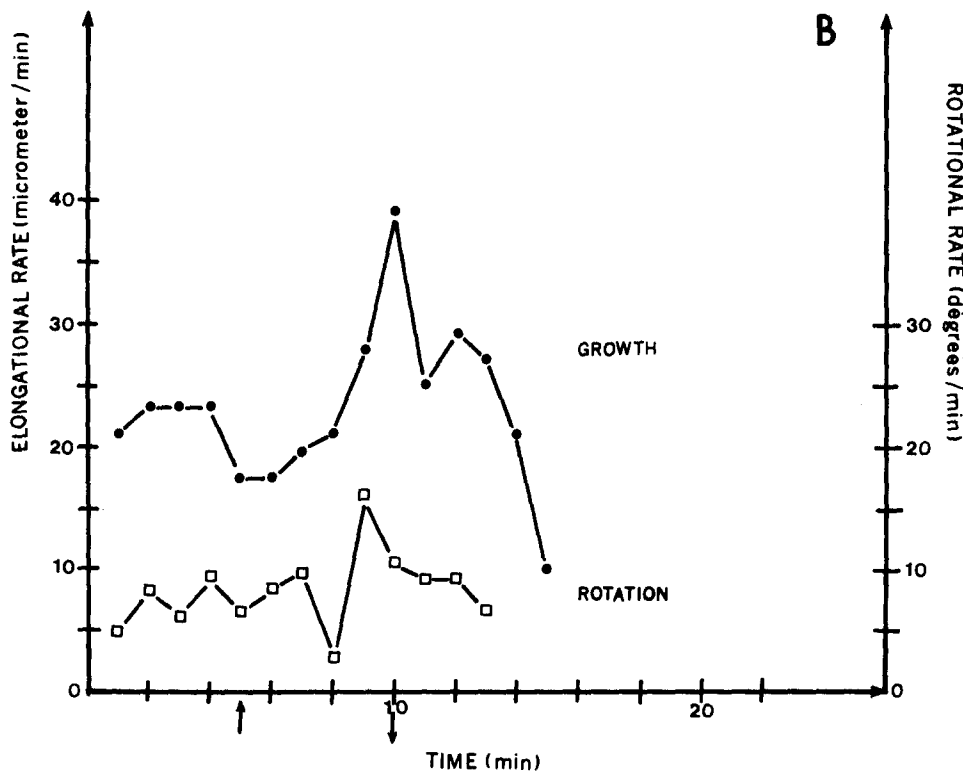

FIGURE 3.

requirement being that the average minute-per-minute extension is greater on the barrier side. This absolute difference in cell wall extension can be quite small.

For us, what is quite unexpected is the apparent lack of correlation between the fluctuations that occur in both stretch and twist components. It is clear from the work of Cohen and Delbrück (1958) and Ortega et al. (1974) that growth and rotation are not proportional to one another, but these results in no way led us to expect that a sudden increase in the stretch vector would not also be reflected in some similar change in the twist vector. This finding leads

us to suspect that at least two processes, both of which could cause twist and stretch, may be occurring simultaneously but randomly in the GZ. There is evidence suggesting that two types of twist and stretch can occur in the mature GZ of the growing stage IVb sporangiophore. Firstly, Oort (1931) reported in stage IVb that "the spiral can change its direction and pass from right handed to left handed or from left handed to right handed." He notes that rotation often reverses direction when the sporangiophore is quite old (20 h) and growing very slowly. We (Gamow, 1979) have found that sporangiophores growing at either abnormally high or low temperatures slow down more in rotation than in elongation. The GZ of these rather poorly growing sporangiophores were quite short, and we speculated that if the upper region of the GZ rotated counterclockwise in respect to the sporangium (a [−] direction), then subtracting the more extensive lower growing zone, which rotates clockwise (a [+] direction), would yield the observed effect at the two extreme temperatures, i.e., a net decrease in [+] rotation. This is true if the entire GZ still stretches and if the upper GZ rotates in the direction opposite the clockwise direction of the lower GZ. Cohen and Delbrück (1958) in their elegant analysis of the GZ observed this [−] rotation in the upper region of the GZ. In their 1958 paper they write, "The curious negative values of the twist in the uppermost portion of the GZ are undoubtedly real." Thus, it appears that the direction of twist in any one GZ not only changes parity but that the two directions can occur simultaneously in one GZ. That the stretch occurs in only one direction, i.e., cell wall contraction does not occur but twist occurs in either direction, may explain the apparent lack of correlation between the net twist and the net stretch fluctuation as well as the nature of the growth fluctuations themselves. For this explanation to be valid, one of the two twist directions must dominate; this is, of course, the case—the dominant twist direction in stage IVb is clockwise. The absolute movement of a point on the surface of the GZ depends on both the magnitude and the sign of the twist and the magnitude of the stretch. We suggest that these two phenomena occuring simultaneously but randomly in one GZ account for the irregular growth patterns.

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