SPIRAL GROWTH AND SPIRAL STRUCTURE

IV. GROWTH STUDIES AND MECHANICAL CONSTANTS IN THE CELL WALL

by

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INTRODUCTION

Since the first observation by OORT[1] of the spiral growth of the sporangiophore of *Phycomyces*, many theories have been put forward to explain this phenomenon. OORT himself thought that it was possibly linked with spiral structure in the chitin cell wall and, in harmony with the conceptions at that time, that protoplasmic streaming played an important part in orienting the chitin micelles. These were said to lie in a steep spiral in the secondary wall, and predominantly transversely in the growth zone. It was later shown (MIDDLEBROOK AND PRESTON[2]) that in the growth zone a flat spiral exists alone only at the very tip.

POP[3] enlarged upon the possible role of protoplasmic streaming in this cell, noting that, although streaming was normally axial, different velocities in the stream, carefully measured by him at different positions and therefore ages in the sporangiophore, could have different effects upon the orientation of an asymmetrical chain molecule. High velocities of streaming in the adult wall would tend to produce an almost vertical alignment of chains while a thicker, sluggish layer of cytoplasm in the growth zone, at a position where the stream was "turning", could give a transverse orientation.

Another theory to explain spiral growth was put forward by HEYN[4] who carried out the first X-ray work upon *Phycomyces*. From his evidence, he found that the secondary wall was wound with a spiral whose winding made an angle of 13.5° to the cell axis. Since this angle agrees well with that found by OORT[1] for the angle of spiral growth, HEYN postulated that growth takes place by slippage along inclined crystal planes, to consolidate the plastic extension of the cell wall. To explain the occurrence of either left or right hand spiralling in Stage 4 cells, HEYN said that there was a possibility of two orientations in the wall, and also of varying proportions of chitin. This theory, though ingenious, is based on work on the secondary wall, where extension and spiral growth have ceased, and would not account for the very wide variation in angle of growth for *Phycomyces*, or for the changes at different times during the growth of a single cell.

In 1937 CASTLE[5] was studying the structure of the growth zone of the *Phycomyces* sporangiophore in relation to the spiralling of the cell, and his work led him to conclude that the transverse orientation of chitin chains was due to the interplay of forces caus-

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ing growth with the elastic properties of the wall. He pointed out (CASTLE[5]) that most extending cells are approximately tubular in form and that, in such a hollow cylinder, under uniform internal pressure the tensile stress in the wall is twice as great across an imaginary longitudinal section as across a transverse section. He suggested that in a turgid cell, this greater circumferential tension acting at right angles to the axis of the cell is the cause of the observed micellar orientation in the expanding primary wall.

At about the same time, VAN ITERSON[6] published observations on the primary wall of the staminal hairs of *Tradescantia*, and reached very much the same conclusions.

PRESTON[7], however, pointed out that although this hypothesis appears at first to be more promising than the others, the orientation of fibrils in the secondary wall of both *Valonia* and *Cladophora* exhibit a crossed fibrillar system, although the shapes of the cells are completely different. It seemed hardly conceivable that stresses could exist which would initiate these two different directions of cellulose chains. It is further to be noted that in these two algae the growing regions are not in any case tubular in form, and the same now appears to be true of *Phycomyces* (PRESTON AND MIDDLEBROOK[2]).

In 1942, CASTLE[8] published further investigations upon the growth of the sporangiophore, and reported for the first time that there is a rotational component in the stage 1 cell. The progress of spiral growth could then be delineated as follows. The spiralling during stage 1 is in a left-hand* direction, and at the onset of stage 2, when the sporangium begins to form, rotation as well as elongation ceases. It is only in stage 4 that growth in length and rotation of the cell recommence, but at first the spiralling is in a right-hand direction, and this continues for about 1½ hours, slows down, and then reverses to the normal left-hand direction which continues for several hours until the cell reaches maturity. The establishment of this reversal caused CASTLE to subdivide stage 4 into 4a and 4b, to denote the difference in direction of rotation. On the discovery of this phenomenon, CASTLE realised that his own attempted explanation of growth was now inadequate.

In the first paper of this series (PRESTON[9]) an explanation of spiral growth was given which was capable of quantitive checking against the data known at the time. This fitted in with all the known details of growth and of structure, and included an explanation of reversals. Since this is the hypothesis upon which the following work was based, it might be summarised here with advantage. The criticisms made by ROELOFSEN[10,11] and his alternative explanation will be dealt with later in this paper.

The growth zone of the sporangiophore was envisaged in a very simplified form, its shape, which is very variable, being considered in the form of a cylinder bounded by an apical flat face. The chitin fibrils in the cell wall, known to be predominantly transverse in orientation, with some indication of organisation in a flat spiral (OORT AND ROELOFSEN[12]), were replaced by a hypothetical set; this had properties dependent on the dispersion of the real fibrils present in the growth zone, and was oriented in a flat spiral at $90-\alpha^{\circ}$ from the directrix of the cylinder. This system was compared with a wire spring which, when weighted at one end, will extend and, up to the limit of elastic extension, will rotate in such a direction as to coil upon itself. After the elastic limit has been reached this, of course, does not apply, for the spiral then uncoils with deformation of the wire. The rotation per unit elongation, $\Delta\phi/\Delta L$, during elastic extension may be expressed as: -

* left-hand spiralling follows the direction of the thread of a left-hand screw, *i.e.* looking down on the top of the cell, rotation is in a clockwise direction.

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$$\frac{\Delta\phi}{\Delta L} = \frac{\cos\alpha \sin\alpha \left(1 - \frac{nC}{qD}\right)}{a\left(\cos^2\alpha + \frac{nC}{qD}\sin^2\alpha\right)} \quad \ldots \text{ Equation 1.}$$

where α = the angle made between the spiral winding and the horizontal,
a = the radius of the spiral,
n = the torsional rigidity of the wire,
q = the Young's modulus of the wire
C and D = factors depending on the shape in cross section of the wire.

It is evident that upon this hypothesis the spring will rotate freely except when $1/nC = 1/qD$; in other words the chief factors governing spiral growth are changes in Young's modulus and the torsional rigidity.

This relation was not in contradiction to any of the facts known at the time and had the advantage over all previous theories in being able to explain the change in direction of spiralling. Thus, if nC/qD is less than unity, sinistral spiralling is implied and if nC/qD exceeds unity, dextral spiralling would follow. Changes in the values for n and q in cellulose are large enough to allow of sufficiently great changes to be postulated in the allied substance, chitin. Other points which were in accord with this hypothesis were:–

1. Rotation and elongation always occur together and cease at the same time and in normal left or right-hand rotation, under constant conditions, the rate of rotation is proportional to the rate of elongation.

2. The value of α is known to be small, since the chitin chains are oriented more or less transversely. Since n, which depends on secondary valencies, will probably be small compared with q, which depends on primary valencies, and C and D are of the same magnitude, ($nC/qD \sin^2\alpha$) will be small compared with $\cos^2\alpha$, therefore $\Delta\phi/\Delta L$ will be largely dependent upon $1—nC/qD$. Therefore, if environmental changes affect this term, they should also affect the rotation per unit elongation. Such changes have indeed been observed (CASTLE[13]) and been accounted for (PRESTON[9]).

3. The application of torque to the spore mass by CASTLE[14] around an axis longitudinally down the sporangiophore reduces or stops the rotation. It was also found that the couple required to slow down rotation increased rapidly with an increase in the diameter of the sporangiophore. The application of the values obtained by CASTLE[14] on this subject (see PRESTON[9]) are in agreement with calculations of the order of torque required to stop rotation in cells of chosen diameter, and although elongation does not altogether cease, it, too is slowed down.

Applying this formula to the hypothetical fibrils of chitin in the cell wall, if the cross section of such fibrils is regarded as being circular, then C and D may be written $C = n\,b^4/2$, and $D = n\,b^4/4$, where b is the radius of cross section of the fibrils.

Equation 1 then becomes

$$\frac{\Delta\phi}{\Delta L} = \frac{\cos\alpha \sin\alpha\,(1-2n/q)}{a\,(\cos^2\alpha + 2n/q \sin^2\alpha)} \quad \ldots \text{ Equation 2}$$

Using this formula then, it may be seen that all the factors are capable of quantitative checking, and that some success has been reached in terms of existing data. It is the purpose of the present paper to examine the position with regard to data which have

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recently been obtained in this laboratory. In particular the relation will be checked, firstly, against the determined elastic constants of the sporangiophore wall in terms of the structure of the growth zone already reported (MIDDLEBROOK AND PRESTON[2]) and, secondly, against the prediction that $\Delta\phi/\Delta L$ should vary linearly with $1/a$.

A. GROWTH STUDIES

Cultures of *Phycomyces nitens* + and —, and *P. Blakesleeanus* were used, grown on Malt agar at 20° C. The apparatus used for the studies on spiral growth is shown in Fig. 1. The glass tank contains water kept at 20° C ± 1° by a Sunvic thermostat, and almost submerged in the water was a plate glass box in which grew the sporangiophore under observation. A block of malt agar supporting a suitable sporangiophore was transferred to a small glass phial, the sporangium under observation projecting above the top of the phial. Through the side of the glass tank length increments every 10 minutes were measured through a cathetometer with vertical movement. These readings were accurate to 0.001 mm. Since it was also necessary to measure the rate of rotation the sporangium was viewed from above through an Ultrapak microscope the light directed down the objective serving also to maintain vertical growth of the sporangiophore. The sporangium had markers placed upon it, Lycopodium spores being used most frequently, although small pieces of glass wool, flour and talc particles were also employed on occasion. The movement of these markers was followed, by aligning with them the cross-wires in the Ultrapak eyepiece every 10 minutes. A pointer was attached to the eyepiece, moving with it, which read off upon a circular protractor attached to the microscope tube the angle through which the cell had moved.

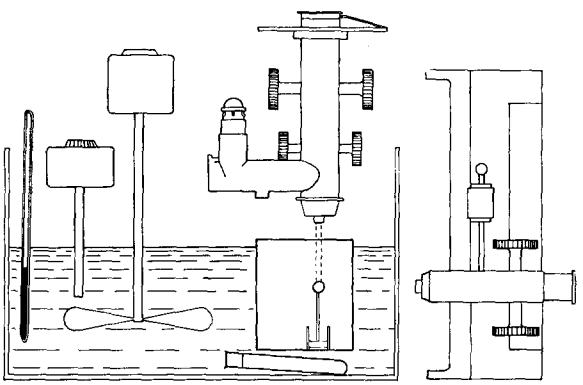

Fig. 1. Apparatus for the studies on spiral growth

This apparatus enabled accurate determinations to be made of the ratio of length increase and of rotation at chosen time intervals. The sporangiophores used in the growth studies were taken whenever possible in an early part of Stage 4b, *i.e.* during the colour change of the sporangium from yellow, through brown to black, in order to make sure that the cells were in a comparable stage of growth and that the maximum growth rate could be observed. Cells which had already become black in the sporangium were avoided since they would have varied more in age. The precise stage of growth of the cells used by CASTLE is not stated, except that they were in Stage 4b, but they might have been older than the ones chosen here.

A series of growth observations was taken on cells of different diameter, and the relationship between $\Delta\phi/\Delta L$ and the diameter established. The measurements of diameter were made by a Zeiss Okular-Schraubenmikrometer in the eyepiece of the cathetometer.

RESULTS

The first observations were made to confirm the stages of growth put forward by CASTLE[8]. These were found to proceed according to his description cited above, i.e. to show left-hand spiralling until the sporangium is formed, then upon resumption of growth a reversal of direction for about 2 hours, followed by normal left-hand rotation until the cell reaches maturity.

The diameter relationship was established, as shown in the graph in Fig. 2, where it is seen that cells having smaller diameters have also greater rotation per unit elongation.

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$$\frac{\Delta\phi}{\Delta L} \propto \frac{1}{a}$$

From the evidence presented here, it will be seen that the value for $\Delta\phi/\Delta L$ increases linearly, with an increase in $1/a$. There is considerable dispersion of points about this line, but to mention only one factor involved, a very slight bending of the cell would cause an alteration in the position of a point in the graph, and for biological material this would seem to be a clear relationship. It is, however, important to point out at this stage, that these values for $\Delta\phi/\Delta L$ (and therefore for values of Θ, see below) were obtained from observations on growth over a period of several hours (Table I). The determinations of rotation and elongation per unit time given by CASTLE[15] were, on the other hand, made after only an hour in the standard apparatus used by him to measure these values. In his graphs showing elongation and rotation plotted respectively against time, CASTLE uses these figures over the period of one hour (after a previous hour of "conditioning" in the apparatus) to work out the "constant" $\Delta\phi/\Delta L$ for the particular cell observed. Actually, his lines do show divergence even after this time. When graphs are plotted in this way from figures taken over a longer period (Fig. 3) it may be seen that the divergence becomes greater. Since these figures are the ones used in the calculation for $\Delta\phi/\Delta L$ and Θ, it will be seen that these values must also vary during the experiment, and in fact that rotation per unit elongation and therefore the angle of growth vary also. Table I has been compiled to show these changes. It was therefore considered safer to use a mean angle of growth over a long period to apply when testing the formula, than to use values obtained after only one hour.

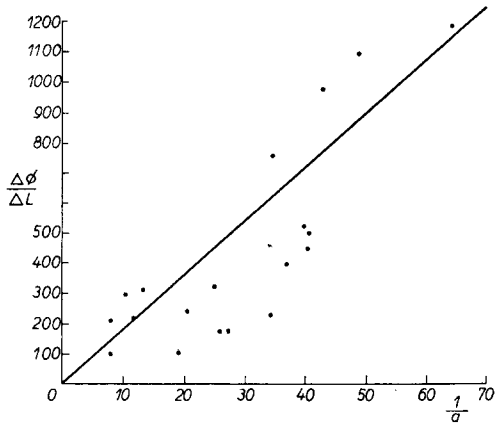

Fig. 2. Relationship between $\Delta\phi/\Delta L$ and $1/a$

From the values obtained for the rotation per unit elongation, the angle of spiral growth, Θ, could be calculated,

$$\Theta = \tan^{-1}\frac{\pi a \Delta\phi}{180\,\Delta L} \quad \ldots \text{ Equation 3.}$$

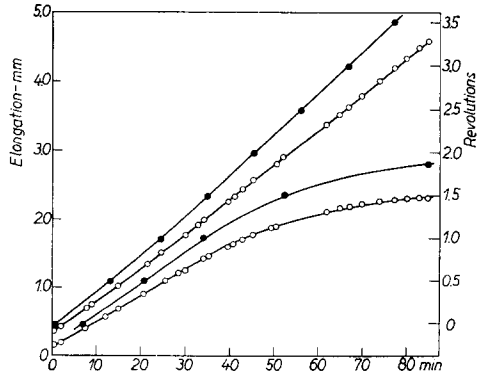

Fig. 3. Relationship between elongation and rotation with time

A series of observations upon 25 sporangiophores of different diameters grown at 20° C, gave a mean value of 13.9° with a variation from 3.8° to 27.8°. The mean value reported by CASTLE[13] on 12 sporangiophores of varying diameters was 12.9°, though the temperature at which the determinations were made was not stated. These results agree well, but vary from OORT's figure of 5.8° at 17.5° C. In part this difference may be explained by the difference in temperature, since it was established by CASTLE[15] that the rotation per unit elongation increases with an increase in temperature.

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TABLE I

VALUES OF Θ (ANGLE OF GROWTH) FOR INDIVIDUAL SPRANGIOPHORES FROM READINGS TAKEN AT HOURLY INTERVALS

	Hour of duration of experiment									*Mean*
	1	2	3	4	5	6	7	8	9	
A	1.9	2.0	14.0	13.3	13.0	13.1	12.5			10.0
B	8.4	10.6	10.2	12.6						10.4
C	4.7	5.5	4.6	5.2	5.4	4.2	5.0			4.9
D	7.5	3.5	6.1	5.8	5.1	6.3	3.3			5.4
E	27.3	39.6	27.4	23.3	24.7	23.2				27.8
F		14.5	25.3	14.4	6.7	6.1	6.1			12.2
G		24.3	29.3	19.6	16.0	16.4	18.1			22.3
H	11.5	22.4	12.9	15.0	32.5	30.2	28.9	19.3		21.6
I	7.3	15.4	22.2	32.7	26.0	21.3	22.6			21.1
J	29.1	9.4	8.3	12.1	7.8					13.5
K	5.0	11.2	14.0	15.8	19.2	12.9	15.0			13.3
L		5.6	6.6	6.8	7.9	8.1				7.0
M	1.8	5.3	7.7	8.4	9.1	6.4	6.8			6.5
N		2.1	3.5	4.4	5.1					3.8
O		14.7	17.3	20.0	20.9	18.0	22.3			20.4
P		8.9	12.0	16.0	13.2	11.9				12.6
Q		8.7	14.0	14.7	23.0	20.6	22.6			17.3
R			13.9	10.9	9.7					11.5
S			23.7	27.8	27.6	21.9				25.3
T					23.9	13.5	13.5	16.1	13.5	16.1
U				13.8	31.1	18.0	9.5			18.1
V				1.9	6.6	7.8	11.0			6.8
W				15.5	14.9	8.1	12.0			12.6
X	15.5	6.7	6.6							9.6
										Mean 13.9

B. THE MECHANICAL CONSTANTS OF THE SPORANGIOPHORE

It may be seen from equation 2 that the values of n and q (torsional rigidity and Young's modulus respectively) should exert a profound effect on the rotation and it was obviously necessary to obtain numerical values for these factors in the chitin fibrils of the cell wall. No data on these points were available, and even for cellulose, of which the physical properties have been studied far more intensively, the evidence was very scanty.

The values for n and q needed are those of the fibrils in the growth zone region of the cell but so far no technique has been evolved for their determination in this region. This is because the growing wall is extremely thin and difficult to handle, and also that its shape is difficult to define and varies with the cell diameter. It was therefore decided to determine these parameters on the adult wall, as a first approximation. Presumably the chitin fibrils here will have the same physical properties as those in the growing wall, though variation in the nature and the amount of incrusting substances may well cause some differences.

Both n and q had to be measured on dried wall material, for if fresh had been used, the drying out which would ensue during the process of evaluation would have an effect upon the cell wall such as to invalidate the result. The severe wrinkling of the wall oc-

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curring when moisture is lost, either from evaporation or the direct loss of cytoplasm, would certainly affect n and q. The cell was therefore removed from the culture medium and dried between two glass plates, which were weighed. This gave a flat ribbon whose dimensions were accurately measurable and which were therefore suited for observation after the removal of the growth zone.

It was possible to use the same sporangiophores for the evaluations of both n and q, this being especially useful because of the wide range of values obtained.

METHODS

a. *Torsional Rigidity*

The technique for measuring the torsional rigidity was adapted from LOCHNER[16], who used various textile fibres as experimental material. The sporangiophore ribbon was fixed at one end to a No. 1 circular cover glass and at the other to a wire hook in a rubber bung, Durofix being used as a cementing material. Then the sporangiophore and coverslip were placed in a glass specimen tube, and held in a vertical position from the rubber bung (Fig. 4). A light beam was reflected from the cover slip on to a centimetre scale, so that as the fibre twisted readings could be taken as the light spot moved along the scale. Readings were taken in air since LOCHNER had previously shown that air resistance has a negligible effect when the fibre used exhibits a high damping capacity such as shown by these sporangiophores. As the disc oscillated the period of oscillation was measured with a stopwatch and the distance that the beam moved for each oscillation was determined. From this information, the decay curve was plotted for a number of fibres, *i.e.* the distance moved by the light beam per oscillation for several consecutive swings, and since the value decreases rapidly (Fig. 5), the damping capacity was evidently sufficiently high. All further measurements were therefore taken in air.

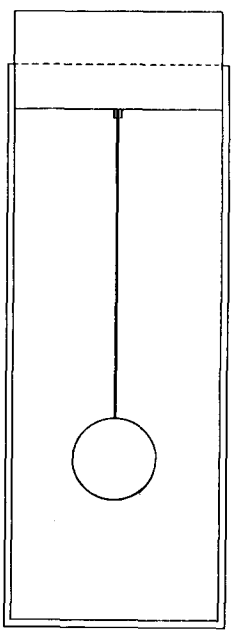

Fig. 4. Apparatus for torsional rigidity determinations

Other information needed to calculate the value for n was found as follows; the length of the fibre was measured as it hung in the position for oscillation, using the cathetometer microscope. The cover glass was weighed to $\pm$ 0.0001 g and its diameter measured. The sporangiophore ribbon was measured across both large and small diameters by mounting it in a suitable position on the stage of the microscope, and a Zeiss Okular-Schraubenmikrometer was used. The torsional rigidity was then given by;

$$n = \frac{128\,\pi\,I_m\,l}{T^2\,D\,d^3}$$ (MORLEY 1940)

Where I_m = moment of inertia of the disc about its diameter, *i.e.* $Mr^2/4$ (M = mass of the glass disc, r = radius of the glass disc),

l = length of the fibre,
T = period of oscillation,
D = ribbon width,
d = ribbon thickness.

(b) *Young's Modulus*

The sporangiophores used were ones for which the torsional rigidity had been found, since they were unaffected by this evaluation. n and q are therefore given for the same cell wall.

The method used was a dynamic one (LOCHNER[16]), in which the sporangiophore was excited by being clamped at one end to the diaphragm of an earphone, which was made to vibrate at known frequencies (Fig. 6). The frequency was controlled by a beat frequency oscillator, which had a range of 1–2000 cycles per second. The lowest frequency at which the resonance of the material was reached was found by observing the tip of the sporangiophore ribbon

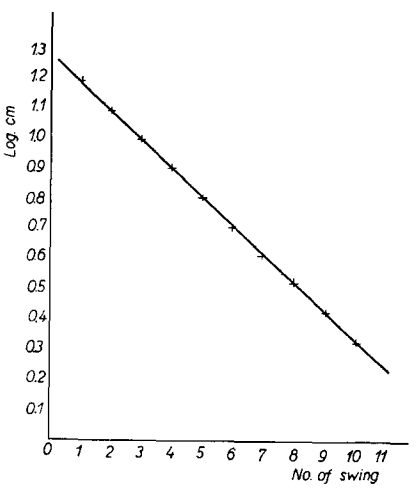

Fig. 5. Degree of damping for *Phycomyces* sporangiophores

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through the cathetometer, and the figure at which it vibrated vigorously was taken as the fundamental resonance frequency. The length of the sporangiophore was measured in position, using the same cathetometer. The determinations were made under laboratory conditions, the only precaution being to exclude outside factors such as air currents, by enclosing the whole sound apparatus in a glass box.

A range of values of the frequency F was found by cutting off the tip of the sporangiophore ribbon after each reading, and measuring the shortened length each time. The relation between length and $1/\sqrt{F}$, was found to be linear (Fig. 7) for all fibres. The line should clearly pass through the origin; any deviation from the origin may be due either to the imperfections in measuring the length, which is often difficult because of (a) the cementing "Durofix" being an uneven distance up the sporangiophore, and (b) very slight bending of the fibre, or to errors in the reading of F since there is a small range of frequency over which resonance is obtained.

The density, ϱ, was ascertained for the walls of the sporangiophores by cutting up cells which had been used in the measurement of F, and centrifuging them in liquids of varying specific gravities. The density of the fibre was taken as that of the liquid in which the cell neither floated on the top of the liquid nor was flattened against the bottom of the tube when centrifuged at 1650 revs/min in a mixture of xylol and α-monobromonaphthalene. This gave a figure of 1.288.

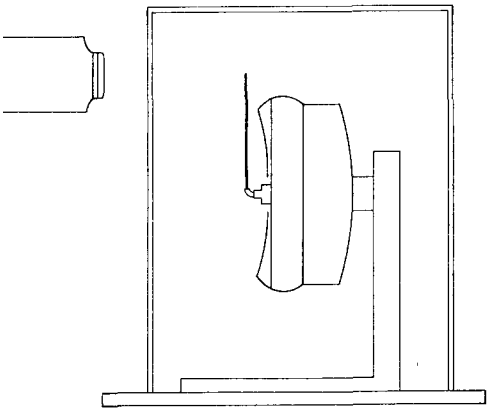

Fig. 6. Apparatus for measurement of Young's modulus

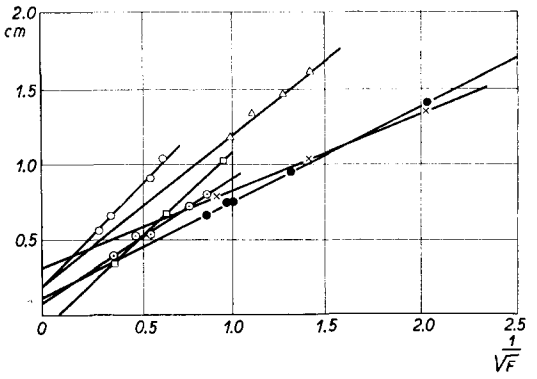

Fig. 7. Relationship between length and $1/\sqrt{F}$ for individual sporangiophores

Young's modulus was calculated according to the following relation

$$F = \frac{m^2 k}{4\pi l^2} \cdot \sqrt{\frac{q}{\varrho}}$$

where, F = fundamental frequency of the sporangiophore,
m = 1.875 for the first mode (MANLEY[17])
l = length of ribbon,
ϱ = density,
q = Young's modulus,
k = radius of gyration of the cross section of the ribbon. The radius of gyration of a flat ribbon such as that used is given by,

$$k = \frac{d}{2\sqrt{3}}$$

where d = the width of the *edge* of the flattened sporangiophore (WOOD[18], MANLEY[17]).

RESULTS

Table II gives the results obtained using these techniques to evaluate n and q on the sporangiophores. From the figures thus tabulated it will be seen that the values of n/q can vary up to fifty fold, even in the stage 4b sporangiophore which is grown under constant conditions. Because of this wide variation, it is easy to conceive of great

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changes during the differentiation of the sporangium, when growth ceases and a new spore-bearing cell is formed at the tip. Therefore, from this evidence from the adult wall, we have some indication of the order of values of n and q, and variation in n/q is sufficiently wide for the needs of the theory proposed. However, it is most desirable to make such evaluations on the growth zone of the cell to ascertain these values in the primary wall, which may vary considerably from the adult wall, and about which the theory was formulated.

TABLE II

VALUES FOR THE TORSIONAL RIGIDITY (n) AND YOUNG'S MODULUS (q) OF INDIVIDUAL SPORANGIOPHORES

n ($dynes/cm^2$) 10^{10}	q ($dynes/cm^2$) 10^{11}	n/q
1.471	1.71	.08604
.506	.826	.06132
.019	.0972	.0197
.424	.665	.06377
.332	13.93	.00231
.996	3.422	.0291
.922	.590	.1563
6.769	81.75	.00828
.873	21.84	.003995
.166	43.91	.000378
3.801	8.859	.0429
2.404	2.456	.0979
9.634	28.02	.03443
6.931	27.64	.02508
.999	64.61	.05176
		Mean = .0911

The application of new values found to the formula

Since all the factors in Equation 2 have been examined in the work reported above, the values of α, and of the mean n/q can be substituted from these new figures, and the calculated value for Θ is found to be

$$\tan \Theta = \frac{\sin 15.3 \cos 15.3 \, (0.8178)}{\cos^2 15.3 + 0.1822 \sin^2 15.3}$$

i.e. $\Theta = 13.9°$

This is exactly the figure found experimentally for the angle of growth (Table I), an agreement which is astonishing in view of the large number of variables involved in the calculation. The value for α substituted above was that found in stage 4 sporangiophores (MIDDLEBROOK AND PRESTON[2]). When the figure $\alpha = 11.01°$ found in stage 1 and 2 growth zones is used, the value for Θ is calculated to be 10.0°, again exactly that found by CASTLE[8] as the angle of spiral growth in the stage 1 sporangiophore.

That the calculated and experimentally found values for Θ agree so well is no doubt fortuitous, but this striking agreement does appear to prove quantitatively that the formula proposed by PRESTON still fulfils the requirements for all the known facts regarding the spiral growth in Phycomyces.

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DISCUSSION

Before making any attempt to assess the merits of the theory underlying the present paper in terms of these newer results, it will be well first to deal with some criticisms which have recently been levelled at it by ROELOFSEN[10,11] and to comment upon the alternative theory which he suggests. ROELOFSEN points out that, since the chitin microfibrils in the growth zone appear to be oriented along a flat spiral, then if the intussusception of new material into the wall occurs in a suitable way and in suitable positions in the growth zone, spiralling might result as a consequence of growth; and the connection with elastic properties could be quite fortuitous. This alternative, although based upon observations published in two long papers, will hardly bear inspection either on general grounds or on examination of the evidence and arguments proposed. On general grounds, it could be said that spiralling by intussusception would need to occur in a very special way indeed in order to produce rates of rotation so completely in harmony with a relationship developed in terms of elastic properties. The more detailed criticisms which can be levelled at this alternative explanation are, however, much more serious.

In the first place, ROELOFSEN's explanation demands a right hand structural spiral in the wall of the growth zone whereas ours demands a left-hand spiral. It has already been shown that ROELOFSEN's attempts to indicate a right-hand structural spiral are hardly convincing, and our own observations (MIDDLEBROOK AND PRESTON[2]) show clearly that the spiral is in fact left-handed. ROELOFSEN's demand for a right-hand structural spiral arose first from his observation that if a coil of iron wire 5 cm in diameter (the wire being 2 mm thick and the spring having 10 turns) is loaded at one end (with a weight which appears to be of the order of 2 kg), then, as the wire spring extends, the free end rotates in such a way as to uncoil the spiral. As ROELOFSEN points out correctly, this rotation is in the sense opposite to that demanded by our basic theory—but so it should be! For, in order to demonstrate any rotation at all in a spring of such dimensions, it is necessary to load the wire beyond its elastic limit; the extension is then plastic and our considerations no longer apply. Inspection of equation 2 shows most clearly that, in order to show *elastic* rotation, the spiral must be kept of very small diameter. That used by PRESTON[7] was 50 cm long and 0.5 cm (wrongly reported in ROELOFSEN's paper as 0.5 mm) diameter. Such a spiral loaded with a few g rotates so that the winding coils up on itself, as simple physical considerations demand. The fact that such a spiral bears little relation to the dimensions of the sporangiophore is entirely beside the point. ROELOFSEN's criticism of our use of a left-hand structural spiral to produce a left-hand growth spiral rests, therefore, on a misunderstanding of the physical principles involved. A similar criticism can be levelled at his further model experiments using cellophane cylinders.

There are, however, a number of other arguments which can be raised against ROELOFSEN's alternative explanation, of which perhaps the more cogent may be quoted. The whole growth zone is expanding, but it is by no means clear that intussusception is occurring over the whole region. On the contrary, the indications are (MIDDLEBROOK AND PRESTON[2]) that new material is deposited only at the very tip of the growth zone. Again, in an attempt to refute our explanation using the sporangiophore itself, ROELOFSEN adopts the following procedure: the sporangiophore bearing a sporangium is severed at the base and attached to a glass tube by wax; pressure is then applied inter-

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nally as nearly as possible equivalent to the predetermined turgor pressure of the sporangiophore, and the rotation per unit elongation noted. It is argued that, if our theory applies, the rotation should be the same as that occurring during growth. Concerning this experiment and its results, it may first be noted that the wax used melts at 70° C which must deliver a marked shock to the living cell. In point of fact, ROELOFSEN attempts to determine the effect of this treatment by observing also the effect of internal pressure on dead cells killed by immersion in water at ± 80° C. He finds rotation to occur at the same rate as in his experimental material and concludes (ROELOFSEN[11]): "This cannot possibly be the result of changes in the wall after the cells have been pulled out, for it also occurs in cells which were killed with hot water immediately after having been pulled out", *i.e.* because the experimental cells behave exactly as do killed cells, then the former must have been alive! The major point to be noted is, however, that cells caused to expand in this way by purely mechanical forces show rotations up to 80% of that occurring during the previous growth. Far from constituting a major criticism of our theories, as claimed by ROELOFSEN, we regard this figure almost as a verification; particularly since in such excised sporangiophores growth must have stopped, and therefore presumably little or no chitin was being produced during the observational period so that renewal of the structural spiral from the cell apex must have almost ceased.

With regard to the implications of the new data recorded in the present paper, there is little need for further statement since some discussion has already been made in the body of the paper. Perhaps, however, it may be profitable to summarise their significance here.

In the first place, the elastic properties of the wall, when applied to the relation expressed in equation 2 in terms of the known structure of the growth zone, do give a good quantitative prediction of the rate of rotation. Certainly the criticism could be made that these elastic properties refer to the secondary and not to the primary wall, but is it to be questioned whether the properties of chitin fibrils will differ markedly in two wall types. In any case, it is satisfactory that the figures are not in complete disharmony. It is further especially to be noted that the spread of the values of the elastic constants is amply sufficient to allow not only the wide variation from cell to cell of the rate of rotations per unit elongation, but also to allow the possibility of reversal in spiralling.

Secondly, the prediction from equation 2, that the rate of rotation should be inversely proportional to the diameter of the sporangiophore, is completely confirmed to a degree which is most striking since the model on which the theory is based is a hollow cylinder differing to some extent therefore from the shape of the sporangiophores themselves.

These newer observations, therefore, coupled with the success achieved in the explanation of the course of spiral growth as described in a very voluminous literature, seems to us to make it certain that the interplay of turgor forces and the properties of the flat molecular spiral in the wall of the growth zone play a very prominent part in the genesis of spiral growth. Spiralling through intussusception as proposed by ROELOFSEN can obviously play only a minor role.

Further discussion of these matters will be postponed until other observations have been reported in a subsequent paper.

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SUMMARY

This paper deals with methods for checking quantitatively the hypothesis on spiral growth in *Phycomyces* (PRESTON[9]). Values are found for all the factors concerned, and the methods of doing this are described. These include the observation of the rotation per unit elongation of the cell, an evaluation of Young's modulus and the torsional rigidity of the cell wall material. The results reported here show striking confirmation of the hypothesis.

RÉSUMÉ

Cette communication traite de méthodes pour vérifier quantitativement l'hypothèse de la croissance en spirale chez *Phycomyces* (PRESTON[9]). Des valeurs ont été trouvées pour tous les facteurs concernés et les méthodes employées décrites. Il s'agit entre autres de l'observation de la rotation par unité d'élongation de la cellule, de la détermination du module de Young et de la rigidité à la torsion de la matière dont la paroi cellulaire est constituée. Les résultats rapportés confirment l'hypothèse de façon frappante.

ZUSAMMENFASSUNG

In dieser Arbeit werden Methoden zur quantitativen Prüfung der Hypothese vom Spiralwachstum bei *Phycomyces* (PRESTON[9]) behandelt. Für alle beteiligten Faktoren wurden Werte gefunden und die verwendeten Methoden beschrieben. Diese umfassen die Beobachtung der Drehung pro Einheit des Länge-Zuwachses der Zelle, die Bestimmung des Modulus von Young und der Torsions-Starrheit des Zellwand-Materials. Die angeführten Resultate bestätigen die Hypothese in auffallender Weise.

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Received September 3rd, 1951