CELL-WALL STRUCTURE IN THE GROWTH-ZONE OF *PHYCOMYCES* SPORANGIOPHORES

I. MODEL EXPERIMENTS AND MICROSCOPICAL OBSERVATIONS

by

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INTRODUCTION

The sporangiophores of *Phycomyces Blakesleeanus* grow exclusively at their tips. As they grow the sporangia usually show rotations, which are clockwise when seen from above. (OORT 1931). We shall call this rotation right handed. OORT AND ROELOFSEN (1932), on the basis of an investigation of the birefringence of the wall in the growth-zone (to be called in this article primary wall or meristematic wall), came to the conclusion that this wall possesses a flat Z-spiral structure*. Thus: "Die Spirale verläuft meistens rechts, bisweilen auch links. Genaue Winkelmessungen waren nicht möglich". In one figure the largest index of refraction is pictured as making an angle of 8° with the transverse direction.

These data were of too scanty a nature to establish beyond doubt the existence of a Z-spiral. A survey of the recent literature does not yield further observations on this matter, but confirms the fact that the double wall has a negative birefringence with regard to the longitudinal direction; consequently it must possess either a transverse structure or a spiral structure making an angle of less than 45° with the transverse direction. PRESTON (1948) writes: "It is not clear in the literature even whether the spiral is left- or right-handed". We therefore thought it necessary to elucidate this point, and this was done by means of: (1) experiments on models; (2) microscopical observations; (3) examinations of birefringence and (4) electronmicrographs. The results of (1) and (2) will be described in part I, those of (3) and (4) in Part II of this publication**.

Elsewhere (ROELOFSEN, 1950), the author discusses the question, whether spiral-growth is due to the mechanical properties of the cell wall only, or whether it is also the result of intussusception of the micro-fibrils in some special way.

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* Following the usage common in the American textile industry, we shall speak of a Z-spiral, if the part of the spiral nearest to the observer runs in a direction parallel to the middle part of the letter Z, the main axis of the spiral being vertical. Its mirror image is an S-spiral. Mathematicians and physicists call a Z-spiral left-handed, many botanists, a.o. OORT AND ROELOFSEN (*l.c.*), call this right-handed. Much confusion has arisen from this.

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§ 1. MODEL EXPERIMENTS ON THE ROTATION THAT OCCURS WHEN SPIRAL SPRINGS ARE STRETCHED OR WHEN THE PRESSURE IS INCREASED IN TUBES WITH SPIRAL STRUCTURE

a. *Stretching of spiral springs*

PRESTON (1948) has drawn attention to the fact that a spiral spring shows a rotation of one end relative to the other when stretched. The degree and sense of the rotation depend mainly on the steepness of the spiral and the magnitude of the quotient $\frac{n}{q}$, where n is the torsional rigidity and q the YOUNG's modulus of the material. In cotton fibers a quotient $\frac{n}{q}$ of 0.155 was found.

The cylinder in Fig. 1 represents a sporangiophore in the growth zone. AA'' is the growth spiral. In other words, a point A will reach the position A'' after a growth over the distance Δl. The angle of the growth spiral is given by $\tan \beta = \frac{\pi a \Delta\gamma}{180 \Delta l}$, where a = radius, $\Delta\gamma$ = rotation in degrees corresponding to an elongation Δl. For β CASTLE found values between 4° and 18° and OORT an average of 5.8°.

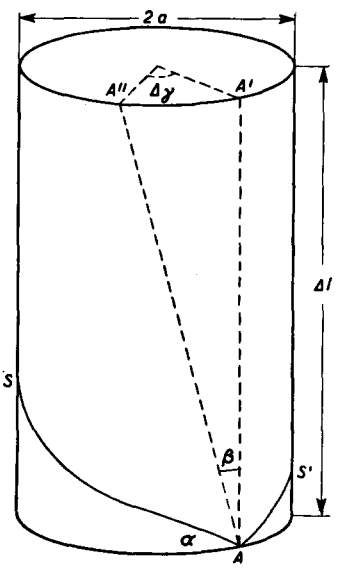

Fig. 1. Growth spiral and structural spiral

Assuming that the sporangiophores of *Phycomyces* rotate as the result of the stretching of spirals, that the growth zone possesses a spiral structure with an average angle of 10° with the transverse and that these cells have a growth spiral making an angle of 5.8° with the longitudinal axis, PRESTON calculates an $\frac{n}{q}$ of 0.2 for chitin.

According to him, a spiral would, on being extended, rotate in the direction of the spiral itself in those cases where $\frac{2n}{q}$ is less than unity ("the spiral coils up on itself"). If $\frac{2n}{q}$ becomes greater than unity, the spiral will unwind when stretched. Thus PRESTON explains the normal, clockwise rotation during growth *i.e.* the growth spiral AA'', by assuming a fibrillar structure running parallel to the spiral AS, *i.e.* an S-spiral.

However, when we studied (1949) the rotation of wire spirals, it became evident that PRESTON's interpretation could be valid only under certain conditions. We used spirals of iron wire that were extended beyond the elastic limit and also spirals of steel wire that were extended below the elastic limit. According to the current tables $\frac{2n}{q}$ here equals 0.6–0.8, *i.e.* less than unity. Both reversible and irreversible stretching resulted in a rotation in a direction opposite to that of the spiral itself; in other words a clockwise rotation for a Z-spiral*. We have previously published a sketch illustrating these results and Fig. 2 shows a spiral before and after extension. The rotation can be

* For instance AS' in Fig. 1.

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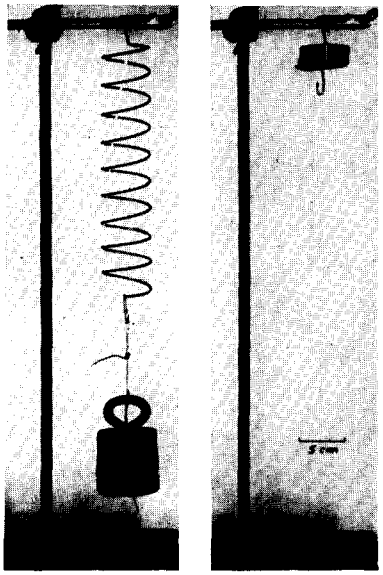

Fig. 2. S-spiral of iron wire, before and after stretching. Note the white paint dots arranged in a Z-spiral

followed here by examining the position of the white dots of paint on this S-spiral.

According to PRESTON (private communication) the formula employed by him is only valid for narrow and flat spirals, lightly stretched below the limit of elasticity. The models used by PRESTON to test his theory were 50 cm long and 0.5 mm wide. We used spirals 5 cm wide. In our opinion, this dimension is in better agreement with the relation between cell lumen and fibril thickness than that of very narrow spirals.

However, we are of the opinion that a cell wall possessing spiral structure cannot be compared with any kind of simple spiral spring. PRESTON (*l.c.*) also suggests that this analogy is open to doubt. Electron micrographs show a network of interwoven micro-fibrils running in all directions but with a preference for a transverse or nearly transverse orientation, as is normal for the meristematic cellulose cell wall and (as will become apparent in Part II) also for the chitin cell wall of *Phycomyces*. These microfibrils are unlikely to be independent of each other and may therefore not be compared with the single spiral of the model experiments, so that, quantitatively, large differences from the theory are to be expected.

However, if one wishes to compare a growing cell having spiral structures in its wall with a simple spiral spring that is being stretched, then our model experiments tend to support the existence of a Z-spiral structure in the meristematic wall of *Phycomyces*.

b. *Increase of pressure in tubes possessing spiral structure*

An important difference between the extension of spirals and the elongation of cells is that in the latter case elongation is the result of internal pressure. CASTLE (1937) and VAN ITERSON (1937) have pointed to the fact that the tension in the wall of a tube-shaped, turgid cell is larger in the transverse than in the axial direction. Therefore the wall reacts in a quite different way to axial stretching than it does to an increase of pressure. The following are striking examples illustrating this difference.

VAN ITERSON (*l.c.*) observed that cuticle-free cells of the well-known staminal hairs of *Tradescantia virginica* can, under certain circumstances, rapidly increase in size by endosmosis to 2–3 times their original length within a couple of minutes, during which process the statistical preference of the fibrils for a transverse orientation is not lost, but, on the contrary, is rather increased, since the negative birefringence increases.

Another example is that of the very rapidly growing stamens of grasses, in which the negative birefringence is likewise retained during elongation (SCHOCH-BODMER, 1939)*.

* The wall has to be in a special, extensible condition to demonstrate this phenomenon. FREY-WYSSLING AND SCHOCH-BODMER (1938) have reported that in the grass-stamens mentioned, an axial orientation of the fibrils occurs towards the end of the period of cell-elongation. Probably this applies to the outer layer of the wall only (ROELOFSEN, 1950).

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We consider these cases striking because elongation takes place so rapidly that the deposition of new cell-wall material can, to all intents and purposes, be neglected; the increase in surface area takes place at the expense of the thickness of the wall. These examples therefore demonstrate the exclusive influence of turgor pressure on the fibril orientation in the cell wall.

The influence of extension is a quite different one, since this always results in a reversion of orientation of the fibrils and consequently a reversal of the sign of the double refraction. This has been stated to occur in many cases, *e.g.* by BONNER (1935) for the *Avena* coleoptile. We ourselves have observed the phenomenon in young extended cotton hairs (15 days after flowering), an object in which the change had already been established by SISSON (1938) with the aid of its X-ray diagrams. The *Tradescantia*-hairs mentioned above and also the growth-zone of *Phycomyces** likewise show the phenomenon.

For this reason it seemed desirable to carry out additional model experiments on the twisting of tubes possessing spiral structure of the wall and in which the pressure could be increased.

We performed the first experiments with a piece of inner tube of a bicycle tyre, around which a jute string was wound in a Z-spiral. When air was pumped into this tube the string unwound, the tube twisting in the same direction as found by us for a stretched Z-spiral spring. A similar model-experiment has already been described by STEENBERG (1947).

An objection which might be raised against this model is the fact that the spiral structure within the wall can not very well be compared to the string wrapped around the tube. For that reason we prepared cellophane tubes.

Commercial cellophane in surface view usually shows double refraction in which the long axis of the refractive index ellipsoid coincides with the so-called machine-direction, the latter being evident from the weak striation that is to be observed in the cellophane. Both the cellulose-crystallites and the amorphous cellulose-molecules show a preference for orientation in the machine-direction. In accordance with this, the greatest extensibility occurs in a direction transverse to the machine-direction and the smallest in a direction coinciding with the latter (EBBINGE 1932). It is a general and long-accepted fact that in cell-walls too the greatest extensibility is to be found in a direction perpendicular to that of the fibrils.

Though the structure of regenerated cellulose is certainly different from that of native cellulose, there is reason to suppose that the mechanical properties may compare favourably with those of the meristematic cell-wall. In cellophane as well we find a network of micellar chains and molecules with a statistical preference for a certain direction.

We prepared cellophane cylinders with a spiral structure by cutting out strips at a certain angle to the machine-direction, subsequently rolling these strips around a

* This phenomenon caused us difficulties when sporangiophores (including the sporangium) were washed in warm NaOH under a coverslip. A contraction then takes place, but is hampered in these preparations because the sporangium is fixed between coverslip and slide, while the basal end usually protrudes from under the cover slip and adheres there onto the slide. In preparations treated in this way, part of the growth zone becomes, as a rule, positively birefringent or isotropic. Cotton hairs and *Tradescantia* hairs may be stretched by putting them in a humid condition on an slide covered by a dried-out layer of gelatine, and pulling them across that layer with a needle until they break. When the hairs have dried, positively birefringent extended cells may be seen as well as negatively birefringent unextended ones.

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glass cylinder and gluing the edges together with a water-resistant adhesive. After that, the cellophane cylinder obtained was pushed off the glass tube except for one end which was tied onto the tube. The other end was fastened to a cork disc or just tied up. The pressure of the air in the cylinder was now increased via the glass tube and the rotation of the free end of the cellophane cylinder was followed. The most convincing rotation was observed in cylinders of a small diameter, because in these pressure can be raised to a higher level before they burst (bursting pressure inversely proportional to the diameter). It is also desirable to render the cellophane more extensible by wetting it with water or preferably with dilute NaOH.

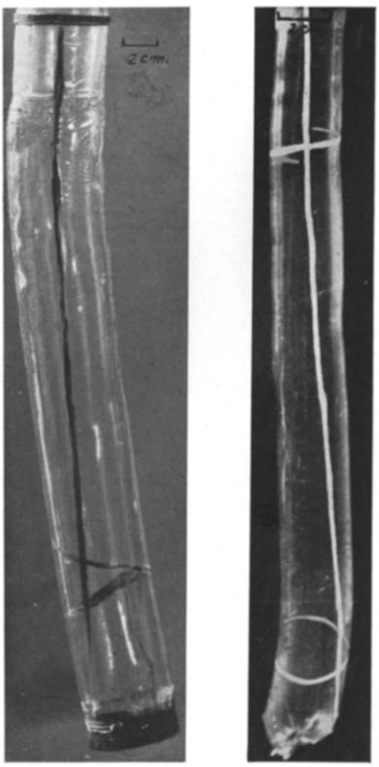

Fig. 3. Rotation of cellophane tubes with S-spiral-structure (left) and Z-spiral-structure (right) by increase of internal pressure

The cylinders were made in such a way that the machine-direction followed a spiral course. In various models the angle between machine-direction and the transverse direction of the cylinder varied from 10° to 90°, either in S-spiral or in Z-spiral direction Both machine-direction and longitudinal axis of the cylinder were indicated by a stripe of paint applied before we started raising the pressure.

Without exception these cylinders twisted when the pressure was changed. See Fig. 3. (The curvature of the tubes is due to the lower extensibility of the seam). When the pressure was increased, cylinders with Z-spiral structure, when seen from the free end (*i.e.* from below in the photograph), showed a clockwise twisting of this free end just as *Phycomyces* sporangiophores. Cylinders with S-spiral structure twisted in the opposite direction.

This is evident in view of the greater extensibility perpendicular to the machine-direction. However, considering the difference in tension in the wall in transverse and axial direction, a quantitative explanation of this phenomenon is not so simple. The question whether the steepness of the spiral is changed by inflation is also connected with the relation between these tensions in the wall. A superficial examination does not reveal any changes in steepness. It is unnecessary to go any further into this matter, because these model experiments only serve to illustrate that if the mechanical properties of the cell-wall of *Phycomyces* in the growth-zone are comparable to those of cellophane, the twist of the sporangiophores of *Phycomyces* during growth would point to the existence of a Z-spiral in the wall. This spiral must needs be less steep than 45°, because the birefringence of the double wall is negative with regard to the longitudinal axis of the cell.

It is of course still possible that the wall contains layers with both S- and Z-spirals, but then the Z-spirals are predominant in a mechanical sense. In Part II it will be shown, that there is an outer isotropic layer and an inner nearly transversely orientated one.

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§ 2. MICROSCOPICAL OBSERVATIONS ON WALL STRUCTURE

Because communications on this subject have already been given by OORT AND ROELOFSEN (1932) and by CASTLE (1938), only a few additional observations will be recorded here.

a. *Unwinding of a spiral*

When efforts were made to detach a sporangiophore in growth stage 4b* from the slide onto which it had stuck while drying out, the cell almost broke in two in the growth zone. A narrow ribbon, however, remained connecting the two parts, one of which, *viz.* the tip, still stuck to the slide. When the basal part was now pulled, the ribbon unwound in the manner indicated in Fig. 4. This proved that a flat spiral structure was present in the wall.

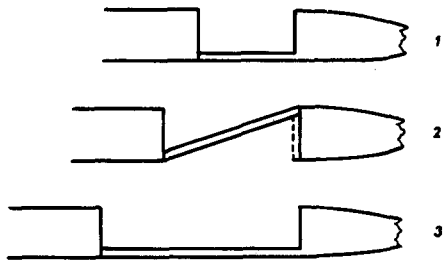

Fig. 4. Unwinding of a spiral in the growth zone

We have many times tried to repeat these observations, but the cell then usually broke completely in two. We have observed it in only three cells, originating from the same culture. We received the impression that it can only be seen in strongly twisting cells, for this was the case with other cells originating from the same culture-vessel.

All three times the unwound spirals turned out to be Z-spirals making angles of 8°, 9° en 13° respectively with the transverse. The last case is shown in Fig. 5b, which clearly proves the presence here of a Z-spiral. The ribbon is in the process of unwinding at the bottom side, for the top side of the cell, characterized by its minute axial folds appearing darker than their background between crossed nicols, is well in focus. Fig. 5a shows a case in which the growth zone has deliberately been torn into three parts; in all four places the spiral was a Z-spiral, which could either be observed at the place where rupture had occurred, in the same way as shown in Fig. 5b, or could be deduced from the Z-spiral twist occurring in the unwound ribbon.

ASTBURY AND PRESTON (1940) show a photograph of a similar phenomenon in *Cladophora.*

b. *Cuticular wrinkles*

VAN ITERSON drew our attention to the fact that CARNOY (1870) has already described a cuticle that makes the cell-wall of *Phycomyces* (called *Mucor romanus*)

* ERRERA has described 4 stages, stage 4 being split in two further stages (4a and 4b), by CASTLE (1942).

Thus stage 1. no sporangium, longitudinal growth;
2. growing sporangium, no longitudinal growth (very small yellow sporangium);
3. full-grown sporangium, no longitudinal growth (big yellow sporangium);
4a. renewed longitudinal growth, left hand rotation (big yellow sporangium);
4b. longitudinal growth, right hand rotation (big yellow, yellowish-brown, or brown sporangium).

We shall employ mostly the criteria recorded between brackets, which, as will be evident, do not differentiate between the stages 3, 4a and 4b as long as the sporangium is still yellow. A yellowish-brown or brown sporangium is only found in stage 4b. In special experiments we have established the fact that growth is resumed (4a) while the sporangium is still yellow and that cells with big yellow sporangia are even present at the beginning of stage 4b.

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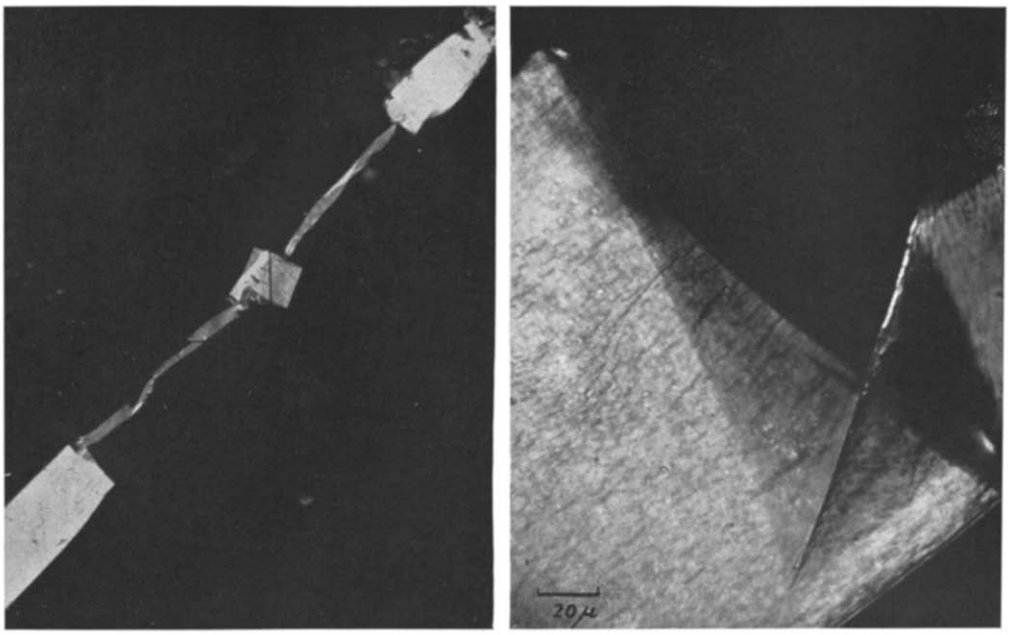

a b

Fig. 5a. Unwinding of spirals by tearing growth-zone into three parts. Growth stage 4b. Crossed nicols. Fig. 5b. Same as 5a; focussed on upper wall. A Z-spiral, making an angle of 13° with the transverse, is in the process of unwinding in the lower wall of the cell

impervious to reagents like IKI. He stated that the cuticle is very loose and can easily be rubbed away locally; in places where this had occurred reagents could stain the remainder of the wall without difficulty.

In cells which were in stage 1 and less obviously in those being in stage 4, CASTLE (1938) observed cuticular wrinkles orientated transversely in the upper part of the growth zone. These changed, via an isotropic network, into longitudinal folds below the growth zone; this he pictures as in Fig. 6. These wrinkles were indeed observed only in cells dried out on the slide, but apparently CASTLE tacitly assumes that they were also present in the turgid cell. They are said to be stretched in a positive sense by longitudinal growth and this he interprets as a demonstration of the theory of reorientation of transverse fibrils in cells growing longitudinally, a theory proposed but soon abandoned by FREY-WYSSLING.

In our opinion it is more likely that the wrinkles find their origin in a contraction of the cell wall caused by loss of turgor pressure, so that the direction of the wrinkles and that of least contraction coincide. Since the orientation of the fibrils is a transverse one in the growth zone and a preponderantly axial one below that zone and since, naturally, the contraction is greatest perpendicular to the direction of the fibrils, the direction of the cuticular wrinkles in and below the growth zone can be completely explained.

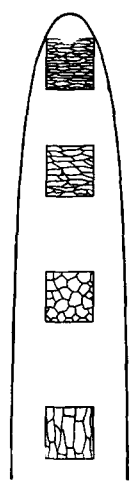

Fig. 6. Diagram (CASTLE, 1938) showing cuticular pattern at different locations in the growth zone in stage 1

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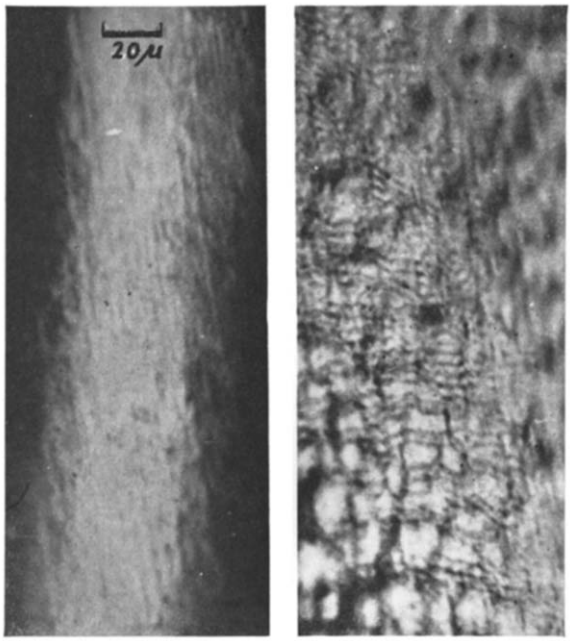

Fig. 7. Cuticular pattern in the growth-zone of stage 4b. Illumination from above. a. Steep S-spiral in turgid cell. b. Same cell in the process of contracting. Appearance of transverse wrinkles besides the steep S-spiral

Our surmise turned out to be correct. Sporangiophores in various stages of growth were carefully removed and were put on a slide. By this procedure they did not lose their turgor, since the basal part was very thin and plugged with protoplasm. When these cells were examined in or below the growth zone, under a microscope with illumination from above, we observed a rather irregular surface, in which (and this is very remarkable) we could always discern a steep S-spiral-shaped striation or folding but not the wrinkles described by CASTLE. We saw those appearing very clearly when the cell was cut in two in the middle. This caused a conspicuous contraction of the cell wall in the growth zone. Fig. 7a gives the picture of the cell wall surface in the growth zone of a cell in stage 4, whereas Fig. 7b has been taken in the same place immediately after the cell had been cut in two and while the wall was still in the process of contracting. For that reason the cuticular wrinkles are not in focus. The steep S-spiral retains its original orientation. Apparently the contraction follows the same S-spiral, otherwise the S-spiral-like folds would change their slope.

The cuticular folds may be studied also by making replicas in gelatin. For this purpose we applied the following technique, which is a modification of the procedure usually employed in the study of the scale-structure of animal hairs.

A drop of liquefied 12% gelatin was placed on a slide lying on a luke-warm copper plate and was subsequently smeared out in a layer of uniform thickness with the aid of a razor blade resting on two narrow strips of paper on the long sides of the slide (see Fig. 8). After that, turgid sporangiophores were placed in the layer of gelatin, so that they were half covered by this substance and the slide was put on a small ice cube for ½ minute in order to solidify the gelatin. As soon as the gelatin had dried in the air the cells were pulled off (if this is postponed too long the cells will break in the process)*.

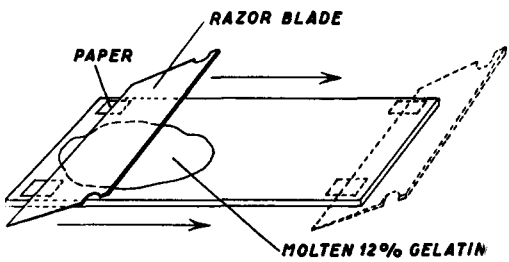

Fig. 8. Smearing out of gelatin prior to making replicas

* In the hope of obtaining a replica of the cell in the turgid condition we first removed the cells immediately after the gelatin had solidified, but in that case the replica turned out to be completely devoid of wrinkles. For that reason this method is also unsuitable for replicas of animal hairs.

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Fig. 9. Gelatin-replica of growth-zone of contracted cell in stage 4b just beneath sporangium. Mirror image of steep S-spiral and flat Z-spiral

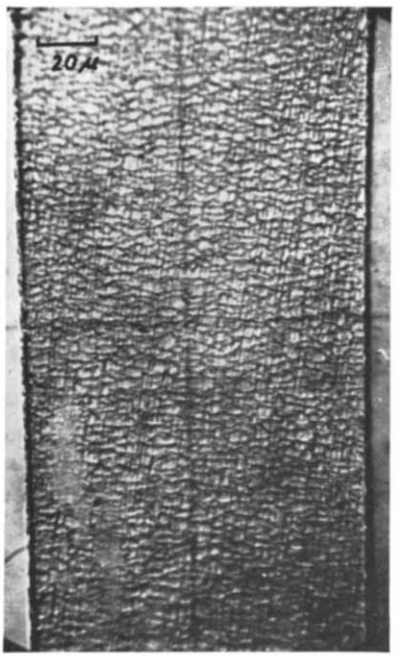

Fig. 10. Same cell as in Fig. 9, 1 mm beneath sporangium

Though the solidified gelatin embedding the turgid cells will counteract the shrinkage of the wall occurring on drying out of the cell, still a considerable shrinkage occurred, for in the growth zone the replicas always showed the two directions of folding we have just described for cells that had lost their turgor, *viz.* a steep S-spiral folding and the transverse folding described by CASTLE. The replicas may be studied by means of transmitted light, but better with the aid of illumination from above, especially by dark field illumination (Reichert's 42 × Epilum objective).

Finally, we have also employed a third method to study cuticular folding, *viz.* the one applied by CASTLE (*l.c.*) by allowing the cells to dry out on a slide. The results were similar except that the wrinkling could not be observed so well, despite the use of surface illumination.

Though both striation-systems could be observed fairly well in cells of stage 1, they were much more distinct in cells of stage 4, where, strangely enough, CASTLE (*l.c.*) could not discern them so well. Figures 9, 10 and 11 are pictures taken with the aid of illumination from above of replicas of the growth zone. Figures 9 and 10 are from the same cell, just below and 1 mm below the sporangium respectively. Fig. 11 shows another cell immediately below the sporangium. Because these are replicas we have a mirror image of the real situation and the direction of the spirals is reversed.

The steep S-spiral can be seen both in and below the growth zone; the mean angle with the axis of the cell was 9° and varied from 5–11°. Obviously, this folding is more or less parallel to the growth-spiral and also parallel to the direction of the greatest

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contraction occurring on loss of turgor pressure. CASTLE (1938) has also described these steep cuticular folds, but only "within the lower part of the growth zone and below" (Fig. 7 of his publication). He demonstrated that these folds run parallel to the growth-spiral. In the growth zone itself he also saw steep S-spiral striation but only after staining with congo red (Figures 10 and 11). Obviously then, staining enhanced the wrinkles in the growth zone, a fact that may be the result of the larger surface in the profile of the wrinkles. CASTLE's explanation of the origin of the striation-like coloration is not clear; he speaks of tiny cracks in the cuticle and also of an interfibrillar non-chitinous wall substance. This latter deduction is based on the disappearance of the striations on heating in acid. We do not think this last assumption is necessary; the disappearance may be the result of a more uniform penetration of the dye due to the chemical pretreatment.

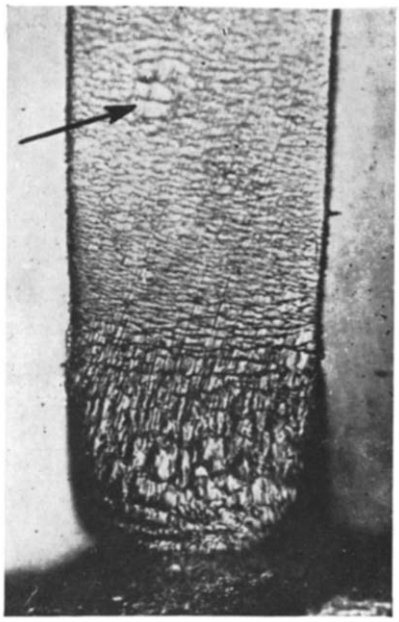

Fig. 11. As Figs. 9 and 10, just beneath sporangium. Arrow indicates spot where a bubble was present

We wish to point out that cuticular folds running in the direction of most active growth, have also been described in the hairs of *Tradescantia* (MARTENS 1934, VAN ITERSON 1937).

In the majority of cases it was very hard to decide whether the more or less transverse network of cuticular folds did, on the average, run in a transverse direction, or in flat spiral fashion, but in some preparations a spiral structure was very clear. Then it was always a Z-spiral. This can be seen in Fig. 9 and 11. This too supports the idea that the direction of greatest contraction follows a steep S-spiral course.

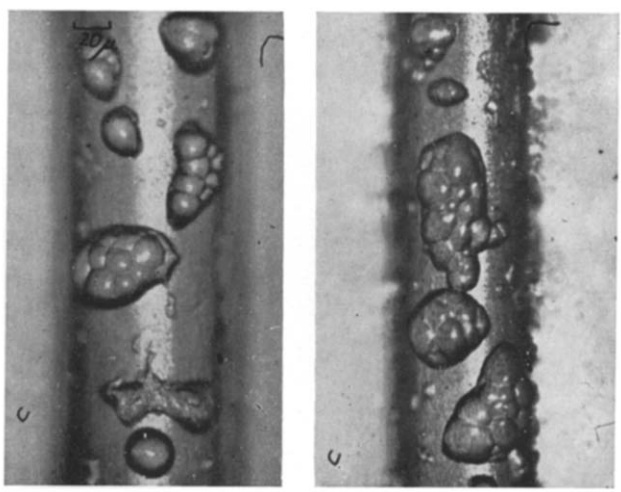

Fig. 12. Bubbles between cuticle and cell wall in stage 1. In the right picture cuticular films have spread out on the water surface

The great looseness of the cuticle also became apparent from the remarkable fact that drops of fluid seen to be excreted in such large numbers on the cell-surface below the growth zone in the growth-stages 1–3, proved to occur between cell-wall and cuticle. This became evident immediately from their shape, when examined in light from above. Fig. 12 gives a picture of this. In a cell which is half under water, the bubbles sometimes

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burst on the waterline and the cuticle spreads out on the water surface. Electron micrographs have been made of these cuticular films (Part II). In dried-out cells and in gelatin replicas, we were able to establish the fact that the cuticular pattern, as might be expected, is different in those places where bubbles had been present; this can be seen in Fig. 11 near the marked spot.

A loose cuticle is relatively rarely found. MARTENS (1933) mentions these cases, and he himself described one in petals and staminal hairs of *Tradescantia virginica.*

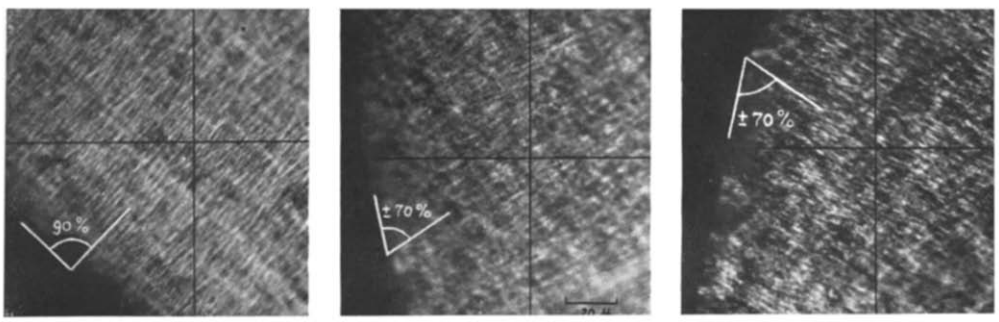
c. *Wrinkles and striations in the cell wall itself*

These are only visible between crossed nicols in the cleaned, preferably single, cell wall, after staining with congo red or chlorzinc iodine. The cells were put in a drop of 5% NaOH on a slide (or first in water and afterwards in NaOH) and then cut through obliquely beneath the sporangium. By tapping softly on the coverslip the cell was made to roll through about 90° and at the same time the contents were squeezed out. In this way a small diamond shaped area of single cell wall was made visible at the cut end of the cell. After that the preparation was heated for 15 minutes on a steam bath and water was added from time to time, to make up for losses due to evaporation. By this procedure the wall became permeable and proteins and fats for the greater part went into solution. Moreover the ribbon-like cell became fairly rigid and could be moved about on the slide without difficulty. Then, with a dissecting needle, the cell was pulled away from under the coverslip into a drop of 1% congo red; it was subsequently transferred to water and then to glycerol on the same slide. It was examined in glycerol.

More or less transverse striations

In the meristematic wall kept in a diagonal position between crossed nicols minute transverse striations are to be seen, giving the impression of very small undulations or folds. Sometimes these are more pronounced than at others. (See Fig. 13a and 14a). The fact that the smallest index of refraction is oriented radially accounts for the stronger birefringence of wrinkles in the wall.

Because it was our hope to demonstrate the existence of a spiral structure, we studied the way the wrinkles were arranged with respect to the transverse direction.



a b c

Fig. 13. Misleading orientation (indicated) of striations (wrinkles) in the primary wall, as seen on the inside of the single wall between crossed nicols. a. Diagonal position; transverse striation. b. Angle of 12.5° between cell axis and vibration-plane of polarizer. Striation apparently in S-spiral. c. As b but in other quadrants; striation apparently in Z-spiral

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However, it became clear that their direction apparently changed when the microscope-stage was turned round, so that, according to the position of the cell, we could observe folds varying in direction between a Z- and S-spiral, at an angle of about 20° with the transverse. This can be seen in photographs of Fig. 13b and 13c and, schematically, in Fig. 14b and 14c. The photographs are of one of the not infrequent cases where the wrinkles were very pronounced. The apparent change in direction is caused by the fact that the wrinkles run not only in a purely transverse direction, but also at angles with the transverse, so that, when the stage is turned round, those wrinkles running diagonally to the planes of vibration of the nicols (or showing the nearest approach to that position), become most pronounced.

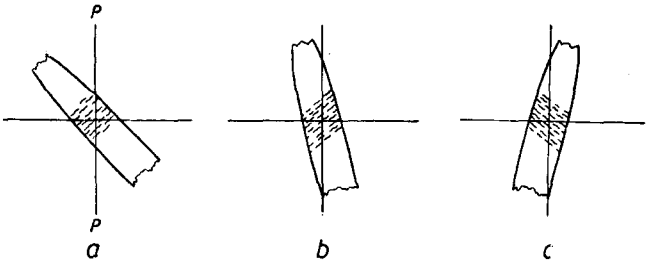

Fig. 14. Diagram showing how misinterpretations of apparent striations may arise

It is a striking fact that in the diagonal position (Fig. 13a) no wrinkles arranged in an axial direction are visible, but only bands of alternating lighter and darker tones. However, this fact alone does not warrant the conclusion that they are absent, for the bi-axial index-ellipsoid in the primary wall is orientated in such a way, that the smallest index, $n\alpha$, has a radial direction, $n\beta$ an axial one and the largest index, $n\gamma$, a transverse one. When the cell is in a diagonal position an inclination of the wall with $n\gamma$ as its axis (transverse undulation) will always result in a stronger birefringence, however large this inclination may be. On the other hand an inclination with $n\beta$ as its axis (longitudinal undulation) will first become isotropic and will only brighten up at larger inclinations. Thus, when the undulations run in all directions, one will still get the impression that there are mainly transverse wrinkles and wrinkles arranged in a flat spiral. In spite of what has been said we can assume a preference for a more or less transverse orientation, because the bright wrinkles indicated in our Figs. 13b and 13c, are not exactly diagonal but deviate from that position by 7.5 degrees.

This is in good agreement with the picture obtained at a 400–500 fold magnification by oblique, unilateral illumination of dried-out cells from above. On turning the stage, one sees many small wrinkles in transverse and nearly transverse directions, while in the axial direction only a few larger folds may be noticed as well as a few small ones.

From the electron micrographs described in Part II it also appears that there are many small wrinkles in the transverse direction, while in the axial direction only a few coarse folds are to be seen.

It thus became evident that the appearance of shrunken thin cell walls seen between crossed nicols may be a misleading one, giving the impression of the presence of several striation systems. An example of such misinterpretation, in which a series of investigators confirmed one another's observations so that finally their conclusions were accepted as established facts, is that of the alleged spiral structures in the primary wall of the young cotton hair. In such hairs stained with congo red and kept between crossed nicols, BALLS (1923) described two striations in the wall, each making an angle of about 70° with the cell axis, *i.e.* an S- and Z-spiral in one and the same wall. Later investigators (ANDERSON AND KERR, 1938; HOCK *et al.*, 1941; KERR, 1946) saw yet

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a third and transverse system of striation as well as these two. BALLS is the only one to remark that the striations seem to change their direction when the stage is turned. HOCK *et al.* see the transverse striation in the diagonal position only and the flat spiral striations only in positions making an angle of about 15° with the directions of vibration in the nicols. Both phenomena as well as the photographs given by HOCK *et al.* are in such complete agreement with what we described in *Phycomyces* that the explanation must be the same. Under the circumstances described, we were indeed able to establish in young cotton hairs phenomena identical in every respect with those in *Phycomyces* and, using illumination from above, we could also see here a system of folds running mainly in the transverse direction, although axial wrinkles were present as well.

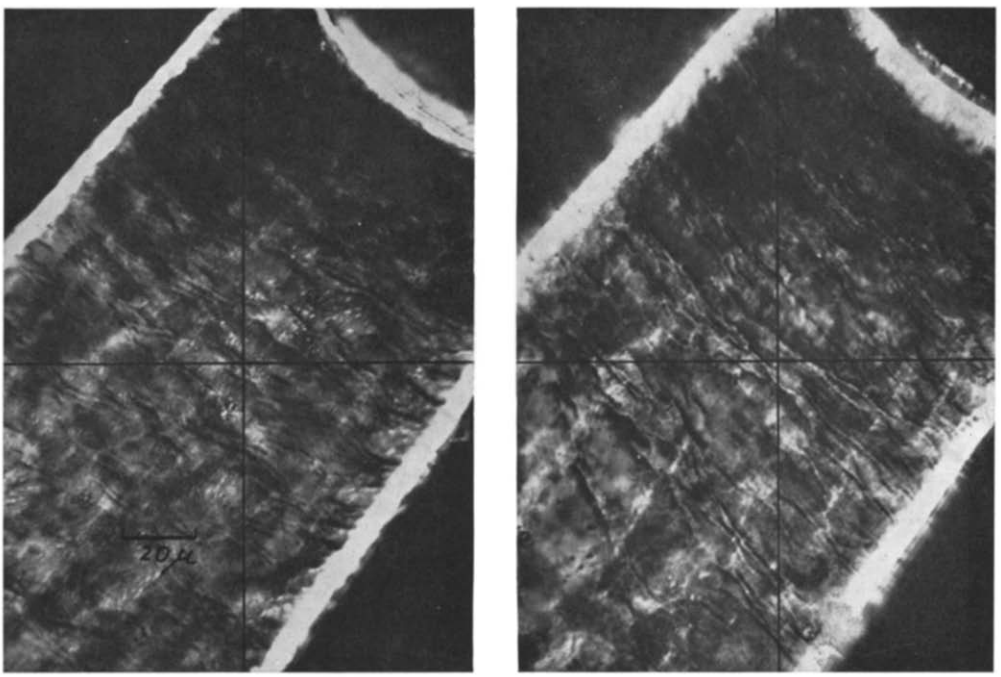

Fig. 15. Coarse folds in a Z-spiral in the growth zone of stage 4b. Stained with congored. Crossed nicols. Left: upper wall, right: lower wall

In some preparations of *Phycomyces* there appeared, for some reason as yet unproven, conspicuously coarser and more or less transverse folds, which appeared bright between crossed nicols. They could be observed in both the upper- and the lower wall of the cell. In Fig. 15 it can be seen that such folds followed a flat Z-spiral course. They did not change their direction when the stage was turned.

Axial striations

Apart from the more or less transverse undulations and wrinkles already described, one usually finds axial structures also in the growth zone. In the first place the rather broad lighter and darker bands to be seen for example in Fig. 13a, which may possibly be ascribed to undulations.

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VII

METABOLISM BY A SUSPENSION OF FRESH MINCE

μmoles acetoin				μmoles lactate			
9 No addit.	10 5 μg TPP	11 6 μg thiam.	12 5 μg TPP 6 μg thiam.	13 No addit.	14 5 μg TPP	15 6 μg thiam.	16 5 μg TPP 6 μg thiam.
+4.74	+4.88	+4.01	+5.56				
+3.16	+3.40	+3.31	+3.74				
+3.93	+4.21	+3.94	+4.97				
+5.05	+5.00	+4.04	+5.84				
+4.52	+5.09	+5.33	+5.73				
+4.75	+5.60	+4.45	+5.96				
				+1.67	+0.78	+1.67	+1.67
+4.03	+4.44	+4.04	+5.03	+0.89	−0.78	+2.89	+2.11
10 − 9 = 0.02 12 − 11 < 0.001 12 − 10 < 0.001							

As, however, acetate is consumed in our experiments we cannot decide this question before more knowledge is gained concerning acetate metabolism under the prevailing conditions.

The product of the reaction in which pyruvate and CO_2 are utilized is also unknown. It is not oxaloacetate, which is very unstable and would have been decomposed again. As acetate is utilized, citrate determinations were carried out, but with negative result.

The correlation coefficients show some interesting features. The second decarboxylation reaction has a strong negative correlation with the formation of acetoin, in the absence and in the presence of TPP. Perhaps this correlation might suggest that a two-carbon compound is formed, which—depending upon conditions as yet unknown—can either condense to acetoin or form acetate. Whether acetoin is a physiologically occurring intermediate or more or less an artefact, whose presence is perhaps due to a lack of some necessary factor, is not yet clear.

The highly significant linkage between the conversion of lactate to pyruvate and the fixation reaction deserves special comment. As the conversion of lactate to pyruvate is catalyzed by an enzyme which can cooperate with either DPN or TPN[14], this reaction might perhaps be a source of the TPN-H_2 necessary for the fixation of CO_2 to pyruvate by the "malic enzyme" as discovered in pigeon liver by MEHLER, KORNBERG, GRISOLIA AND OCHOA[14, 15]. Whether a similar enzyme operates in pig heart muscle remains, however, to be demonstrated. It also seems possible that lactate rather than pyruvate is the direct precursor of the fixation product by yielding on oxidation some form of "active pyruvate".

Addition of TPP has no significant influence on the "oxidative decarboxylation". This does not mean that this reaction is not catalyzed by a TPP bearing enzyme. It might be that TPP is not split off from this enzyme during storage. Even if the same intermediate is formed as in the decarboxylation to acetoin, which is strongly TPP-dependent, addition of TPP would not necessarily enhance the "oxidative" decarboxylation, as the formation of "acetate" might already proceed at the maximal rate.

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We also employed walls stained beforehand with congo red and we tried saturated $ZnCl_2$, 60–80% sulphuric acid and SCHWEITZER's reagent. Finally, we also studied microtome cross-sections, made after embedding in paraffin and swollen as indicated. In not a single case did we see lamellae, either in ordinary light, or between crossed nicols or in a phase-contrast microscope. We did however, observe a conspicuous outer layer, which was more resistant and probably cutinized and also the strongly swollen hyaline remainder of the cell-wall. The cuticle proper must have been dissolved. Especially by using too narrow a diaphragm, conspicuous diffraction-lines that might be taken for lamellae, were observed. Again, by focusing at a higher or lower level one could see longitudinal stripes in the wall. However, we cannot believe that a skilled microscopist like CARNOY should have been mistaken in this and we do not, therefore, consider ourselves justified in denying the presence of lamellae in the wall. They might become apparent under conditions not realised by us.

Electron micrographs do not show the presence of many layers either. Besides the cuticle two layers could be found in the growth zone (Part II).

(To be continued)

SUMMARY

OORT AND ROELOFSEN's original opinion (1932) that the cell wall in the growth zone of *Phycomyces* sporangiophores in growth-stage 4 (ERRERA) possesses a flat Z-spiral structure, was corroborated by the following facts and considerations.

1. If it is assumed that spiral growth is comparable to a simple extension of spirals, model experiments on wire spirals show that the clockwise rotation of the sporangium occurring during growth or on increase of pressure is in agreement with a Z-spiral structure (section 1a, Fig. 2).

2. If spiral growth is deemed comparable to an extension due to internal pressure of tubes with spiral structure in the wall, the clockwise rotation of the sporangium likewise favours a Z-spiral-structure (section 1b, Fig. 3).

3. The presence of a Z-spiral structure in the growth zone is demonstrated by the unwinding of Z-spirals when the cell is torn apart (section 2a, Figs. 4 and 5).

4. As may be expected for a flat Z-spiral structure, the extensibility of the wall is greatest in the direction of a steep S-spiral. This follows from the Z-spiral direction of folds formed after shrinkage in the cuticle (Figs. 7, 9, 11, section 2b), and sometimes in the wall itself (Fig. 15). Often, however, it is difficult to judge whether the folds run transversely or in spiral fashion (Fig. 10).

The cuticle of the sporangiophores is very loose; the drops of moisture on young sporangiophores are located between cuticle and cell wall (section 2b, Fig. 12).

Between crossed nicols the small wrinkles resulting from shrinkage of thin cell walls such as those of *Phycomyces* and young cotton hairs, give the false impression that spiral systems are present (section 2c, Fig. 13 and 14).

The lamellar structure of the wall, described by CARNOY (1870), could not be confirmed (section 2d).

RÉSUMÉ

Les faits et considérations suivants nous ont permis de confirmer l'opinion que OORT ET ROELOFSEN ont publiée en 1932 au sujet de la paroi cellulaire de la zone de croissance des sporangiophores de *Phycomyces*. Cette paroi posséraît, en effet, dans le 4e stade de croissance (d'après ERRERA) une structure fibrillaire en forme de spirale en Z.

1. Admettons qu'une croissance en forme de spirale soit comparable à une simple extension de spirales, des expériences modèles avec des spirales en fil métallique montrent alors que la rotation du sporange dans le sens des aiguilles de la montre, qui a lieu pendant la croissance ou lorsque la pression augmente, est en accord avec une structure en forme de spirale en Z (§ 1a, Fig. 2).

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2. Si l'on considère la croissance en forme de spirale comme comparable à l'extension sous l'influence d'une pression interne de tubes, ayant une paroi avec structure en forme de spirale, alors la rotation du sporange dans le sens des aiguilles de la montre est également en faveur d'une structure en forme de spirale en Z (§ 1b, Fig. 3).

3. La présence d'une structure en forme de spirale en Z dans la zone de croissance est démontrée par le fait que, lorsqu'on déchire la cellule en pièces, quelquefois des spirales en forme de Z se déroulent (§ 2a, Fig. 4 et 5).

4. Ainsi que l'on pouvait s'y attendre pour une structure en forme de spirale en Z, la paroi s'étend le plus dans la direction d'une spirale en S montant rapidement. En effet, les plis qui se forment lorsque la cuticule (Fig. 7, 9, 11) ou parfois la parois elle-même (Fig. 15) se ratatinent, ont la direction d'une spirale en Z. Il est souvent difficile, cependant, de décider si les plis sont transversaux ou en spirale, (Fig. 10).

La cuticule des sporangiophores est très lâche; les gouttelettes d'eau sur les jeunes sporangiophores sont situées entre le cuticule et la paroi cellulaire (§ 2b, Fig. 12).

Entre nicols croisés les petites rides qui prennent naissance lorsque des parois minces telles que celles de *Phycomyces* et de coton jeune se ratatinent donnent la fausse impression que des systèmes en forme de spirale y existent (2c, Fig. 13 et 14).

Nous n'avons pas réussi a confirmer l'existence d'une structure en forme de lamelles telle que l'a décrite CARNOY en 1870 (§ 2d).

ZUSAMMENFASSUNG

Die ursprünglich im Jahre 1932 von OORT UND ROELOFSEN geäusserte Ansicht, dass die Zellwand des Meristems der Sporangiophoren von *Phycomyces* im vierten Wachstumsstadium (ERRERA) eine flache Z-spiral-Struktur besitzt, wurde durch die folgenden Tatsachen und Betrachtungen bestätigt.

1. Wenn man annimmt, dass Spiralwachstum mit einer einfachen Ausdehnung von Spiralen vergleichbar ist, dann zeigen Modellversuche an Drahtspiralen, dass die Drehung der Sporangien im Uhrzeigersinn die während des Wachstums oder bei zunehmendem Drucke vor sich geht, mit einer Z-Spiral-Struktur in Einklang gebracht werden kann (§ 1a, Fig. 2).

2. Wenn Spiralwachstum als vergleichbar angesehen wird mit einer Ausdehnung von Röhren mit Spiralstruktur im Wande, unter dem Einfluss von innerem Druck, dann weist auch Drehung des Sporangiums im Uhrzeigersinn auf eine Z-Spiralstruktur hin (§ 1b, Fig. 3).

3. Die Gegenwart einer Z-Spiralstruktur im Meristem wurde durch das abwickeln von Z-Spiralen beim Zerreissen der Zelle bewiesen (§ 2a, Fig. 4 und 5).

4. Wie für eine flache Z-Spiralstruktur zu erwarten war, ist die Dehnbarkeit der Wand in der Richtung einer steilen S-Spirale am grössten. Dies folgt aus der Z-Spiral-förmigen Richtung von Falten, die sich beim Schrumpfen in die Kutikula (Fig. 7, 9, 11, 2b) und manchmal auch in der Wand selbst (Fig. 15) bilden. Ofters allerdings ist es schwer zu beurteilen, ob die Falten transversal oder spiralförmig verlaufen (Fig. 10).

Die Kutikula der Sporangiophoren ist sehr lose; die Feuchtigkeitstropfen auf jungen Sporangiophoren sitzen zwischen Kutikula und Zellwand (§ 2b, Fig. 12).

Zwischen gekreuzten Nicols erwecken die kleinen Runzeln, die beim Zusammenschrumpfen dünner Zellwände, wie z. B. von *Phycomyces* und jungen Baumwollhärchen, entstehen, den falschen Eindruck, dass Spiralsysteme vorhanden sind (§ 2c, Fig. 13 und 14).

Das Vorhandensein der von CARNOY im Jahre 1870 beschriebenen Lamellenstruktur konnte nicht bestätigt werden (§ 2d).

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