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CELL WALL TEXTURE AND CORTICAL MICROTUBULES IN GROWING STAMINAL HAIRS OF TRADESCANTIA VIRGINIANA

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SUMMARY

Cell wall texture and orientation of cortical microtubules of staminal hairs of *Tradescantia virginiana* were studied. A new method for determining of the microfibril orientation was applied. On the outer surface of the cell wall the microfibrils undergo a shift from preferentially transverse in young cells to preferentially axial in older cells. On the inner surface microfibrils are deposited in transverse direction throughout the growth period of the hair. The same applies to the cortical microtubules. The results are in agreement with the multinet growth hypothesis of Roelofsen and Houwink.

1. INTRODUCTION

Cell walls of staminal hairs of *Tradescantia virginiana* have been extensively studied by VAN ITERSON (1937) and ROELOFSEN & HOUWINK (1951). According to the latter authors, the orientation of the microfibrils on the inner surface of the wall is predominantly perpendicular to the axis of the cell, whereas on the outer surface the orientation is more or less isotropic. To explain this type of wall texture in growing cells ROELOFSEN & HOUWINK (1953) formulated the so-called multinet growth hypothesis (MGH). According to this theory the microfibrils are deposited on the inside of the wall in a direction perpendicular to the cell axis. During growth these microfibrils will be shifted in an outward direction and stretched in an axial direction. Although the outer surface of the cell wall of *Tradescantia* staminal hairs did not show this reorientation of microfibrils, ROELOFSEN & HOUWINK (1953) and ROELOFSEN (1959, 1965) considered these cells to constitute an example of multinet growth.

For more than 30 years, this theory was subject to both criticism and approbation (PRESTON 1974, FREY-WYSSLING 1976), but no reasonable alternative was available. Recently, however, BOYD 1985 published his new strain theory which implies that microfibrils in growing cell walls remain in the same position in which they are deposited, which would mean that there is no reorientation of microfibrils at all. In view of the existing confusion we decided to carry out a series of experiments in order to determine quantitatively the passive reorientation in a suitable system i.e. *Tradescantia* staminal hairs. In addition, this study investigates the correlation between the orientation of microfibrils and that of cortical microtubules in staminal hairs.

2. MATERIALS AND METHODS

2.1. Materials

Tradescantia virginiana plants were grown under greenhouse conditions (20 °C). Flowers in various stages of development were collected and stamens with hairs were obtained for examination.

2.2. Light microscopy

Staminal hairs were fixed in 3% formaldhyde in phosphate buffer (0.1 M, pH 7.2) for 10 min. and photographed with a Leitz Dialux 20 EB light microscope. From the microrgraphs lengths and widths of the cells were measured.

2.3. Electron microscopy

2.3.1. Cell wall extraction and preparation

In order to visualize the microfibrils, stamens with hairs were extracted in 10% NaOH for 30 min. Subsequently they were immersed for 45 min. in a mixture of equal amounts of hydrogen peroxide and glacial acetic acid at a temperature of 90 °C. After rinsing in distilled water the hairs were severed of from the stamens and attached to poly-L-lysine-coated grids. Some hairs were air-dried on the grids, other were dehydrated, critical-point-dried and cleaved (TRAAS 1984). All preparations were shadowed with platinum at an angle of 45°. Examination was carried out with a Philips EM 300 or 201.

2.3.2. Preparation for examination of microtubules and microfibrils in thin sections

Microtubules and in some cases microfibrils were visualized by means of the freeze-substitution procedure. Stamens with hairs were rapidly frozen by plunging them in liquid propane. They were transferred to a substitution fluid of anhydrous acetone containing 2% OsO_4 and 0.01% uranyl-acetate at -78 °C. The vials, containing the specimens, were transferred to a freeze-drying device with a temperature of -80 ± 5 °C for 20 h. For the next 6 h. the material was very slowly brought to room temperature, rinsed several times with anhydrous acetone, infiltrated with Spurr's resin and embedded as a flat layer.
Individual hairs showing no evidence of gross ice cristal damage were selected under the light microscope and thin sections were made with a Sorvall Porter Blum MT 5000. The sections were mounted on formvar coated grids and stained with uranyl-acetate/lead-citrate.

2.4. Quantitative analysis

2.4.1. Analysis of microfibril orientation

Micrographs of processed cell walls, showing the microfibrillar skeleton in different growth stages of the staminal hair cells, were printed at a final magnification of 92000 times. A plastic sheet with a regular point lattice (distance of points in horizontal and vertical direction 1 cm) was placed over the photographs and at each point the orientation of the underlying microfibril was traced. Next,

the angles of the microfibril directions with reference to the axis of the cell were measured with a Kontron Videoplan computer in 2 quadrants. In the graphs, the X-axis of the quadrants is drawn parallel to the axis of the cell.

2.4.2. Analysis of microtubule orientation

Thin sections were photographed and the microtubules were traced on sheets of plastic. The orientation of the microtubules with reference to the axis of the cell was measured using the Kontron Videoplan computer. In the graphs, the X-axis of the quadrants is drawn parallel to the axis of the cell.

3. RESULTS

3.1. Growth pattern of staminal hairs

Multicellular staminal hairs of *Tradescantia virginiana* develop from epidermal cells by repeated division of the top cell.Young hairs consist only of a few cells of roughly uniform size, measuring 10 μm in length and 10 μm in diameter. In full-grown hairs, consisting of about 18 cells, there is a differentiation in size from the top to the bottom. The youngest cells at the top measure about 75 μm in length and 50 μm in diameter, while the full-grown cells at the basis of the hair have a length of about 240 μm and a diameter of 70 μm. Thus the length of the cell increases up to about 24 times and the diameter up to about 7 times. Three stages in the development of a staminal hair will be referred to below viz. a cell of a young hair (stage 1), a cell from the top region of an older hair (stage 2) and a full-grown cell from the basis of an old hair (stage 3).

3.2. Microfibril organisation in growing cell walls

Figures 1A, 1B and *1C* represent the outer surface of cell walls of the stages 1, 2 and 3 respectively. The orientation of the microfibrils is gradually changing from preferentially transverse in stage 1, via more or less random in stage 2, to preferentially axial in stage 3. Since differences in texture are not easily recognized in photographs, the frequent distribution of the microfibrillar angles was measured quantitatively. The results are shown in *tables 1, 2* and *3*, which correspond to *figures 1a, 1b* and *1c*.

In *table 1* the highest frequency is found at about 90 degrees, which means that the main orientation of the microfibrils in young cells is transverse. *Table 2* shows the frequency distribution of microfibrils in cell walls of stage 2, showing no distinct peak, which means that the microfibrils have a random distribution. The graph representing stage 3 (*table 3*) shows 2 peaks, one between 30 and 40 degrees and another between 150 and 160 degrees, which can be interpreted as a preference on the part of the microfibrils for two oblique directions. Thus we conclude that there is a gradual shift of microfibrils from the transverse to the longitudinal direction of the cell during cell growth.

On the inner surface of the cell walls of differentiating hair cells the orientation of microfibrils has a different appearance. In young cells (stage 1) a predominantly transverse orientation of microfibrils is visible (*fig. 2A*). Most fibrils are

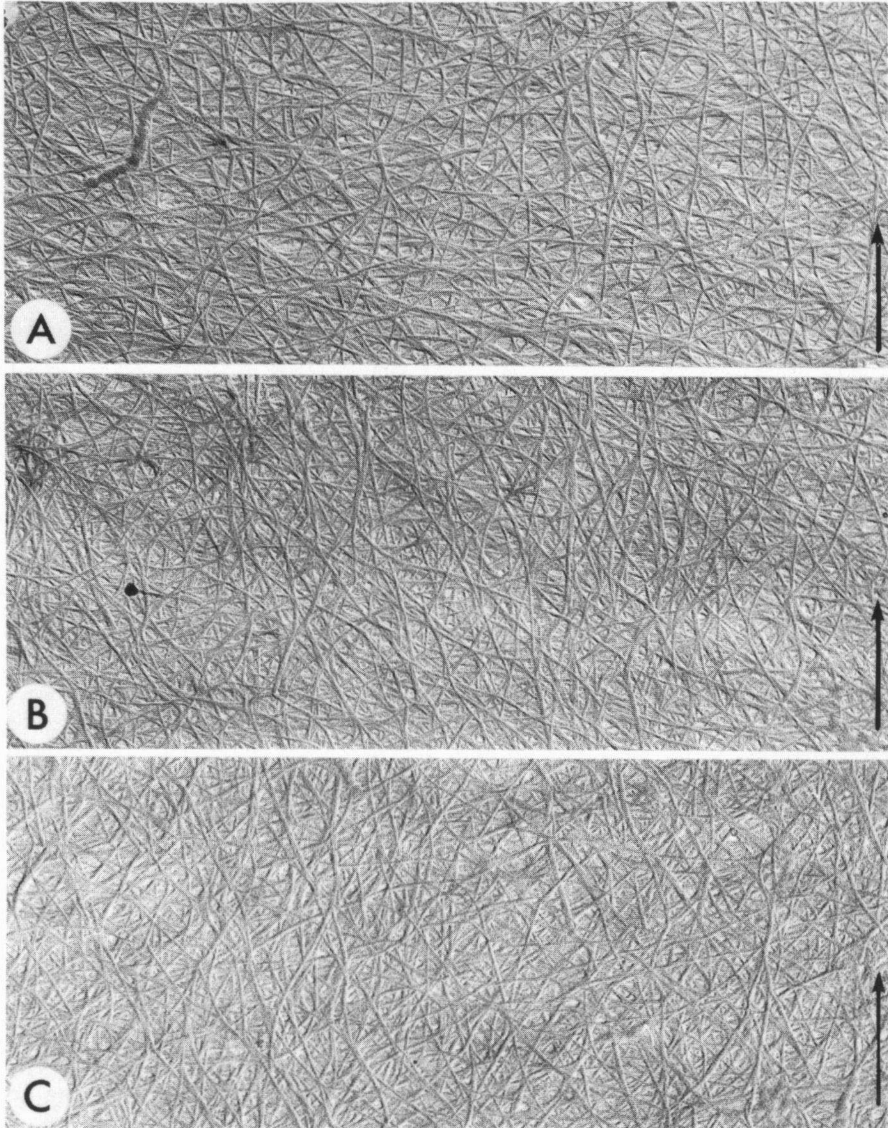
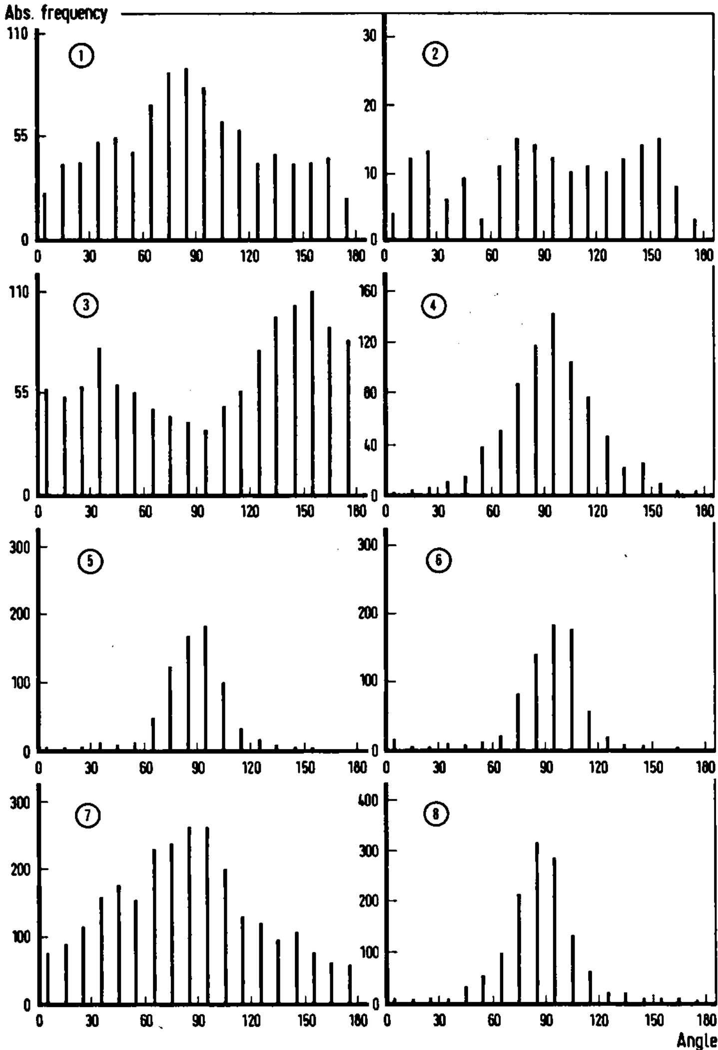


Fig. 1. Staminal hairs of *Tradescantia virginiana*. Microfibrillar organisation on the outer surface of the cell of a young (A), an older (B) and a full-grown hair (C). Cell axis indicated by arrow. 43.000 ×.

seen separately and only a few form small bundles at this stage. *Fig. 2B* represents the interior surface of the wall of stage 2 and stage 3. The most striking difference with the inner surface of the wall of stage 1 is the increase in bundle formation and the more pronounced transverse orientation of these bundles of microfibrils.



Tables 1–8. Absolute frequency of microfibrillar and microtubular angles in staminal hair cells of *Tradescantia virginiana*. Tables 1–3: frequency of microfibrillar angles on the outer surface of growing cell walls. Tables 4–6: frequency of microfibrillar angles on the inner surface of growing cell walls. Tables 7 and 8: frequency of microtubular angles in growing cells. The X-axis of the quadrants is parallel to the axis of the cell.

Microfibrils of the outer part of the wall are visible through the openings between the bundles. The frequency distribution graphs (*tables 4–6*) for the 3 stages are in agreement with the visual information obtained from the photographs of these stages. They all show a distinct peak at about 90 degrees, suggesting that the microfibrils are predominantly transversely oriented.

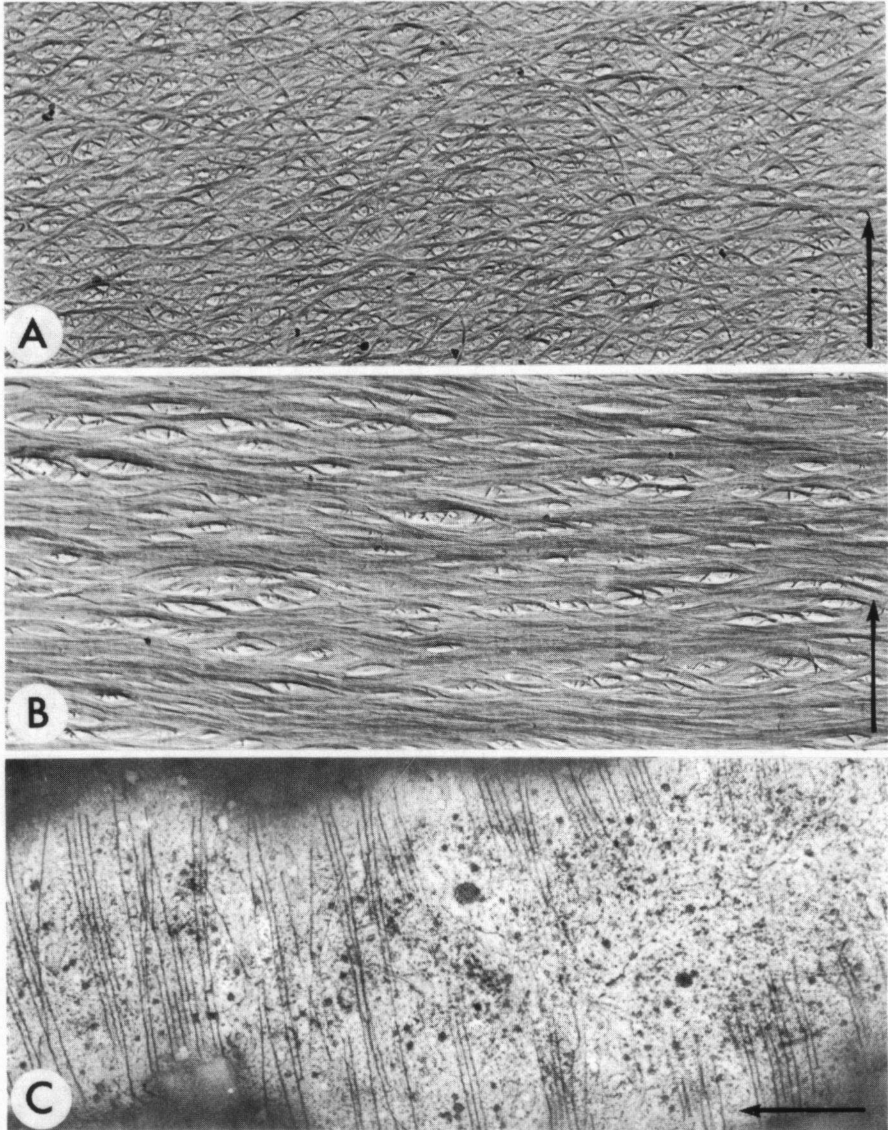


Fig. 2. Staminal hairs of *Tradescantia virginiana.* A and B: microfibrillar organisation on the inner surface of a young and an older cell wall respectively. C: arrangement of cortical microtubules in a young cell. Cell axis indicated by arrow. A and B 43.000 ×; C 20.000 ×.

3.3. Microtubular organisation in growing cells

Microtubular orientation was studied in tangential sections of cells in the above mentioned 3 different growth stages. Cells of stage 1 generally show parallel

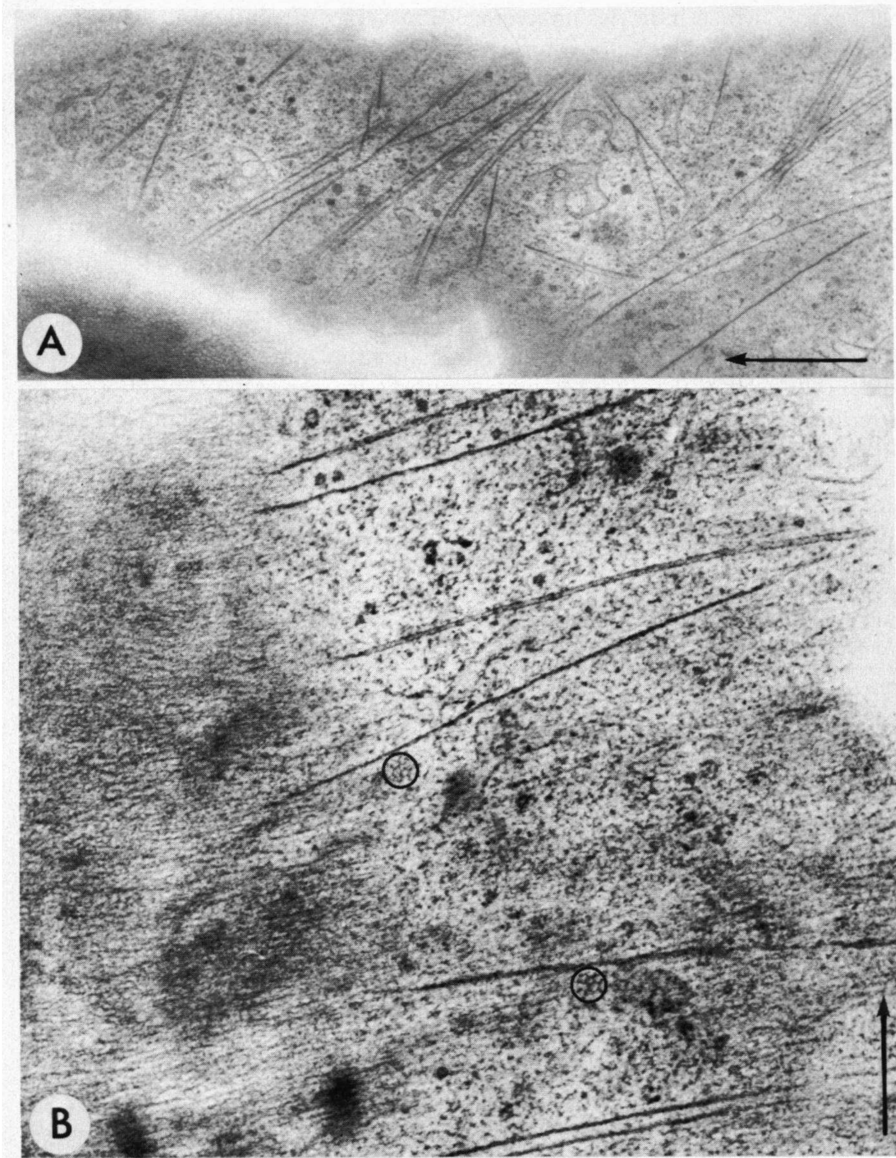


Fig. 3. Staminal hair of *Tradescantia virginiana*. A: arrangement of cortical microtubules in a cell of a young hair. B: cortical microtubules and last deposited microfibrils in a cell of a full-grown hair. Coated pits encircled. Cell axis indicated by arrow. A 20.000 ×; B 45.000 ×.

arrays of microtubules with transverse orientation (*fig. 2C*), but irregular orientation patterns occur as well (*fig. 3A*). In a frequency distribution graph of more than 2500 microtubular angles from young hairs (*table 7*) a peak is seen at about

90 degrees, which indicates an average transverse orientation of the microtubules. In cells of older hairs, representing stages 2 and 3 (*fig. 3B*), microtubules are less frequent and the orientation is transverse in all cells of the hair. This is expressed by the sharp peak at 90 degrees in the frequency distribution graph (*table 8*).

3.4. Correlation between microfibrils and microtubules

Generally, a correlation exists between the orientation of microtubules and that of microfibrils, especially in the older cells of a hair. The frequency distribution graphs of microtubules (*tables 7, 8*) and of microfibrils in the inner part of the wall (*tables 4–6*) both show peaks at about 90 degrees. An alignment between microtubules and microfibrils is also visualized in tangential thin sections of older cells, where microtubules and microfibrils are seen in the same photograph (*fig. 3B*). For one hair the angles of fibrils and tubules of the same cells have been measured with reference to the axis of these cells (*table 9*), and in this case too a correlation was found. In the photograph (*fig. 3B*) a strictly parallel orientation between single microtubules and the microfibril arrays is not always observed. A striking feature of this photograph is also that the microfibrils are very straight, and not grouped in undulating bundles as seen in extracted and shadowed material (*fig. 2B*). Furthermore, in tangential sections coated pits are frequently found (*fig. 3B*, encircled). The fact that coated pits are found in the same section with microtubules and microfibrils proves that the microfibrils are situated near the plasmalemma and are thus the last microfibrils to be deposited.

Table 9. Correlation between angles of microfibrils and microtubules of 9 cells of one staminal hair of *Tradescantia virginiana*. Top of the hair at left.

Angle of microfibrils	70	85.5	92	92.5	88	88	89	92.5	89
Angle of microtubules	76.2	86	90	83	82	88	94	95	96

4. DISCUSSION

When ROELOFSEN & HOUWINK (1951) published their study of staminal hairs of *Tradescantia*, their MGH had not yet been postulated. Nevertheless they expected to find a transverse orientation of microfibrils on the outer surface of young cells and an axial direction on the outer surface of older cells. On the basis of examination of cells with a polarization microscope the authors expected to find a transverse orientation on the inner surface of both young and old hairs. As far as the inner surface of the cell wall was concerned, they succeeded in finding a transverse orientation. However, for the outer surface of both young and old cells they described a random texture. In our study we observed a preferentially transverse orientation for outer surface of young cell walls and a preferentially axial direction for the outer surface of old cell walls. This discrepancy

may have been caused by the fact that ROELOFSEN & HOUWINK lacked an appropriate method for measuring the microfibril orientation of textures which superficially seem to be random. In fact the outer surface of the cell walls in growing staminal hairs behaves exactly as postulated in the MGH. As far as the inner surface of the cell wall is concerned, we were able to confirm the transverse orientation of the microfibrils as found by ROELOFSEN & HOUWINK (1951), but what these authors did not mention is that the percentage of transversely oriented microfibrils increases during the growth of the cells. Whether these microfibrils undergo a reorientation in an oblique direction is not clear. The undulating of microfibrillar bundles in extracted material may be caused by the axial elongation of the cell wall during deposition. Alternatively, the undulation could be an artefact caused by the treatment, since in thin sections of freeze substituted materials microfibrils are fairly straight and bundles of microfibrils are not frequently seen. In tangential thin sections, however, we can only see the last deposited fibrils and therefore we cannot draw conclusions concerning the more outwardly situated microfibrils. If, as the MGH predicts, microfibrils deposited in a transverse or nearly transverse direction are stretched in an oblique direction, the maximum reorientation can be calculated and should depend on the total increase of a cell in length and width (PRESTON 1982). For staminal hairs of *Tradescantia virginiana* the maximum expansion of the length of a cell is about 24 times and the maximum increase in width about 7 times. Microfibrils with an initial angle of 87 degrees with reference to the axis should undergo a reorientation of at most 8 degrees. Since the last deposited microfibrils were not subject to reorientation their direction did not change at all. From this we can conclude that the inner surface of growing cell walls of *Tradescantia* hairs will show more or less transversely oriented microfibrils in all growth stages.

The outer surface of the wall, however, behaves differently, because the initial orientation of the microfibrils is not transverse, although there is a certain preference for a transverse direction. Calculations of microfibril orientation in this part of the cell wall show a higher frequency of angle distribution at about 30 and 150 degrees after extension. This is in agreement with the MGH and confirms the generally accepted possibility of reorientation of microfibrils in growing cells (WARDROP et al. 1979, PRESTON 1982, NEVILLE & LEVY 1984, RYSER 1985).

The expression 'passive reorientation', suggested by GREEN and lateron adapted by PRESTON (1982) instead of MGH, is perhaps more generally applicable to growing cells (NEVILLE & LEVY 1984), but we prefer MGH because it is an accepted term with a known content and using another term might in fact cause more confusion.

In our study of *Tradescantia* hairs a good correlation was found between microtubules and microfibrils. This is in agreement with current ideas concerning microtubular influence on microfibril deposition (GUNNING 1981, HEATH & SEAGULL 1982). However, exceptions to the coalignment of microtubules and microfibrils have been reported (EMONS 1982, EMONS & WOLTERS-ARTS 1983, HAHNE & HOFFMANN 1985). The idea formulated by BOYD (1985), that strains, generated

in the cell wall, orient the cellulose synthesizing machinery in the plasmalemma and thus influence the orientation of the microfibrils, is very attractive. In our opinion the same forces could orient the cortical microtubules, and in that case no causal relationship needs to exist between the coalignment of microtubules and microfibrils.

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REFERENCES

BOYD, J. D. (1985): *Biophysical control of microfibril orientation in plant cell walls. Aquatic and terrestrial plants including trees.* Martinus Nijhoff - Dr. W. Junk Publishers, Dordrecht - Boston - Lancaster.

EMONS, A. M. C. (1982): Microtubules do not control microfibril orientation in a helicoidal cell wall. *Protoplasma* **113**: 85–87.

— & A. M. C. WOLTERS-ARTS (1983): Cortical microtubules and microfibril deposition in the cell wall of root hairs of Equisetum hyemale. *Protoplasma* **117**: 68–81.

FREY-WYSSLING, A. (1976): *The plant cell wall.* Borntraeger, Stuttgart.

GUNNING, B. E. S. (1981): Microtubules and cytomorphogenesis in a developing organ: the root primordium of Azolla pinnata. In: O. KIERMAYER: *Cytomorphogenesis in plants.* Springer-Verlag, Wien - New York.

HAHNE, G. & F. HOFFMANN (1985): Cortical microtubular lattices: absent from mature mesophyll and necessary for cell division. *Planta* **166**: 309–313.

HEATH, I. B. & R. W. SEAGULL (1982): Oriented cellulose fibrils and the cytoskeleton: a critical comparison of models. In: C. W. LLOYD (ed.): *The cytoskeleton in plant growth and development,* Academic Press, London.

ITERSON, G. van (1937): A few observations on the hairs of the stamens of Tradescantia virginica. *Protoplasma* **27**: 190.

NEVILLE, A. C. & S. LEVY (1984): Helicoidal orientation of cellulose microfibrils in Nitella opaca internode cells: ultrastructure and computed theoretical effects of strain reorientation during wall growth. *Planta* **162**: 370–384.

PRESTON, R. D. (1974): *The physical biology of plant cell walls.* Chapman and Hall, London.

— (1982): The case for multinet growth in growing walls of plant cells. *Planta* **155**: 356–363.

ROELOFSEN, P. A. (1959): *The plant cell wall.* Gebrüder Borntraeger, Berlin - Nikolassee.

— (1965): Ultrastructure of the wall in growing cells and in relation to the direction of growth. In: R. D. PRESTON (ed.): *Advances in botanical research.* Academic Press, London - New York.

— & A. L. HOUWINK (1951): Cell wall structure of staminal hairs of Tradescantia virginica and its relation with growth. *Protoplasma* **40**: 1–22.

— & — (1953) Architecture and growth of the primary cell wall in some plant hairs and in the Phycomyces sporangiophore. *Acta Bot. Neerl.* **2**: 218–225.

RYSER, U. (1985): Cell wall biosynthesis in differentiating cotton fibres. *Eur. J. Cell Biol.* **39**: 236–256.

TRAAS, J. A. (1984): Visualization of the membrane bound cytoskeleton and coated pits of plant cells by means of dry-cleaving. *Protoplasma* **119**: 212–218.

WARDROP, A. B., M. WOLTERS-ARTS & M. M. A. SASSEN (1979): Changes in microfibril orientation in the walls of elongating plant cells. *Acta Bot. Neerl.* **28**: 313–333.