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SUBMICROSCOPIC STRUCTURE OF THE CELLULOSE IN THE CELL-WALLS OF SPIROGYRA AND NITELLA

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1. INTRODUCTION

The presence of cellulose in the cell-walls of both *Spirogyra* and *Nitella* has been demonstrated by several investigators (cf. ROELOFSEN, 1959).

The presence of microfibrils in the cell-wall of *Spirogyra* was discovered by Vogel in 1950 by means of the electron microscope. It was not published but one of his micrographs is to be seen in FREY-WYSSLING's book (1953 p. 128). Since, however, the fibrillar orientation with respect to the cell axis is still unknown, this was studied in the investigation, reported here. We used *Spirogyra setiformis* Kütz.

The microfibrillar orientation on the *inner surface* of the cell-wall of *Nitella axillaris* Braun has been studied extensively by GREEN (1958). A predominantly transverse orientation was observed. This was corroborated recently by PROBINE and PRESTON (1961) with *N. opaca* Ag. However, they also observed a fairly large number of microfibrils lying roughly at right angles to the main direction, thus presenting a very imperfect crossed-fibrillar structure.

GREEN (l.c.) did not study the fibrillar orientation on the *outer surface*, but later (1960) he deduced from polarization microscopy that here axial orientation must predominate. It was our intention to verify this with the electron microscope by making replicas from the surface of cleaned cells. However, before our results could be published, PROBINE and PRESTON (l.c.) presented an electron micrograph in which axial fibrillar orientation is clearly visible in a lamella torn off from the outer surface of *Nitella opaca*. Since, however, we used another species viz. *N. mucronata* (H. Br.) Miq. and moreover studied replicas of the intact cell-wall instead of lamellae separated from the wall, it still seemed worth-while to mention our findings here.

2. METHODS USED

Spirogyra filaments were suspended in water and blended until disintegration in small pieces was obtained. These were centrifuged

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and extracted at boiling temperature for one hour with 2 % HCl and 2 % NaOH respectively. After frequent washing with double distilled water they were put on formvar coated grids, shadowed with Pt and observed with a modified Philips 100 kV electron microscope. For the study of transverse cell-walls the disintegration was simply continued until there appeared pieces in which a transverse wall had apparently been separated from the corresponding tubular piece of outer wall and could be seen protruding at one end of it. The transverse sectioning of paraffin embedded filaments with a microtome is another useful but more elaborate method of obtaining transverse walls.

Since with *Nitella* we were only interested in the outer surface of the cell-wall and since the cell-walls were rather too thick to be studied directly, we used the carbon-replica technique as described by ROELOFSEN, *et al.* (1953), with the only difference that the cell-wall was finally dissolved carefully in concentrated sulfuric acid instead of in cuprammonium. Previously the intact cells had been cleaned with HCl and NaOH as with *Spirogyra*, which was followed by treatment with 50 % ethanolamine and subsequently 5 % Na_2SO_3, both at 80° C for 1½ hr. The latter treatments were necessary to remove some unknown amorphous material which completely obscured the fibrillar structure at the surface. The direction of shadowing was perpendicular to the cell axis and at 45° to the plane of the slide.

3. DISCUSSION OF RESULTS

Spirogyra setiformis

In Fig. 1 both the outer and the inner surface of the cell-wall are visible. Evidently the main direction of the microfibrils is axial on the outer surface and transverse on the inner one and the texture is more dense in the latter case. These structures are in full agreement with multinet growth (see ROELOFSEN, 1959). However, both on the inner and on the outer surface there are microfibrils running in other directions. Of course the structure shown is that of the cellulose skeleton only, the native cell-wall contains in addition more than 50 % amorphous non-cellulose material.

Fig. 1 is typical for all micrographs we obtained. We could not find differences that might be ascribed to the cells being either young or old. In the filaments these occur side by side.

Fig. 2 shows the structure of the cellulose in the transverse cell-walls. The direction of the microfibrils is completely at random. They obviously tend to aggregate in flat bundles, a phenomenon seen in many cellulose preparations. One is not sure to what extent this bundling occurred before or arised after elimination of non-cellulose material.

Although there seem to occur some minute perforations of the wall, the difference with the transverse walls in filaments of land plants is very striking. In the latter there always are numerous perforations from plasmodesmata, e.g. in the septa of the staminal hairs of *Tradescantia* (see ROELOFSEN and HOUWINK, 1951). This difference may be

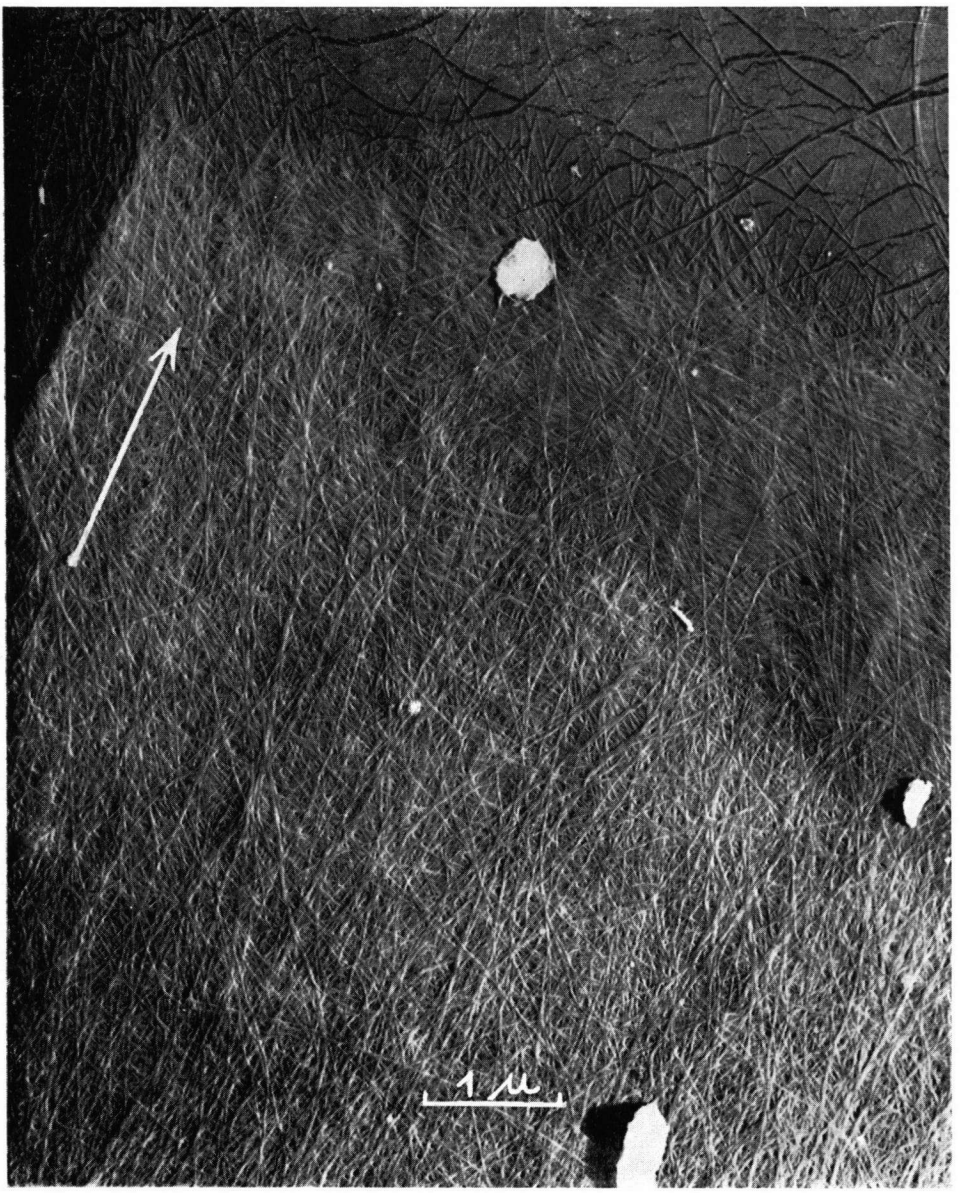


Fig. 1. Cellulose structure on the outer and the inner surface of the cell-wall of *Spirogyra setiformis*. Arrow indicates cell axis.

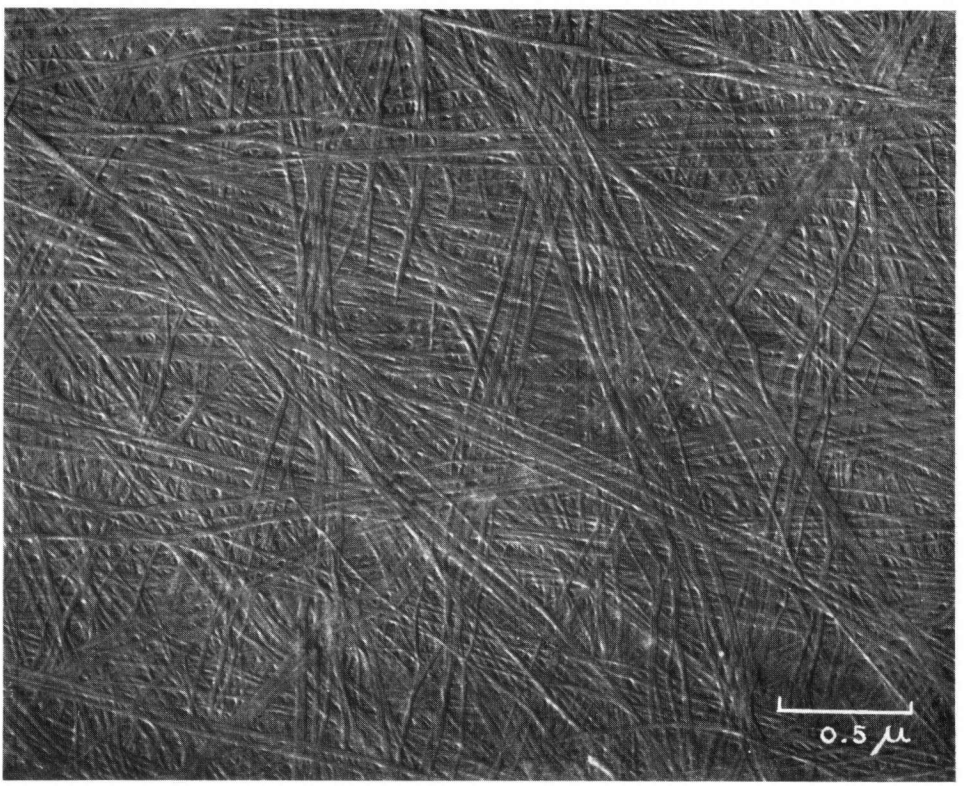
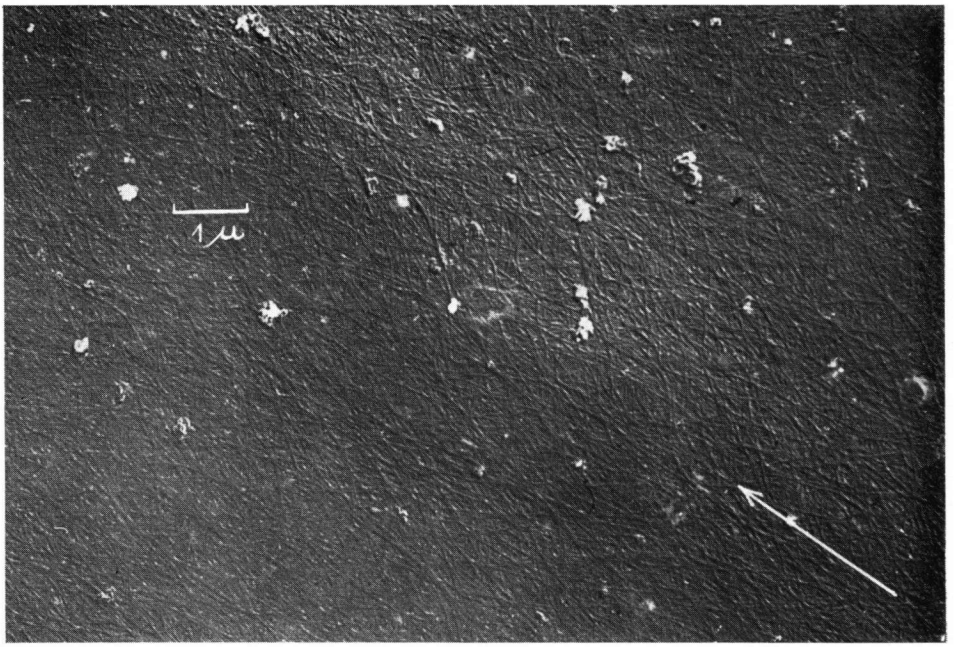


Fig. 2. Cellulose structure in a transverse wall of *Spirogyra setiformis*.



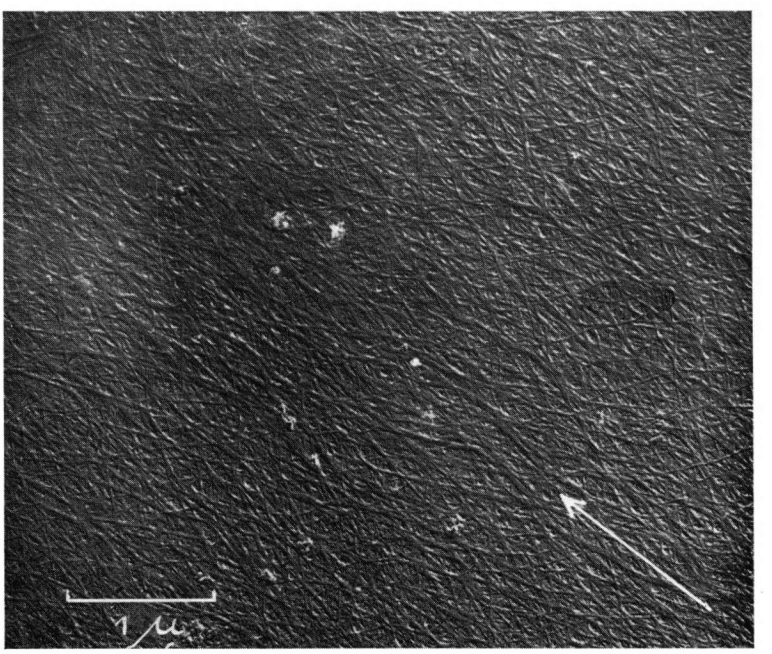


Fig. 3 and 4. Cellulose structure on the outer surface of the cell-wall of *Nitella mucronata;* arrow indicates cell axis.

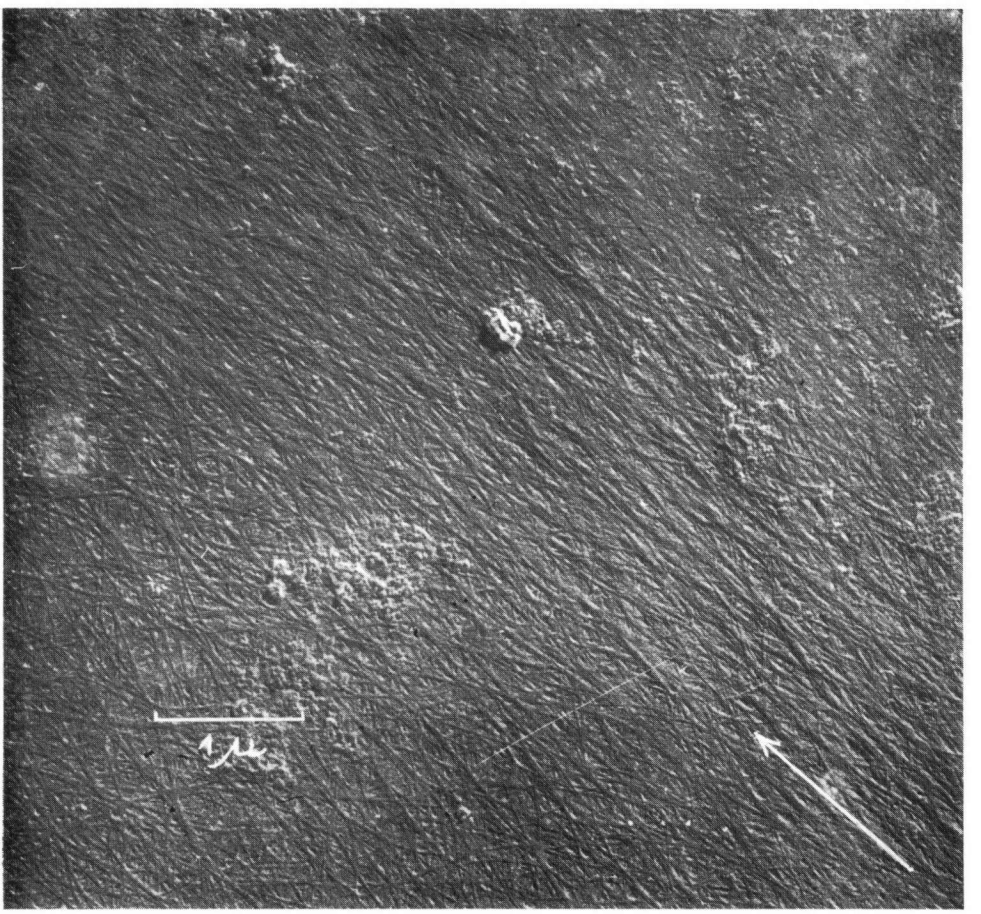


Fig. 5. A piece of the outer surface of *Nitella* showing a broad band with more axially oriented microfibrils.

related with the probability that every cell in the filament of *Spirogyra* can live as a single independent unit, whereas in the staminal hairs all nutrients and water have of course to be transported along the hair through the transverse walls.

Nitella mucronata

Fig. 3 and 4 show the cellulose structure on the outer surface of a cell having a length of about 1,5 cm. The same structure was seen in replicas of all cells studied which varied in length between 1 and 3 cm. As could be expected from the findings of GREEN (1960) and of PROBINE and PRESTON (1961) the main direction is axial or nearly so. In Fig. 4 the microfibrils tend to be aggregated in flat bundles. We believe that the structure as seen in Fig. 3 is the more natural one.

In Fig. 5 one can see a broad band of microfibrils being oriented more nearly axial. Such bands were observed very often. They were so frequent that they cannot correspond with the two striations which are conspicious in the light microscope and with according to PROBINE and PRESTON (l.c.) are regions of disorder in the cellulose structure. On the outer surface, however, we could not find any difference in structure that could be related to these striations.

SUMMARY

The cellulose structure of the cell-walls of *Spirogyra setiformis* and of *Nitella mucronata* was studied by means of the electron microscope.

In *Spirogyra* the main direction of orientation of the microfibrils is axial on the outer surface and transverse on the inner surface, which is in line with growth according to the multinet theory. The transverse walls have a random microfibrillar orientation and differ from transverse walls from filaments of land plants in the lack of plasmodesmata.

In *Nitella* the outer surface of the cell-wall shows a pre-dominant axial orientation of the microfibrils. This is in line with the findings of others who used other species and with multinet growth.

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