SPIRAL FIBRILS IN NERINE FOTHERGILLI

FURTHER EXAMINATION WITH POLARIZED LIGHT

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RESUMEN

Las fibrillas espiraladas de « Nerine Fothergilli ». Observaciones adicionales realizadas con luz polarizada. — El autor amplía su trabajo anterior *Remarkable Spiral Arrangement of Fibrils in the Cell Walls of « Nerine Fothergilli »* aparecido en esta misma Revista, tomo V (1940) 213-217. Describe e ilustra otras observaciones realizadas mediante el uso de luz polarizada sobre la notable disposición espiralada de las fibrillas que forman las paredes celulares de esa planta.

INTRODUCTION

The structure of the fibrous tissue of *Nerine Fothergilli*, a South African lily of the Amarillis family, is so remarkable that I called attention to it in a previous article published in *Lilloa*, V, 1940, pgs. 213-217, entitled *Remarkable Spiral Arrangement of Fibrils in the Cell Walls of « Nerine Fothergilli »*.

Further occasional study with greatly improved facilities and with the help of a skilled technician, J. P. Limbach, has clarified much which was then obscure. With use of accurately prepared specimens in polarized light, excellent photomicrographs have been obtained showing the spiral fibrils in their

* Maintained at Madison., Wis., in cooperation with the University of Wisconsin.

natural state, intact within the tissue of the leaves, as well as the stretched-out fibrils.

The hypothesis discussed in the previous article and proposed by a number of other writers; namely, that fibrils appeared to be a universal unit structure of cell walls in general, and that fibrils are of a uniform diameter of about one micron (10,000 Å), must be abandoned. Findings substantiate I. W. Bayley's conclusion that fibrils grade down in size to the very limit of visibility the light microscope (2,000 Å). In fact, some parenchyma cell walls themselves are 2,000 Å in thickness and therefore could not consist of larger fibrils. For example, if pith tissue of sunflower be viewed in brilliant sunlight or other parallel-ray illumination, it shows soap-bubble colorings indicating a thinness of the walls comparable with the half wave length of light.

Whether or not the finest fibrils might be of unit size in all cellulosic tissue and the larger ones made up of groups of the finer ones is still undetermined.

RECENT DETERMINATIONS OF FIBRIL SIZE IN GENERAL

Preston (11) states that fibrils were described by Grüger as long ago as 1854. Their diameter appears to be 4,000 Å in the unswollen condition, and to consist of micelles arranged in parallel order. In *Valonia* the walls have been spread flat and examined singly. The wall consists of 30 layers alternating in slope of fibrils with an angle of 80° between the two directions.

Eisenhut and Kuhn (6) contend that natural cellulose fibers consist of fibrils a few tenths of a micron in diameter (1 micron = 10,000 Å). These are further divided into microfibrils with diameters as small as 100 Å.

Wergin (13), after examination with and electron microscope, states that cellulose fiber is shown to be fibrillar and is made of bundles of elemental microfibrils 80 to 100 Å in diameter. From X-ray, optical, and electron microscope investigations, microfibrils appear to be 400 Å long. Each fibrillar bundle represents an individual structural element.

I. W. Bailey (2) states that fibrils are held together, not by a cementing substance, but by branching and lateral anastomosing connections (somewhat as proposed by Hermann, Gerngross, and Abidz in 1930 for structure of gelatine, and referred to as cellulose fibrillae by van Iterson (10) and vary in size, grading down to the limite of microscopic visibility (2,000 Å) even in much swollen material. They may be arranged into concentric or radial lamellae of various widths and porosity. Furthermore, many cell walls are less than 4,000 Å in thickness and hence cannot be composed of fibrils of greater diameter.

Wanda K. Farr (7) found what appeared to be unit-sized particles composed of crystalline cellulose approximately 10,000 Å in diameter which were remarkably uniform in size in various plant material. In a more recent study (8) she found uniformly sized cellulose particles, 1,1 × 1,5 microns, forming in cotton fiber.

C. Hock, R. Ramsey, and M. Harris (9) state that in cotton fiber, treatment with Congo red caused alternate coloring of lamellae which varied in thickness from 0,1 to 0,2 microns, determined from material swollen in dilute cuprammonium solution but reduced proportionately to unswollen size. They conclude from studies on *Valonia* that it is unlikely that fibrils are of unit size.

Florence Barrows (3) found cotton fibers without swelling to be built of layers which measure 2,35 microns center to center of adjacent lamellae. The width of the lamellae vary from 1,05 to 1,3 microns. Each lamella is made of a single row of cellulose ellipsoid particles held together by cementing substances. The particles are arranged end to end to form fibrils.

A. J. Bailey and R. M. Brown (1) measured thousands of apparent fibrils, in wood cell walls, which averaged 0,9 to 1,0 micron in diameter. They concluded that all natural cellulose was made of fibrils about 1,0 micron in diameter.

S. H. Clarke (4) states that fibrils are estimated to be 1,000 to 5,000 Å in diameter.

G. van Iterson (10) gives a comprehensive review of work done on cell walls up to 1933, with 79 references, and also discussion of his own extensive research on cell walls. He also

worked with *Valonia* and *Cladophora*. The walls may easily be split into 20 to 40 lamellae with parallel striations at nearly 90° with each other in the alternate layers. These striations in direction correspond exactly with the axes of the crystallites. Occasionally there is a slight indication of some at intermediate inclinations. As the walls are only 10 microns thick, the layers must be less than half a micron. The lamellae are easily disintegrated into fibrils by slight pressure. He asks why the fibrils are so much easier to separate than the crystallites themselves, as crystallites can only be separated by strong chemical treatment, such as dissolvinging cuprammonia. The way in which crystallites are bound together to form fibrils is still unsolved, but he suggested that their ends may be fringelike and interlace. These fringes are supposed to hold together with the same kind of force that binds molecules to the crystallites. It is certain that there is intermicellary matter between the fibrils. It is not certain, however, that this intermicellary matter plays a role in the cohesion of the crystallites into fibrils. In 1879 Nägeli accepted the possibility of the uniting of micelles into rodlike bodies in the same way that single crystals may unite into compound ones.

In addition to the works referred to on the existence and size of fibrils in cell walls, much has been done by Astbury, Bernal, Berkley, Clark, Frey Wyssling, Kundu, Marks, Marwick, Meyer, Preston, Sisson, Sponsler, Seifriz, and others on the atomic structure of cellulose, by means of X-ray diffraction, spectra. X—ray diffraction however, gives no information, other than orientation of the crystal axes, on the existence of the fibrils, which are too large to affect the spectra. It does, however, offer means of showing the orientation of the crystallites and of the planes of diffraction of the cellulose molecules with respect to the cell walls. Polarized light, on the other hand, offers means of determining the grosser structure, still below microscopic visibility, of the fibrils and their orientation.

FIBRILS EN NERINE

The spiral fibrils in *Nerine Fothergilli* are now clearly shown in the accompanying photomicrograph (Plate 1) as they naturally exist intact within the leaf tissue itself without being pullet out. The use of polarized light greatly improves the appearance. Plate 1 is a longitudinal section of a green leaf perpendicular to its broad surface, in the blackout position of the analyzer. The long direction is, and therefore the striations are, at 45° to the vibrations. Four distinct types of banding are apparent: (1) A tube or vessel with rings spaced far apart, (2) a tube with rings spaced near together, (3) a tube with a single spiral springlike thread, (4) three tubes with considerably finer single-pitched threads. The threads are brilliant in the 45° position to blackout. When the tubes were cut longitudinally through their walls, the cross sections of the threads attached to the side walls were clearly seen but are not shown in the photograph. The thread cross sections appeared as embossed rounded bumps on the inner surface of the wall, somewhat oval in shape. As the spirals are very small, it is difficult to measure their thickness accurately; but in one or two instances, the measurements taken indicate center-to-center distances (pitch of the thread) as 3,5 microns. If the spiral threads occupy the same distance as the spaces, they would be 1,7 microns in diameter. Apparently they are considerably narrower than the spaces. An attempt to measure them directly seemed to give 1,5 microns for the diameter of each thread.

In the onionlike skin from the bulb, these spiral tubes (5) are much more numerous than in the leaves, as shown in Plates II and III. Microscope sections of bulb skin were not made.

When stretched out, these spirals unwind into twisted ribbons or into loose worstedlike skeins which fibrillate into many separate fine filaments of astonishing length as shown in the photomicrographs in my previous article on *Nerine Fothergilli* (12). In breaking apart a piece of skin from the bulb, the filaments may be stretched to a length of 5 or even 10 centimeters. By polarized light with direct sunlight illumination, the twist-

ed filaments as pulled-out spirals were easily traced far into the tubes from which they originate. In one examination the stretched out portion was clearly seen within a tube as a continuous thread from the spiral lining to the narrowed stretched-out filament.

The appearance of the stretched filaments from the bulb skin does not seem to agree with the appearance of the spirals seen in the section of the leaves. The filaments into which the fibrils separate, in the case of the bulb skin, form multiple spirals of 4 to 10 or more filaments arranged side by side in parallel, but the longitudinal view in the leaf section does not indicate sufficient slope to accommodate bands of that width. The only explanation of this seems to be that the tissue of the bulb skin differs from that of the green portion of the leaf in having tracheids with many pitched spirals. Viewed in polarized light, in the blackout position, the fine threadlike filaments are distinctly visible twisting around in ribbon form as shown in plates II and III.

An attempt was made to determine by polarized light whether it was the cross banded or spiral thickenings in the leaf, or the spaces between which turned brilliant in the blackout position of the analyzer. In the longitudinal view (plate I), the cross bandings are brilliant when the fibrils lie in the 45° position to the blackout, whereas the walls of the tubes or vessels are dark. Moreover, the walls are not brilliant in any position. There can be no doubt as to the identity of the stretched-out filaments with the spiral fibrils in the tubes, and that they are composed largely, if not entirely, of cellulose crystallites whose axes are arranged parallel to the long direction of the fibrils.

Cross sections of the leaf at two magnifications are shown in plates IV and V. In the first, the analyzer was turned just enough away from the blackout to permit the outlines of the surrounding tissue to be seen. The tubes having the spiral or annular linings are the only tissue showing birefrigence, and they are localized in the leaf. The black cross in the vibration planes of polarizer and analyzer is clearly evident, which in connection with the great brilliance of fibrils, shows clearly

that the crystallite axes lie in concentric directions on the inside of the tubes. This agrees with the appearance of these fibrils in the longitudinal section. The intensity of the birefringence of these fibrils in contrast to all the other tissue is remarkable, since in ordinary light they are almost indistinguishable from the other tissue.

In intense sunlight by polarization when in the 45° position to blackout, the fine stretched-out filaments appear as if made of many smaller elongated rods sharply outlined by interference colors with black margins. The effect is probably due to interference from refraction around invisible particles of submicroscopic size or to the formation of Newton's rings by reflection across interstices less than the wave length of light in diameter. In either it indicates the existence of minute components of approximately the same shape and arrangement as the colored rings and rods. It is possible that these may actually be the crystalline micelles of which the filaments are composed. X-ray diffraction spectra tell nothing of these particles other than their orientation with respect to the axis, since the interference spots in the X-ray spectra are produced by diffraction from the very much finer cellulose molecules within the micelles. The edges of the filaments are intensely brilliant when in the 45° position. The individual particles appear in size to be of a length from about twothirds the width of the filament and perhaps one-fifth or one tenth as wide as long, to nearly round. In a general way the appearance resembles the pearl markings on an abalone shell.

Examination of isolated fibers of wood pulp showed almost exactly the same effects in bright light, being made of sharply defined, short, rodlike color rings outlined by black lines.

Plates II and III show the stretched-out filaments from the lower leaf tissue in polarized light sloping at 45° to the blackout. The intense brilliance of the filaments in this position indicates that the cellulose crystallites are well oriented in the axial direction. When turned parallel to the vibrations, they appear dark. It also suggests that they are nearly pure cellulose.

Ends of broken *Nerine Fothergilli* filaments are seen to fray out into still finer filaments down to the limits of visibility of

the microscope. This agrees with I W. Bailey's observations as to fibrils.

The transparency of the fibrils varies with their dryness and probably with their age. Those from the green leaves are more transparent than those from the bulb skin; this makes a great difference when viewed in polarized light. Feeble effects may be due to opaqueness.

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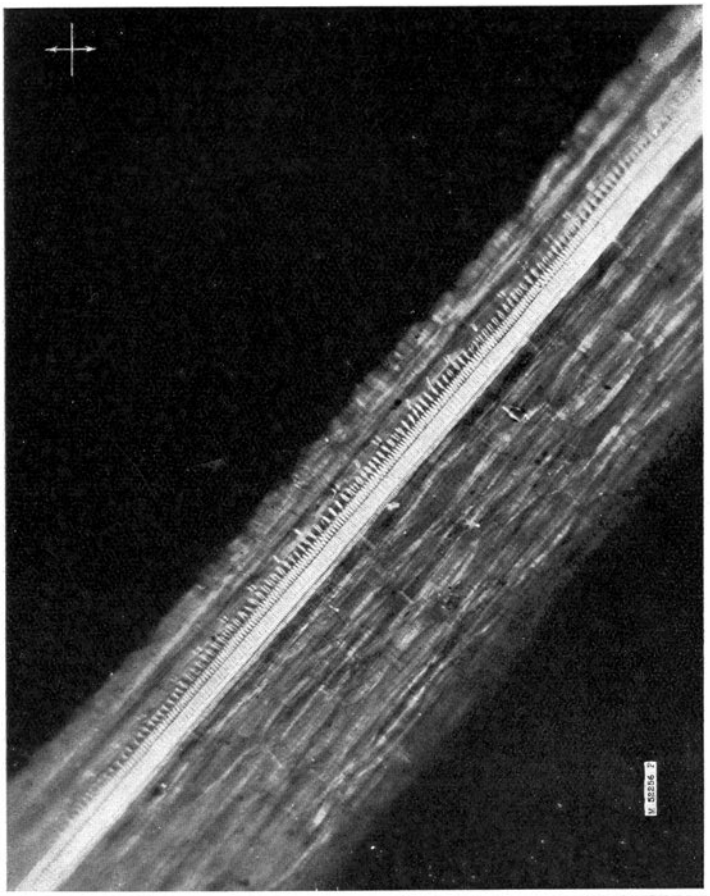
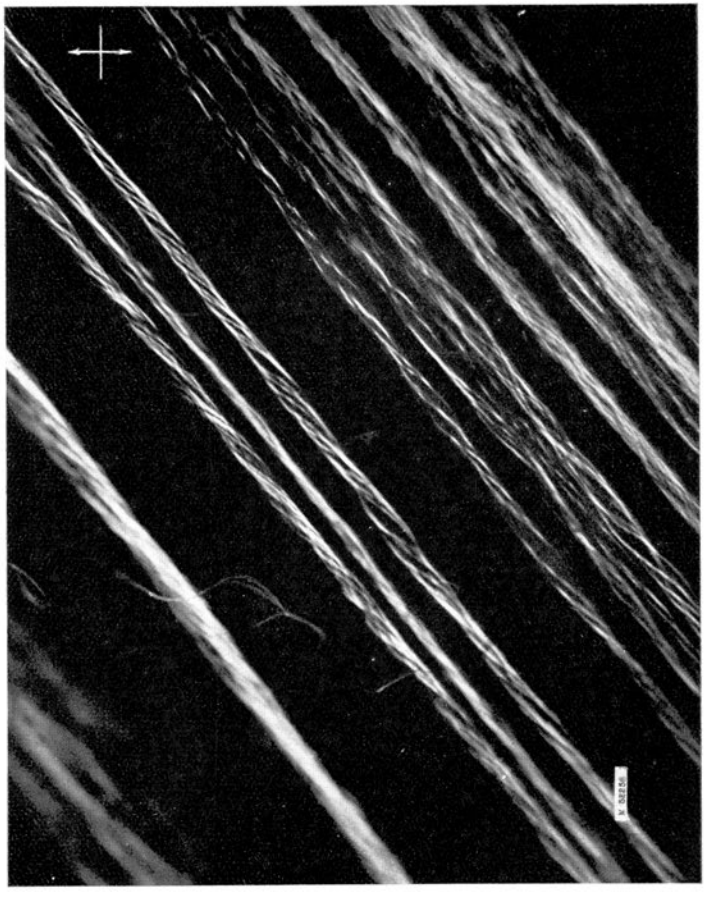
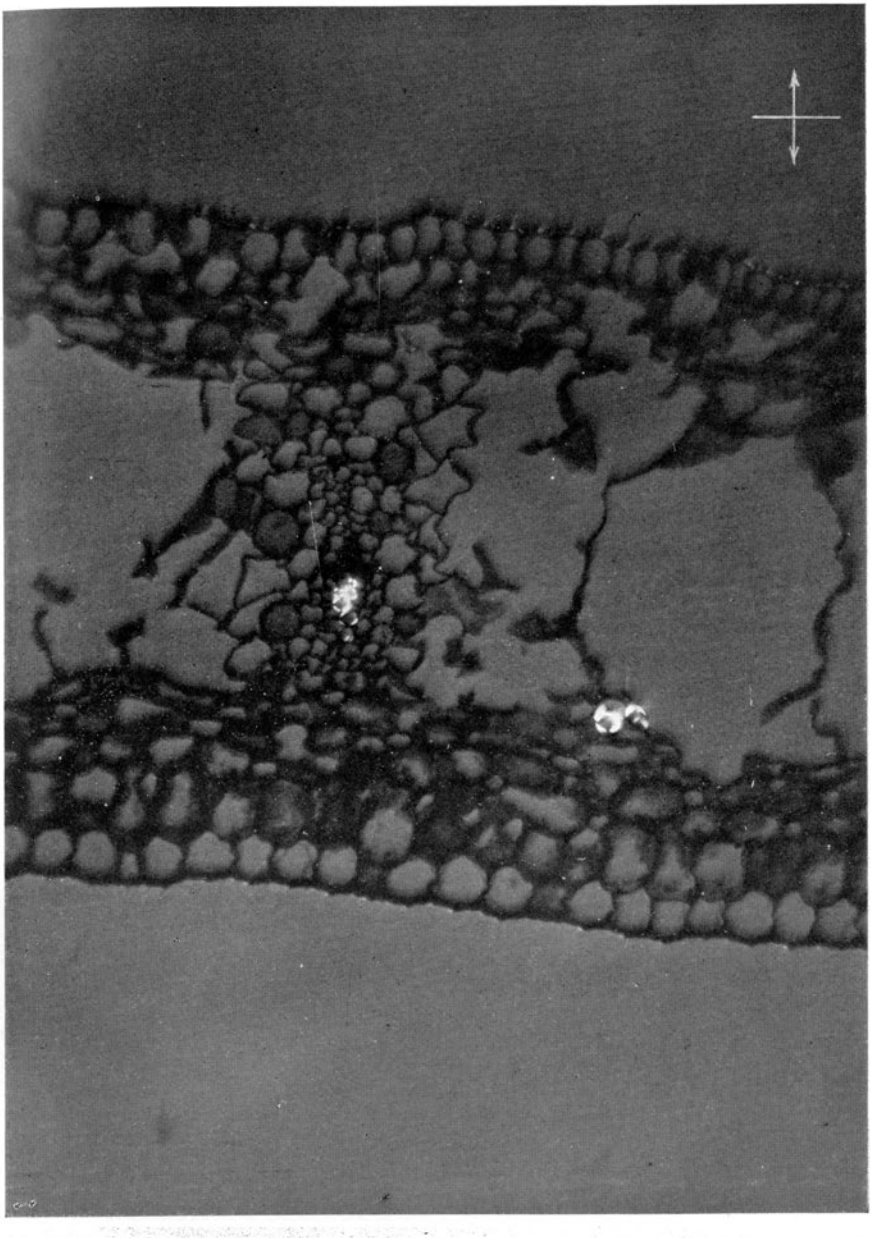


Photomicrograph of a longitudinal section perpendicular to its flat surface of a green leaf of *Nerine fothergilli* in polarized light showing bandings with rings spaced far apart, rings spaced near together, a single spiral, and three considerably finer single-pitched threads.

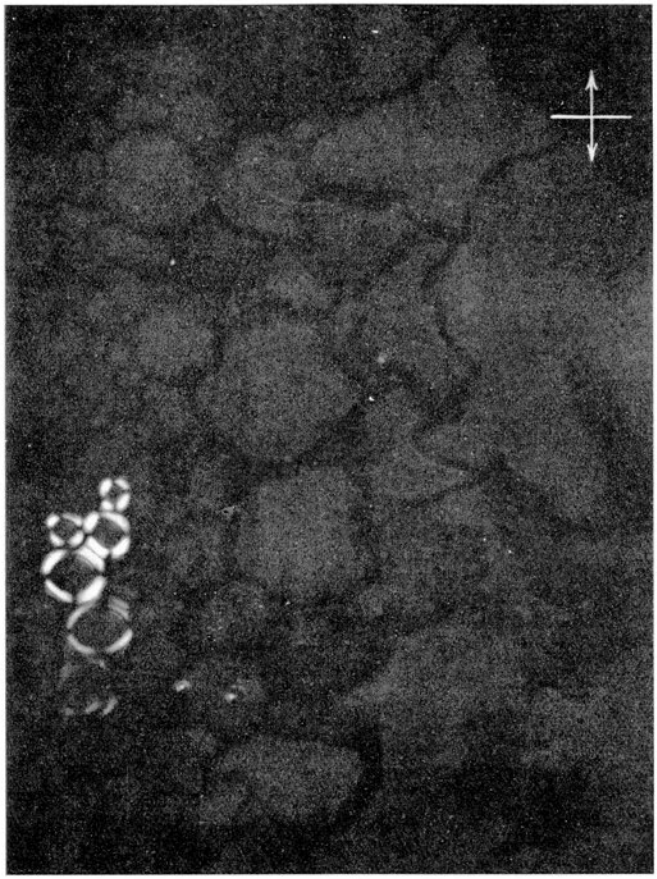
Photomicrograph of the stretched-out spiral threads from the bulb-skin tissue of *Nerine fothergilli* in polarized light showing the enormous number of individual filaments.



Photomicrograph of the stretched-out filaments from the bulb-skin tissue of *Nerine fothergilli* in polarized light showing the skeinlike arrangement into which the threads have separated.



Photomicrograph of a cross section of the green leaf of *Nerine fothergilli* in polarized light showing location of two groups of cellulose-lined conducting tubes



Photomicrograph in polarized light showing a cross section of one of the groups visible in plate IV for a comparison with the longitudinal view of plate I. The black cross in the direction of vibration indicates that the fibrils run concentrically with respect to the tubes.