DEVELOPMENT AND GROWTH OF THE SCLERENCHYMA FIBERS AND SOME REMARKS ON THE DEVELOPMENT OF THE TRACHEIDS IN SOME MONOCOTYLEDONS

by

A. D. J. MEEUSE.

CONTENTS:

1. *Introduction.*

As far as we know, the first investigations on the origin of the sclerenchyma fibers in Monocotyledons are due to HABERLANDT (9). In his extensive study, illustrated with fine figures, he compared young and older stages of fiber bundles and arrived at the conclusion that the fibers originate from parenchyma cells which divide into a bundle of cells which show the same length as the original parenchyma cells, but which possess a

smaller diameter. The division-products grow out until they have reached their definite length which is a multiple of their initial length. The transition from young to old fibers was not traced by him.

GREVE (8) mentions that the sclerenchyma fibers of *Musa ensete* also originate from parenchyma cells which divide into a bundle of cells.

In 1886 KRABBE (13) published his essay on "gliding growth". His concept of gliding growth was rejected later in the case of the bast fibers of *Linum* by TAMMES (23) and ALBADA (1) and of *Boehmeria* by ALBADA.

The appearance of the secondary thickening of the cell wall of the sclerenchyma fibers is described neither by HABERLANDT (9) nor by GREVE (8). In the bast fibers of *Linum* and *Boehmeria* it arises in a very peculiar way by successive "telescopic" lamellae, the apex of the growing fiber remaining unchanged [see ALBADA (1)].

In the following investigation it has been tried to answer especially the following questions:

a. Where, whence and in which way do the sclerenchyma fibers originate in the objects studied?

b. In which way does the fiber grow before it matures and is this growth accompanied by gliding growth between or along other elements?

c. Where, when and how does the secondary thickening of the cell wall develop and does the thickened fiber still show longitudinal growth?

d. Which wall substances occur in the several stages of development of the fiber?

e. Which is the direction of the long axis of the index ellipsoid in the various stages and in the various lamellae of the fiber? [1])

f. Do the tracheids, in the cases examined, elongate by means of sliding growth?

2. *Methods.*

The objects examined had been selected from a great number of Monocotyledons, in order to enable us to work with material, containing many bundles of long (though not too long) sclerenchyma fibers, these fibers being present moreover in an ample

[1]) It is known by experience that, ordinarily, in cell walls the shortest axis always has a radial position and therefore the situation of the index ellipsoid is determined by the direction of the long axis only.

quantity. *Sanseviera guineensis* Willd., *Agave americana* L. var. *albomarginata* and *Musa sinensis* Sweet proved to be satisfactory.

The parts of the plants used were either studied in living state or fixed directly; eventual macerations were also performed with fresh material. Young and growing parts of leaves or leaf sheaths were always used, as these parts show every stage of development of the sclerenchyma.

The anatomical investigation was carried out using:

a. stained microtome sections of young and growing fiber bundles,

b. macerations and

c. hand-sections, some of them cleared in a mixture of chloral hydrate and water 8 : 5.

The submicroscopic structure of the cell wall was studied by means of the polarisation microscope; the chemical composition of the wall by means of the usual reagents.

As a fixative JUEL's mixture was very satisfactory, as the preservation of very delicate cytological structures was not aimed at.

The slides were cut 5—15 μ in thickness, in our case 8 μ slides proved to be the most satisfactory. Part of the paraffin ribbons, obtained by cutting, was transferred into xylene to dissolve the paraffin, the sections were freed and filtered off on a microfiltration apparatus. After rinsing with xylene, absolute alcohol and water these sections were used for microchemical reactions.

In trying several stains the combination haematoxylin HEIDENHAIN and "Light Green" was most satisfactory, as nuclei as well as cytoplasm and cell walls become stained; "Bismarck Brown" cleared in carbol-xylene appeared to be very good for staining only the cell walls.

Macerations were performed in several ways:

a. with boiling concentrated KOH-solution after ALBADA (1) (until sufficient separation has been obtained),

b. with javelle water at room temperature (until sufficient separation occurs),

c. with 1% potassium-phtalate or -citrate at 40°—50° for 3—4 days [after SLOEP (21)],

d. with H_2O_2 at 50° after KISSER (12), but in much higher concentration than is mentioned by this author (10—20%, also for 3—4 days).

The methods c and d proved to be most satisfactory, as these maceration agents exert their action very gradually. Sometimes the process was followed by a short maceration in javelle water,

after which the thickened fibers may easily be teased apart, while they, moreover, become bleached.

3. *The developmental processes of the sclerenchyma fibers in Sanseviera guineensis Willd.*

The leaves contain, besides "fibro-vascular strands" fiber bundles, which are not connected with a vascular strand. These separate sclerenchyma bundles are extraordinary suitable for the study of the development of the fibers, because there are, in the youngest stages of the ontogeny, no disturbing young elements of a vascular strand to be found in the neighbourhood of the fibers.

The average length of the sclerenchyma fibers [1]) is not constant in every leaf, but depends upon the length of the leaf. In the foliage shoots of *Sanseviera* on the outer side short, scale-like leaves, only some (5—8) cm in length, are found; more towards the centre longer leaves are present, reaching in a mature shoot a maximum of 30—40 cm; inside of them, at last, immature leaves are present, which are shorter, as they are situated nearer to the growing point of the plant. The short, scale-like leaves were formed, when the shoot was still young; at that time the leaves were much shorter than in a mature shoot. When the shoot grows old, the average length of the leaves formed increases until the maximum of 30—40 cm has been reached; the length of the mature leaf then remains nearly constant until the shoot dies.

The average length of the sclerenchyma fibers increases with the length of the mature leaf: in a leaf of 30—40 cm the sclerenchyma elements measure ± 1800 μ, in the shorter leaves their length is less, to a minimum of about 70 μ.

In order to eliminate eventual small differences between the fibers of different leaves, always the fibers out of a single leaf were compared with one another. If possible the fibers out of different parts of the same bundle were compared.

In the leaf-base the growing zone is found; in this growing zone the youngest stages of development of the fibers may be studied best in microtome sections.

For some reason repeated cell divisions appear in distinct parts of this growing zone, which is built up by a more or less meristematic parenchyma. As a rule these divisions begin in a nearly mature leaf in those parenchyma cells, which are

[1]) With "(sclerenchyma) fibers" in the following always is meant *elementary fibers.*

situated at the very base of young parts of a fiber bundle already present. Sometimes, however, new bundles may derive from the divisions of one or more parenchyma cells, which show no connection with any existing fiber bundle.

These cell divisions are directed in such a way, that from a dividing parenchyma cell a bundle of parenchyma cells is derived; these division-products elongate later into fibers. At their origin every cell has about the same length as the original parenchyma cell, but has a much smaller diameter (see fig. 1 and 2).

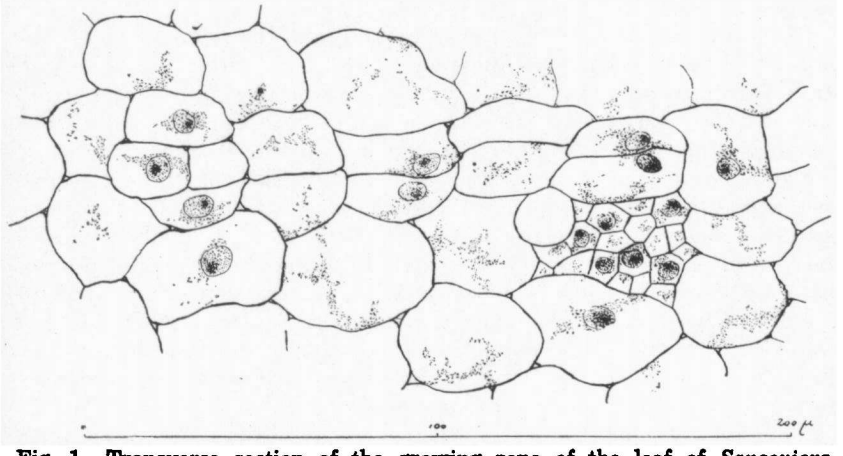

Fig. 1. Transverse section of the growing zone of the leaf of *Sanseviera*. Formation of two bundles of sclerenchyma fibers by repeated divisions of parenchyma cells. At left 4-celled, at right multicellular stage. Between the two initial bundles an anastomosis may be observed.

In the meantime the leaf has not ceased stretching. The rest of the parenchyma cells of the growing zone is divided in the "ordinary" way (the new walls formed after these divisions have a horizontal position), so that the initial fibers are lifted to a somewhat higher level in the growing zone when these divided parenchyma cells stretch after mitosis. The cells situated at the base of the initial fibers now begin to divide repeatedly, the new formed fiber-initials are lifted to a higher level by the surrounding parenchyma, dividing in the "ordinary" way, etc. This process is continued as long as the leaf is increasing in length and in this way the long coherent fiber bundles are formed (see fig. 3).

The young fibers do not divide any more. They are and remain uninucleate and very thin-walled in the beginning; their transverse section is polyhedrical, the cells being obviously flattened against one another by their turgor (fig. 2).

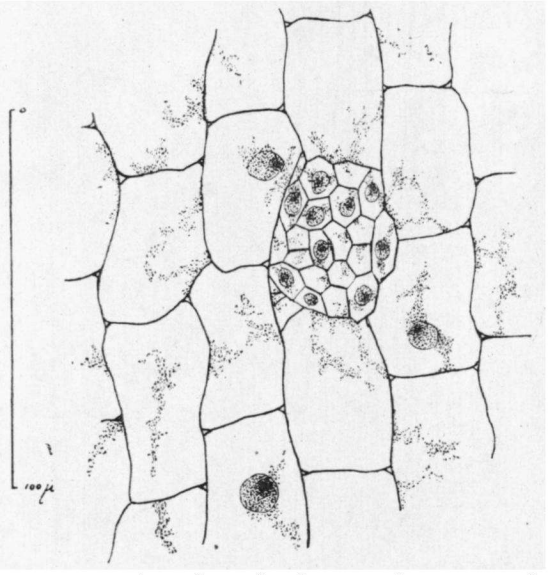

Fig. 2. Transverse section through the growing zone of the leaf of *Sanseviera*, showing a somewhat later stage of development of a sclerenchyma bundle.

When the young fibers are formed and do not divide any more, they begin to stretch, whereas the surrounding parenchyma still goes on dividing. The young fibers are stretching continuously as long as they find themselves in the growing zone of the leaf.

The divisions in the parenchyma keep pace with the stretching of the fibers; there is no indication that the fibers are elongating with gliding growth between — or along — the parenchyma cells (see fig. 4).

The apices of the forming fibers are not in the least acute, as a rule only somewhat angular; they assume a more acute shape during their elongation. As the examination of the slides permits no other conclusion but that the fibers are stretching in every part of the wall in the same degree, the explanation of the increasing sharpness of the fibers is easy. In the schema-

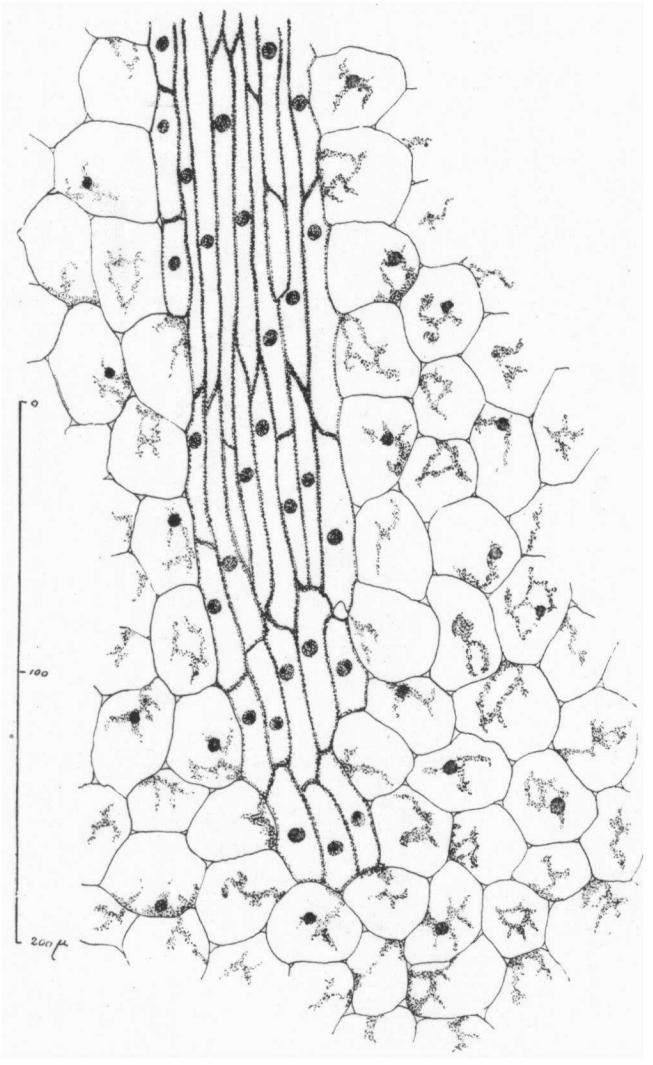

Fig. 3. Longitudinal section of the growing zone of the leaf of *Sanseviera*. Formation and beginning of stretching of initial fibers.

tical drawing given in figure 5 we assume that a parenchyma cell divides into a number of initial fibers (fig. 5 A, B). The transverse walls ab and cd of one of those fibers abcd (represented separately in fig. 5 C) have an oblique position with regard to the walls ad and bc; the wall bc is somewhat longer than the wall ad, namely the sum of the distances a'b and d'c. During the stretching of the cell ad reaches n times its initial length, as does bc; the parts a'b and d'c of the d walls also become n times longer. The growth of the cell in width is slight in proportion to the elongation, so that the fiber obtains the apex a"a'b', if we suppose n (= the degree of longitudinal growth) = 10 and the growth in width is 2 times the initial width aa'.

That the apices of the fiber-primordia possess such somewhat oblique transverse walls, is shown clearly by the preparations (fig. 3).

The growth of the cell wall in width is, as said before, by no means as important as the growth in length; it amounts to less than two times the original width, as was shown by measurements. This growth in width ceases, moreover, much quicker than the growth in length, so that in the final stages of the stretching period longitudinal growth occurs exclusively. During their growth the fibers remain very thin walled and uninucleate, the protoplasm being found against the cell wall in a thin layer (fig. 4).

A young growing fiber bundle soon shows distinct thickened edges of the cell wall. These thickenings (*"Zwickel"*) are not present from the beginning (see fig. 2); they may be clearly distinguished from other parts of the wall, which are extremely thin, expecially in stained preparations by their great affinity to stains (e.g. Light Green).

When the growing fibers have reached a given length, it is no longer possible to trace them over their full length in microtome slides; the older stages had to be studied therefore in macerated preparations.

In the macerated parts of the plants the thickened parts of the plants were located and traced to their growing parts in the growing zone of the leaf. This tracing may be easily performed as the parenchyma is less coherent after maceration than the young sclerenchyma.

After this the parenchyma was eliminated as much as possible. The fibers which already show a secondary cell wall may be separated by slight pressure after treatment with javelle water; if an other macerating agent was used, the fiber bundles

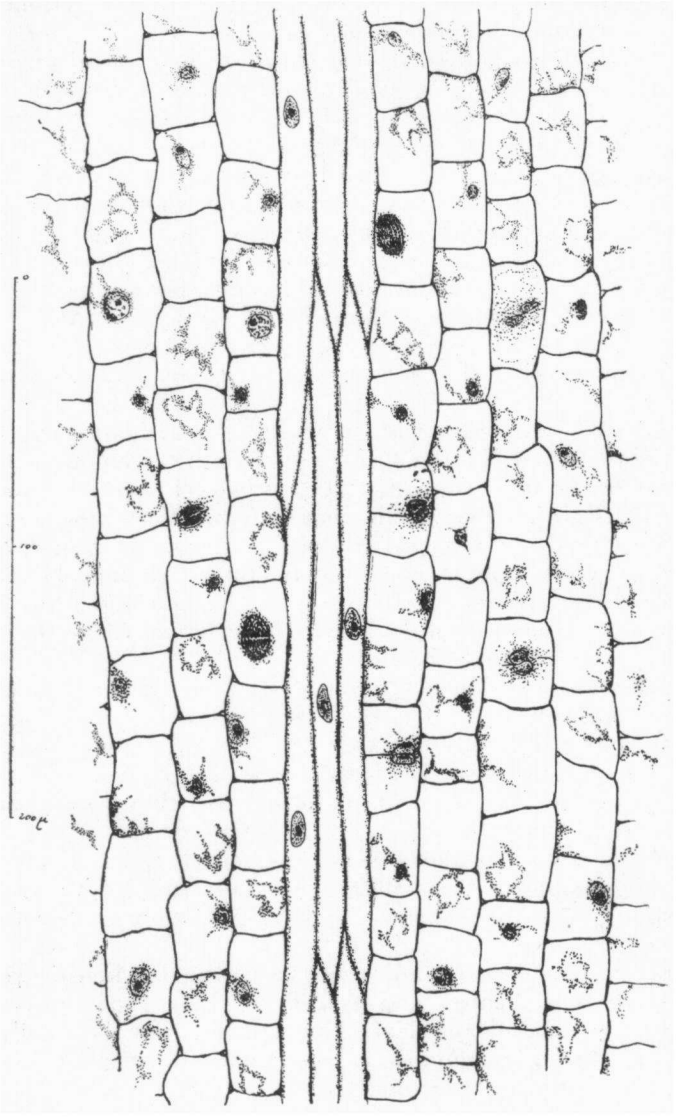

Fig. 4. As fig. 3., but showing an older stage of a young sclerenchyma bundle. Elongation of the young sclerenchyma elements, whereas the parenchyma cells keep pace with them by continuous cell divisions.

were treated afterwards with javelle water. Unthickened fibers cannot stand a treatment with javelle water and have to be separated in another way. Sometimes I succeeded in teasing apart one or more of them by a careful mechanical treatment (picking with needles etc.), when as a macerating agent H_2O_2

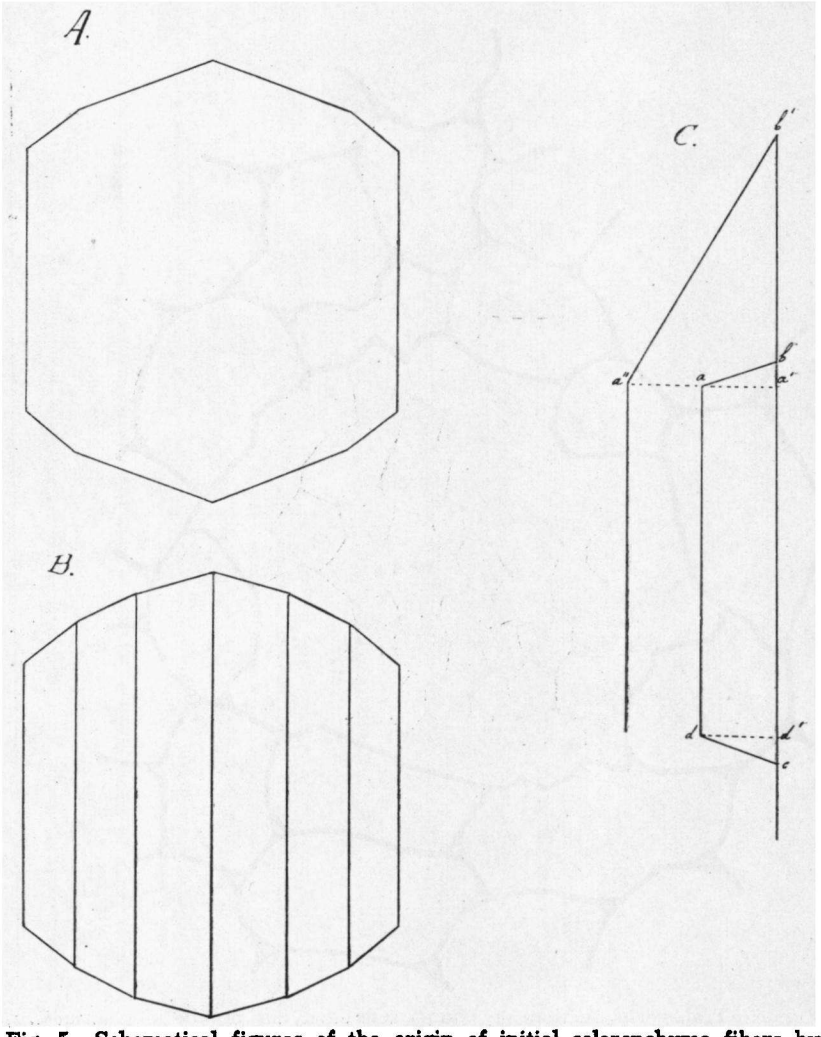

Fig. 5. Schematical figures of the origin of initial sclerenchyma fibers by divisions of a parenchyma cell and of their elongation. Explanation see text.

had been used. When a fiber starts thickening, its length agrees with the average length of the fibers of the same bundle, which are completely thickened. From this it may be concluded that *the sclerenchyma fibers keep growing until they reach their final length without showing any secondary thickening of the cell wall.*

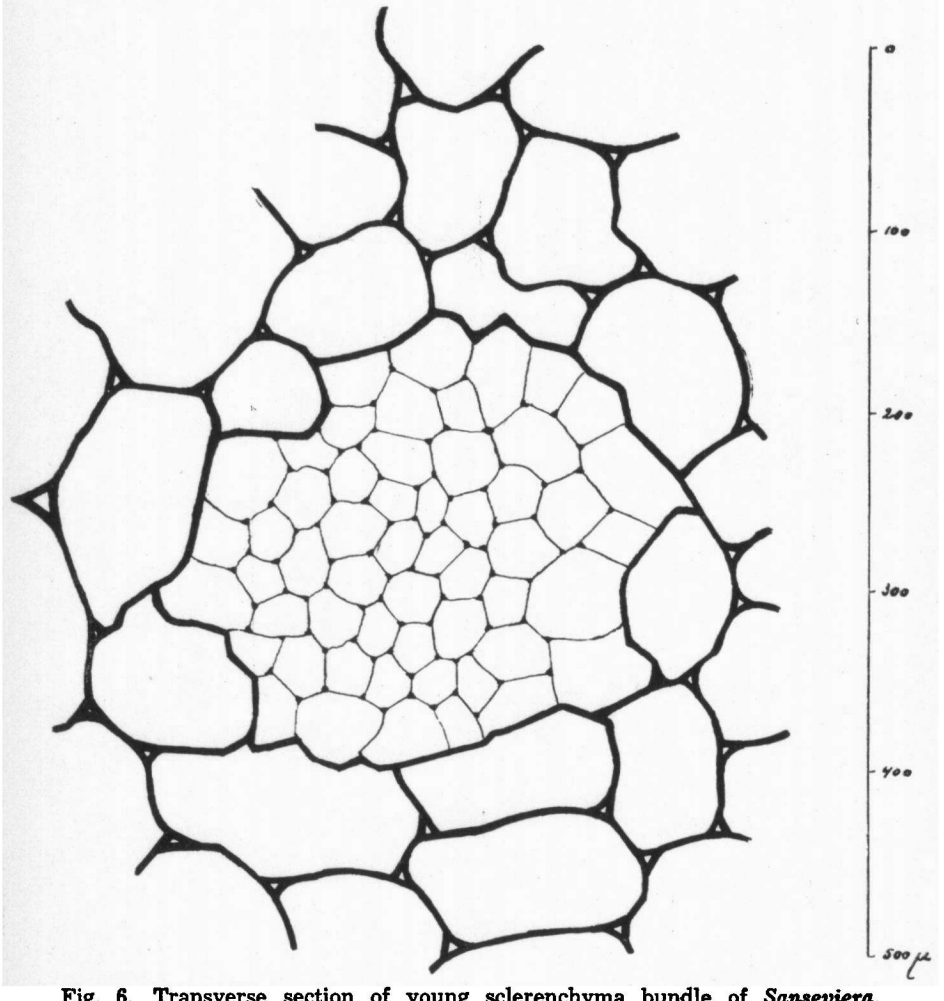

Fig. 6. Transverse section of young sclerenchyma bundle of *Sanseviera*, an older stage than figured in fig. 3. One should notice the very thin primary cell wall and the distinct thickened edges of the cell walls.

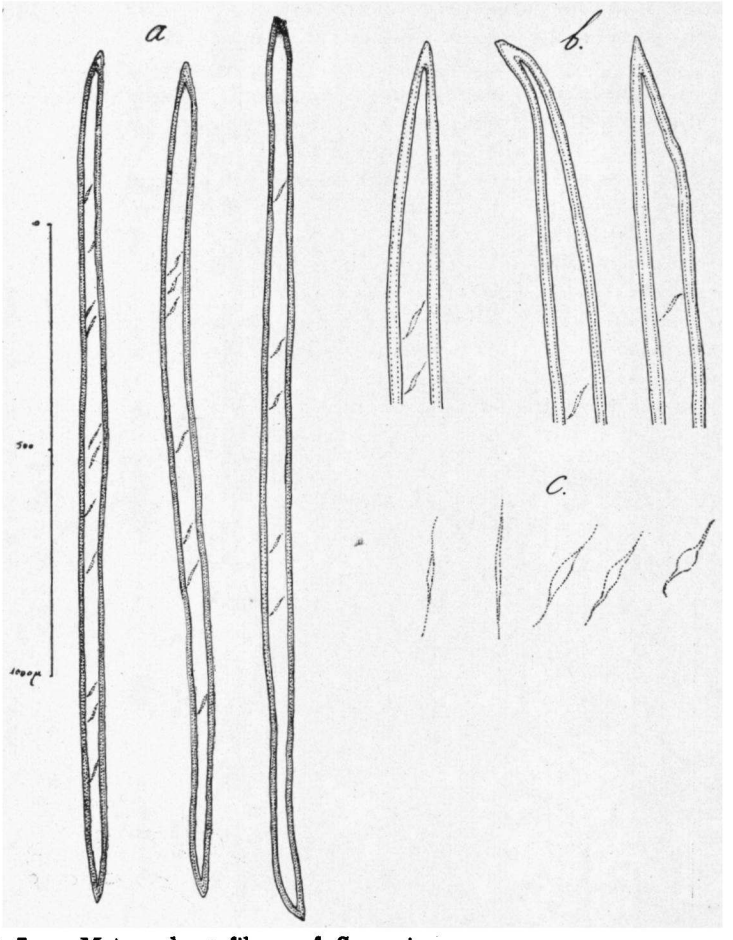

Fig. 7. a. Mature long fibers of *Sanseviera*.
b. Apices of fibers of the same kind.
c. Pits in the wall of such fibers (the long axis of the fiber is assumed to be vertical in the plane of the paper).

After this stretching process the fibers may reach a maximum length amounting to more than 60 times the length of the initial parenchyma cells in the growing zone of the leaf from which they have been derived.

The thickening of the cell wall in the completely stretched fiber is initiated simultaneously along its entire wall, as it

appears from the absolutely homogeneous colour shown by a fiber in any stage of secondary thickening of the cell wall under light first order red. Nor could I observe, at close examination of the fiber wall under very high magnifications, any differences in the thickness of the secondary wall at different spots of the wall of a single fiber.

The process of secondary thickening of the cell wall does not take a long time and starts, as said before, in the central fibers of a bundle.

In the carefully macerated fibers a single nucleus appears to be present even when they have reached their definite length.

The parenchyma cells have been stretching as well, but their elongation is, relatively as well as absolutely small when compared with the enormous stretching of the sclerenchyma.

In *Sanseviera* the developmental processes described above occur in all fibers; this is the case in the sclerenchyma of

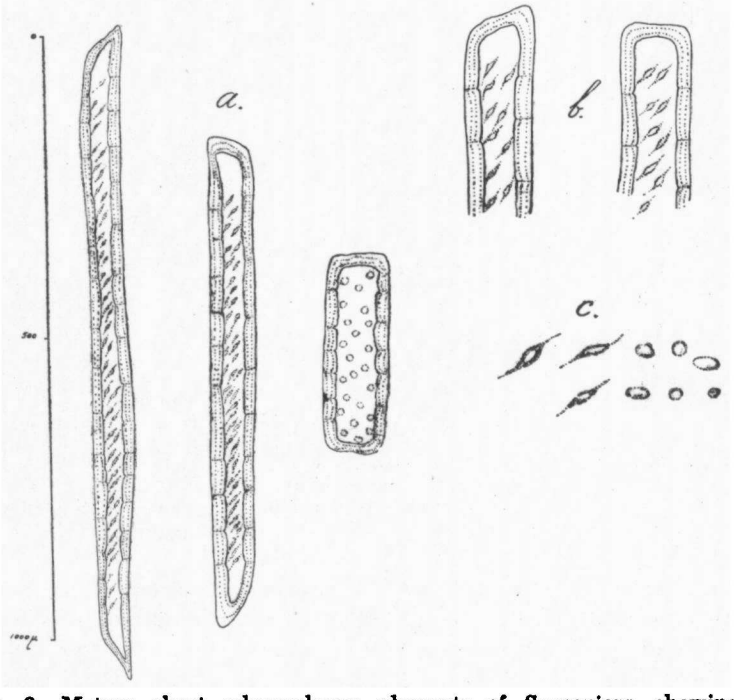

Fig. 8. Mature short sclerenchyma elements of *Sanseviera*, showing the same peculiarities as the fibers in fig. 7.

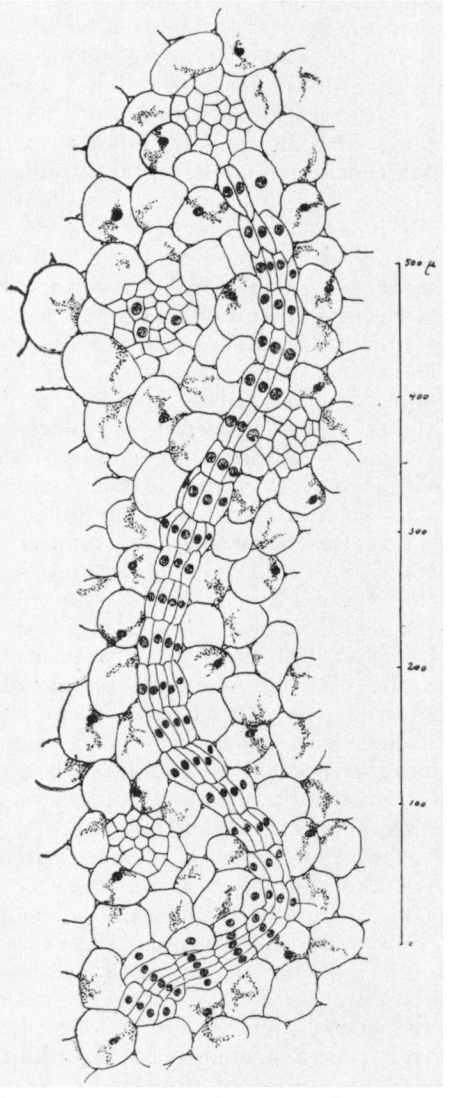

Fig. 9. Initial fiber-anastomoses with surrounding tissues in a transverse section of the growing zone of the leaf of *Sanseviera*. Four vertical fiber bundles are cut transversely, two of them being connected by the anastomoses.

the fibro-vascular bundles as well as in the separate sclerenchyma bundles, in the fibers of the long leaves as well as in those of the shorter ones.

In studying the short leaves it appears that the fibers reach a smaller length than they do in the long leaves and become less acute. The very short fibers are blunt (for long fibers see fig. 7, for shorter sclerenchyma elements see fig. 8).

The shortest sclerenchyma elements are found in the anastomoses running in the leaf in more or less horizontal direction from one fiber bundle to another.

All elements of such an anastomosis are derived simultaneously from parenchyma cells, the developmental processes of the single elements are quite the same as those of all other sclerenchyma elements (for initial stages see fig. 9, for mature stage see fig. 10). These elements cannot be called "fibers" any more, in fact; they are but little longer than mature parenchyma cells. This is to be expected as the elongation of the entire anastomosis can — without any gliding growth — only be equal to the total growth in width of the leaf in the direction of the anastomosis, so that each cell can only show extremely little stretching. For the occurrence of gliding growth in the anastomoses I could not find any indication, as may be also concluded from the figures given. In the sclerenchyma elements, discussed here, the acute apices are wanting; only oblique transverse walls are found at both ends. A number of these transverse walls from a bundle of these elements appear to be placed at about the same level (fig. 10). If the short elements of the shorter leaves, or the elements of the anastomoses should stretch by means of gliding growth, they could reach indefinite lengths and they should not remain always shorter than the fibers of the long leaves, as appears to be the case.

The length of the sclerenchyma elements depends probably upon the ability of longitudinal growth, presented by the growth of the part of the plant in which these elements occur. If there is little growth a fiber bundle shows small ability to stretch (because there is no gliding growth); the extreme case is shown by the anastomoses (see above); leaves with considerable longitudinal growth always contain long elementary fibers.

The gliding growth is improbable for yet another reason. In every fiber bundle a number of fiber-apices may be found, situated at about the same level which level is perpendicular to the long axis of the bundle (for a young stage see fig. 4, for a mature one see fig. 11); the shorter the fibers are, the smal-

ler the distances between the endwalls of the fibers. In the extreme case of the elements of the anastomoses mentioned above, these distances are very small, so that the transverse walls are found situated practically at the same level (fig. 10).

Considering that from one parenchyma cell a number of initial fibers are derived, the ends of which are situated at nearly the same level, representing the transverse cell wall of the parenchyma cell (see the scheme in fig. 5B), as may be seen from fig. 3, fig. 4 and fig. 9, I cannot find the slightest indication that this configuration is disturbed later on by gliding growth of the elements along one another.

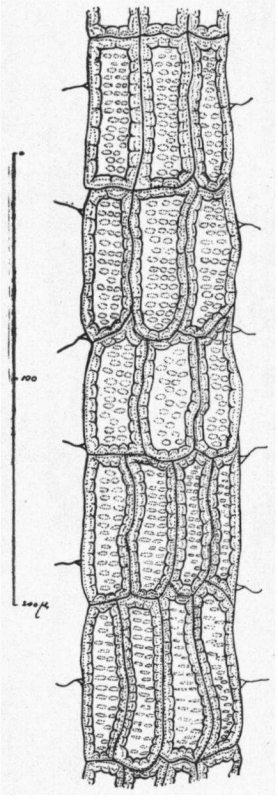

Fig. 10. Mature fiber anastomosis of *Sanseviera* in longitudinal section. One should notice the frequent obtuse end walls, the small length and the direction of the long axes of the pits.

In the initial phase of development, as may be seen from the preparations (see fig. 6), there is no evidence at all of gliding growth, therefore it is not to be expected that in the older stages of development than the one figured in fig. 6 any gliding growth does occur. The small distances between the apices of the mature fibers are due to the increase of the very small distances already present in the initial stage, this increase being proportional to the "stretching factor" of the fiber, which amounts to about 60 in the case of long fibers.

As may be concluded from the preceding, a separate fiber bundle is derived originally from a single parenchyma cell, which divides into a group of small cells (fig. 1, fig. 2), this groups being recognizable in the mature bundle as a number of fibers with their apices situated at about the same level. Besides this type of fibers however, fibers are present with their apices arbitrarily located in relation to the apices of the neighbouring fibers. These fibers are always found in the periphery of a bundle, their length is always smaller than the average length of the fibers of the same bundle, as

I could establish by measurements. Their apices are, as a rule, less acute than those of the central fibers of the bundle. It follows from this — and it could be confirmed experimentally — that the central parts of a seperate sclerenchyma bundle are derived from a single cell, which divides into a group of initial

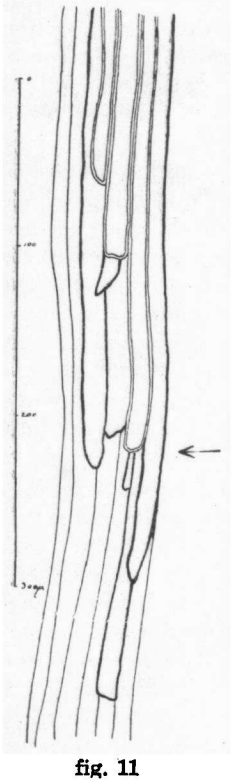

fig. 11

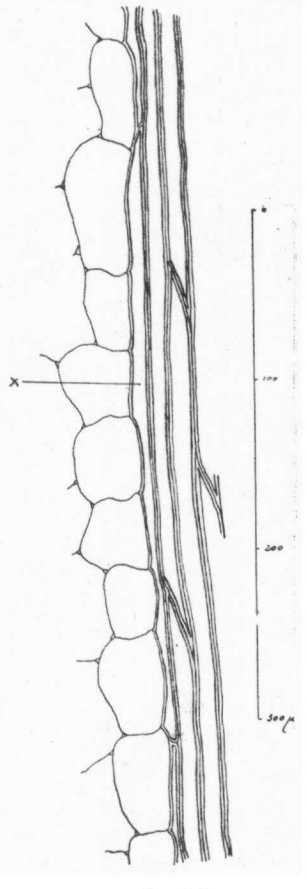

fig. 12

Fig. 11. Fiber bundle of *Sanseviera* seen from the side. Beginning of the secondary thickening of the cell wall, at the level of the arrow a number of fibre-apices situated close together.
Fig. 12. Longitudinal section of a mature part of a fiber bundle of *Sanseviera*. Notice the fiber (x) situated peripherally (see text).

fibres, whereas the parenchyma cells neighbouring the initial sclerenchyma bundle split off parts of their cell bodies later, broadening in this way the bundle. The division of these neighbouring cells occurs, as far as could be found, in the earliest stages of development of the fiber bundles; yet the peripheral fibers do not occur until the central ones have already shown some, ever so very little, growth in length. The concommittant difference in length explains the differences in length between the central and the peripheral fibers of the mature bundle and also explains the presence of more obtuse apices of the peripheral fibers compared with those of the central ones and also the fact, that the apices of the peripheral fibers are irregularly distributed (fig. 12).

The addition of such peripheral fibers is demonstrated nicely in an abnormal case, figured in fig. 13B. For in this case a fiber has been split off below the bundle, whereas the parenchyma cell from which it has apparently been derived has produced a longitudinal series of parenchyma cells, separating the fiber from the bundle; one of these cells is visible in the figure in transverse section.

The preceding not only applies to the peripheral fibers of a separate bundle but also to the fibers of a fibro-vascular bundle, if one considers only as "central" the part of the sclerenchyma bordering the phloem and as "peripheral" the one bordering the leaf-parenchyma (see fig. 14).

These broadenings of the fiber bundles seem to occur generally, in the long leaves as well as in the short ones, but they are lacking in the horizontal anastomoses (compare fig. 9 with fig. 10). The question whether the sclerenchyma of the fibro-vascular bundles is derived originally from one ore more parenchyma cells which divide entirely into initial fibers, cannot be answered with absolute certainty, because of the initial vascular elements neighbouring the fiber-initial. The initials are not clearly distinguishable from each other in the youngest stages of development in longitudinal section.

It seems however, that this sclerenchyma in contradistinction with the separate fiber bundles, originates from more than one parenchyma cell, because, studying transverse sections, it appears that the sclerenchymatous cover of the vascular bundle is already arched in a very early stage of development. This should not be expected if the sclerenchyma bundle were derived from a single cell.

4. *The submicroscopic structure of the cell wall of the sclerenchyma fibers in Sanseviera guineensis Willd.*

On surface view of the walls of the parenchyma cells from which the fibers originate, the active refractive-index ellips is situated with its longest axis in transverse position. The initial fibers, derived from this parenchyma, show the same position of this ellips in the primary wall and this situation remains unchanged until the fibers are quite stretched.

With the occurrence of the secondary thickening the birefringence changes. The way in which it changes depends upon the length of the completely stretched fiber.

In the longest mature fibers (average length about 1800μ) the long axis of the active refractive-index ellips is, on surface view of the wall, directed more or less longitudinally, as is the case in all normal cellulose fibers. During the development of the secondary thickening of the cell wall the sign of birefringence of the cell wall changes in this kind of fibers consequently.

The birefringence changes first to a stage in which the fiber is apparently isotropic and shows, consequently, the colour of the field between crossed nicols with added gypsum plate of first order red light in every position. In this "isotropic" stage the birefringence of the primary cell wall and of the (still thin) secondary wall apparently neutralize one another, but, finally, as the secondary thickening of the cell wall increases, the birefringence of the secondary wall predominates. The birefringence of the secondary cell wall finally amounts to several times the birefringence of the primary wall.

The position of the pits in the secondary wall of long fibers is a very steep one; the long axis of the refractive-index-ellipsoid in the single cell wall consequently has a direction which differs but little from the direction of the long axis of the fiber (see fig. 7).

Considering a mature shorter fiber (average length about 900μ) less steep pits are found, the long axis of the fiber forming an angle of about 45° with the long axis of the fiber. These fibers are apparently isotropic, they show between crossed nicols with added gypsum plate the colour of the field in every position. Considering the direction of the pits this may be accounted for by the phenomenon of „crossed crystal-plates" as represented by the upper and lower cell wall; the long axes of the refractive-index ellipsoids in these two walls being perpendicular to one another. As a control oblique sections were cut from such fibers, so that only a single cell wall could

be viewed. The single wall shows a considerable birefringence, the long axis of the index ellipsoid actually forming an angle of ± 45° with the long axis of the fiber. The birefringence of the primary wall is too small to be of importance.

During the process of secondary thickening no marked decrease of the birefringence is observed, the observed birefringence is very small on surface view of the fiber or the wall may even be completely isotropic. This is due to the slight thickness and consequent small birefringence of the primary wall and the very small resulting birefringence of upper and lower secondary wall (see above).

In still shorter mature sclerenchyma elements (70-200μ), the pits approach the horizontal position with the decrease in length. The form of the pits is not the same as in the longer fibers, but they are less stretched and wider, roundish-oval or slightly spindle-shaped (see fig. 8, shortest elements).

During the process of secondary thickening the sign of birefringence does not change, only the birefringence increases. The direction of the long axis of the refractive-index ellipsoid is the same in the primary cell wall and in the secondary wall, i.e. it is about perpendicular to the long axis of the cell, as is shown by the horizontal position of the pits in the secondary wall (see fig. 10).

Between these three instances mentioned all transitions can be found, so that it may be said: the longer the fiber, the steeper the position of the pits and the position of the long axis of the refractive-index ellipsoid in the single fiber wall and the more considerable the "positive" birefringence [1]). Although these gradual transitions are present, it is better not to call the shortest elements "fibers" because of the lack of the typical prosenchymatous shape (there are no acute apices!) and their "negative" birefringence [1]).

5. *The cell wall substances of the sclerenchyma fibers of Sanseviera guineensis Willd.*

The mother parenchyma of the fibers shows with ruthenium

[1]) With "positive" birefringence of a cellulose cell wall is meant: the long axis of the active refractive-index ellips is situated in the direction of the long axis of the cell, with "negative" birefringence: the long axis of the active-index ellips is directed perpendicular to the long axis of the cell. This nomenclature is actually misleading, as *cellulose* always shows positive birefringence, and is based on the analogy with positive and negative birefringent crystals. Sometimes, however, as in the case mentioned, it still seems advantageous to use this nomenclature.

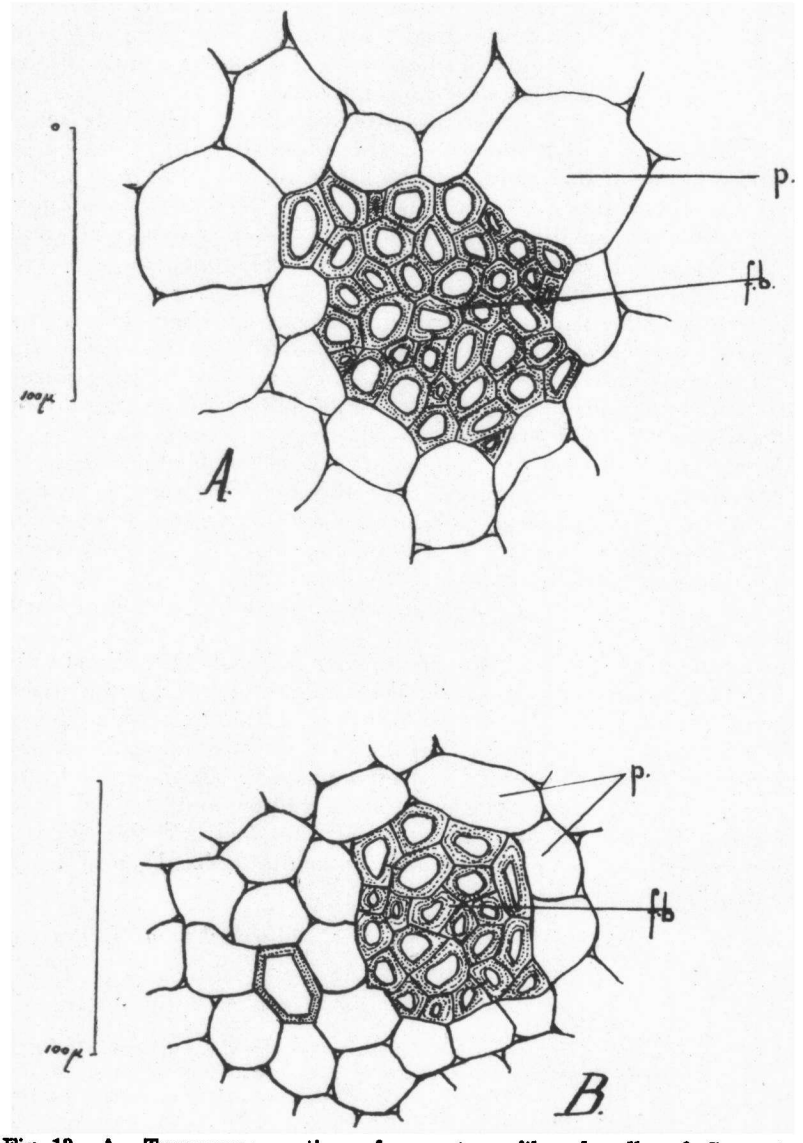

Fig. 13. A. Transverse section of a mature fiber bundle of *Sanseviera*.
B. As A, section of a bundle beside which a separate fiber is present.
f.b. = fiber bundle, p = parenchyma.

red a clear coloration and with chlor-zinc iodide a slight blue coloration. After a treatment of the tissue with cuproxam for 36 hours it appeared, as far as could be established, not to have lost much of its solidity (a 10μ section has, before and after treatment as it seems about the same resistance against slight pressure and against picking with needles etc.). This seems to be due to the relatively small amount of cellulose.

This seems to be due to the relatively small amount of zone the reaction on "amyloid" in the sence of ZIEGENSPECK (24); this reaction disappears more apically in the growing zone and is replaced gradually by the reaction on "collose" (ZIEGENSPECK 24). This reactions are carried out as follows:

a. amyloid-reaction. The slides, which should be of the proper thickness, are placed under a cover glass in water, a drop of highly concentrated iodine-KI solution is put at the margin and the reagent is enabled to run under it by sucking away the water partly with a strip of filter paper, so that a gradually decrease of concentration of the iodine-KI solution is obtained. The preparation is examined for a spot where the concentration is the proper one to give the reaction. One should examine (as well as in the collose-reaction) the slide under not to high magnifications with wide open diaphragm after the reagent has been in contact with the section at least for five minutes.

b. collose-reaction. The slides should be treated first with an (exactly) 15% solution of HCl; the reaction runs further as described for the amyloid-reaction.

It may be noticed here that the colour of the cell wall, when the reactions are positive, is a pale steel-blue, so that it requires much practice (obtained from objects which show this reactions very well, see for this matter ZIEGENSPECK 24) to actually recognize this shade. Yet it may occur, that the reaction does not succeed at the first time, so that repeated efforts must be made before we may conclude to either a positive or a negative reaction.

Experience has taught me that these reactions, when positive, give a dichroitic stain (from steel-blue to colourless).

The fiber-initials show the same reactions as the surrounding parenchyma; in the youngest stages the amyloid-reaction is clear, but is replaced later on by the reaction on collose. No other cell wall constituents of organic source could be found.

According to ZIEGENSPECK (24) the presence of positive reactions on "amyloid" and "collose" in the young and growing

cell walls should point to the existence of certain special cell wall constituents, which should have to be considered as pre-existing stages of the cellulose (in a series amyloid → collose → cellulose).

In my opinion it is more probable that the cellulose itself is present in an initial state, which may only signify another constellation of the micelles, or a relatively smaller amount of them, or both. This opinion is also held by BONNER (5), as, after analysis, young and growing cell walls appear to contain only 10—12% cellulose. Moreover, HOPMANN (11) has found very gradual transitions between cell walls giving the amyloid-reaction, and cell walls giving a blue coloration only after treatment with H_2SO_4 and iodine-KI-solution; he also describes cell walls giving both reactions (on amyloid and on cellulose) and growing cell walls in which the amyloid changes into cellulose very slowly, as is the case in my object.

The dichroism shows that in such young cell walls a structure of a "Mischkörper" is present (see also FREY 7). When a low percentage of cellulose is present, much more room between the micelles is available for the (complex) iodine of the reagents, so that swelling is not necessary for staining blue amyloid-reaction. A somewhat higher quantity of cellulose requires little swelling by 15% HCl ("collose"-reaction). Still higher quantities require swelling by stronger swelling-agents, e.g. $ZnCl_2$, H_2SO_4 ("cellulose"-reaction).

Although the parenchyma of the mature leaf parts of *Sanseviera* still gives the reaction on "collose", this reaction is not obtained with elongating fibers still being far from completely stretched, so that the number of micelles in the elongating cell wall increases, as is also clearly shown by the increasing intensity of the cellulose-reaction (with chlor-zinc iodide). The collose-reaction decreases as the reaction on cellulose is clearer.

In the quite stretched, but unthickened fiber pectic substances and cellulose seem to be the only organic cell wall-constituents.

During the very rapid secondary thickening of the wall new cellulose layers are deposited; these layers being, at the beginning, highly impregnated with pectic substances. Other wall substances, especially "lignin", could not be found.

Immediately after the formation of the secondary wall the lignification begins (as a reaction on lignified walls phloroglucinol-HCl was employed). The lignification starts in the primary cell walls and middle lamellae. The central fibers of a sclerenchyma bundle lignify sooner than the peripheral ones; this is to

be expected as the central fibers are the first to form secondary walls.

It is remarkable that the reaction on pectic substances decreases as the lignification proceeds, the more considerable the lignification, the less pectic substances may be found so that, finally, this reaction is absent when the process of lignification ceases. The pectic substances seem to be necessary for the formation of "lignin", for the lignification ceases exactly when the amount of pectic substances is reduced to zero.

The mature fiber wall consists consequently — as far as was established — of cellulose and "lignin". The degree of lignification is rather high, as is shown by the intensity of the reaction with phloroglucinol-HCl. During the lignification the secondary wall does not increase in thickness; this does not agree with the results obtained in other objects by ALEXANDROV & DJAPARIDZE (2).

6. *The developmental processes of the sclerenchyma fibers and their walls in Agave americana var. albomarginata.*

In *Agave* the development of the fibers shows so much resemblance to the developmental processes in the preceding object, that it suffices to briefly mention the points in which it is different.

The fibers occur exclusively in the sclerenchymatous covers of the fibro-vascular bundles. Even the transverse fiber-anastomoses in the leaf are combined with a vascular anastomosis, which is of rare occurrence in *Sanseviera*. Just like in *Sanseviera* long fibers are found in the longer leaves and short fibers in the shorter leaves.

The sclerenchyma also originates from divisions of parenchyma cells in the basal growing zone of the leaf, after which the division-products stretch until they have reached their final length, without any sliding growth or secondary thickening of the cell wall, the cells remaining uninucleate. The arguments for this are the same as those mentioned in the case of *Sanseviera*.

The fibers may reach a maximum length making up to 70 times the initial length of the parenchyma cells from which they have derived.

The secondary thickening of the cell wall occurs rapidly and in all parts of the fiber wall simultaneously. When it is finished, lignification begins.

Form and growth of the fibers is similar to those of *Sanseviera*. Sliding growth is also highly improbable.

The direction of the long axis of the refractive-index ellipsoid in the primary and secondary cell wall of a long fiber and of a shorter sclerenchyma cell is similar to the various directions described in the different kinds of sclerenchyma elements of *Sanseviera*.

The cell wall constituents are the same as in *Sanseviera*-fibers, so "amyloid"- and "collose"-reactions are found in the younger stages of development of the growing primary wall, etc. The

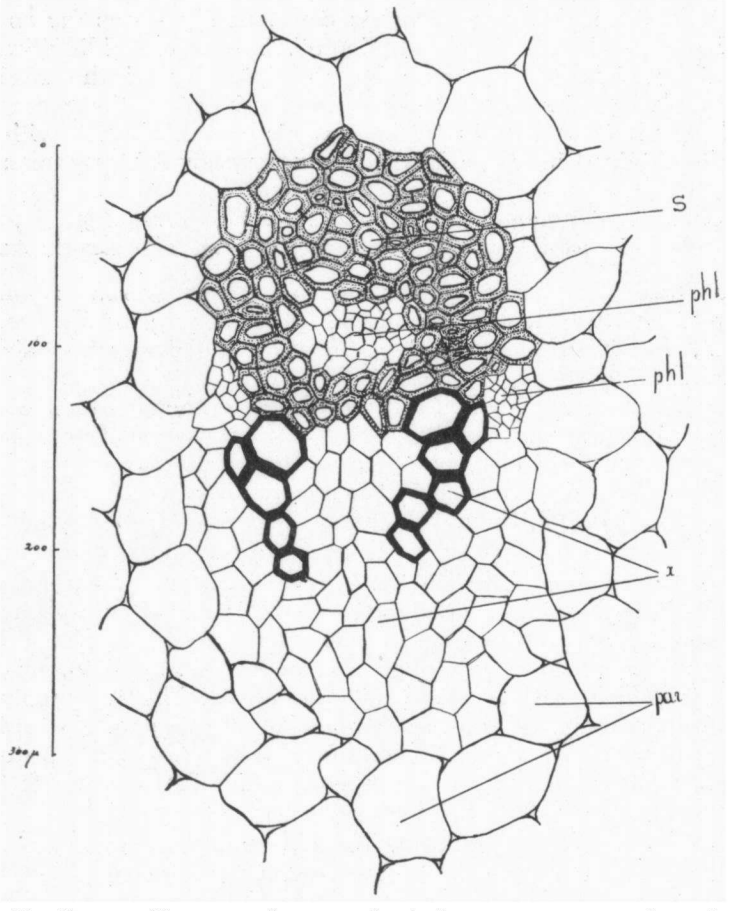

Fig. 14. Mature fibro-vascular strand of *Sanseviera*. s = sclerenchyma, phl. = phloem, divided into three parts, x = xylem (tracheidal walls drawn quite black), par. = parenchyma.

only difference is the rather small amount of pectic substances in the unlignified secondary thickening layers of the wall. It is remarkable, that the lignification also starts in the primary cell walls and middle lamellae and that the quantity of "lignin" is rather small in the secondary wall, as is shown by a faint red staining with phloroglucinol-HCl and by a greyish-yellow tinge obtained with chlor-zinc iodide.

In the primary cell wall and middle lamellae the pectic substances have not entirely disappeared when the lignification of the fiber ceases; in the secondary wall, however, they have, as in the cell wall of *Sanseviera*-fibers, disappeared at that moment. The amount of pectic substances also seems to limit the amount of "lignin" formed as described above for *Sanseviera*-fiber walls.

Just like in *Sanseviera* the secondary wall does not appreciably increase in thickness during the process of lignification (2).

7. *The developmental processes of the sclerenchyma fibers and their walls in Musa sinensis Sweet.*

This object shows much resemblance to both preceding ones in the development of the sclerenchyma, so that a short discussion will be sufficient.

Fibers were studied from the pseudo-stems of young plants of about 40 cm in length, and also from leaf sheaths of the mature plant.

It appeared that the peripheral leaf sheaths of a pseudo-stem do not stretch any more, so that the fiber bundles in these sheaths do not possess young elongating parts, it is, therefore, necessary to examine the more central leaf sheaths in order to study initial stages of development.

The anatomy of *Musa* is described in detail by SKUTCH (18, 19, 20).

GREVE (8) states that the fibers originate from divisions of parenchyma cells, but does not consider this question in detail.

Just as in *Agave* the sclerenchyma is present exclusively in combination with a vascular strand, this strand being either differentiated into phloem and xylem, or not. Anastomoses of sclerenchyma elements do not seem to occur, they were neither found in sections, nor in macerated preparations, though transverse vascular anastomoses frequently are present.

The length of the fibers of a single pseudo-stem seems to be rather constant. It is, on the average, smaller in the young plants than in a mature *Musa*-stem. The fiber bundles originate, as

they do in the other objects studied, by repeated division of parenchyma cells; most probably the smaller, peripheral bundles (see SKUTCH 18) may be derived from one parenchyma cell, the larger bundles (being arched) from more than one.

A secondary widening of the bundle may not be excluded, but if it occurs, it should be in the very early stages of development, as the peripheral fibers of a bundle are not considerably shorter than the central ones. The stegmata, moreover, are not disturbed in their position by the addition of new parts to the sclerenchyma bundle, though they arise already in a very early stage of development of the bundle as small, cubical cells, limiting the initial fibers.

The development of the fiber and its wall is the same as in the other objects studied, but only fibers with "positive" birefringent secondary walls are present, as the length of the fibers in a single pseudo-stem is rather constant and as anastomoses are wanting. The fibers in *Musa* consequently resemble the fibers of the "long" type of *Sanseviera* and *Agave*.

The cell wall substances are quite the same as in the other objects, but the initial fibers do not show the amyloid-reaction of ZIEGENSPECK, therefore this reaction does not seem to be present in all young cell walls. The presence of a greater percentage of cellulose was indicated by staining the walls with chlorzinc iodide. The blue colour was more intense than it was in the walls of the young fibers of *Sanseviera* and *Agave* in the same stage of development. After elimination of the pectic substances by oxidation with H_2O_2 (30%) for some days the primary cell wall of *Musa* had lost less of its original solidity than the primary wall of *Sanseviera* and *Agave* does under these circumstances.

The "collose"-reaction of ZIEGENSPECK is less intense than in the other objects and disappears quicker.

The process of lignification is the same as described above for *Sanseviera* and *Agave*. In young *Musa*-stems the pectic substances in the secondary wall are not completely replaced by "lignin". In an older *Musa*-stem, however, the pectic substances disappear in the primary cell wall and middle lamellae as well as in the secondary wall; even then the lignification is not considerable. During the lignification no marked increase in thickness of the secondary wall was noticed (2).

8. *Some remarks on the development of the tracheids.*

KRABBE (13) states, that many prosenchymatous cells originate

from a single cell by stretching and pushing between neighbouring cells. This process was called by him "*gliding growth*" ("*gleitendes Wachstum*"), which expression is generally employed in literature. This gliding growth really occurs in the formation of the libriform from cambium initials as well as in the growth of the latex vessels of *Euphorbia*, as here is no other possibility. A stretching wood fiber must glide between other initials, because it elongates until reaching its mature length which amounts to many times its initial length and because the parts of the stem in which the libriform is formed do not show appreciable growth in length. In *Euphorbia* only 8 latex vessels are present in a single plant; they reach from the root to the leaves. As they have been present as initials in the embryo, we must also accept stretching by means of gliding between other cells.

KRABBE mentions that the tracheids also originate from a single cell which cell has been stretching by means of gliding growth. This should occur in Dicotyledons as well as in Monocotyledons (*Dracaena*). This opinion was also held by STRASBURGER (22).

SCOTT & BREBNER (16) confirmed the observations of KRABBE for *Dracaena, Yucca* and *Aristea,* all Monocotyledons *with secondary growth,* in a careful anatomical investigation. The length of the mature tracheid is a multiple of the length of the cambium cell from which it has been derived. Because of the cessation of longitudinal growth of the stem, a cambium initial, when passing into a tracheid, should lengthen itself and this may be explained only by the occurrence of sliding growth of the cell along and between other elements surrounding the initial tracheid, for cellfusion never occurs, the tracheids being uninucleate in every stage of development.

CHEADLE (6) obtains the same results for a number of Monocotyledons *with secondary growth.* The mature length of the tracheids amounts on the average to 20—40 times the initial length of the cambium cell. The gliding growth was traced in microtome sections. Moreover, an added argument was found in the fact that after maceration branched and forked tracheids were observed.

It may be noticed here that from the data of the last two publications (both from text and figures) I think it is very likely that the tracheids in these Monocotyledons elongate until they have reached their definite length without forming any secondary thickening of the wall, though the authors do not

mention this fact especially.

SKUTCH (20) assumes, in analogy with the data of SCOTT & BREBNER, that in the leaf sheath of *Musa sapientum* the tracheids elongate by means of gliding growth as well. The tracheids and the sieve tubes develop simultaneously and should start to stretch at the same moment. In mature state the elements of the sieve tubes situated beside one another are nearly equal in length and the sieve plates of these neighbouring sieve vessels are situated at the same level, so that sliding growth apparently does not occur amongst the sieve tubes. The tracheids, on the contrary, being much longer than the sieve tubes in the mature state should grow out by means of gliding growth. SKUTCH's conclusions were drawn from anatomical data of mature parts of the leaf-sheaths only.

In studying the development of the vascular strands of *Musa* (and the same results were obtained in *Sanseviera* and *Agave*) it appeared that SKUTCH's view is untenable. These three Monocotyledons still show longitudinal growth in the zone in which the tracheids are formed in contradistinction to the objects of SCOTT & BREBNER and CHEADLE. Therefore the assumption of sliding growth depends upon the simultaneous beginning of the stretching of sieve tubes and tracheids. In my objects, however, the tracheids always start growth earlier than the sieve tubes; when the latter start to stretch, the former are already somewhat longer; this difference in length becomes more and more enhanced during the process of elongation. It appears, moreover, that in the horizontally traced anastomoses between the vascular strands the apices of neighbouring tracheids are situated at nearly the same level with the sieve plates of neighbouring sieve tubes (fig. 15); tracheids and sieve tube elements are nearly equal in length, they are but little longer than the surrounding parenchyma cells. If gliding growth should occur it would appear more probable that the tracheids in these anastomoses should reach various lengths and that they should not stretch only as far as is permitted by the available room. This also makes the occurrence of gliding growth in the longer tracheids less probable.

The primary cell wall of the elongating pro-tracheids in the objects studied shows the reaction on pectic substances with ruthenium red, the cellulose reaction and, when the cell is still initial, the "amyloid"-reaction. The secondary thickenings of the cell wall consist first of cellulose and pectic substances. If the secondary thickenings of the wall occur simultaneously in the tracheid or if they are formed succedaneously, could not be

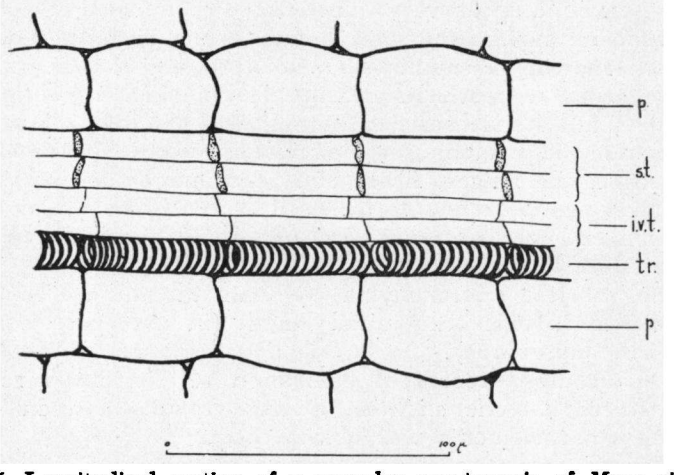

Fig. 15. Longitudinal section of a vascular anastomosis of *Musa sinensis*. One should notice the almost equal lengths of parenchyma cells, tracheids and sieve tube elements. — p. = parenchyma, tr. = tracheids, s.t. = sieve tubes, i.v.t. = undifferentiated initial vascular tissue.

established with certainty [see also F. M. SCOTT (17)].

During the lignification which is rapid, the pectic substances disappear gradually as the reaction on "lignin" becomes more intense. Finally the reaction on pectic substances is negative when the reaction on "lignin" reaches its maximum.

I could not find an increase in thickness of the secondary wall during the process of lignification as established by ALEXANDROV & DJAPARIDZE (2) in other objects.

9. *Summary.*

1. The average length of the sclerenchymatous elements in different leaves or leaf-sheaths of the plants examined is not constant, but depends in *Sanseviera* and *Agave* upon the length of the leaf in which the elements occur, and seems to depend in *Musa* upon the length of the pseudostem. The longer leaves (or leaf-sheaths) contain longer fibers, the shorter leaves (or sheaths) shorter sclerenchymatous elements. The shortest elements are found in the anastomoses running across in the leaf and connecting the longitudinal strands in *Sanseviera* and *Agave*.

2. The sclerenchyma fibers originate by repeated divisions of parenchyma cells in the growing zone of the leaf (or leaf

sheath) as bundles of very thin walled, uninucleate elements, these elements having the same length as the initial parenchyma cells, but showing a smaller diameter.

3. In order to reach its definite length such a young fiber elongates until it has reached many times its initial length; as a maximum of stretching an elongation up to 70 times was observed (in the longest fibers of *Agave* and *Sanseviera*). As a rule the elongation amounts to 40 to 60 times the initial length in the longer leaves of *Sanseviera* and *Agave* and in the pseudostem of *Musa*.

In the shortest sclerenchyma elements the mature length is sometimes only twice the initial length (in the anastomoses of *Sanseviera* and *Agave*).

4. During this process of elongation of the initial sclerenchyma fibers not the slightest gliding growth between — or along — other elements was observed.

5. The acute apices of the longer fibers are formed during the elongation of the cells from the transverse walls, which walls show in the beginning a slightly slanting position. The acuteness is the result of the difference in longitudinal and transverse growth-rate of the fiber. The longest fibers always show the most acute apices.

6. The secondary thickening of the cell wall occurs only when the fiber has reached its final length. The thickening occurs in every part of the cell wall simultaneously and equally. The thickening process soon ceases, after which the lignification begins.

7. In the primary cell wall of the sclerenchyma elements the direction of the long axis of the refractive-index ellipsoid is transverse in surface view of the cell.

In the secondary cell wall of the longest fibers the direction of the long axis of this ellipsoid differs only little from the longitudinal axis of the fiber. In fibers of average length the long axis of the index-ellipsoid forms an angle of about 45° with the longitudinal axis of the fiber. In the shortest sclerenchyma elements the long axis of the index-ellipsoid shows a transverse direction. Between these three typical directions of the index-ellipsoid all transitions are present, if fibers of various lengths are examined. When the long axis of the refractive-index-ellipsoid forms an angle of about 45° with the long axis of the fiber, the latter is apparently isotropic, this phenomenon being caused by the crossed systems in the upper and lower wall of the fiber.

8. The direction and shape of the pits in the secondary wall is also variable. In the longest fibers very steep inclined long pits are present, in fibers of average length the (long) pits form angles of about 45° with the long axis of the fiber and in the shortest sclerenchyma elements short, roundish-oval pits are found, which are placed in transverse direction. Between these forms and directions of the pits all transitions are also present.

9. The primary cell wall of the sclerenchyma fibers mostly shows in the initial stage, besides reactions on cellulose and pectic substances, the "amyloid"- and "collose"-reactions after ZIEGENSPECK. It seems highly probable that these reactions occur when the amount of cellulose is small (e.g. 10—12%), which may often be the case in young cell walls.

10. The secondary wall originally shows reactions on pectic substances and on cellulose. When the secondary wall is completely formed, lignification begins, occurring at first in the primary wall and middle lamellae and later on in the secondary thickening layers.

11. In the primary fiber wall as well as in the secondary one the pectic substances disappear during the process of lignification entirely or in part; the reaction on "lignin" with phloroglucinol-HCl becomes the more intensely red, the less the walls are stainable with ruthenium red. The process of lignification, as a rule, ceases as soon as the pectic substances have disappeared; sometimes the lignification ceases earlier, but never later.

During the lignification in none of the three plants examined a considerable increase in thickness of the fiber wall was observed; this statement does not agree with the data of ALEXANDROV & DJAPARIDZE (2), obtained from other objects.

12. The tracheids of the plants studied do not stretch by means of gliding growth along the sieve tubes, though they are, as a rule, longer than the elements of those sieve tubes; the differences in length are to be ascribed to the fact that the tracheids always begin to grow out earlier than the elements of the sieve tubes do.

13. In the unlignified secondary thickenings of the walls of the tracheids only cellulose and pectic substances could be indicated. During the lignification the reaction on pectic substances decreases as the amount of "lignin" becomes more considerable. The lignification ceases at the moment in which the pectic substances have disappeared completely. The lignifying cell wall does not increase in thickness; this fact does not agree

with the data of ALEXANDROV & DJAPARIDZE, obtained in other objects.

14. From the preceding it may be concluded that:

a. in Monocotyledons which do not show secondary growth gliding growth does not occur, or, at least, occurs less generally than could be expected from the existing literature and

b. during the process of lignification in the Monocotyledons studied probably no new cell wall substance ("lignin") is laid down, but that this substance is formed on the very spot out of substances already present (the pectic substances).

This investigation was started at the Botanical Laboratory of the Government University, LEIDEN, Director Prof. Dr. L. G. M. BAAS BECKING and continued at the Laboratory for Technical Botany, DELFT. The author desires to express his indebtedness to Prof. Dr. G. VAN ITERSON JR. for hospitality and for his continuous interest in this subject.

DELFT, Laboratory for Technical Botany, September 1937.

LITERATURE.

1. ALBADA, V. C. (1927). The structure and development of the cell wall in plants: I. Bast fibers of *Boehmeria* and *Linum*. Amer. Journ. Bot. 14: 16.
2. ALEXANDROV, W. G. and DJAPARIDZE, L. I. (1927). Ueber das Entholzen und Verholzen der Zellhaut. Planta 4: 467.
3. AMBRONN, H. and FREY, A. (1926). Das Polarisationsmikroskop. Leipzig.
4. ANDERSON, D. B. (1927). Ueber die Struktur der Kollenchymzellwand auf Grund mikrochemischer Untersuchungen. Sitzgsber. Akad. d. Wiss. Wien, Mathem. — Naturw. Kl., Abt. I, 136: 429.
5. BONNER, J. (1936). Zum Mechanismus der Zellstreckung auf Grund der Mizellarlehre. Jahrb. wiss. Bot. 82: 377.
6. CHEADLE, V. I. (1937). Secondary growth by means of a thickening ring in certain Monocotyledons. Bot. Gaz. 98: 535.
7. FREY, A. (1928). Das Wesen der Chlorzinkjodreaktion und das Problem der Faserdichroismus. Jahrb. wiss. Bot. 67: 597.
8. GREVE, G. (1909). Beiträge zur physiologischen Anatomie von *Musa ensete*. Dissert. Kiel.
9. HABERLANDT, G. (1879). Die Entwickelungsgeschichte des mechanischen Gewebesystems der Pflanzen. Leipzig.
10. HESS, K., TROGUS, C., and WERGIN, W. (1936). Untersuchungen über die Bildung der Pflanzlichen Zellwand. Planta 25: 419.
11. HOPMANN, O. (1930). Das Vorkommen und die Mikrochemie des Amyloids. Dissert. Muenster.
12. KISSER, J. (1926). Mazeration parenchymatischer Gewebe bei vollständiger Behaltung des Zellinhaltes. Planta 2: 325.

13. KRABBE, G. (1886). Das gleitende Wachstum bei der Gewebebildung der Gefässpflanzen. Berlin.

14. LOUIS, J. (1935). L'ontogénèse du système conducteur dans la pousse feuillée des Dicotylées et des Gymnospermes. La Cellule 44: 87.

15. SCHWENDENER, S. (1882). Die Stutzscheiden und ihre Verstärkungen. Abh. d. Königl. Akad. d. Wiss. Berlin 4: 25.

16. SCOTT, D. H. and BREBNER, G. (1893). On the secondary tissues in certain Monocotyledons. Ann. of Bot. 7: 21.

17. SCOTT, F. M. (1937). Differentiation of the spiral vessels in *Ricinus communis*. Bot. Gaz. 99: 69.

18. SKUTCH, A. F. (1927). Anatomy of the leaf of the banana, *Musa sapientum L. var. hortorum Gros Michel*. Bot. Gazette 84: 337.

19. SKUTCH, A. F. (1930). On the development and morphology of the leaf of the banana *(Musa sapientum L.)*. Amer. Journ. Bot. 17: 252.

20. SKUTCH, A. F. (1932). Anatomy of the axis of the banana. Bot. Gazette 93: 233.

21. SLOEP, A. C. (1928). Onderzoekingen over pectinestoffen en hare enzymatische ontleding. Dissert. Delft.

22. STRASBURGER, E. (1891). Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. Jena.

23. TAMMES, T. (1908). Der Flachsstengel. Natuurk. Verh. d. Holl. Mij. van Wetensch. te Haarlem, Sér. 3, Reeks VI: 4de stuk.

24. ZIEGENSPECK, H. (1925). Ueber Zwischenprodukte des Aufbaues von Kohlenhydrat-Zellwänden und deren mechanischen Eigenschaften. Bot. Archiv. 9: 297.