SPIRAL GRAIN AND XYLEM POLARITY IN RADIATA PINE: MICROSCOPY OF CAMBIAL REORIENTATION

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ABSTRACT

Spiral girdling of young radiata pine (**Pinus radiata** D. Don) was used to study the anatomical changes in cambium as initials move into alignment with the girdle. Light and electron microscopy showed that cambial initials are capable of considerable plastic deformation, initiated primarily by differential growth in fusiform initials. Ray initials appear to play a passive role in developing spirality, but eventually respond to pressures from the surrounding cambial elements. The frequency and directions of pseudotransverse divisions were also examined, but realignment of the daughter cells of multiplicative division could account for only a minor part of the grain angles that developed. It is believed that cytoplasmic stress arising during differential growth in fusiform initials determines the orientation of the mitotic spindle during anticlinal division. The spindle axis, in turn, determines the direction of chromosomal separation at anaphase, and the direction of pseudotransverse division. Hence, the direction of pseudotransverse division is thought to be symptomatic of differential growth rather than a primary cause of spiral grain. Analysis of the component features of cellular reorientation gives support to the concept of a symplastic pathway being the route of auxin movement through cambial cells.

INTRODUCTION

In previous studies of spiral grain in radiata pine (*Pinus radiata* D. Don), spiral girdling and other methods of changing the direction of metabolite movement down young stems were used to induce corresponding changes in the orientation of newly-formed xylem elements (Harris, 1969). Serial tangential-longitudinal sections through regions of reorientating xylem were used to examine the anatomy of wood in which grain angle was changing. From these experiments, it was concluded that cells in the cambial zone must be capable of considerable plastic deformation to account for the rapid changes of grain angle that can take place—up to 45° over 4 mm radial growth.

The type of cambial reorientation that results in changes in alignment between successive xylem cells along radial files was illustrated by tangential sections cut 100 μm apart (here reproduced as Fig. 1). Most of the differences between these two sections can be interpreted as arising from "intrusive growth" of tracheid ends (Sinnot and Bloch, 1939). There is also an indication that rays are "under pressure" from reorientating

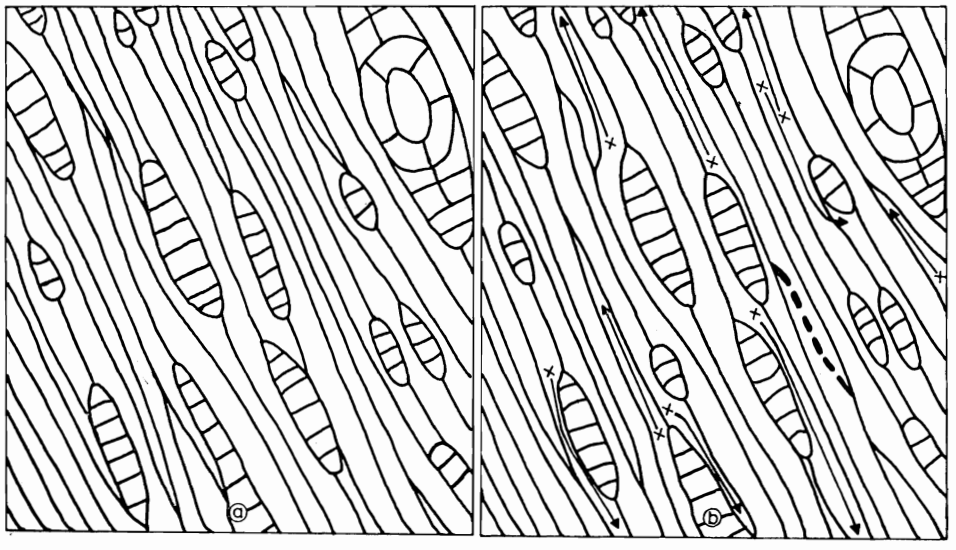

FIG. 1—Detail from sections cut 100 μm apart to show anatomical adjustments (intrusive growth, pseudotransverse division, and ray splitting) associated with rapid change in grain angle. (Reproduced from Harris, 1969—Fig. 4.)

tracheids, in that the wood ray at bottom centre is split into two (Fig. 1b) by the end of a tracheid that is pressed to one side of the ray in Fig. 1a.

Several workers (Bannan, 1950, 1954, 1964a, b, 1966; Hartig, 1895; Hejnowicz, 1961, 1964) have observed that the cross walls in multiplicative (anticlinal or pseudotransverse) divisions of cambial initials are laid down in a direction that will supplement changing grain angles. Though no intensive study was made of this feature in radiata pine, it was observed that pseudotransverse divisions were not exclusively in the direction that would favour an increase in grain angle. The frequency of such divisions was, in any case, inadequate to make any significant contribution to changes in grain angle of the magnitude observed. This is in agreement with the conclusions of Bannan (1966, p. 1535), who states that "the role of multiplicative division and subsequent cell elongation in the development of spiral grain, as proposed by various authors during the past several decades, has been oversimplified."

Although spiral girdling severely disrupts normal stem growth, it nevertheless has merits as a manipulative technique. In radiata pine, wood formation a short distance above the girdle continues with relatively little change other than realignment of cambial elements and their derivatives into the new direction of metabolite flow (Harris, 1969 pp. 206-7). Furthermore, reorientation is so rapid that the method provides excellent material for examining the anatomical basis of changing grain angles.

Even so, the maximum observed rate of change requires realignment of the cell axis of a fusiform initial by no more than half a degree between successive periclinal divisions. It is, therefore, probable that the effects of reorientation will be more apparent at the cumulative level than in the very minor adjustments that need take place at each cell division.

The observations described in this report were designed to find out how pine

cambium can accommodate rapid changes in grain angle at the anatomical level, and how these changes arise.

MATERIAL AND METHODS

Tree Girdling

Four-year-old radiata pine trees were spirally girdled in a right-hand spiral in October 1970 by removing a single 12 mm strip of bark and cambium around the stem between branch whorls, at a level corresponding to 2 years' growth below the stem apex. Stem diameter at this level was approximately 35 mm at the time of girdling. The vertical interval between spiral cuts was 120 mm, and spiral angle was, therefore, approximately 35° to the stem axis (Fig. 2).

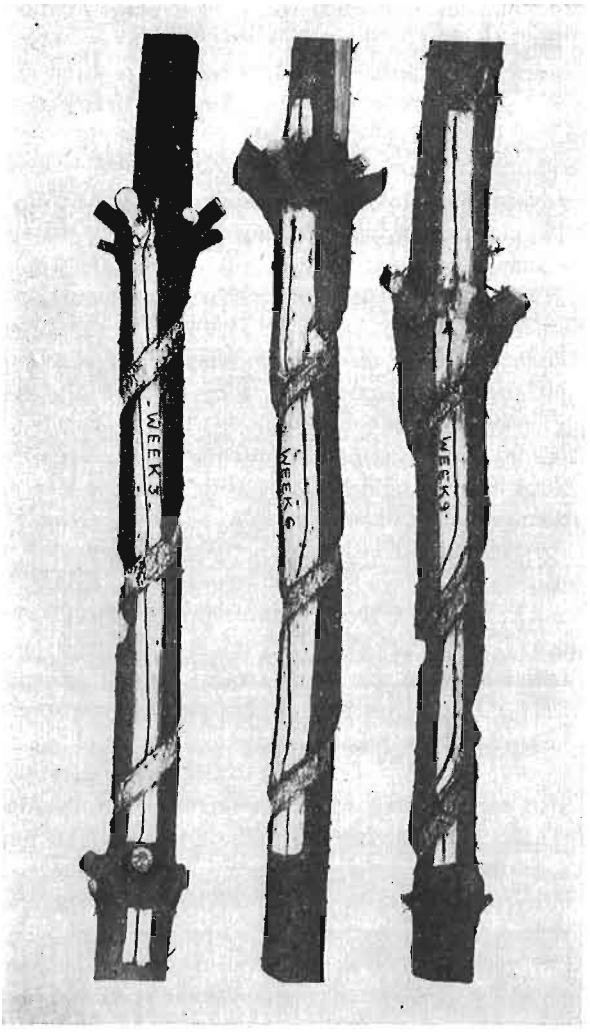

FIG. 2—Spirally girdled stems with strip of bark removed to show grain angle. From left to right, harvested 3, 6 and 9 weeks after girdling.

Trees were sampled 3, 6 and 9 weeks after girdling, and also towards the end of the growing season, 29 weeks after girdling. The effect of a full season's growth was to increase stem diameter in the region of maximum growth, just above the girdle, to approximately 55 mm. Because the vertical interval between revolutions of the spiral remained constant, circumferential growth increased the effective spiral angle to approximately 50°.

Light Microscopy of Cambium

Blocks of stem tissue for examination were removed from standing trees as follows: transverse cuts were made with a scalpel into the newly-formed wood above and below the region of maximum growth. A block of wood and bark approximately 25 mm square and extending up to 5 mm into the wood was then removed. Similar blocks were cut from ungirdled (control) regions of the same stems. Samples for microscopy, consisting of wood and bark, were cut immediately with a razor blade from the centres of these blocks. In this way the more drastic effects of releasing sap stream tension were confined to the outer margins of the large blocks, which were discarded.

Small blocks for light microscopy measured approximately 4 mm longitudinally by 1.5 mm tangentially and included bark, cambium, and 3-4 mm of wood. These were placed directly into 4% glutaraldehyde in Sorenson's phosphate buffer (pH 7.2). After not less than 24 hours' fixation, samples were dehydrated through an ethanol series and embedded in Epon (Barnett, 1971). They remained in pure Epon 812 for at least 2 weeks before polymerisation at 65°C.

Tangential-longitudinal sections of cambium 4 μm thick were cut from embedded samples with an LKB ultratome using a glass knife. Location of the cambial zone was facilitated by the presence of tannin-filled cells in the phloem. These are identifiable up to a few cells from the cambial initials, so that the approach towards the few critical sections that included cambium could be made with some confidence. Identification of the cambial zone in tangential sections was made on the basis that the cells within it:

1. Lay interior to the tannin-filled cells or other phloem cells with their distinctively thickened cell walls;
2. Contained large nuclei that were broadly elliptical in section or which were showing evidence of mitosis;
3. Lay exterior to cells with walls that showed early signs of secondary thickening.

Using these criteria, it is believed that observations of the cambial zone were restricted to those cells that lay within four to six cells on the xylem side of the true cambial initial.

Sections were dried onto the slide where they remained firmly attached, without the use of adhesives, throughout the staining and washing schedules. Staining in 1% aqueous safranin for 1 hour was done by placing a small, glass ring around the sections. This held a few drops of safranin in place by surface tension, and evaporation was minimised by placing a coverslip on top. After thorough washing, sections were differentiated in fast green for 10 to 20 minutes. They were then washed again, dried in an oven at 65°C and mounted in Epon 812, which was polymerised at the same oven setting for 24 hours. A Zeiss Photomicroscope II was used for microscopy and photography.

Electron Microscopy of Cambium

Slivers of wood for electron microscopy, measuring 1 mm tangentially, were cut from the blocks fixed in glutaraldehyde, and post-fixed in 2% osmic acid as small slivers 1 mm

square (Barnett, 1970). After embedding in Epon, tangential-longitudinal and transverse sections of cambium 60-80 nm thick were cut on an LKB III Ultrotome and examined with the Philips EM 300.

Serial Sections of Xylem

Other blocks, cut from above the girdle in the tree harvested at the end of the growing season, were used to examine the directions and frequency of pseudotransverse divisions. Tangential-longitudinal sections 25 μm thick were cut with a sledge-type microtome from the zone in which grain angles were changing most rapidly, 1.5-3 mm outside the girdle. Photomicrographs were made by direct projection onto photographic paper at 120x magnification. These were taken so that some identifiable feature that appeared on every section, such as a characteristic grouping of wood rays, was located at a similar place on each photograph. Cell divisions and lossess of cells within radial files were then identified by comparing photographs of adjacent sections, and these events were plotted in sequence by the method of Bannan (1964a).

RESULTS

Gross Effects of Girdling

Grain angles within and on either side of the girdled internode were measured by removing from the stem a longitudinal strip of bark about 15 mm wide (Fig. 2). A slope-of-grain detector (freely pivoted needle) was dragged down the exposed wood and the track that it followed was marked with pencil.

Three weeks after girdling, the left hand (LH) spiral normally encountered in young stems is still visible in the wood below the girdle (in this example about 5°), but this changes to right hand (RH) sloping grain of about 4° in wood laid down near to the upper edge of the girdle. Six weeks after girdling, grain angle in the region above the girdle has increased to 18° RH but grain angles below the girdle vary from 2° LH at the top of the treated internode to 5° LH in the lower half of the internode. By week 9, newly-formed wood is nearly straight grained below the girdle and 38° RH (parallel with the girdle) in the region above it.

Thus, in the zone of most rapid radial growth, within 20 mm of the upper side of the girdle, grain angle had swung from approximately 4° LH to 38° RH over a period of 9 weeks.

Because grain angle was changing most rapidly about 6 weeks after treatment, most of the electron microscopy and all the observations of pseudotransverse divisions were concentrated on cambium at this stage of development, or on the zone of wood, 1.5-3 mm outside the girdle, that was being laid down at this time.

Reorientation of Cambium

(1) *Light Microscopy*

The most obvious evidence of the cumulative effects of changing grain angle within the cambial zone was provided by the relative orientation of fusiform and ray initials. The line at the top of Fig. 3a marks the edge of a block cut parallel to the stem axis of the tree sampled 6 weeks after girdling. The angle between the longitudinal axes of fusiform initials and this line indicates the RH grain angle (18°) induced by the treatment. The longitudinal axes of many wood rays are, by comparison, at a much smaller

angle to the stem axis, the difference being most marked in large fusiform rays, and less so in the biseriate and in the longer of the uniseriate rays.

Short uniseriate rays, being more directly influenced by the orientation of the radial walls of adjacent fusiform initials, tend to conform with the initials in their orientation.

The fact that the two major components of cambium—ray initials and fusiform initials—respond "independently" when grain angle changes is striking evidence of the plasticity of cambium and of its capacity to adapt to major changes without total disruption. Closer examination of the manner in which this adaptation occurs provides evidence that fusiform initials are the dynamic component of reorientating cambium,

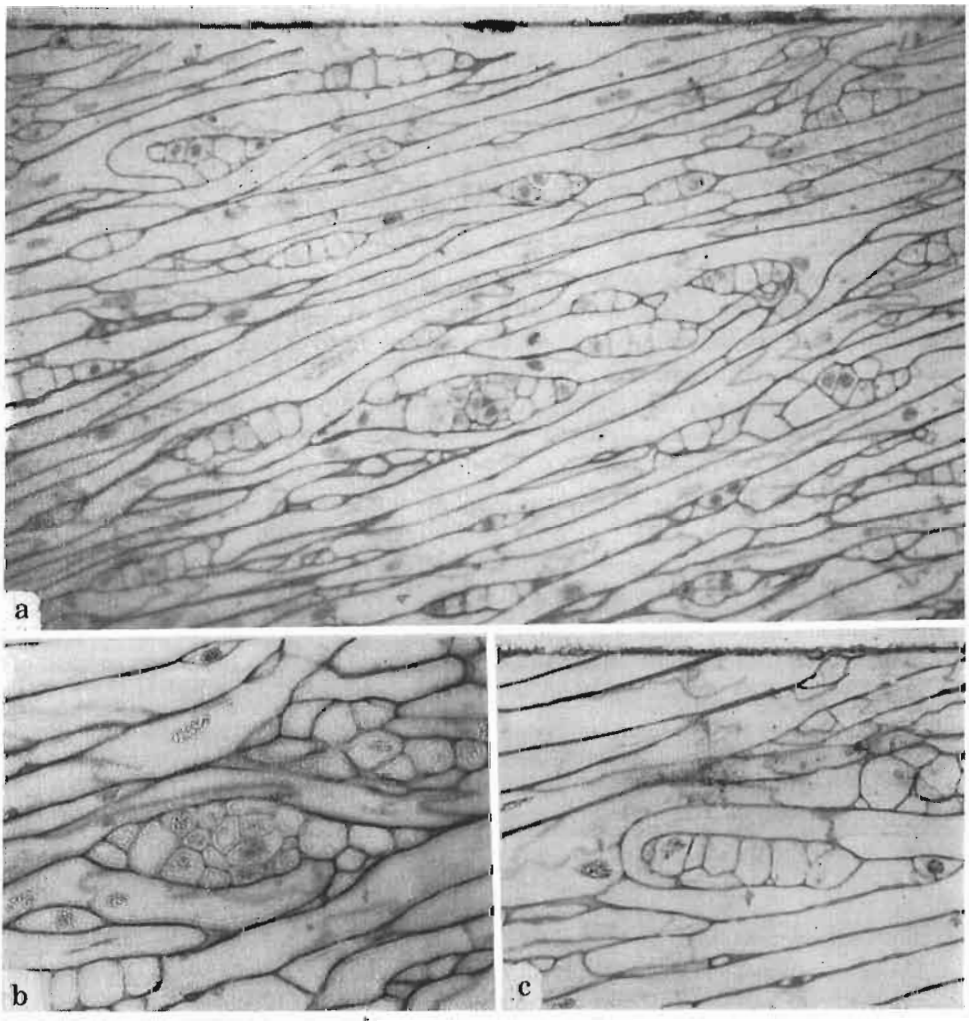

FIG. 3—(a) TLS of cambium from spirally girdled stem. The upper edge of this section is parallel to the stem axis.
(b) Fusiform ray "under pressure" from fusiform initials.
(c) End of fusiform initial wrapped around a uniseriate ray.

and that they have to overcome considerable inertia on the part of the larger groups of ray initials. It is not difficult to envisage the turning moment being applied to the fusiform ray in Fig. 3b by growth and movement of fusiform initials around it. Similar stresses can be attributed to the fusiform ray towards the centre of Fig. 3a. The greater mobility of fusiform initials is also illustrated by the tendency for ends of these cells to become wrapped around uniseriate rays (Fig. 3c). A similar situation can be seen in the top left of Fig. 3a.

Although these differential movements were most marked in cambium sampled 6 weeks after girdling, similar trends could be detected in other samples of reorientating cambium. However, the complexities of cell shape and orientation made it difficult to assess rate of realignment visually or to obtain clear evidence as to how it came about. A series of measurements was, therefore, made on cambial sections from all the trees examined and from untreated (control) samples.

A graduated rotating microscope stage was used to measure the angle between the stem axis and various cell axes. The axes of rays were measured in three groups: (i) fusiform and biseriate rays, (ii) uniseriate rays five or more cells in length, (iii) uniseriate rays four or less cells in length. The orientation of fusiform initials was measured in four ways (Fig. 4): (a) the overall orientation of the cell along the direct line from tip to tip, (b) the orientation at the centre of the cell in the region of the nucleus, (c) and (d) the lines bisecting the angles at the upper and lower cell tips respectively. Observations on fusiform initials were made only on those which appeared to be complete within the

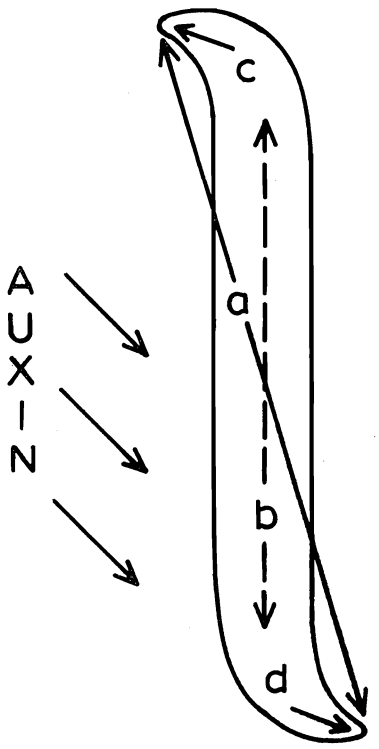

FIG. 4—Diagram to illustrate measurements made on the axes of fusiform initials and their configuration in relation to the direction of auxin movement.

plane of the section, that is, on those initials in which the nucleus could be seen and in which both ends of the cell tapered to a point with distinct cell walls. It was observed that if the end of an initial did not lie in the plane of the section the cell ends tended to "fade out" as an oblique cut through the thinner tangential wall.

The results of these measurements are summarised in Table 1. At week 6 in particular, the tendency for tips of fusiform initials to assume a greater angle to the stem axis than the main body of the cell is most marked. During the most active period of reorientation a picture is built up of a cell with distinctly sigmoid curvature, caused by cell tips adjusting more rapidly to the new alignment than the central region of the cell. But, in week 9, as soon as reorientation is completed and the fusiform initials lie parallel to the girdle, this distinction is lost.

Table 1 also demonstrates the relative inertia shown by groups of ray initials, particularly for the multiseriate (fusiform and biseriate) and large uniseriate rays. It is interesting that the figures indicate a slight (though not statistically significant) "lag" in the orientation of the larger rays compared with that of the fusiform initials, even at weeks 9 and 29.

The frequency of cell divisions in the cambium provided excellent examples of mitosis both in the periclinal and in the anticlinal directions. In view of the influence on grain angle that some authors have ascribed to the direction of anticlinal divisions, particular interest attached to the way in which pseudotransverse divisions arise.

In the early stages of mitosis it is impossible to distinguish between a periclinal and an anticlinal division because during prophase the chromosomes are not orientated (Fig. 5a). By early metaphase, however, a distinction can usually be made between chromosomes coming into line with the longitudinal axis of the cell prior to multiplicative division (Fig. 5b), and those that will lie at right angles to it prior to periclinal division (Fig. 5c). At late metaphase and sometimes into early anaphase of an anticlinal division (Figs. 5d, e) the chromosomes lie in the equatorial plane of the cell, but by late anaphase and through telophase (Fig. 5f) chromosomes move apart at an angle to the cell axis so that the cell plate initiates a pseudotransverse cell wall. Thus, it is the skewing of the poles of the spindle in the cytoplasm, and formation of the cell plate between these poles at telophase, that determines the direction of pseudotransverse division.

(2) *Electron Microscopy*

From previous studies (Harris, 1969) it was concluded that the direction of movement of auxin down a stem is responsible for differential growth and realignment of cambial cells. The way in which auxin changes the plasticity of cell walls during growth and differentiation is still a matter of dispute (Thimann, 1969; Cleland, 1968). Because spirally girdled stems provide such extreme examples of cambial reorientation, it was felt that studies of cytology and wall structure in cambium might provide some clue as to the *modus operandi* of auxin in these circumstances.

Fusiform initials of radiata pine are highly vacuolate (Barnett, 1971). Throughout most of their length they are characterised by very narrow peripheral cytoplasm. Only in the region of the nucleus and in the cell tips does cytoplasm occupy most of the cell cross section. For this reason, and because reorientation was most marked in cell tips, particular attention was paid to the distribution of cytoplasm, and to the distribution of organelles within it, in the tips of fusiform initials.

TABLE 1—Angle to the stem axis of cambial elements observed at various periods after girdling

Weeks after girdling	Statistic	Fusiform Initials				Ray Initials		
		Overall (tip to tip)	Upper tip	Lower tip	Centre of cell	Multi-seriate	Uniseriate 5 cells or more	Uniseriate 4 cells or less
0	Av. angle (°LH)	3	3	4	3	3	3	3
(control)	s.d.	1.35	2.39	2.84	2.01	2.77	2.44	2.85
	n	50	50	50	50	60	60	60
3	Av. angle (°RH)	4	4	6	2	2	2	4
	s.d.	3.07	5.52	6.32	2.98	3.67	3.34	4.24
	n	50	50	50	50	65	65	65
6	Av. angle (°RH)	18	20	18	15	10	12	16
	s.d.	3.99	6.40	6.31	3.95	5.83	5.14	4.09
	n	50	50	50	50	54	79	79
9	Av. angle (°RH)	38	37	37	38	36	37	33
	s.d.	4.16	5.76	7.93	5.07	6.33	6.63	7.17
	n	50	50	50	50	52	77	83
29	Av. angle (°RH)	50	48	49	50	48	49	51
	s.d.	2.38	6.16	4.80	2.84	2.93	3.19	5.62
	n	50	50	50	50	70	70	70

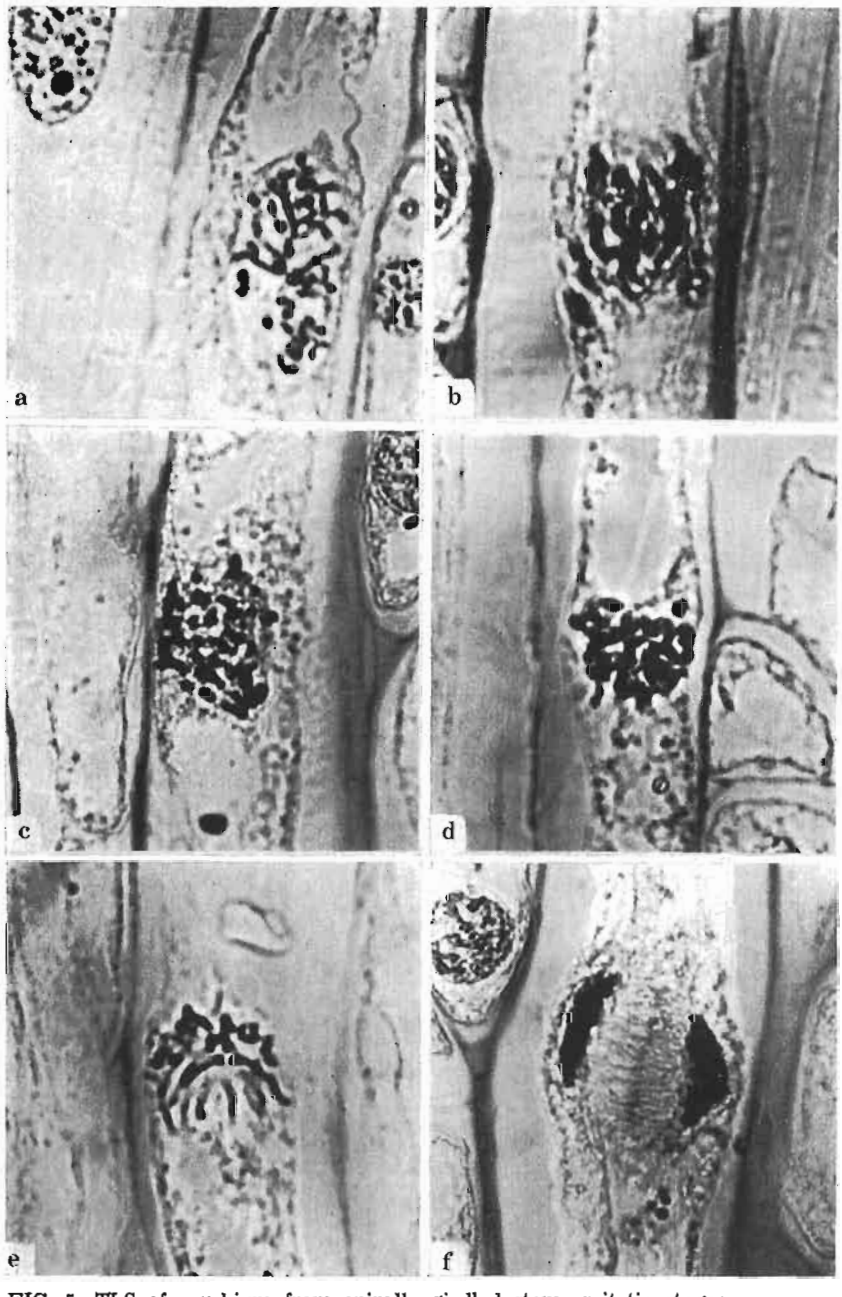

FIG. 5—TLS of cambium from spirally girdled stem: mitotic stages.
(a) Prophase
(b) Early metaphase of anticlinal division
(c) Metaphase of periclinal division
(d) Late metaphase of anticlinal division
(e) Early anaphase of anticlinal division
(f) Telophase of anticlinal division

The results were negative. None of the cambial cells, even during the most rapid period of realignment, showed any consistent cytological differences between one side of the cell and the other. Fig. 6 shows the tip of one such cell. The section was cut in TS as nearly perpendicular to the stem axis as possible, so that oblique cuts through the radial walls reflect the inclination of the tip to the stem axis. At this and higher magnifications, cell organelles appear to be distributed "randomly" — without any indication of greater or lesser cytological activity in the plane of curvature.

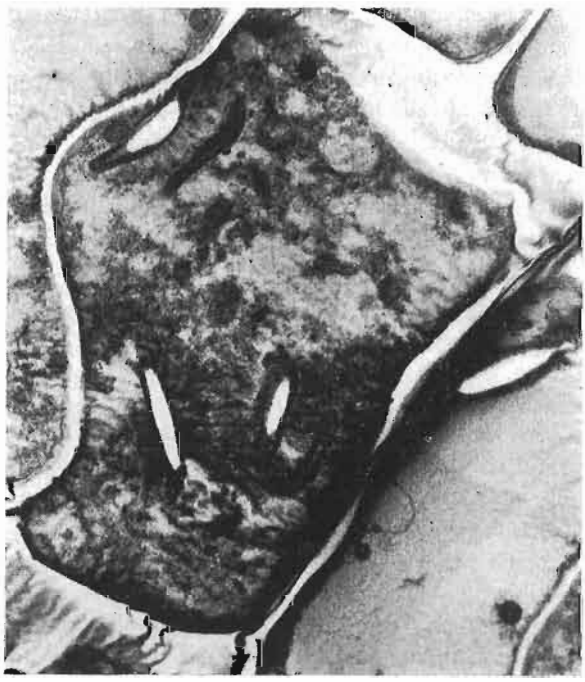

FIG. 6—TS of the tip of a fusiform initial from a spirally girdled stem.

Frequency and Direction of Anticlinal Divisions

The frequencies of anticlinal divisions and losses of initials were expressed as events per centimetre of radial growth, in keeping with the results summarised by Bannan (1966).

In the ungirdled stem, 3.6 anticlinal divisions were recorded for each 1 cm of radial growth. The potential gain in numbers of cells around the circumference was largely offset by loss of initials (through abortion or parenchymatisation) at the rate of 3.4/cm. The ratio of left-handed to right-handed pseudotransverse divisions was 6 : 5.

In the girdled stem the ratio of left- to right-handed pseudotransverse divisions was 1 : 4. The rate of anticlinal divisions was 5.9/cm, but this again was largely offset by loss of initials amounting to 5.3/cm of radial growth.

DISCUSSION

Reorientation of Fusiform Initials

When envisaging the changes that occur in cambium during rapid reorientation of its various elements, it helps to remember that the cambium is not a static tissue that simply cuts off phloem to one side and xylem to the other. A natural consequence of cambial division is that the cambium must also renew *itself* in successively widening concentric sheaths. After each periclinal division the cell that retains the function of cambial initial must grow, mainly by extension of its radial walls, before redividing.

However, growth of fusiform initials is by no means restricted to the radial direction. Elongation of the initials occurs as a general trend in young conifer cambium: this gives rise to the characteristic increase in tracheid length from the pith outwards in young stems. In addition, individual initials are repeatedly elongating after anticlinal divisions or in occupying "spaces" created by the loss of neighbouring cells.

Though the mechanism of cell growth is imperfectly understood, there is no doubt that it is influenced by the presence of auxin. In the present study it has been observed that, when metabolites flow down a stem at an angle to the axes of fusiform initials, differential growth induces a sigmoid curvature that tends to bring the initial into alignment with the direction of flow of metabolites. Previous studies (Harris, 1969) have suggested that auxin is the metabolite most directly responsible for cambial reorientation.

Without specifying the means by which auxin activity leads to cell extension, observations of radiata pine cambium suggest a sequence by which fusiform initials could change their orientation. Non-axial auxin movement is envisaged as providing the stimulus for differential growth in the radial walls of fusiform initials so that the tips tend to move in the tangential plane into alignment with the direction of auxin flow. Because the average angle between the tips and central regions of fusiform initials never exceeds 5° (Table 1) some adjustment must take place as the curvature of the tips increases. It seems probable that this adjustment would follow periclinal division, and that the newly-formed initial would (a) relieve stresses arising from its new sigmoid conformation by straightening up between cell tips (i.e., overall realignment of the cell axis) and (b) further adjust to non-axial flow of auxin by additional curvature of cell tips during subsequent growth. Reorientation is, therefore, envisaged as occurring through differential growth of the cell tips of fusiform initials, with partial adjustments of the cell axis after each periclinal division.

Reorientation of Rays

The appearance of the cambium in Fig. 3 suggests that reorientation of fusiform initials is frequently retarded by the presence of rays, especially the multiseriate and larger of the uniseriate rays. Because ray initials are nearly circular in tangential section, and because they are incapable of any significant elongation in the tangential plane, they lack the essential characteristics to respond to growth stimuli along the lines postulated for fusiform initials. Consequently, a group of ray initials behaves passively in the presence of an auxin cross-flow, and change in the alignment of a ray can take place only in response to pressures from surrounding fusiform initials.

The processes outlined above are adequate, by themselves, to explain any degree of cambial reorientation or plastic deformation which does not exceed the limits that can

be tolerated by a fusiform initial between one periclinal division and the next. As has already been pointed out, a change of grain angle of 45° over 3 mm radial growth requires the cell axis to move through less than half a degree between successive periclinal divisions. Fig. 3 suggests that this would lie well within the tolerance of a cambial cell.

Importance of Anticlinal Divisions

Turning now to the possible importance of multiplicative divisions for developing spirality, it is obvious that a pseudotransverse division will bring about some reorientation of the resultant initials only if both survive. This is not the usual outcome, and Bannan (1966, Table VI) shows that it happens in rather less than one-third of all anticlinal divisions. Observations of girdled and ungirdled cambium of radiata pine confirm this estimate.

If both cells do survive and grow to full length, the resultant reorientation is unlikely to exceed one degree, even when the ratio of cell diameter to cell length is comparatively large—say, 1 : 60 (30 μm by 2 mm). The observed rate of anticlinal division in the girdled stems (5.9/cm) would, therefore, account for a change in grain angle of no more than 0.2°/mm of radial growth as compared with at least 10°/mm for the maximum observed value.

However, it is worth noting that spiral girdling did give rise to an increase in the rate of anticlinal divisions, and that the direction of division did favour developing spirality at the time of maximum rate of change. The increased rate of multiplicative division is in keeping with previous observations of decreasing tracheid length in girdled stems (Harris, 1969, p. 206). On the other hand, girdling was observed to have only a minor influence on the direction of pseudotransverse divisions in the previous studies. This is probably because anticlinal divisions were originally observed over the whole year's growth, including the period when reorientation was complete, whereas the present study concentrated on the zone of most rapid reorientation.

If the direction of pseudotransverse divisions favours increasing spirality, but fails to account for more than a very small part of it when changes of grain angle are rapid, the question arises as to what the real significance of pseudotransverse divisions may be. It has been shown that the direction of an anticlinal division is determined by skewing of the poles of the mitotic spindle away from the longitudinal axis of the cell. It is now suggested that the cytoplasmic stresses that cause late metaphase skewing may well reflect the forces arising during curvature and reorientation of the fusiform initial as a whole. If this is so, the orientation of pseudotransverse divisions will certainly favour developing spirality, but will be symptomatic of stresses arising from reorientation of the cell rather than being a primary cause of realignment.

The conclusions

1. that spiral grain arises mainly through differential growth of fusiform initials,
2. that the direction of pseudotransverse division is a consequence of this differential growth and not a primary cause of spirality,
3. that ray cells play a passive role, at least so far as the dynamics of cellular reorientation are concerned,

serve only to raise the larger question of how polarity is expressed by non-axial flow of auxin.

Auxin and Differential Growth

The classical experiments with seedling hypocotyls suggest that polar transport of auxin is a result, and not the cause, of longitudinal polarity in plants (Goldsmith, 1969). Nevertheless, other experiments with young plants (e.g., with *Coleus* — Jacobs and Morrow, 1967) have shown that normal xylem differentiation appears to be controlled by auxin.

Because so little is known about auxin transport it is difficult to interpret the polar aspects of differential growth in the presence of auxin cross-flow. For example, the naive representation of Fig. 4, which corresponds in principle with direct cellular transport, gives no indication as to why the upper tip of the initial should move *towards* the direction of auxin flow. It would seem more logical for the recipient cell wall on the left in the diagram to elongate more readily than that on the right, so that the cell would develop a convex surface to the left and both tips would curve to the right.

Alternatively, because auxin transport has frequently been shown to be metabolically dependent (Goldsmith, 1969), it is tempting to look for evidence of metabolic transport in cells, or regions of cells, that are rich in cytoplasm, and which are, therefore, presumably metabolically active. For example, the "over-correction" of cell tips in the vicinity of ray cells (Fig. 3c) invites speculation that the rays may control auxin movement in some way even if they do not themselves respond in terms of differential growth. But any analysis of this possibility makes it clear that cell polarity and the direction of auxin movement must also be involved in differential growth.

This is illustrated in Fig. 7. There are equal chances that an upper or a lower cell tip may be in contact with a ray: for development of LH spirality the upper tip should curve around the ray and the lower tip should curve away from it. If the cell tips in the diagram had been applied to the opposite side of the ray, or if RH spirality were to be developed, the directions of movement would, of course, have to be reversed.

Quite obviously, change in the rates of auxin movement through metabolic activity could not, by itself, bring about differential growth of this type in fusiform initials.

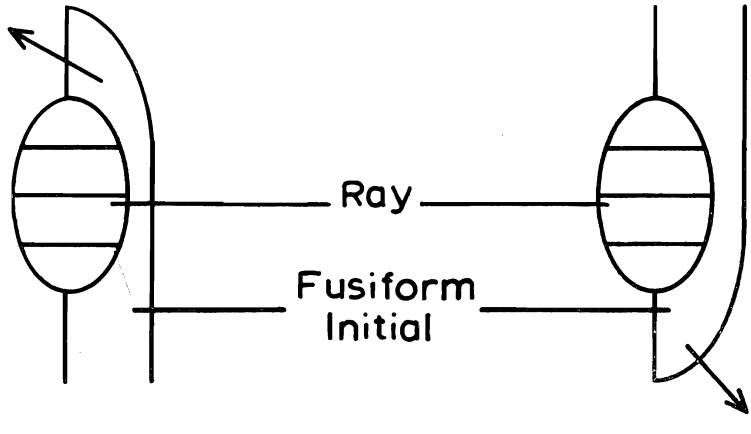

FIG. 7—Diagram to illustrate the anomalies that arise when direct association is sought between the presence of wood rays and differential growth in tips of fusiform initials.

Curvature in the upper end of a fusiform initial must proceed in the opposite sense to curvature in the lower end—"into" the auxin flow rather than "with" it—and curvature of both must respond to the *direction* of flow.

These considerations support the concept of a symplastic path rather than a cellular one for movement of auxin through cambium. The attractive feature of symplastic transport in this situation is that it would contain vector components related both to cell polarity and to the direction of auxin flow—i.e., in by the up-stream side at the top of a cell, and out by the down-stream side at the bottom. The fact that fusiform initials are poorly endowed with plasmodesmata need not necessarily rule out some degree of cytoplasmic intimacy between cells. The areas of future bordered pit formation can be detected very early in the development of cambial cells and could provide preferred pathways for intercellular transport.

CONCLUSIONS

1. Spiral girdling of young radiata pine trees is an effective means of manipulating the alignment of cambial initials to study the anatomy of cellular reorientation.
2. Spiral grain arises mainly from differential growth of fusiform initials. This produces curvature that is most marked in the tips of the cells. Partial adjustment of the longitudinal axis of the cell is believed to take place after periclinal division.
3. It is suggested that the direction of pseudotransverse divisions is determined by cytoplasmic stress induced by differential growth. Stresses in the cytoplasm are envisaged as determining the direction in which the mitotic spindle is skewed from the cell axis. This in turn determines the direction of chromosomal separation at anaphase and subsequently the orientation of the new cell wall.
4. The direction of pseudotransverse divisions is, therefore, symptomatic of differential growth and not a primary cause of spirality.
5. Ray initials in the cambium are seen to play a passive role as far as the dynamics of cellular reorientation are concerned. The rays are slow to change their alignment as spiral grain develops and appear to do so only under pressure from reorientating fusiform initials.
6. Analysis of the component features of cellular reorientation lends support to the concept of a symplastic pathway for auxin movement through cambial cells.

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