THICKNESS VARIATION IN ULTRAMICROTOMED WOOD SECTIONS[1]

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ABSTRACT

Electron micrographs of ultrathin sections reveal variation in the section thickness of the various cell-wall layers. The variation was present in cell walls that were not parallel to the cutting edge of the knife and absent from cell walls that were parallel to the knife edge. Although only a speculative explanation for this cutting artifact is offered, its presence in the various cell types is clearly illustrated.

Keywords: Ultrathin sections, cell-wall layers, ultramicrotome, section thickness, secondary wall, electron microscopy, ultrastructure, anatomy.

INTRODUCTION

The preparation of ultrathin sections of wood cells for study with light and electron microscopes is a common practice. Fergus et al. (1969) utilized thin sections to determine lignin distribution based on UV absorption across the cell wall. Also, Asunmaa and Steenberg (1957) used ultrathin sections to evaluate the relative scattering densities of the middle lamella and adjacent secondary walls to electrons. Quantitive evaluation of X-ray dispersive techniques of elements impregnated within the cell wall will increase as wood researchers gain access to such equipment. All of these techniques rely on known section thickness, small cell-wall thickness variation of the sections, or both. However, Preusser et al. (1961) noted large section thickness variability in ultrathin sections of beech ray parenchyma cells. They observed that if the cell wall is parallel to the knife edge during cutting, little difference in electron density exists between the cell-wall layers. However, if the cell wall is perpendicular to the knife edge, the middle layer on one side of the cell is electron dense whereas on the opposite side it "appears only slightly electron dense" and the "contrast of the interior and exterior layer is very big." In addition, they stated:

> The cuttability of the secondary wall differs considerably due to the varying angles which the microfibrils form with the knife edge in the individual layers. Due to the relatively flat arrangement in the central layer, for instance, the microfibrils slide away under the glass knife; they are cut later than their surroundings, raise, form a thickening of the object and consequently an area of a greater electron density. In this case, the microfibrils of the interior and

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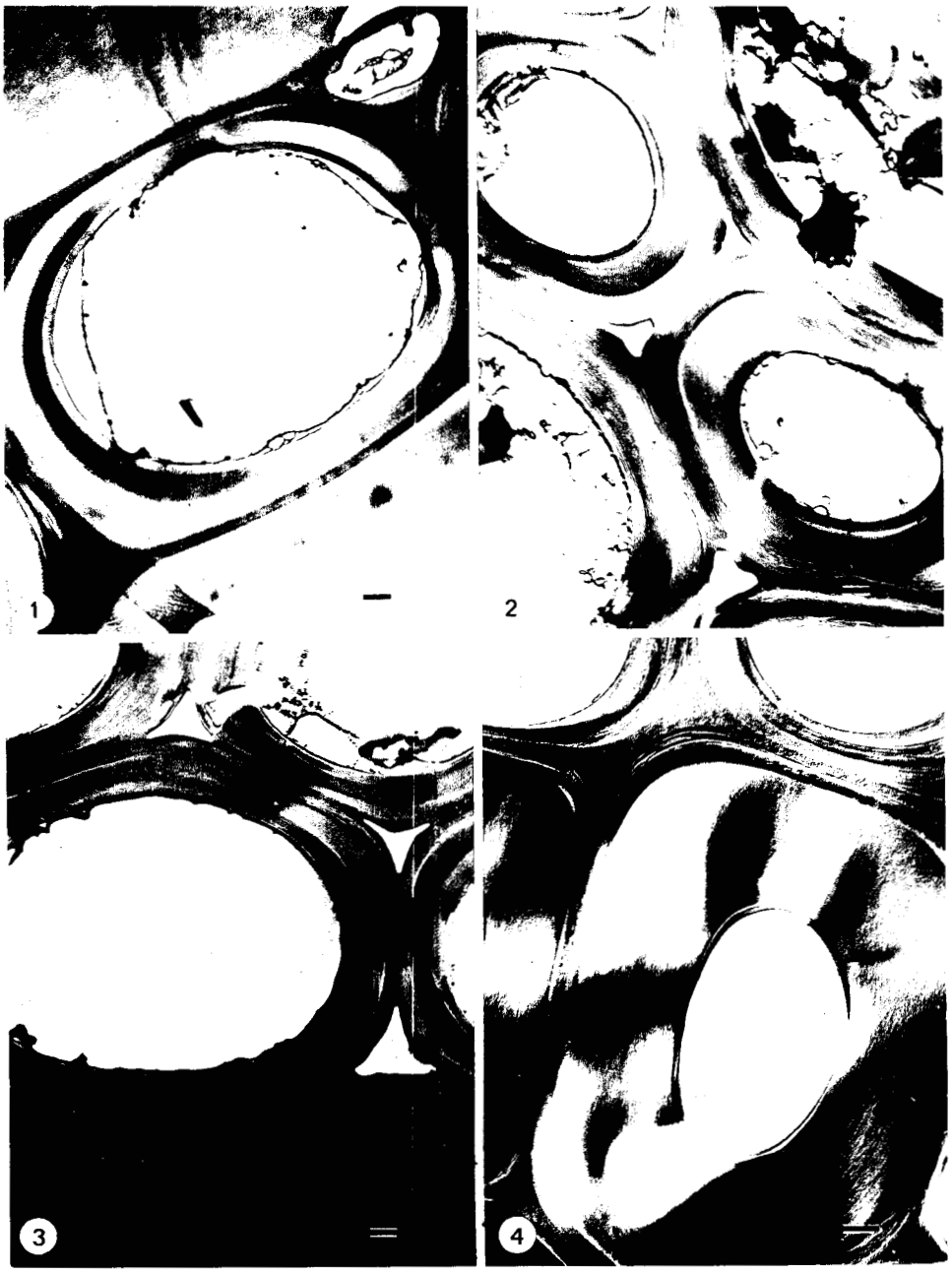

(All bars represent one micrometer)

FIG. 1. Ray parenchyma cell showing electron dense layers within the cell wall. Tangential section, not shadowed. Reason for electron dense regions not apparent. Mockernut hickory (*Carya tomentosa* Nutt.).

FIG. 2. Cell walls of ray parenchyma revealing thick and thin layers that appear to correspond with the S_1, S_2, and S_3 layers. Note reversal of thickness within any given layer from one side of cell

exterior layer form a steeper angle with the knife, are therefore cut earlier and have only a slight increasing effect on the contrast. If the cutting direction is the same, the opposite arrangement of fibrils in the three wall layers can be found in the opposite area of the cell wall of the same cell due to the spiral texture of the microfibrils, so that the central layer appears electron light, the interior and exterior layers appear, however, electron dense. If for instance the knife cuts the cell wall perpendicularly, all the microfibrils are cut in an angle of approximately 90°, so that an evenly electron dispersing secondary wall can be observed. All the discussed fibril arrangements show a different electron density in the general picture.

During a recent study of hardwood ultrastructural features, cell-wall thickness variability due to sectioning was noted not only in ray parenchyma cells, but also in longitudinal parenchyma, fibers, and vessels. Thus, the objective of this paper is to illustrate the presence of this sectioning artifact in all cell types.

MATERIALS AND METHODS

Never-dried specimens of various hardwood species were embedded in methacrylate (7 parts butyl- and 3 parts methyl-methacrylate). The specimens were sectioned with a Sorvall MT-2B ultramicrotome. In some cases, prior to examination of the sections with a Siemens IA electron microscope, the methacrylate was removed with xylene and the sections were shadowed to improve contrast. In others, nonshadowed sections with the methacrylate present or absent were examined.

RESULTS AND DISCUSSION

Electron dense layers within ray parenchyma cell walls are clearly illustrated in Fig. 1. Sections shadowed with platinum reveal that the high electron dense zones are due to an increase in section thickness within certain cell-wall layers (Figs. 2 and 3). Note that on one side of the cells the section thickness of the central region (S_2) is greater than the interior (S_3) and the exterior (S_1), whereas on the opposite side the reverse is true. The change in thickness is easily seen as one layer is followed around the cell wall.

Evidence that this cutting phenomenon exists in cell types other than ray parenchyma was found. Figures 4 and 5 reveal varying section thicknesses for

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to the other side. Tangential section, shadowed. Shadowing illustrates that the electron dense regions are due to increased thickness. Blackgum (*Nyssa sylvatica* Marsh.).

FIG. 3. Direction of cut shown by knife mark (arrow) indicates that only the fiber wall and the ray parenchyma wall adjacent to the fiber were parallel to the knife edge. As a result, uniform wall thickness is present only in these walls. Tangential section, shadowed. Sweetgum (*Liquidambar styraciflua* L.).

FIG. 4. Cell-wall thickness variation in thick-walled fiber tracheids and thin-walled longitudinal parenchyma. Portions of cell walls at top of figure are from longitudinal parenchyma cells. Note the more obvious delineation of the layers in the longitudinal parenchyma cell walls than in the fiber tracheid due to the smaller parenchyma cell wall. Cross-sectional view shadowed. Mockernut hickory (*Carya tomentosa* Nutt.).

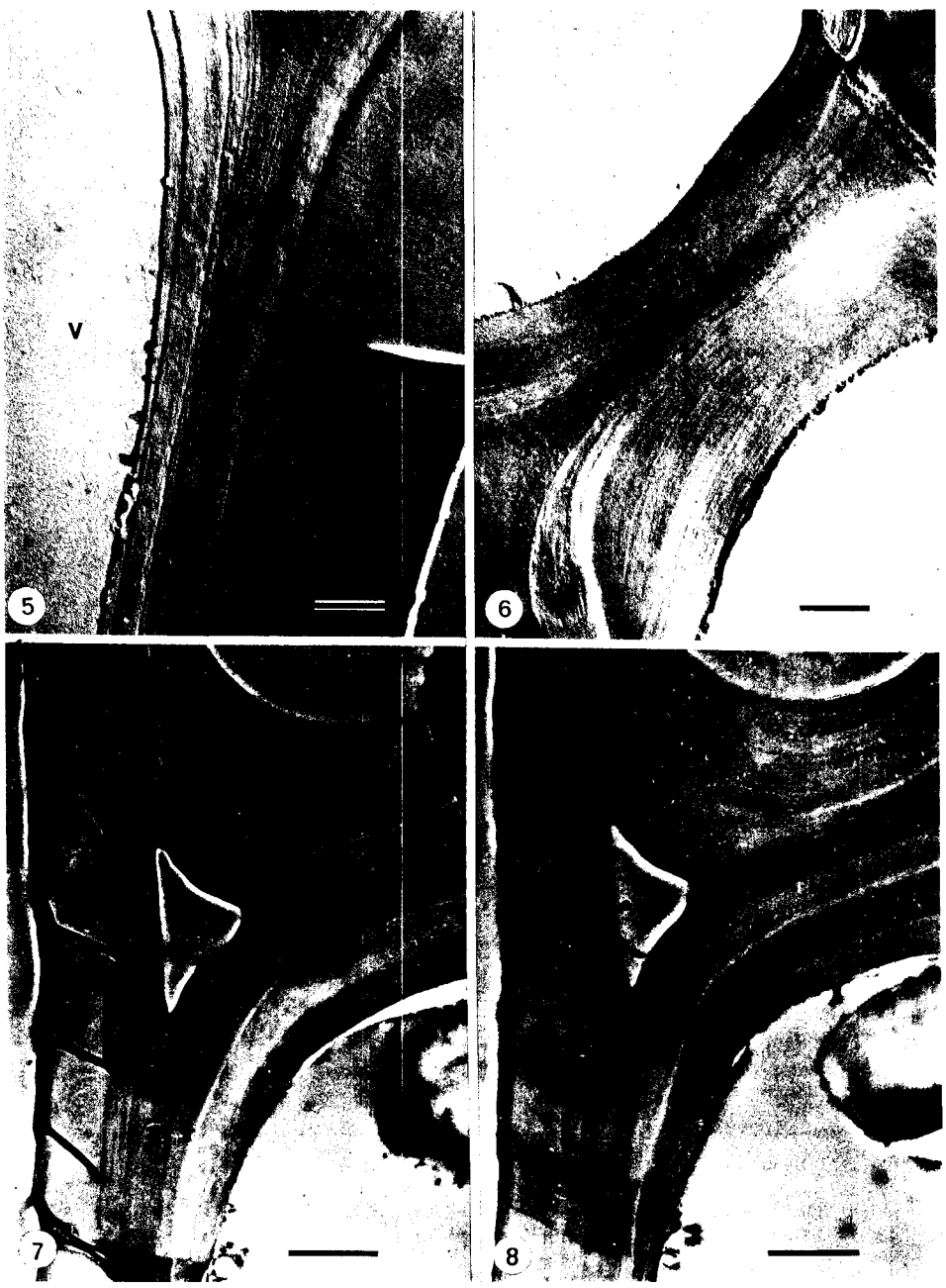

FIG. 5. Portion of a vessel (V) and adjacent fiber tracheid (F) showing thick and thin layers in the cell walls. Note that as the vessel wall turns from near perpendicular to the direction of cut (as indicated by knife marks), the wall layering becomes more distinct. Cross-sectional view, shadowed. Sweetgum (*Liquidambar styraciflua* L.).

FIG. 6. Ray parenchyma cell walls without obvious delineation of cell-wall layers due to thickness changes. As indicated by the knife mark (arrow), the cell wall was parallel to the knife edge during cutting. Tangential section, shadowed. Blackgum (*Nyssa sylvatica* Marsh.).

fibers, ray parenchyma, and a vessel. Published papers concerned with softwoods illustrate the same cutting phenomenon in tracheid cell walls (for examples see Côté and Day 1969). The difficulty in noting this cutting artifact in vessels, fibers, and softwood tracheids can be attributed mainly to their large cell diameter relative to ray parenchyma cells. As a result, generally only portions of the cell walls are depicted in micrographs, and consequently, the change in section thickness of the layers around the cell wall is not as noticeable. Also, in fibers and tracheids, the much larger cell wall characterized by a wide S_2 layer tends to obscure the differences in section thickness of the various cell layers (Figs. 4 and 5). However, in the small diameter parenchyma cells with thin walls, the cutting artifact is very obvious (Figs. 2, 3, and 4).

Cell walls parallel to the knife edge have a uniform thickness of wall layers (Figs. 3 and 6). It is often difficult to ascertain cutting direction, but the knife marks in Figs. 3 and 6 clearly indicate the exact cutting direction. In Fig. 3, only the ray parenchyma cell wall adjacent to the fiber and the fiber cell wall were parallel to the knife edge. Neither of these walls exhibits a thickness variation between the wall layers. The central portion of ray parenchyma cell walls depicted in Fig. 6 was exactly parallel to the knife edge during cutting and shows a uniform thickness. Note, however, that the part of the wall in the lower left corner of the micrograph, which was not exactly parallel to the knife edge, reveals an S_1 layer with reduced thickness.

Additional evidence, which shows that a uniformly thick cell wall is obtained only when the wall is exactly parallel to the knife edge, is presented in Fig. 5. Note that the cell walls have numerous fine knife marks that clearly indicate the direction of cut. Also notice the layering in the vessel cell wall despite the fact that the wall was almost parallel to the knife edge ($\pm 16°$) during the cutting. It is also obvious that the layering becomes more distinct as the vessel cell wall curves such that the angle between the cell wall and the knife edge increases.

Notice that similar cell-wall layers in adjoining ray cells alternate in section thickness, i.e. if the S_1 and S_3 layers are thicker than the S_2 layer in one cell, the reverse occurs in the adjacent cell (Figs. 2 and 3). Also, when a given section reveals a thick S_2 with thin S_1 and S_3 on one side of the cell wall, the same wall of the same cell from the following section shows reverse thickening, i.e. a thin S_2 with thick S_1 and S_3 (Figs. 7 and 8).

Since the thick and thin layers in the cell wall are reversed from one side of the cell to the other, as well as in contiguous cell walls, and the cell walls parallel to the knife edge do not show section thickness differences, it appears that both the helical arrangement and the angle of microfibrils dictate the resulting thick-thin pattern. If the knife edge is parallel to the cell wall, so that the microfibrils of all cell-wall layers are at right angles to the knife edge, then the various layers in the cell wall are of approximately uniform thickness. However, when the cell

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FIGS. 7 and 8. Serial sections of ray parenchyma cell walls revealing the reversal of thickness within the wall layers. Note in Fig. 7 the S_2 is thick and the S_1 and S_3 thin. In Fig. 8, the S_1 and S_3 are thicker than the S_2. Tangential section, not shadowed and methacrylate not removed. Sweetgum (*Liquidambar styraciflua* L.).

wall is not parallel to the knife edge, all the microfibrils are oriented at angles other than 90°. Thus, cell-wall layers with the microfibrils leaning away from the knife edge are cut at a different thickness than the layers with the microfibrils leaning towards the knife edge.

This difference in section thickness of the layers within the cell wall might be explained by the microfibrils that leaned away from the knife being pushed downwards, and possibly stretched also, so that this layer would be thinner than the nominal value for the section. The microfibrils that leaned towards the knife would be bent upwards and the knife would tend to cut low, giving a layer thicker than nominal. The surface of the block exposed for the next cut would therefore be elevated where the section had been cut thin and depressed where the section had been cut thick. Thus, the second and subsequent cuts would be expected to produce stepped sections, but the knife would still be expected to cut high where the microfibrils lean towards it and to cut low where the microfibrils lean away from it. Consequently, a given layer of the cell wall should be of similar thickness in successive sections. However, examination of micrographs of serial sections shows that a layer that was thin in one section is thick in the succeeding section and thin again in the next section (Figs. 7 and 8).

One possible explanation of the alternations in thickness of a cell-wall layer in serial sections is that the resistance of the section to deformation by the knife depends on the thickness of the block above the cutting plane. For example, assume that the variation in thickness within the section is directly proportional to the thickness of the block above the cutting plane and that k is the constant of proportionality. Also assume that the nominal thickness, i.e. the displacement of the knife between successive cuts, is equal to T, and that the microfibrils are oriented so that the section will tend to be thin. Then on the first cut, the actual thickness of the section at this part of the cell wall would be equal to $T - kT$. The depth of the block above the cutting plane for the next cut would be $T + kT$. Hence the thickness of the second cut would be $T + kT - k(T + kT) = T - k^2T$. Similarly, the corresponding thickness for the third cut would be $T + k^2T - k(T + k^2T) = T - kT(1 - k + k^2)$, and so on.

Substitution of any suitable value for k in the above expressions for the thickness yields values that are alternately thin and thick; e.g. if $k = 0.1$, then the sections are successively 0.9, 0.99, 0.91, 0.98, . . . times T in thickness. It may be similarly shown that on the opposite side of the cell wall where the microfibrils of that same layer are oriented to produce thick sections, the sections would be 1.1, 1.01, 1.09, 1.02, . . . times T in thickness. There would thus be a significant difference in thickness of a given layer in successive sections, but these differences become less as the number of sections increases. Further, the differences between the thickness of a given layer on opposite sides of the cell are alternately small and large. Consequently, a simple assumption of the above kind is inadequate, as we did not observe any such changes between sections.

Another possibility is that the first cut causes substantial damage to the material just below the cutting plane with the result that very little resistance is offered to the next pass of the knife so that the surface of the block is cut plane rather than stepped. The third cut would then be a repetition of the first and would produce a stepped surface and further subsurface damage. This explanation more closely fits our observations.

CONCLUSION

To our knowledge, little research has been done on the cross-cutting of wood by a microtome, and speculation on the cause of the variations in thickness of the sections is perhaps futile without more knowledge of the mechanisms of the cutting action. Although this paper offers only a speculative explanation for the cutting artifact, its presence in various cell types is clearly illustrated. Therefore, caution should be utilized in any studies of the cell wall that depend on a uniform section thickness.

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