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ON THE SHAPE OF DEVELOPING VESSEL ELEMENTS IN FRAXINUS EXCELSIOR L.*

P. D. BURGGRAAF

Botanisch Laboratorium, Leiden

SUMMARY

In thick transverse sections through carefully sampled pieces of the developing early-wood zone of *Fraxinus excelsior*, the shape of the developing vessel elements is circular.

On the basis of this shape and of some considerations on the mode of cellular adjustments and preliminary counts of cell-division figures in this zone, it is concluded that a two-stage process for the enlargement of the vessel elements, as proposed by ZASADA & ZAHNER (1969), is unlikely.

1. INTRODUCTION

In his latest book KOZLOWSKI (1971) shows an illustration of transverse sections through the developing early-wood zone of a red oak tree, originally published by ZASADA & ZAHNER (1969). These authors concluded, in agreement with CHALK (1930) who studied *Fraxinus*, that the radially flattened appearance of the young vessel elements in their sections was not due to distortion resulting from sampling and subsequent microtechnical handling of the tissues but presents a true picture of the shape of these elements in the living tree: "The development of this shape in the vessel element occurs in two stages: first a rapid enlargement to nearly full tangential dimension, followed by a slow radial expansion to the final dimensions". Zasada and Zahner proposed a mechanism for this mode of growth of the vessel element, based on:

1. differences in turgor pressure, permitting the vessel element to "expand readily into the less turgid derivative tissue with soft primary walls tangential to it", while "enlargement in the radial direction at this time must be prevented by the adjacent mature latewood on the inside and by cambial meristem of high turgor pressure, backed up by lignified bark tissue on the outside";
2. "intrusive wedging by the vessel element" between the elements tangential to it;
3. "temporary inhibition of the production of new derivatives by the cambium located radially outward to the enlarging vessel elements until vessel elements reach full radial dimensions";
4. "division of xylem mother cells and enlargement of new derivatives on either side, tangentially, of the enlarging vessel elements", providing space for their radial expansion by outward displacement of the cambium.

* Dedicated to Professor Karstens on the occasion of his retirement.

Working on the differentiation of the vessel elements in *Fraxinus excelsior* L., on the wood of which a preliminary report appeared recently (BURGGRAAF 1972), I initially obtained pictures of these elements very similar to those given by Zasada and Zahner. A different sampling technique gave, however, entirely different results. In view of the importance attached to the apparent shape of the vessel elements by the authors mentioned, it seemed relevant to discuss this discrepancy.

2. MATERIAL AND METHODS

Samples were taken from vertical six-year old shoots growing rapidly on old stumps on rather wet soil in the Royal Domains "De Horsten" at Voorschoten. The sampling technique (NEWMAN 1956) consisted of:

a. slicing off most of the bark and outer tissues of the sampling area, keeping the underlying tissue wet;
b. making two parallel longitudinal incisions, 4 cm long and 4 mm apart and reaching into the mature wood: this releases existing tangential tensions in the bark tissues remaining on the sample and is done by placing two thick, single-edged razor blades in narrow slits fashioned in a copper guide-strip and hammering the blades into the shoot, after which the strip is used for extracting the blades;
c. making transverse cuts marking the upper and lower ends of the sample, with a narrow chisel;
d. wedging the sample carefully off the stem with the chisel.

The sample was then immediately immersed in a formalin-acetic acid-propionic acid-alcohol mixture, evacuated on return to the laboratory, and stored.

After washing in 50% alcohol the material was wet-sectioned (50 μm thick) on a bench microtome with a razor blade. (The microtome and blade holder were both produced by the Technical Department of the Botanical Laboratory: the former as a larger version of a very old model Leitz hand microtome (No. 1214, Leitz catalogue 1930), the latter after an existing Leitz razor-blade holder modified to accomodate Weck hair-shaper blades, the sharpest and hardest thick blades that could be found.)

By making a slicing cut and keeping the knife edge well flooded with 50% alcohol, series of good-quality transverse sections were obtained. As judged from the appearance of the delicate walls, the cambial zone in these sections is only slightly compressed.

The sections were mounted unstained in glycerol.

3. RESULTS AND DISCUSSION

Figs. 1 to *8* show transverse sections of samples taken on May 4th (*figs. 1, 3, 4, 7*) and May 12th (*figs. 2, 5, 6, 8*), photographed at different magnifications with ordinary light (*figs. 1, 2, 3, 5, 7, 8*) or polarized light (*figs. 4* and *6*). Vessel elements in different stages of development are present, as judged from the

diameter of the elements and the thickness of the walls: in corresponding parts of mature annual rings the radial diameter of 90% of the single vessels is 140 to 200 μm and the walls are thick and lignified.

The shape of the young vessel elements is roughly circular, even in these somewhat compressed sections. The diameter in the radial direction is as large as that in the tangential direction, sometimes even larger. This oval form is typical for the transverse section through large mature spring vessels.

It is evident from the photographs that there are enough cells in the radial rows tangential to the vessel elements at all developmental stages to insure unhampered expansion in a radial direction. In the radial files tangentially adjacent to the expanding vessel elements, the continuity of the rows is broken during the enlargement of the vessel element.

Figs. 4 and *6* show the same sections as *figs. 3* and *5* but photographed with polarized light, and reveal a very slight birefringence in the walls of the developing vessel elements. This indicates that these elements still have very thin primary walls (KERR & BAILEY 1934), even thinner than the walls of the tangentially adjacent cells.

The few clear pictures of developing vessel elements that could be found in the literature also show circular shapes of these elements, i.e. in *Boehmeria nivea* (ESAU 1965), *Carya illinoensis* (ARTSCHWAGER 1950), and *Acer pseudoplatanus* (CATESSON 1964).

Beside the shape of the vessel elements in the transverse sections there are also other objections to the mode of enlargement proposed by Zasada and Zahner:

The question of turgor pressure in growing cells presents great difficulties, which seems to be about the only point on which there is consensus; for the rest, the relevant literature is highly contradictory (BURSTRÖM 1961, 1971; RAY, GREEN & CLELAND 1972). It seems to me, however, that large differences in turgor pressure between the thin-walled cells in the cambial and differentiation zones are not likely to occur.

Since the cells of the tissue tangential to the growing vessel elements are themselves enlarging, expansion by pressure of the vessel elements into this tissue will displace these cells. The net result of such displacement must be a radially outward pushing of the cambial and outer tissues by these cells, which would mean that the vessel elements would also be able to push in the same direction.

These considerations on turgor also raise doubts concerning the remarks made by ZASADA & ZAHNER (1969) and ESAU (1965) on intrusive growth of the vessel element as a mechanism by which the cells adjacent to it are forced apart. It seems impossible for an enlarging element to force its way tangentially in between two adjacent, also growing, elements, unless there is already some space between them. Such space could only arise from radial displacement of the outermost of these two elements, and this could only occur if the elements tangential to them grew in the radial direction.

The only other possibility is that the adjacent cells do not grow. Then they

could be flattened by the enlarging element, as sometimes seems to happen in mature tissue.

In these considerations it is assumed that sliding of the radial rows along each other in the radial direction does not occur. Modern authors agree that sliding growth does not normally play an important part in cellular adjustments during growth (BROWN 1963; ESAU 1965). But a temporary inhibition of the production of new elements in the radial rows in which vessel elements are expanding (ESAU 1965; ZASADA & ZAHNER 1969) would inevitably cause such radially-directed sliding of the radial rows. The production of cells is thought to continue in adjacent rows of xylem mother cells but not in the rows containing the enlarging vessel elements.

Preliminary counts of mitoses and periclinal cell divisions in transverse sections of our material indicate that there are as many divisions in the cells in the radial rows outside the expanding vessel elements as in other radial rows. Moreover, periclinal divisions were found in the tissue tangential to the enlarging vessel elements.

On the basis of the shape of the developing vessel elements in transverse sections of *Fraxinus excelsior* as well as the aforementioned considerations, I would conclude that a two-stage process for enlargement of the vessel elements as proposed by ZASADA & ZAHNER (1969) is unlikely. It seems improbable that intrusive wedging by the expanding vessel element and temporary inhibition of cell production in the radial rows peripheral to the vessel elements are important mechanisms in the enlargement of these elements.

Periclinal divisions and radial growth, and also longitudinal growth in the cells tangential to the expanding vessel elements but not in the immediately adjacent cells, would provide the conditions for unhampered enlargement of the vessel elements in both the radial and the tangential directions simultaneously.

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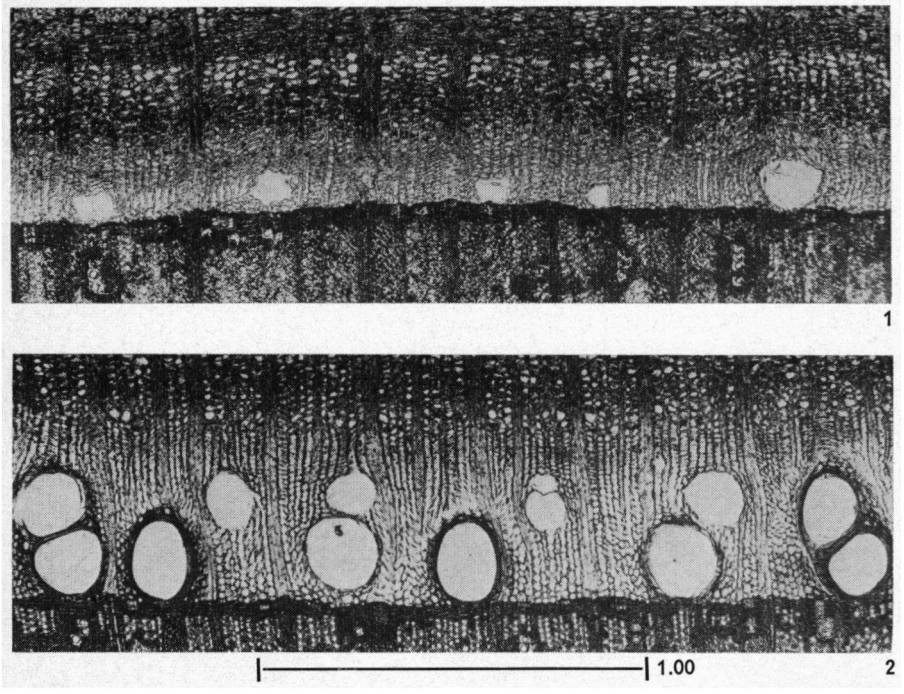

Figs. 1 and 2. Transverse sections through the cambium of *Fraxinus excelsior*.
1. Sample taken on May 4, 1970. 2. Sample taken on May 12, 1970.
The young vessel elements are roughly circular. The radial diameter is at least as large as the tangential diameter. Bar = 1.00 mm.

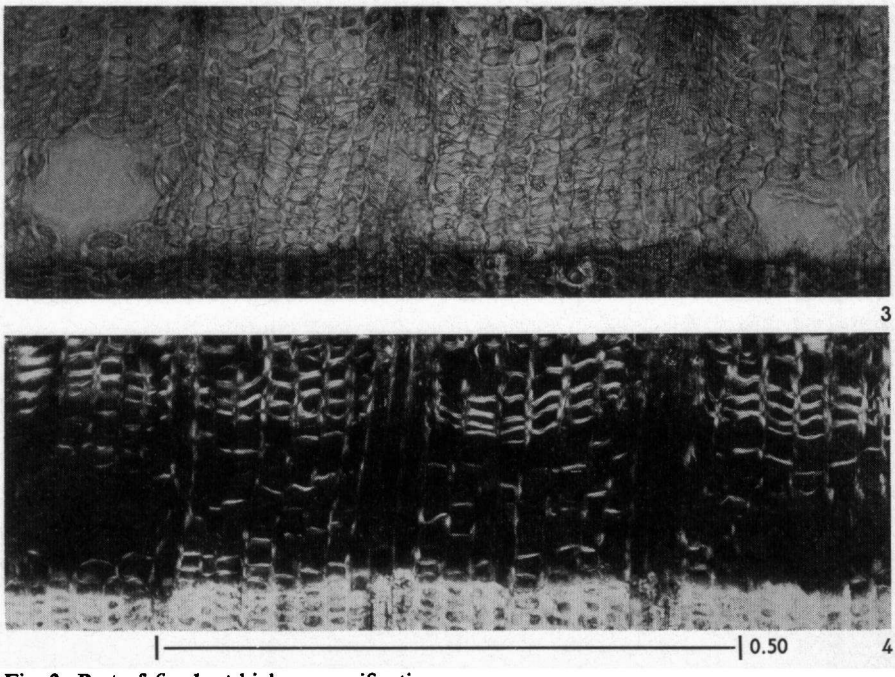

Fig. 3. Part of *fig. 1*, at higher magnification.
Fig. 4. As *fig. 3*, but photographed in polarized light.

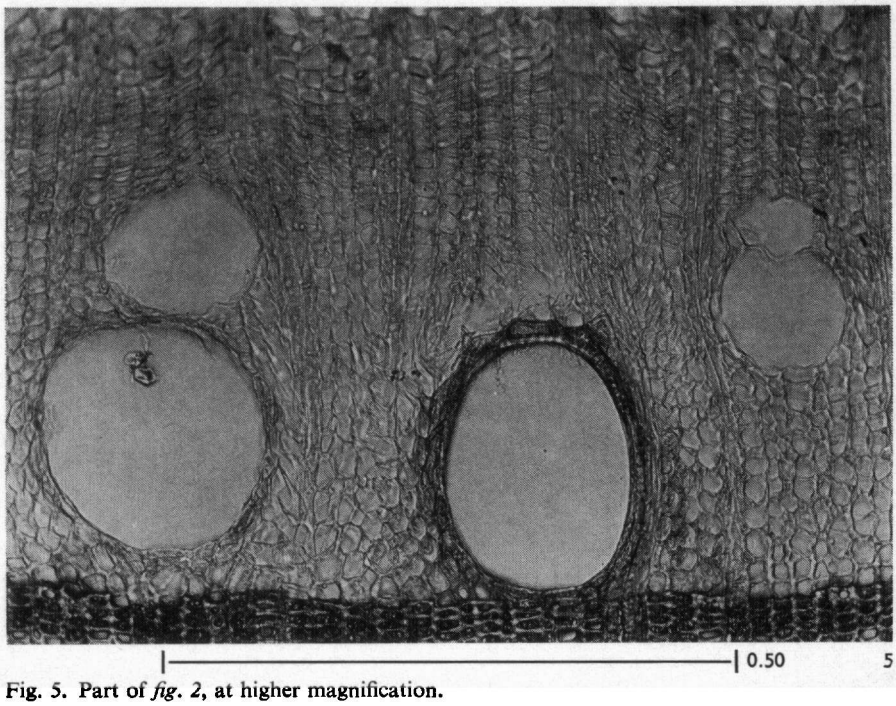

Fig. 5. Part of *fig. 2*, at higher magnification.

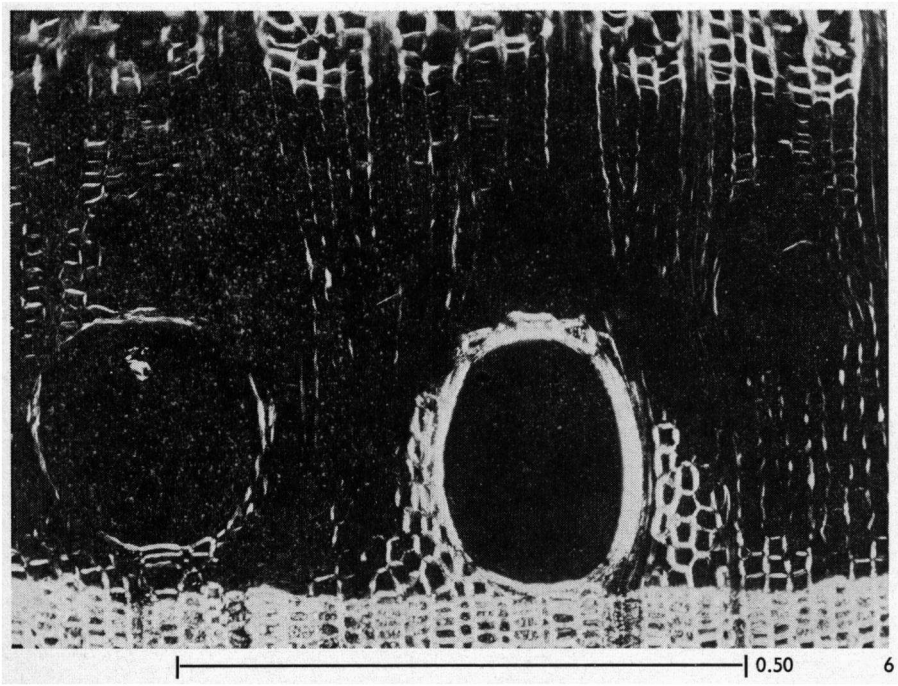

Fig. 6. As *fig. 5*, but photographed in polarized light.
Figs. 3 to 6. The walls of the enlarging vessel elements show less birefringence than those of the cells tangential to them.
Bar = 0.50 mm.

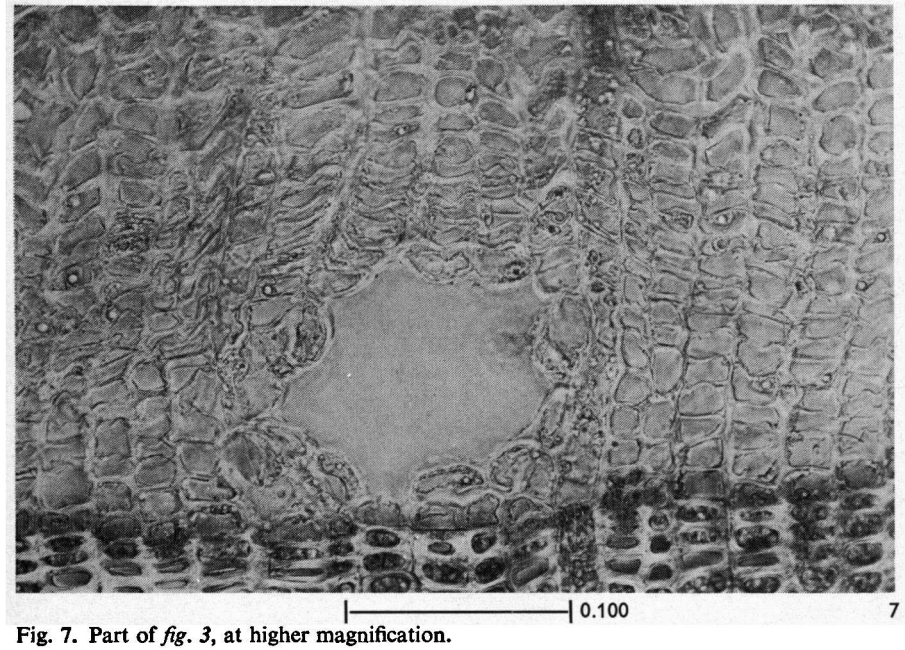

Fig. 7. Part of *fig. 3*, at higher magnification.

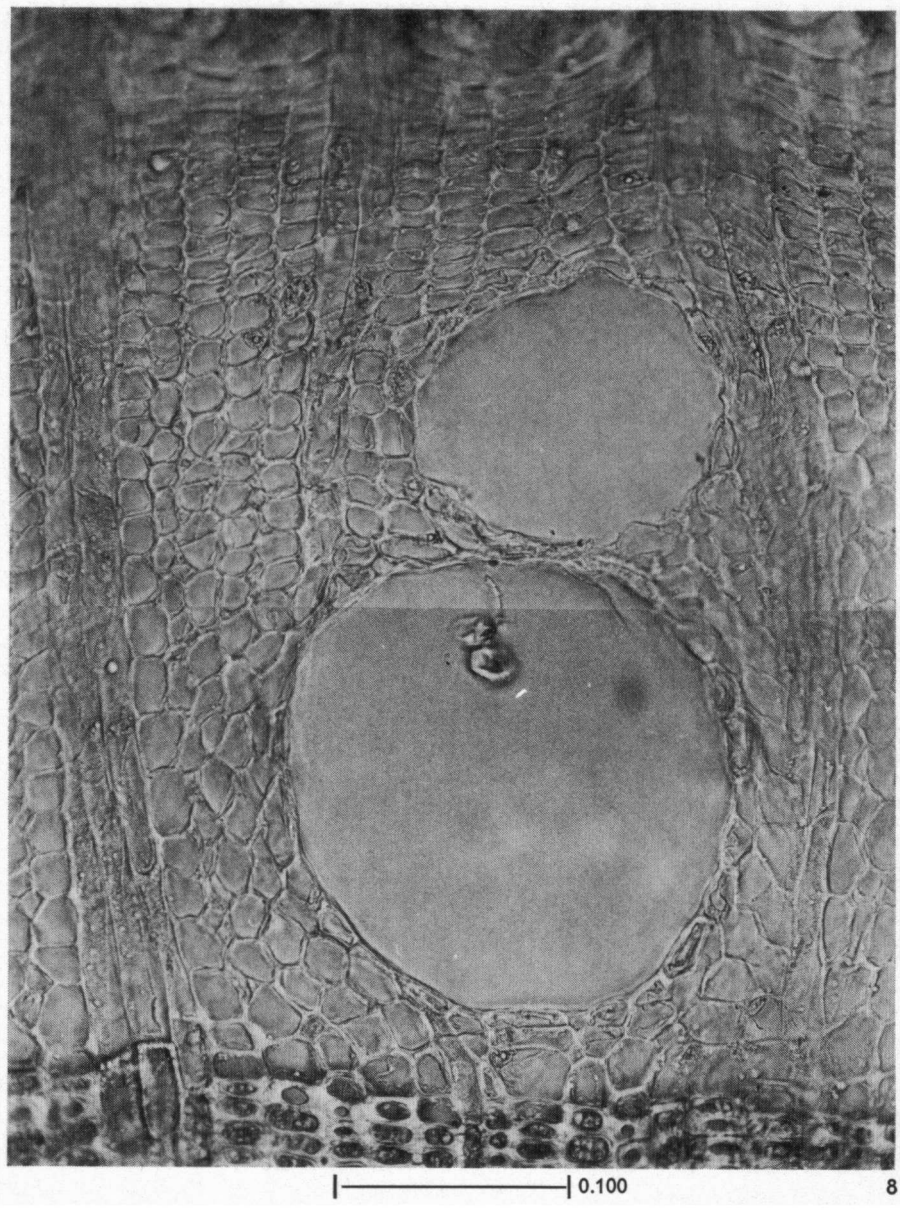

Fig. 8. Part of *fig. 5*, at higher magnification.
Figs. 7 and 8. The radial rows of cells contain enough elements tangential to the developing vessel members to insure unhampered radial expansion of the latter.
Bar = 0.100 mm.