Acta Bot. Neerl. 21 (1): 32-47. 1972

SOME OBSERVATIONS ON THE COURSE OF THE VESSELS IN THE WOOD OF FRAXINUS EXCELSIOR L.

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SUMMARY

The vessels in the secondary xylem of *Fraxinus excelsior*, while running through the wood in a longitudinal direction, show a tangentially and radially curved course. Vessels are in contact over widely differing distances. The result is a rather loose three-dimensional vascular network. The phenomena are discussed from an ontogenetical point of view, in relation to prevailing concepts of cambial activity.

1. INTRODUCTION

In the wood of *Populus*, BRAUN (1959) described a three-dimensional network of vessels. These vessels show a course curved in tangential and radial directions, making contact with other vessels in the same annual ring and also with vessels in adjacent annual rings. The network therefore includes not only the complete annual ring but also extends beyond its boundaries.

To determine whether ring-porous wood, which differs anatomically and ontogenetically from the diffuse-porous wood of *Populus*, also contains such a vascular network, CHRISTINA W. VAN DER VOET (internal report 1963) made a preliminary investigation of the wood of *Fraxinus excelsior*.

In 1963 BRAUN reported the occurrence of a vascular network in other species, including *Fraxinus excelsior*. Being particularly concerned with problems pertaining to the water transport in the wood, this publication gives few anatomical data on this network. In order to obtain more detailed information the investigations of VAN DER VOET were extended.

2. MATERIAL AND METHODS

From a five-year-old, vertically growing branch of *Fraxinus excelsior* (Botanic Garden, Leiden) samples were taken in October of 1963 and fixed in formalin-acetic acid-alcohol (JOHANSEN 1940).

Several not entirely successful efforts were made to clear samples of the wood, the most satisfactory agent being benzylalcohol ($n_d = 1.54$). To contrast the vessels, air and India ink gave the least unsatisfactory results. The method finally chosen for most of the work in this investigation was to project the microscopical image of 100 μm serial transverse sections for tracing on translucent paper. For the orientation of the drawings in the three-dimensional reconstruction of the course of the vessels, a few holes were drilled through the specimen as exactly as possible in the longitudinal direction.

The following descriptions are based on observations made in a fragment measuring 10 mm longitudinally, 2 mm tangentially, and 1.5 mm radially, from a larger serially sectioned sample (*Plate I A, fig. 1*).

In this sample the apical direction was not recorded. This is probably irrelevant, because the anatomical structure of the wood is unpolarized and no difference could be found between this sample and others in which the apical direction was known.

3. GENERAL STRUCTURE OF THE WOOD

For the sake of completeness, a short prefatory description of the wood structure is given. The terminology is based on METCALFE & CHALK (1950).

The wood consists mainly of fibres (libriform) with a length of 0.25 to 0.70 mm (average 0.45 mm). No tracheids were found, in agreement with GREGUSS (1947). The spring wood shows wide vessels with a radial diameter of 80 to 170 μm. The summer wood contains small to extremely small vessels, 10 to 70 μm in radial diameter. The length of the vessel members in spring wood and

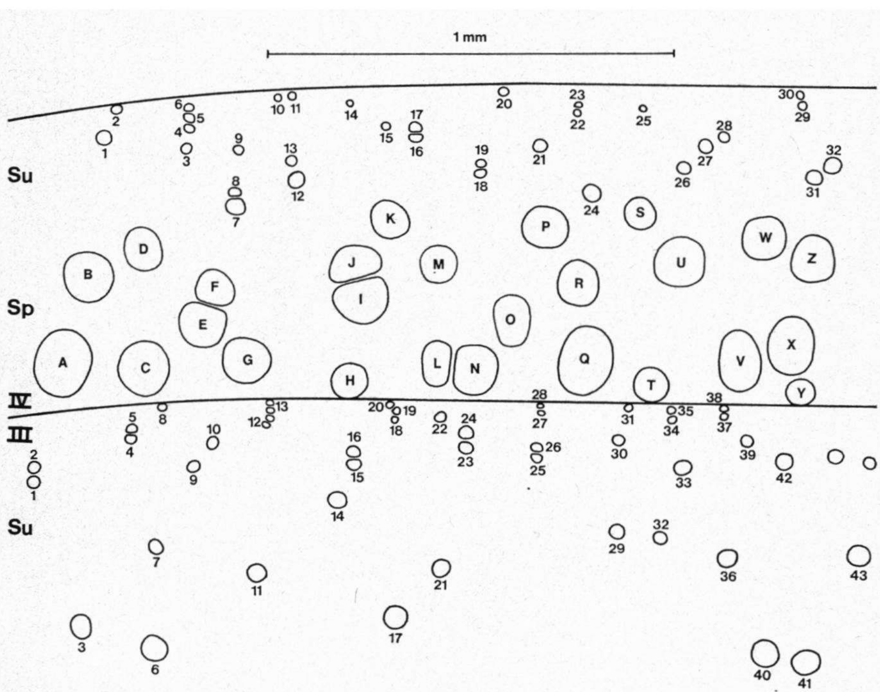
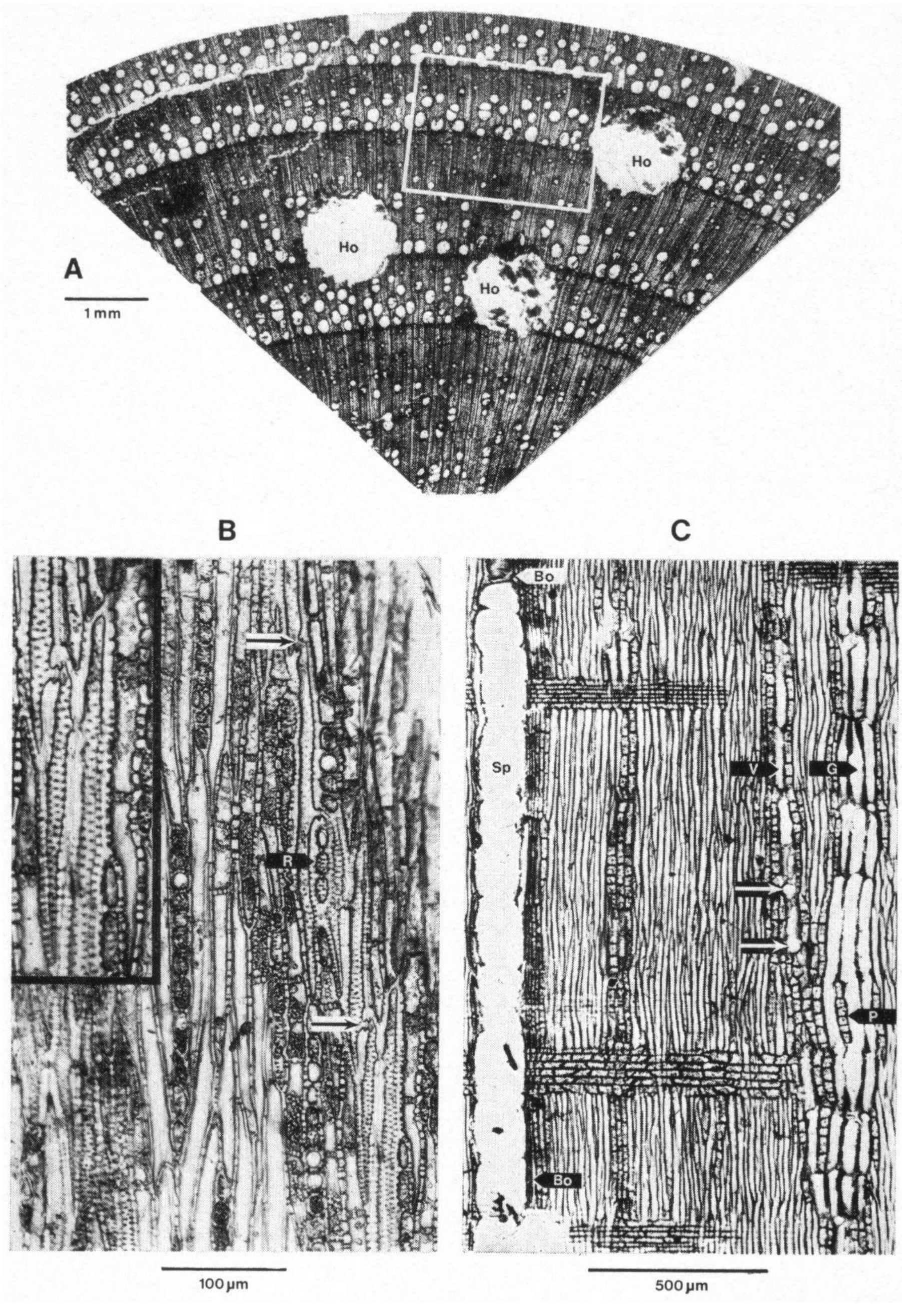


Fig. 1. Chart of the vessels in the rectangle indicated in Plate I A. III, IV: third and fourth annual rings. Su: summer wood; vessels indicated by numbers. Sp: spring wood; vessels indicated by symbols.


A
1 mm
Ho
Ho
Ho
B
R
100 µm
C
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Sp
V
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500 µm

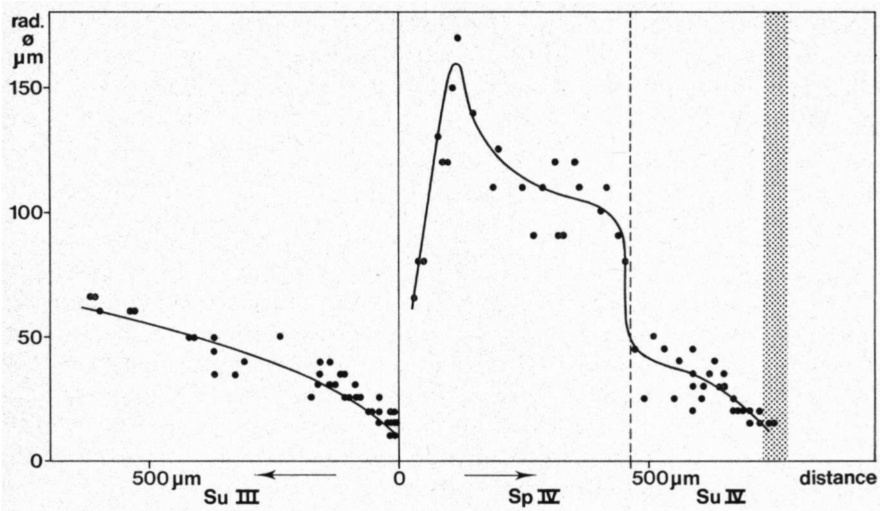

Fig. 2. Relation between radial diameter of vessels and location in the third (III) or fourth (IV) annual ring, expressed as the distance from the centre of the vessel to the boundary III/IV (solid vertical line). Due to slight variations in annual ring width the boundary IV/V is indicated as a stippled area. Su: summer wood; Sp: spring wood; dashed vertical line: transition Sp/Su.

summer wood is 0.15 to 0.30 mm (average 0.25 mm). The perforations are always unmistakably simple in all vessels. As is especially clear in the very small summer-wood vessels, they are situated mainly on the radial walls, at various distances from the tips of the elements (*Plate I B* and *C*).

Usually the vessels are almost or completely surrounded by parenchyma cells. In addition to this paratracheal wood parenchyma there is also diffuse wood parenchyma, and a not quite continuous layer of terminal wood parenchyma is present at the annual-ring boundary.

The size of the rays varies widely: 1 to 3 cells wide and 20 to 800 µm high. These rays are mainly composed of procumbent parenchyma cells.

The ring-porous character of the wood, which is clearly visible in *Plate I A* and in *fig. 1*, is also apparent from *fig. 2*. In this graph the radial diameter of the

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Plate 1

A. First transverse section of the described series. The investigated fragment is outlined. Ho: bore holes.

B. Tangential section near the cambial zone. A very small summer-wood vessel shows perforations on the radial walls (arrows). A ray (R) can be seen lying between blind ends of members of the same vessel. Insert shows a part of this vessel at higher magnification.

C. Radial bifurcation in radial section. Arrows indicate perforations in the radial walls. The indicated vessel (V) leaves vessel group (G) at an angle carrying it radially outward across 7 elements within a short distance. Parenchyma cells (P) cause radial interruption of contact between vessels of the group. Su: summer wood; Sp: spring-wood vessel; Bo: annual-ring boundary.

vessels is plotted against the site in the annual ring. As in *Plate I A* and *fig. 1*, the sudden transition from spring wood to summer wood is conspicuous in this graph. Investigation of other specimens showed that this transition is much less abrupt in wider annual rings.

4. THE COURSE OF THE VESSELS

A three-dimensional reconstruction of the vessels charted in *fig. 1* shows that the course over 10 mm in the wood is highly irregular. A two-dimensional representation of this course is given in *fig. 3*, which was made by connecting the centres of the consecutive section-projections of each vessel. From this diagram it can be seen that many vessels take a very oblique path through the wood, whereas others run almost vertically. Still other vessels show a bent or tortuous course. The shift in the tangential direction is conspicuously greater than the shift in the radial direction, which is also distinctly present. It is also striking that the great majority of the vessels slant in the same tangential direction: in *fig. 3* to the right. Few vessels show a slope in the opposite direction over any considerable distance. Other specimens indicated that this drift in a specific direction is not a general feature but might be an artefact caused by small errors in the determination of the longitudinal direction.

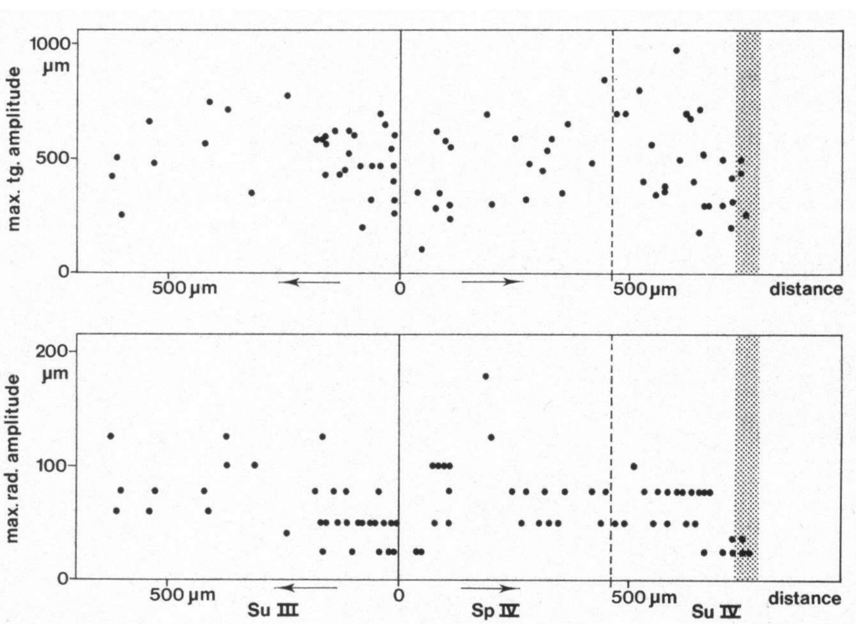


Fig. 4. Maximum amplitude of each vessel in the tangential (A) and radial (B) directions in a 10 mm length of wood, in relation to the site in the annual ring. Su: summer wood; Sp: spring wood; solid vertical line: boundary III/IV; stippled zone: boundary IV/V; dashed vertical line: transition Sp/Su.

In *fig. 4* the maximum tangential (A) and radial (B) "amplitudes" are given for each vessel in µm. Under amplitude is understood the width between the positions of a given vessel at different levels, measured in the tangential or radial directions. There is no obvious relationship between the maximum amplitude and the location of the vessels in the annual ring, but there are suggestions of a tendency for the very large and the very small vessels near the boundary to show a smaller radial amplitude.

Table 1. Radial distance, expressed as the number of cells in one radial file between two vessels or between a vessel and the annual-ring boundary III/IV. Numbers in parentheses pertain to vessels not in the same radial file (cells counted in the radial direction between the outer tangent of one vessel and the inner tangent of the other). Bo: boundary. Numbering of vessels as in fig. 1.

Level of section	Summer wood III vessel number									Spring wood IV vessel number		
	22-Bo	26-Bo	22-26	33-Bo	33-32	11-7	21-29	16-Bo	10-Bo	C-Bo	H-Bo	O-Bo
0	4	10	(5)	10	10			10		1	0	4
0.3	3	10	(6)	10	10			9			0	4
0.6	3	9	(5)	11				10		1	0	5
0.9	3	9	(5)	13				12			0	5
1.2	3	9–12	(5–7)	13				11		1	1	4
1.5	4	12	(7)	15	7			9		1	0	4
1.8	3	11	(7)	13	6			10	9	0	0	5
2.1	4	9	(4)	13	7			11	8		0	4
2.4	4	12	(7)	12	9			10	9		0	3
2.7	4	11	(6)					12			0	1
3.0	4	10	(5)	12	8			9	8			1
3.3	3	10	(6)		8			9	8			1
3.6	3	10	(6)		7		(6)	10	9		0	1
3.9	4	9	(4)					9	8		0	1
4.2	4	11	(6)		7		(7)	8	7			1
4.5	4	12	(7)		6			8	7			1
4.8	5	9	(3)		8	7	(9)	8	9	1	0	1
5.1	5	9	2	13	5	6	(10)	10	8			1
5.4	8	9	0	14	1	7	(8)	8	8	2	0	1
5.7	9	10	0	18	2	6	(8)	9–12	9	2		2
6.0	7	8	0	16	2	4	(6)	9	8	3		2
6.3	7	8	0	18	0	1	(4)					1
6.6	8	9	0		1	2	(2)	11	8	2	1	2
6.9	7	8	0			3	2	9	8	3	0	2
7.2	8	10	1			3	0	8	8	4		3
7.5	6	9	2			1	0	9	8		1	3
7.8	5	8	2			0	0	8	8			3
8.1	6	9	2			0	0	10	8		1	2
8.4	5	9	2			0				5	1	3
8.7	4	9								4	0	4
9.0	4	8								4		6
9.3	3	8								3		6
max. rad. amplitude	6	4	7	8	10	7	10	4	2	5	1	4

For some of the vessels the maximum radial amplitude is expressed as the maximum difference of the number of elements interposed in one radial file of cells between two vessels or between a vessel and the boundary between the third and fourth annual rings (denoted as III/IV), at various levels in the sample (see *table 1*). Numbers in parentheses indicate that the vessels concerned are, at that particular level, not situated in the same radial file as at other levels. In these cases the elements were counted in a radial file between the outer tangent of one vessel and the inner tangent of the other. This table indicates that although some vessels show almost no radial shift, others cover relatively large radial distances: 8 elements up to the boundary or 10 elements between two vessels.

The course of the vessels in part of the summer wood of the third annual ring is reconstructed in a block diagram (*fig. 5*). The vessels, much simplified, are drawn as smooth pipes, although especially the smallest summer-wood vessels hardly approximate this shape. The perforations are frequently situated at a considerable distance from the tips of the vessel members, which then extend far beyond these pores (*Plate I B*). Other elements, e.g. rays, are often found between these blind ends. Furthermore, at one transverse level several members of the same vessel can be seen (*Plate I B*). The resulting irregular shape of these small vessels greatly complicates the reconstruction of their actual course.

The irregularity of the course of the vessels is also shown by the block diagram. In addition the vessels touch tangentially or radially for more or less long stretches. The contiguous walls are always densely set with bordered pits, but no perforations permitting open contact between two vessels were observed.

The contacts between the vessels are schematically represented in *fig. 6* for the summer wood (A) of the third annual ring and for the spring wood (B) and the summer wood (C) of the fourth annual ring. These contacts appear to vary widely in length. Interruption of contact for a rather short distance occurs often. Rays are frequently present in these cases, causing the vessels to separate in a tangential direction, or one parenchyma cell (in rare cases two) separates the vessels radially (see also *Plate I C*). Interruptions of contacts showing only fibres, without parenchyma cells or rays, also occur.

Establishment or disruption of the contacts, frequently "initiated" by the presence of parenchyma cells or rays between the vessels, is especially abundant in the summer wood. "Duplications" (two adjacent vessels are seen where the preceding section showed only one) and the opposite, i.e. "unifications" (two vessels seen in one section are reduced to one in the next section), are also encountered mainly in the summer wood. Here again, no open contacts were found.

In the investigated sample only one case was observed of a vessel which ended (or started) in a group of parenchyma cells, out of touch with any other vessel (nr. 23, summer wood III). Subsequent investigations showed that this was not an incidental occurrence, contrary to the reports of ZIMMERMANN &

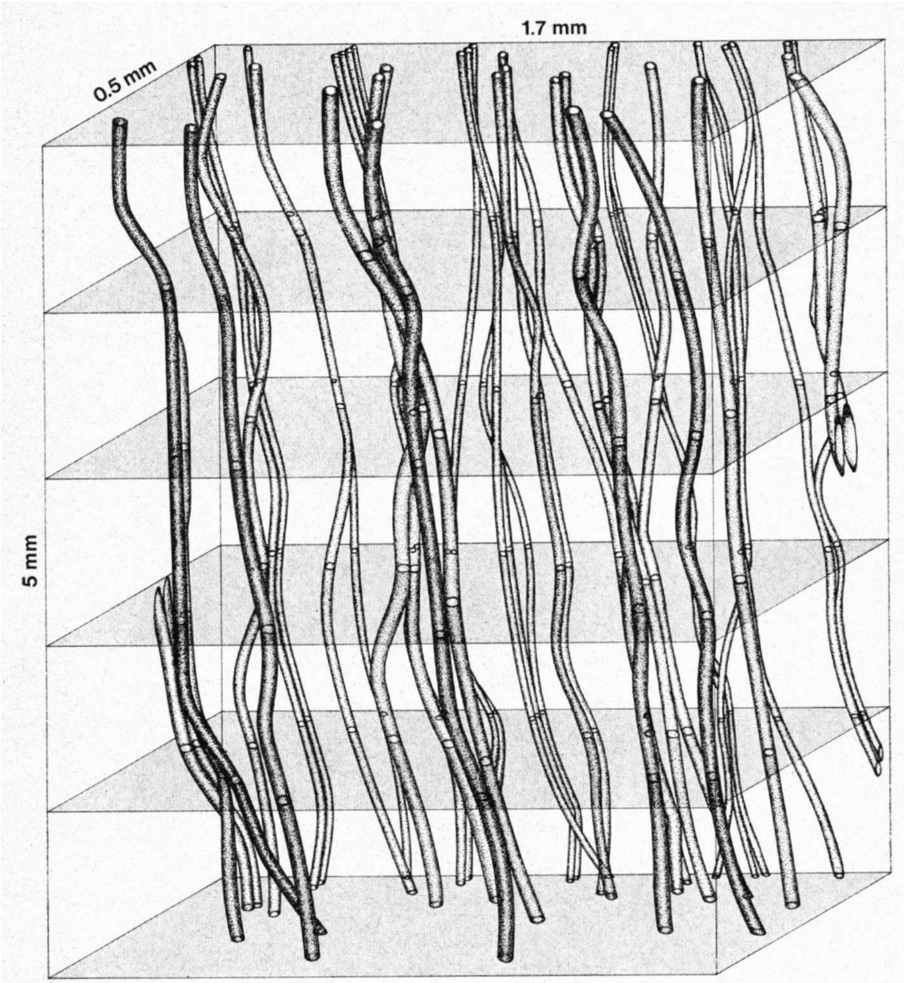


Fig. 5. Block diagram of the course of the vessels in part of the summer wood of the third annual ring. 1-mm levels, with vessel intersections, are shaded. The back face of the block is the annual-ring boundary III/IV. (Note that different scales are used in the three directions.)

TOMLINSON (1967) and of BRAUN (1970).

The contacts between the winding vessels crossing relatively large transverse distances provide a network for the water transport spanning the whole of the annual ring and extending beyond its boundaries. *Fig. 7* shows this transport network schematically and clearly indicates that the network is denser in the summer wood than in the spring wood. In this 10-mm length of wood the summer wood contains hardly any isolated vessels: in the third annual ring only 2 out of 36 investigated vessels (5%); in the fourth annual ring 1 out of 32

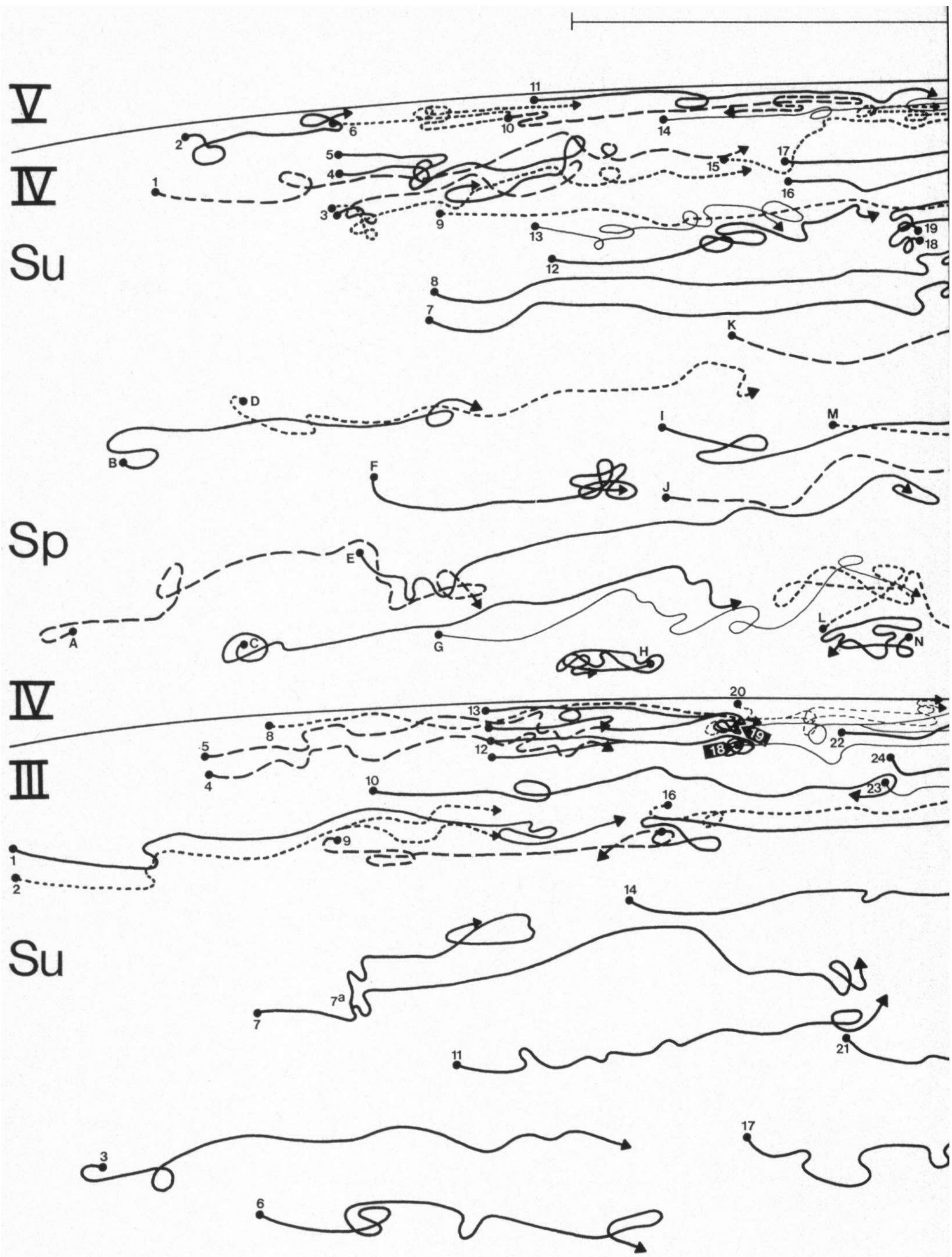
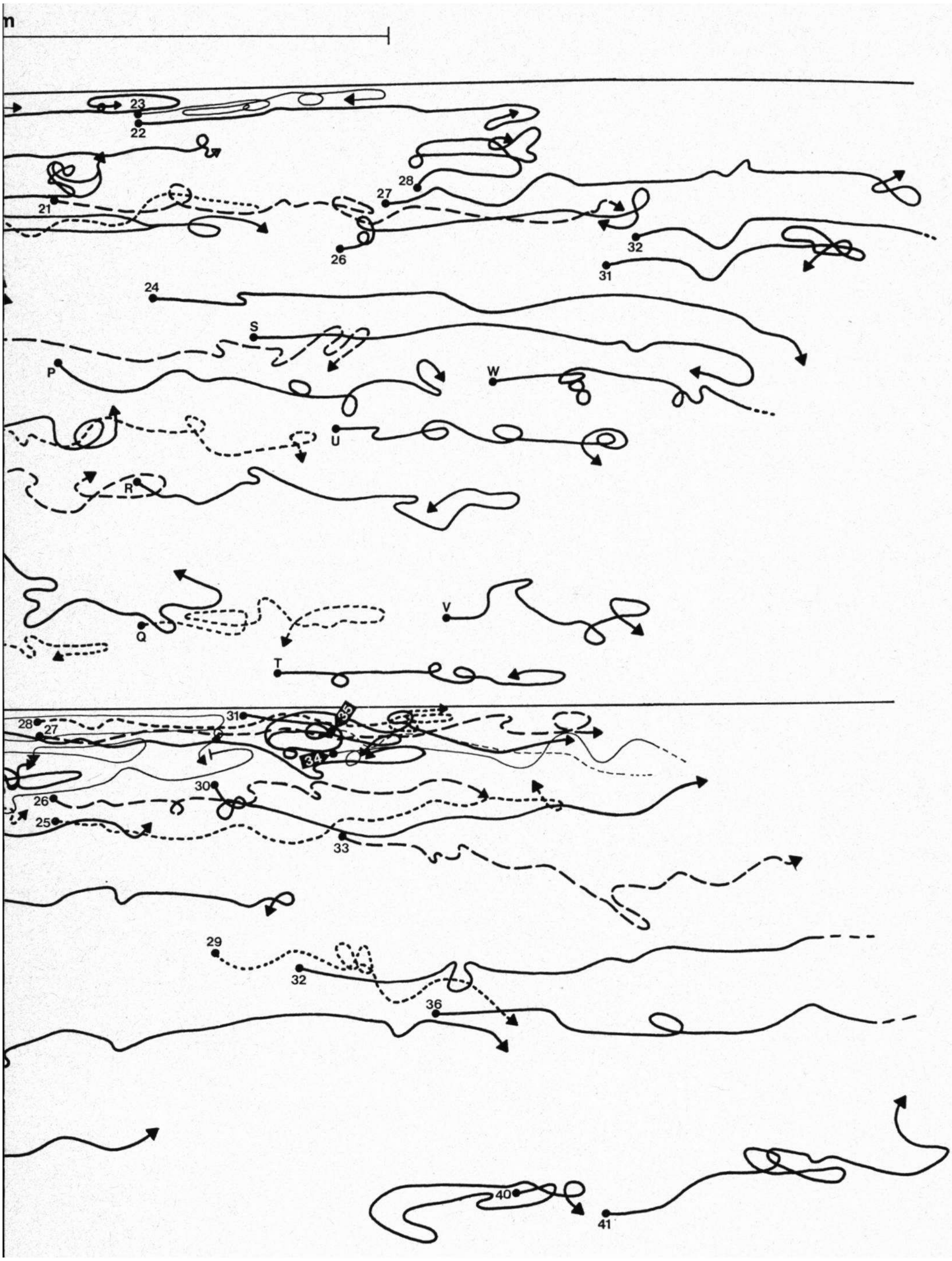
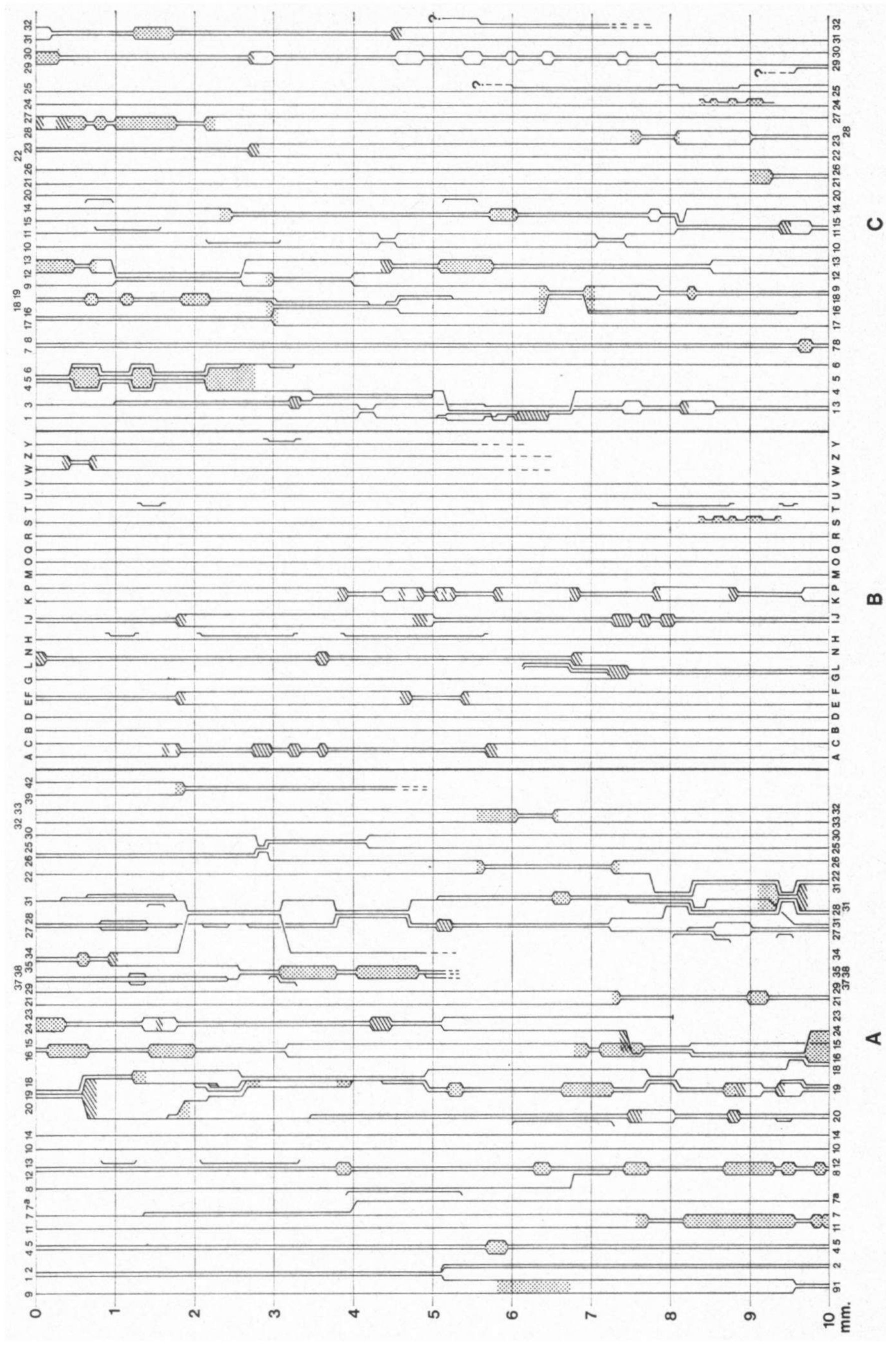


Fig. 3. Two-dimensional representation of the course of the vessels over a distance of 10 mm. For the sake of clarity, individual vessels are indicated by different kinds of lines. Su: summer wood; Sp: spring wood; III/IV, IV/V: boundaries between the third and fourth and between



the fourth and fifth annual rings, respectively. ●: position of the vessels in the first section; ◀ : position of the vessels in the last section. Numbering of the vessels corresponds with fig. 1.


A
B
C
mm.

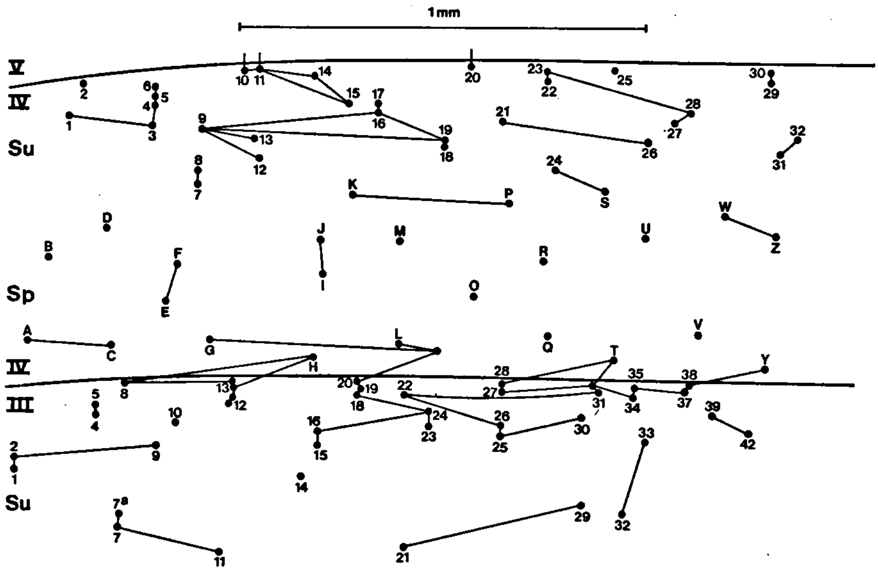


Fig. 7. Two-dimensional representation of the transport network in a 10-mm length of wood. Vessels with direct pit-contact at some level are connected by lines. Su: summer wood; Sp: spring wood; III/IV, IV/V: boundaries between the third and fourth and between the fourth and fifth annual rings, respectively. Numbering of vessels as in fig. 1.

vessels (3%). The spring wood shows many more single vessels: 8 out of 26 (30%).

Computation of the net density quotient for a 5-mm length, according to the method used by BRAUN (1959, 1963) (to the extent that this could be deduced from his descriptions) gives the following values:

	0–5 mm	5–10 mm	average
summer wood III	0.70	1.11	0.91
summer wood IV	1.11	0.87	0.99
spring wood IV	0.46	0.66	0.56

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Fig. 6. Schematic representation of contacts between vessels in a 10-mm length of wood. A: summer wood III; B: spring wood IV; C: summer wood IV. The vessels are represented by single lines. Numbering corresponds with fig. 1.

Y : establishment of contact; ⅄ : disruption of contact; [ray symbol] : ray separating two vessels; [parenchyma symbol] : parenchyma separating two vessels;)| : contact with vessel from another part of the wood; ¦ : course interrupted by bore hole; ⊥ : blind vessel ending among parenchyma cells; [duplication symbol] : duplication; [unification symbol] : unification; ?: untraceable part of the course.

In the calculations no repeating contacts, i.e. two or more contacts between the same vessels without intervening touching of another vessel, were taken into account, but contacts across an annual-ring boundary were included. These results agree with those given by BRAUN (1963) and also demonstrate that the vascular network in the spring wood is more loosely knit than that in the summer wood.

5. DISCUSSION

Research on the three-dimensional course of the vessels in the secondary xylem, on the contacts between these vessels in transverse directions, and on the resulting vascular plexus, has been done mainly in relation to problems concerning water transport in the wood (GNENTZSCH 1888; STRASBURGER 1891; BRAUN 1959, 1963, 1970; ZIMMERMANN & TOMLINSON 1967). But the existence of tangential and radial curvatures, more fully described from the results of the present investigation, also raises many important questions concerning the ontogeny of the vessels. These questions are, of course, closely related to the model of cambial activity and of differentiation of its products generally accepted in handbooks and textbooks.

The "prevailing concept" (ESAU 1965, p. 134) is that the cambium consists of initials, which "are arranged in one layer, one cell in thickness". "Adjacent cambium cells apparently divide at the same time, and the daughter cells belong to the same tissue. In this way the tangential continuity of the cambium is maintained" (EAMES & MACDANIELS 1951, p. 186). The daughter cells differentiate and "gradually assume the characteristics of the various xylem and phloem cells" (ESAU 1965, p. 134).

Im Höhepunkt der Vegetationszeit wird das Cambium vielschichtig, weil die Differenzierung mit der Zellneubildung nicht Schritt zu halten vermag (HUBER 1961, p. 92). This gives rise to a more or less broad zone of cells in which divisions occur, i.e. the cambial zone (NEWMAN 1956). Lacking a sharp boundary, this zone has on either side of it a differentiation zone, in the inside part of which "a gradient of increasing differentiation [can] be traced centripetally from the cambial zone" (WAREING & ROBERTS 1956, p. 358).

In the cambial zone the determination (BÜNNING 1953) takes place, deciding into which type of cell a certain element will differentiate (CASPERSON 1960). This is thought to happen during or immediately after the last cell division, which would then be a "differential division" (TORREY 1966, citing BÜNNING 1952; TORREY & FOSKET 1970).

With respect to the ontogeny of the vessels a vertical influence is very generally assumed. On the one hand this is based on the fact that in ring-porous as well as in diffuse-porous species the resumption of cambial activity in the spring progresses in a downward direction, starting from the buds or from the young shoots, respectively. The velocity of this progression differs widely between the two types of wood (PRIESTLEY *et al.* 1933; PRIESTLEY & SCOTT 1938; WAREING 1951; ESAU 1960; HUBER 1961; WILCOX 1962; ROMBERGER 1963).

On the other hand there is the assumption, made by most authors, that the differentiation of the vessels is initiated by an influence operating vertically from cell to cell. Elements already differentiating act upon other, still undetermined elements causing the same type of differentiation, the so-called homeogenetic induction (BÜNNING 1953, p. 222; JOST 1932; REHM 1937; LANG 1966; WAREING & PHILLIPS 1970; NOZERAN *et al.* 1971). *Und etwas Derartiges muss ja immer vorliegen wenn sich von einzelnen Zellen ausgehend Stränge bilden* (BÜNNING 1953, p. 224). This opinion was based largely on research in primary structures. There were thought to be vessel-forming substances (*Gefässbildende Stoffe*, BÜNNING 1953, p. 224), which, through action on a "file of cells, ready to differentiate" (PRIESTLEY & SCOTT 1936, p. 172), determine the future vessel. "The vessel-forming stimulus clearly moves downward" (SINNOTT 1960, p. 201).

The basipetal spread of cambial reactivation is much more rapid in ring-porous trees than in diffuse-porous ones and the stage of differentiation of the developing vessels in these ring-porous trees is essentially the same over long distances (PRIESTLEY *et al.* 1933, 1935).

In ring-porous species the cambial zone has been reported to be rather narrow, even in the growth season:

7–9 cells in *Fraxinus campestris* (HANSON & BRENKE 1926).
4–9 cells in *Robinia pseudacacia* (WAREING & ROBERTS 1956).
3–4 cells in *Fraxinus americana* (SRIVASTAVA 1966).
7–9 cells in *Ulmus americana* (EVERT & DESHPANDE 1970).

This number includes all undifferentiated elements of the xylem and the phloem as well as the cambial initials.

The development of a three-dimensional vascular network is hard to comprehend on the basis of the above-mentioned model. This model implies that, at least seen over short distances, the cambium is a flat plate of iso-active cells. At the inner face there is a zone of cells with a centripetal gradient of increasing differentiation. In ring-porous species the differentiation of the vessels occurs simultaneously over long distances, after a vertical determination-induction taking place in the narrow cambial zone. Particularly the existence of radially curved vessels whose radial amplitude amounts to 10 elements (see *Plate I C* and *table 1*) necessitates a reconsideration of the model.

Several aspects of this problem are being investigated. Results will be reported in due course.

ACKNOWLEDGEMENTS

The author is much indebted to Prof. W. K. H. Karstens for his stimulating interest and helpful criticism during this investigation. Thanks are also due to Messrs P. Hock and H. Verkijk for their skilled assistance in preparing drawings and photographs, and to Mrs I. Seeger for the correction of the English text.

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