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Terminal vessel and early vessel arrangement in internodes of *Fraxinus excelsior*

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Abstract

Application of auxin to the apical end and to the transverse incision in a dissected internode, led to the formation of early vessels in such a way that the vessels below the incision were aligned with the vessels above it. There was a structural relation between the early vessels and terminal vessels based on the fact that the former overlay the latter. A hypothesis is forwarded stating that a template according to which the early vessels find their positions, exists on the surface of terminal wood.

Key words: terminal vessel, early vessel, template

INTRODUCTION

It is well known that the differentiation of secondary vessels is induced and controlled by polar movement of auxin (Jacobs 1952). While studying the formation of vessels in dissected internodes of *Fraxinus*, it was noticed that if a transverse incision was made on the internode and auxin applied both to the apical end and to the incision, the new vessels below the incision were aligned with those above the incision (Fig. 1). This raised the question: were the new vessels determined before incising or were they formed according to some template? Investigation of the wood surface existing at the moment when auxin was applied revealed the presence of narrow vessels of the terminal wood. It was found that vessels originating under the influence of auxin lay above the terminal narrow vessels. The hypothesis was set forth, that the narrow vessels are the template for the differentiation of new vessels. The investigations presented in this paper were performed taking into account the above-mentioned hypothesis.

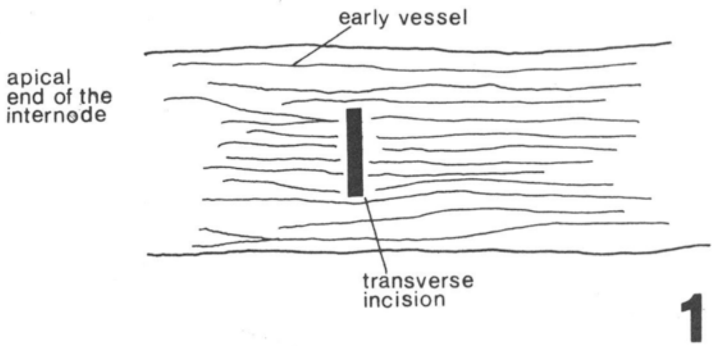


Fig. 1. A schematic diagram of the wood surface of a *Fraxinus* internode piece with a transverse incision after 14 days treatment with the auxin medium. The early vessels below the incision lie on the extention of the early vessel lines above the incision

MATERIAL AND METHODS

One to two year old sprout stems of *Fraxinus excelsior* were used in the experiment. The stems were cut into 10-15 cm long segments comprising one single internode without buds. Material was collected from January through March and in June.

INCUBATION OF INTERNODE SEGMENTS

An auxin paste composed of: 1 part of NAA solution (1×10^{-4} M), 1 part of GA_3 solution (1×10^{-5} M) and two parts of lanolin was used. The control paste was without NAA and gibberelin. The paste was applied to the apical end of the internode and to the incision. The transverse incision was 10 mm long and 4 mm wide. The paste was renewed every three days. The treatment extended over a 14 day period. During this time the internodes were in a vertical position in darkness at room temperature. The basal end of the internode was immersed in tap water which was renewed every day. The experiment was repeated 10 times with 10 internodes in each series.

EXPOSITION AND STAINING OF THE TERMINAL WOOD SURFACE

Twenty internodes of stem were investigated during the rest season. The bark tissue was removed by immersing the internode into concentrated HCL. The wood core was next immersed in an ethanol solution of phloroglucin and subsequently in HCl to stain the terminal vessels. Finally, it was examined under a stereomicroscope. The internodes which were incubated, were stained and examined in the same way.

PREPARATION OF SECTIONS FOR MICROSCOPIC INVESTIGATION

Pieces of tissue including cambium, phloem and late wood were fixed in 2-4 per cent glutaraldehyde buffered at 7.3 pH with 0.2 M phosphate buffer, dehydrated in an acetone series and propylene oxide and saturated with Epon. The material thus prepared was placed in gelatin capsules and after polymerization, cut with an ultramicrotome into semi-thin sections. The succesive sections were put on microscope slides and left to dry for two days after which they stained with leucofuchsin (PAS reaction) and toluidine blue.

RESULTS

THE CHARACTERISTICS OF THE TERMINAL WOOD

The vessels in the terminal layer of wood became visible after exposing the surface of the wood and staining it with phloroglucin (Fig. 2). The vessels were composed of narrow members. They were continuous, but usually were not seen on the surface along the entire length. This apparent discontinuity of the vessels when observed on the wood surface was caused by the fact that they penetrated into the wood leaving the surface. To examine the course of the vessels, the internodes were immersed by their basal ends in red ink for a few hours. Subsequently they were treated with HCl to remove the bark. Examination of the distribution of the ink tincture in the vessels proved the continuity of vessels. A vessel which in a certain position was on the surface, in another position, could penetrate the wood to a depth of 5 cells. In most instances, penetration was to the depth of 1 to 2 cells. Since the wood surface is rather smooth, the variable position of the vessels with respect to the surface means that it is not straight when projected on the radial surface. As can be seen, half of the length of the terminal vessels was on the surface of the terminal wood (Table 1). The projection of terminal vessels onto the surface of the annual ring showed that the vessels were not straight either. Sometimes

Table 1

The depth of vessel penetration under the surface, expressed in terms of cell numbers between the vessels and the boundary of the annual ring

Depth of penetration (number of cells)	0	1	2	3	4	5
The relative length (in %) of vessels at different depths	51	21	17	5	4	2

a vessels bifurcated. The branching was in the acropetal direction, basipetal branching being quite rare. On the periclinal walls of the vessels located on the wood surface, the former being in contact with cambium of an intact stem, there were unilateral bordered pits. The structure of these pits was the same as that of pits on the other walls. The tangential diameter of terminal vessels was 6 to 24 μm, the average 14.5 μm (Table 2) compared with the average tangential width of the other cells on the terminal wood surface. The perforations were simple. It seems that there were no unperforated walls across a file of terminal vessels as far as the internode length is

Table 2

The average diameter of terminal, early *in vitro* and early *in situ* vessels

Vessels	Terminal	Early *in vitro*	Early *in situ*
Average diameter, μm	14.5	38.6	83.7

considered. On the wood surface there were strands of terminal parenchyma along the terminal vessels and the rest of the surface was occupied by fibres. The terminal parenchyma contained starch which enabled easy identification by means of KJ. Their cells were lignified with secondary walls. There were simple pits on the outer tangential walls of these cells.

THE CAMBIUM IN RESTING SEASON

The cambial zone comprised 4 to 5 of the fusiform cells in the tangential file (Fig. 3). The radial walls of these cells were thick. On tangential sections they were characteristically indented due to the presence of thinner primary pit fields. The substance deposited in the radial walls during resting season was not hydrolysed by the action of HCl. Neither was it hydrolysed by pectinase. However, both HCl and pectinase hydrolysed the middle lamellas which resulted in maceration of the cambium. Owing to the thick radial walls, the fusiform cells in macerated state remained straight, which allowed easy determination of their length.

THE CAMBIUM AND NEWLY FORMED VESSELS AFTER 14 DAYS OF INCUBATION WITH AUXIN

A newly formed vessel (which have been formed during the incubation with auxin), called early, in on transection is shown on Fig. 4. The other cells of the newly formed wood were parenchymatous though those surrounding the early vessel were lignified. Typical fibres were never observed in the wood formed *in vitro*. The surface of wood after the rind tissues were removed is shown on Fig. 5. The early vessels began a few mm from the apical end and terminated a few mm before the basal end. The vessel ended basaly with a series of "tear" like structures, each composed of a few tracheary elements arranged in a tangential plane. These "tears" could be connected by bridges composed of single tracheary elements arranged in files running tangentially above the terminal vessel or were more or less obliquely arranged. An early vessel may have begun far from the apical end or may have blindly terminated far from the basal end of the segment. The early vessels bifurcated. Branching was in the acropetal direction, the basipetal direction was very rare. Typically, an early vessel overlaid a terminal vessel (Figs. 4 and 5). In some parts, however, such contact may have been lacking due to the fact that the terminal vessel and the early vessel were not on the surface of the annual ring. Cases in which the early vessels were not in contact with the terminal vessels could be classified into two groups:

a. A terminal vessel which, up to a certain level, was in contact with an early vessel penetrated the late wood. The early vessel either went over a neighbouring terminal vessel lying on the surface or did not change its direction so that it could come in contact with the same terminal vessel again.

b. A terminal vessel remained on the surface, yet the early vessel lost contact with it, changing its direction either in a radial or a tangential plane.

When the early and terminal vessels were in contact, they were connected by bordered pits. The chambers and external apertures of the early vessel pits had a larger diameter than those of the pairs on the terminal vessel side.

After 14 days of incubation with auxin, there were about 6 fusiform cells in a radial row in the cambial zone and about 4 cells in the newly formed wood (Fig. 4). At the site where an early vessel was located, the number of cells in the row to which the vessel belonged, as well as in the neighbouring rows, was lower. The higher tangential diameter of the vessel members than the tangential dimension of the cambial derivative from which it originated was accompanied by narrowing of this dimension in neighbouring rows. Whether this narrowing was caused by the compression of the neighbouring cells due to the pressure exerted by the developing member or by the shortening of the tangential dimension of these cells by releasing tangential tension through expansion of the developing vessel (Hejnowicz 1980) cannot be answered as yet. As already mentioned, during incubation with auxin, no differentiated fibres or no intrusive growth of fusiform cells have been observed. In control specimens incubated in the same conditions but without auxin, there was no differentiation at all.

THE TERMINAL VESSELS AND THE EARLY VESSELS IN THE STEM DEVELOPING *IN SITU* IN NORMAL CONDITIONS

The early vessels which were differentiated in normal conditions overlaid the terminal vessels (Fig. 6). They were characterized by a larger diameter (Table 2). The other wood cells were differentiated into fibres. Their length was greater than that of fusiform cells in the resting cambium, which means that intrusive growth occured.

DISCUSSION

The results of this study lead to the conclusion that exist the structural and developmental relations between early vessels and terminal vessels. Are the early vessels formed in compliance with a pattern existing on the surface of the terminal wood? The distribution of early vessels and terminal vessels is not a matter of chance as evidenced by the following calculation: the surface occupied by early vessels has been estimated as 10% of the whole annual ring surface, whereas the terminal vessels occupy not more than 1% of the total annual ring surface. The probability of chance connections of early vessels and terminal vessels is proportional to the product of these values and is approximately 0.001. In fact, more than 50% of early vessels are in contact with terminal vessels. Thus the question posed must be answered positively. It is inferred that the terminal vessels are a template for the early ones.

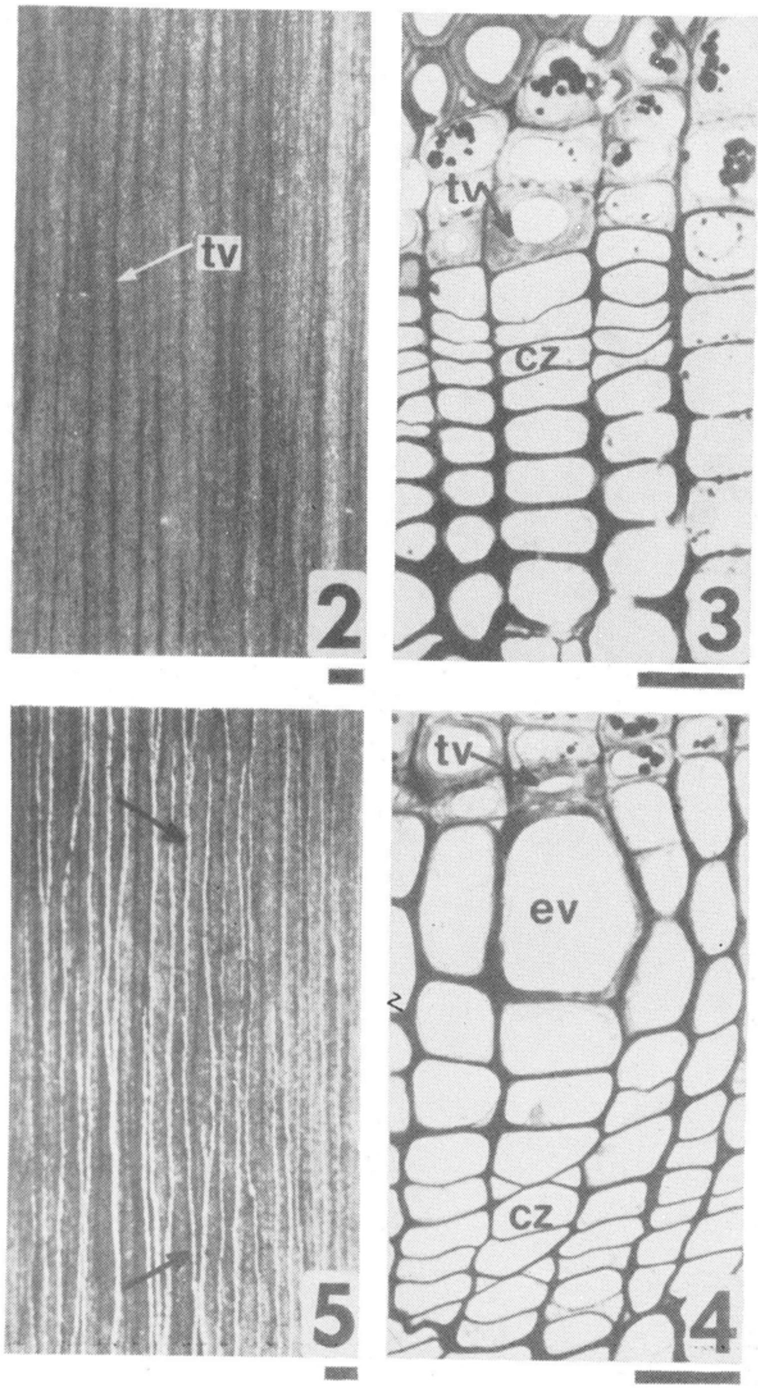
The overlaying of both kinds of vessels can be of functional importance. Namely, the vessels are connected by bordered pits and compose a bridge for the hydrosystem of adjacent ring. Braun (1962) studied the anatomical interdependence of different tissues in wood taking into special consideration the water flow inside the rings and between rings. He divided woody plants

Fig. 2. The vessels on the terminal layer of wood become visible after exposing the surface of the wood and staining it with phloroglucin. The dark lines represent the terminal vessels (tv)

Fig. 3. A transverse section through the sprout stem of *Fraxinus* in the cambium rest season. The narrow terminal vessel (tv) is in contact with the dormant cambial zone (cz)

Fig. 4. A transverse section through a *Fraxinus* internode piece after 14 days treatment with the auxin medium. The early vessel (ev) and the terminal vessel (tv) are in contact across the annual ring boundary (cz — cambial zone)

Fig. 5. The surface of wood treated with auxin medium after removing the rind tissues. The early vessels (white lines) usually overlie terminal vessels (dark lines — see the black arrows). Black sectors on photographs 3 and 4 denote 20 μm. Black sectors on photographs 2 and 5 denote 1 mm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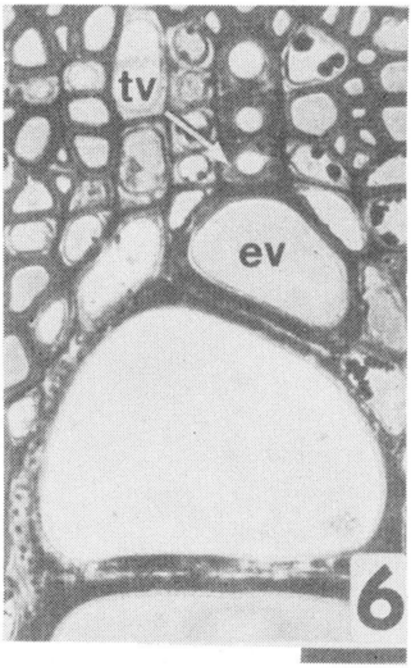

Fig. 6. A transverse section through a *Fraxinus* sprout stem. An early vessel (ev) and a terminal vessel (tv) are in contact across the annual ring boundary. Black sector denotes 20 μm

into two groups. The first group includes plants in which there are no vessels on the border of the annual ring on the side of late wood and the vascular network is limited to one annual ring. The second group includes plants in which vascular systems of individual rings communicate. Such communication is possible because the vessels of late wood occur on the border of the annual ring and there they join the early vessels. Braun et al. (1968) included *Fraxinus excelsior* in the group of plants belonging to the second group. In *Fraxinus*, the early vessels are surrounded by sheaths of paratracheal parenchyma with the exception of that side of the vessel which is in contact with the surface of the annual ring (Braun 1970, Braun and Wolkinger 1970). In these contact spots, the vessels are provided with bordered pits. Only vessels constitute the connection between the tracheary elements on both sides of the annual ring border, which agrees with the statement by Braun (1970). This means that on the side of the late wood, only terminal vessels touching the annual ring surface have bordered pits in the wall adjacent to the surface. Other tracheary elements contacting the surface do not have bordered pits on the walls contacting this surface. As explained by Braun (1970), terminal vessels reach the surface of the annual ring only in discrete parts of their course. The vessels follow an arcuate line in the radial plane deep in the ring. They run into the annual ring where they make connections with other vessels of late wood. This feature was shared by the investigated here vessels though they were inconspicuous as to the diameter. Individual bordered pits occur in terminal vessels on walls tangential to the surface of cambium. The second pit of the pair will appear in the wall of an early vessel in the next vegetative season. This brings up the question whether the bordered pits of terminal vessels situated on the walls coming into contact with early vessels have a different structure than pits situated apart from the contact spot. If such an difference exists, it would mean that the terminal vessel had been informed about what it would contact the next year. No such specialization has been discovered. It has been noticed, however, that the template is more "active" if terminal vessels are surrounded by terminal parenchyma cells with more starch. It is possible then, that the presence of terminal parenchyma favours the overlying arrangement of vessels.

Now the question arises: is the arrangement of vessels present also in other trees? Braun's (1970) anatomical description points to such an arrangement also in *Acer pseudoplatanus* and *Populus* sp. In *Acer pseudoplatanus* and *Populus* sp. the terminal vessels of late wood come into contact with early vessels through bordered pits. It seems however, that not only terminal vessels can be the template. For example, the connection between succeeding annual rings of *Quercus* (Braun 1970) is made through the fiber tracheid. The water flow from one annual ring to the early vessels of the next ring runs through the tracheid. In this case the arrangement of fiber tracheids on the border of the annual ring may play the part of the template. It is possible that the hypothesis presented herein according to which there is a template for early vessel differentiation has a general meaning. This would be also reasonable from the point of view of connections between hydrosystems of adjacent annual rings.

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Rozmieszczenie naczyń terminalnych i wczesnych w międzywęźlach Fraxinus excelsior

Streszczenie

Odcinki międzywęźli *Fraxinus* pobrane w okresie spoczynku zimowego nacinano poprzecznie w środkowej części międzywęźla. Następnie podawano auksynę na aplikalny koniec i na nacięcie. Po około dwóch tygodniach obserwowano położenie naczyń wczesnych, powstałych pod wpływem egzogennej auksyny. Okazało się, że naczynia wczesne pod nacięciem leżą na przedłużeniu naczyń znad nacięcia. Zwrócono uwagę na to, że istnieje powiązanie strukturalne między naczyniami wczesnymi i terminalnymi. Wysunięto hipotezę, że na powierzchni drewna terminalnego istnieje wzorzec według którego naczynia wczesne znajdują swoje właściwe położenie. Wzorcem tym jest wzór naczyń terminalnych na powierzchni przyrostu rocznego. Funkcjonowanie takiego wzorca na powierzchni przyrostu rocznego wydaje się być sensowne z punktu widzenia ruchu wody między słojami przyrostów rocznych.