Estimation of directional division frequencies in vascular cambium and in marginal meristematic cells of plants

P. W. Barlow, P. Brain and S. J. Powers

IACR–Long Ashton, Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol,
Long Ashton, Bristol, UK

Received 8 May 2001; revision accepted 21 August 2001

Abstract. Using simple arithmetical formulae, it is shown that, when the meristematic initial cells of a growing plant organ are arranged in a ring, the cellular dimensions predict the relative frequencies of anticlinal and periclinal divisions which these cells undergo. The pattern of cell file branching which appears during the course of development, and which is predicted by this mathematical model, is validated using data pertaining to the numbers and dimensions of initial cells within the secondary vascular cambium of hybrid aspen trees. Data pertaining to a second, simpler set of initial cells which comprises the outer cellular ring of the thallus of the alga *Coleochaete orbicularis*, and from which all the radial cell files of the circular disc-like thallus are descended, have also been used for model validation. Combining the mathematical approach to division frequencies with data of actual cell sizes permits inferences about the course of the increase of the number of cell files (generated by the anticlinal divisions) and the number of cells within each file (generated by the periclinal divisions) during the earlier stages of secondary tissue or thallus development, and also about how they will develop at future stages. The question whether or not cell division patterns conform to the geometry of the system in which the cells are embedded is also discussed.

INTRODUCTION

The interior woody portion of the trunks and branches of trees is ensheathed within a meristematic vascular cambium which consists of elongated, fusiform cells and smaller, cuboid ray cells. Regardless of whether the fusiform cells of the cambium are arranged around the cambial circumference (or cambial surface) in storied or non-storied layers (Bailey 1923; Iqbal & Ghouse 1990), their descendants are arranged in radial files. Many workers now accept the concept proposed by Sanio (1873) that each cambial cell file consists of an initial cell and two sets of dividing cells which are derived from this initial (e.g. Mahmood 1968; Timell 1980). One set of derivative cells contributes to the phloem tissue, which includes sugar-transporting sieve elements, and the other set contributes cells to the xylem, which includes woody fibres and

Figure 1. Scheme to show planes of cell division and directions of cell production. A cubiform autoreproductive *a* cell (outlined in unbroken lines) is divided at cytokinesis by a division wall (broken lines), the plane of which may be either anticlinal (pseudotransverse) (*a*) or periclinal (*p*). The respective productions, according to the position of whichever of the two daughter cells remains in the *a* state, are said to be directed either east (E) or west (W), or north (N) or south (S). In the case of a vascular cambial initial cell, productions in the N or S direction results in the non-*a* daughter cell differentiating as phloem or xylem cells, respectively, whereas daughters produced in either the E or the W direction retain the autoreproductive *a* state. Occasionally, there are transversal divisions (*t*) which result in upwards (U)- or downwards (D)-directed cell productions. These, however, are not considered in the present paper and hence are not marked.

water-conducting vessel elements. In cross-sections of woody stems, the initials of each radial file are seen to be connected laterally to form a ring of initials which encircles the organ. This ring is considered here to define the perimeter of that portion of the cambium which contributes to the secondary xylem.

According to a recently proposed scheme of organogenesis which involves 'directed' cell divisions (Barlow, Lück & Lück 2001), the cambial initials can be regarded as producing cells in two principal directions, north (N) and south (S), as a result of their periclinal divisions. (In a periclinal division, the new division wall is orientated parallel to the cambial perimeter.) The initials are autoreproductive stem cells (Barlow 1997; Barlow *et al.* 2001) and are designated as *a* cells. Following a periclinal division of an *a* cell, one of the two daughter cells continues to hold the *a* state and hence continues to behave as an initial cell. The other daughter cell, *p*, assumes an alternative state depending on the direction of its production. If produced from the *a* cell in the N direction, the daughter becomes a phloem mother cell, p_N ; if produced in the S direction, the daughter becomes a xylem mother cell, p_S (Fig. 1). Descendants of p_N and p_S complete a variable number of periclinal divisions within the cambial zone and are destined eventually to differentiate. An *a* cell and all its descendants in both the N and S directions may comprise one single radial file of cells. The cambial layer and the secondary vascular tissues which develop from it are comprised of many such files. When productions from the *a* cells are in the N direction, rows of descendant cells are 'pushed out' radially in front of them, towards the exterior of the tissue (i.e. they are centrifugal productions). However, when *a*-cell productions are in the S direction, cells are produced towards the interior of the tissue (centripetal productions). In this last-mentioned case, the initials and the p_S cells leave behind them a file of cellular descendants whose growth and development as woody xylem tissue increase the radius of the stem. Accordingly, the initials are moved outwards, away from the centre of the stem. By contrast, productions from the *a* cell which are in the N direction do not lead to the relocation of the *a*-cell initials, nor is this brought about as a result of the division and growth of the p_N cells and their descendants.

It is a geometrical necessity that, as the radius of a circle increases, the circumference increases also. The implications of this necessarily anisotropic growth for cell production by the cambium have been considered by Lück *et al.* (1970) and Iqbal (1994). Cambial initial cells

which, by their growth and periclinal divisions, produce cells centripetally, in the S direction, accommodate the increase in the radius of the tissue and hence their own centrifugal displacement, by tangential cell growth and anticlinal cell division. [In so-called 'non-storied' cambia, the new division wall of a longitudinal anticlinal cambial cell division is orientated obliquely, or pseudotransversely. By contrast, the anticlinal divisions in 'storied' cambia are orientated longitudinally, parallel to the axis of the stem (Bailey 1923; Romberger *et al.* 1993).] Anticlinal division of an initial cell results in the production of a daughter in either an east (E) or west (W) direction (Fig. 1). However, such cell productions add new cells to the initial zone only. Because both daughters of such an anticlinal division retain the *a* state of their mother cell they are able to initiate new radial files of cells when cell production again commences in the N or S direction. Numerically, one new cell file is gained following the anticlinal division of a cambial initial cell, and the radial file of cells with which this initial is associated branches accordingly, each branch tracing back to one of the two new sister initials.

The first objective of the present paper is to solve what is basically a simple geometrical problem related to the cell biology of cambium. Given that cambial initial cells are distributed in a cylindrical ring which encases the xylem tissue, and that the cells grow and divide in both the periclinal and anticlinal planes, the question is: How many new cambial initial cells should be produced in the E/W direction to accommodate the cambial cell productions in the S direction as well as the accompanying increases in xylem tissue radius and cambial circumference? This is, in fact, an old problem, having first been tackled in a general way over 130 years ago by Nägeli (1864) in a study of rates of xylem production in trees of *Paullinia* and *Serjania* spp. Both Nägeli (1864) and some others who have more recently considered this problem (e.g. Bannan 1962, 1968; Hejnowicz & Brański 1966) have tended to work in terms of increments of radial length in relation to the frequency of branching of the cambial cell files. However, we prefer to work in terms of cell productions because this gives a closer insight into the proliferation of the cambial initials and, possibly, also into the inherent regulation of this process. An important but generally unstated aspect of the solution of the problem is the requirement of maintaining a constant mean size of the cambial initial cells.

A second objective is to establish whether the frequencies of anticlinal and periclinal divisions in the initial zone can be predicted from data of cellular sizes and distributions provided by photomicrographs of stem cross-sections. If so, this approach might provide a convenient way of investigating cell dynamics in this rather intractable secondary tissue. Estimation of the respective division frequencies requires knowledge of the aspect ratio [the ratio of cellular dimensions in two directions of growth which are orthogonal to each other, as seen in two-dimensional histological sections (e.g. Barlow 1994)] of the initial cells, and of the size and number of the mature cells, all of which can be estimated from, or directly measured upon, micrographs.

A mathematical model was derived to deal with the first of these two objectives. It was then applied to data obtained from micrographs of cross-sections of young saplings of hybrid aspen (*Populus tremula* × *P. tremuloides*) with a non-storied type of cambium, thus verifying the second of our objectives. However, because the mathematical approach to an understanding of cambial growth and division is of general application to cellular systems where the initial cells lie on the perimeter of a circle and which produce cells centripetally, we take advantage of the model to examine the behaviour of a much simpler marginal meristem – that which lies around the perimeter of the disc-shaped thallus of the green alga *Coleochaete orbicularis*. [The growth and development of this *Coleochaete* species are precisely described by Pringsheim (1860).] The marginal meristem of this alga consists of a single layer of *a* cells, each of which heads a radial file of thallus cells. The production of *p* cells from the *a* cells is in the S direction only. The mathematical model predicts a pattern of periclinal (circumferential) and anticlinal divisions based

on the dimensions of the *a* cells, which can then be compared with that which can be actually observed and from which the construction pattern of the thallus can be inferred.

MATERIALS AND METHODS

Hybrid aspen

At various times throughout the growing season of the year 1999, 1-cm-long segments were cut from stems of *Populus tremula* L. $\times$ *P. tremuloides* Michx. at a height 10 cm above the ground. The trees were in their second year of growth following vegetative propagation. Two types of tree were sampled, a wild-type (T89) and a transgenic (G1'2'D). In the latter there occurs a new pattern of auxin biosynthesis resulting in elevated levels of the natural growth hormone indole acetic acid (IAA), as described by Tuominen *et al.* (1995). The segments were split longitudinally into quadrants and fixed in 4% glutaraldehyde, buffered in 25 mM sodium phosphate (pH 7.2). After dehydration in acetone and embedding in resin, transverse sections were cut at 3 or 5 μ m. The resin was then removed and the sections stained sequentially in 0.1% acriflavine, 3% safranin O, 1% auramin O, and 2% methylene blue, as described by Puech *et al.* (2000). The sections were examined in a Zeiss photomicroscope at $\times 40$ magnification and the lengths and widths in the radial and tangential directions, respectively, of putative cambial fusiform initial cells in tissues of T89 and G1'2'D trees sampled in May and June 1999, when cambial activity was greatest, were measured with a calibrated ocular micrometer at a magnification of $\times 640$. From these dimensions, mean aspect ratios of cells in the respective initial cell populations were calculated. The numbers of all fusiform cambial cells lying within representative radial files, as well as the numbers and sizes of differentiating and newly differentiated xylem fibres within the files, were estimated from micrographs of cross-sections printed at a magnification of $\times 82$. Whenever a xylem vessel was encountered along a file it was converted into 'fibre equivalents' by counting the number of fibre cells in an adjoining file of fibre cells and interpolating this number into the tally for the file under study. Wherever mean cell numbers for a file of xylem fibres are mentioned (e.g. in Table 2 below), it has incorporated these interpolated numbers, whenever they occurred. Estimates were made of the number of cambial initials and their derivatives within sectors inscribed on the micrographs. The sector whose angles were measured with a protractor. The radii of the sectors were always defined by files of cells (mostly fibres) that ran alongside unbroken rows of xylem rays.

Coleochaete

Radial and circumferential dimensions of cells in the outermost layer of initials (the marginal meristem) of four thalli of *Coleochaete orbicularis* (as seen in surface view) were estimated from micrographs, printed at a magnification of $\times 2080$. The organisms had previously been grown, fixed, stained with light green and mounted upon a microscope slide. The thalli selected for analysis were of similar size (9–10 cells per radial file) and the numbers of cells in the different files and rings of each of the four major sectors of the thallus (these sectors are derived from the first two divisions of the thallus-generating spore cell) were scored. The angle defining the arc of each sector was measured with a protractor. The radial files extend from the centre of the thallus, where the four sectors meet, to its periphery. The files may branch as a result of earlier anticlinal divisions in the initial cells. Rings are comprised of circumferentially arranged cells which occupy a similar position in terms of cell number, counting along a radial file from its origin at the centre of the thallus.

MATHEMATICAL MODEL OF DIRECTIONAL CELL PRODUCTION

The present mathematical approach to predicting the relative frequencies of anticlinal and periclinal divisions in the initial cells of a centrifugally growing cambium or marginal meristem includes three assumptions: (1) the initial cells are grouped in a ring so that they form a circular perimeter surrounding an inner ring of derivative cells; (2) the initial cells increase in size by means of radial and circumferential growth (though the rate of growth need not be the same in each direction) over the course of the mitotic cycle; (3) the initial cells maintain constant mean dimensions and hence a constant aspect ratio.

Let us assume that cell productions in the initial ring are in both the S and the E/W directions, as they would be in *Coleochaete* and in a vascular cambium producing xylem only. Equation 1 estimates the number of initial cells, N_c , in the initial ring using the dimensions of these cells and the number of cells already produced by these initials in the S direction. These derivative cells are assumed to have the same mean dimensions as the initials. Thus,

$$N_c = 2\pi N_r l_r / l_c \quad (1)$$

where l_c = mean dimension of initial cells measured in the circumferential or tangential direction, l_r = mean dimension of initial cells measured in the radial direction, and N_r = mean number of cells within a radial file, estimated from where the file originates at or near (depending on whether or not a pith is present) the centre of the tissue up to the inner surface of the initial cell at the perimeter.

If a sector of tissue with an angle θ is considered, the number of initial cells on the arc of the sector, $N_{c_{\text{sector}}}$, may be estimated from Equation 2:

$$N_{c_{\text{sector}}} = 2\pi N_r l_r / l_c \times (\theta / 360). \quad (2)$$

The total number of cells, N_c , in the ring of initials may then be extrapolated by proportion as $360 \times N_{c_{\text{sector}}} / \theta$.

As a result of growth and periclinal division in the initial ring, with accompanying cell production in the S direction, the initial cells are displaced centrifugally. Associated with this displacement there may be an increase in the number of initials as a result of anticlinal divisions and cell productions in the E/W direction. A consequence of both types of division is that mean cell dimensions maintain a constant value.

Having established N_c by means of either Equation 1 or Equation 2, it follows that the increase in N_c which attends the centrifugal displacement of the initials can also be estimated by considering the addition of a ring of cells at the perimeter. Following the production of one new derivative cell, p_s , the initial cell located in ring k becomes relocated to ring $k + 1$. The number of cells in the k th ring, N_{c_k} , is given by Equation 3:

$$N_{c_k} = 2\pi r_k / l_c, \quad (3)$$

where r_k is the mean radius up to the inner surface of the k th ring. It follows that the number of cells in the $(k + 1)$ th ring, $N_{c_{k+1}}$, which lies on a circle of radius $(r_k + l_r)$, can be estimated using Equation 4:

$$N_{c_{k+1}} = 2\pi(r_k + l_r) / l_c. \quad (4)$$

Figure 2. Relationship between the mean aspect ratio (l_r/l_c) which applies to cells within a ring of initial cells and the number of E/W-directed productions added to the initial cell layer per single S-directed production by each initial cell. In terms of cell divisions, for a given aspect ratio stated on the abscissa, the ordinate states the number of anticlinal divisions which occur simultaneously with one periclinal division in the ring of initial cells.

The difference in the number of cells within the two rings k and $k + 1$ may then be estimated according to Equation 5.

$$Nc_{k+1} - Nc_k = 2\pi l_r/l_c. \quad (5)$$

This difference in cell number indicates the number of anticlinal divisions and E/W productions which have occurred in ring k before cells pass from this ring into ring $k + 1$ as a result of periclinal division and cell production in the S direction.

The number of anticlinal divisions in the initial cell population is dependent upon cell size in the radial and tangential directions (i.e. the aspect ratio of the cells) (Fig. 2). To illustrate this further, suppose that, in cross-section, the initial cells are rectangular with $l_r = 1.0$ and $l_c = 1.4$ units, respectively. Their mean aspect ratio is therefore 0.714. Then, according to Equation 5, and assuming that only the initial cells divide, the difference in initial cell numbers at the time when the initials occupy ring $k + 1$, having been in ring k prior to the periclinal division, would be: $2\pi \times (1.0/1.4) = 4.4$. In other words, the number of cells composing the ring of initial cells would be increased by 4.4 cells due to anticlinal divisions and E/W-directed cell productions as a consequence of all the initials having divided periclinally and having accomplished one complete set of cell productions in the S direction. These 4.4 new initial cells may then initiate, in ring $k + 1$, a corresponding number of new radial files upon commencement of periclinal cell divisions and cell productions in the S direction.

The equations above are suitable for the situation where only the initials divide and the derivative cells are not larger than the initials, as applies to the thalli of *C. orbicularis* (data not shown). However, in the vascular cambium of higher plants, there are many more dividing cells than just the cambial initials. Besides the initial cell (a), the dividing cells include the xylem mother cell (p_s) as well as the descendants of p_s that are still capable of dividing within the cambium. Furthermore, the non-dividing and differentiating descendants of p_s continue to enlarge and attain a mean size greater than that of the dividing cells. Therefore, to estimate anticlinal

and periclinal division frequencies, it is necessary to modify Equation 3 to accommodate the additional radial growth of such a system.

Equation 3 is adapted to estimate the total number of initials on the cambial perimeter when the radius, r_k , of the enclosed internal cell files is known. However, it is now necessary to add on to r_k an increment, z , as a result of the productions and cell growth which are due to each p_s cell and its descendants. This increment displaces the initial cells from ring k to ring $k + N_s$, where N_s is equal to the number of daughter cells produced, in a cell file, by one cell cycle in cell p_s and each of its cambial descendants. The modified Equation 3 is therefore:

$$N_{c_{k+N_s}} = 2\pi(r_k + z)/l_c, \quad (6)$$

where z is the mean length of the cohort of mature cells within a cell file which issues centripetally from the cambium following the completion of one set of S-directed cambial cell productions.

In order to update the total number of cambial initial cells, N_c , thus accounting for the anticlinal divisions which accompany each complete round of S-directed productions by the initials, the p_s cells, and their descendants, it is sufficient to multiply z by the number of rounds of cell production from all the cambial cells within a radial file of cells. The difference between the values of N_c after each round of S-directed cell production (i.e. the difference between N_c and $N_{c_{k+N_s}}$) yields the increase in the number of initials due to the anticlinal divisions. Hence, the numbers of S- and E/W-directed productions in the cambial initial ring can be estimated.

It should be noted that when cell productions from the cambial initials are in the N direction, that is, when the a -cell initials are producing daughters (p_N) whose descendants divide and differentiate later on as secondary phloem, there is no requirement for the initials to divide anticlinally because the initial cell layer is not being pushed outwards by this set of derivatives and the perimeter enclosing the initial cells is not affected. Instead, the initial ring serves as a platform upon which p_N cells divide and produce their phloemogenic derivatives. The production of secondary phloem cells is, however, associated with an increase in the circumference of phloem and bark tissues. The cell divisions which accompany this growth largely correspond to the anticlinal (radial) dilatation divisions which are found in older phloem ray and phloem parenchyma cells (see Esau 1965).

The relative frequencies of periclinal and anticlinal divisions (S- and E/W-directed cell productions) that can be derived from Equations 1–6 presented above (having in mind both the simple marginal meristem of *Coleochaete* and the more complex higher-plant vascular cambium) apply to cell production systems which are in steady-state. Usually, this would mean that the age distribution, cell dimensions and aspect ratio of the dividing cells remain constant as the system grows. In the case of a woody higher plant, the number of cambial cells within each cell file may both increase and decrease throughout the growing season. The increment, z , would therefore vary with time, and this term, as we have shown, is of importance for the estimation of anticlinal cell division frequency by means of Equation 6.

APPLICATION OF THE MODEL

The mathematical model predicts the frequencies of anticlinal and periclinal cell divisions (see Fig. 2). Therefore, the cell numbers actually present in a given ring of initials can be compared with those predicted on the basis of the current cell and tissue dimensions. It is also possible to reconstruct the earlier developmental stages, but this is necessarily somewhat speculative since the dimensions of the initial cells at these earlier stages are unknown. The past divisional history

Figure 3. Micrograph of a thallus of *Coleochaete orbicularis* showing that it is composed of concentric rings of cells produced by periclinal divisions and radially branched files of cells produced by anticlinal divisions. The outermost ring is a marginal meristem consisting of initial cells. The dimensions of the initial cells are listed in Table 1 under the column 'Thallus number 1'. Scale bar = 50 μm .

is, nevertheless, revealed by the numbers of cells recorded in the various files and rings of the sample and these numbers can be compared with predicted numbers. Conversely, this anatomical record of files and rings can be used to predict the aspect ratios of the initial cells at the earlier stages of development. These approaches represent backward looks at the developmental history. It is also possible to predict future states of development on the basis of information contained within the current state of the initial cell. This forward look at the future course of development assumes, just as did the backward look, that it arises from properties of the initial cells that are identical to those present at the time of sampling. The backward and forward views of cellular behaviour are reminiscent of similar backward and forward analyses of cell cycle kinetics that were made by Macdonald (1978) on the basis of cell age distributions observed at a single time during the growth of the cell population.

The predicted and actual developments of the two systems which were analysed are now discussed. The algal system will be dealt with before the secondary vascular system on account of its simpler construction.

Initial cells of *Coleochaete orbicularis*

One of the four thalli of *C. orbicularis* that were analysed is illustrated in Fig. 3. Cells in the outer ring k are the initial, a , cells. The mean dimensions (l_r and l_o) of the meristematic cells in the outer ring of each of the four thalli are presented in Table 1. The numbers of initial cells in the outer cell ring, N_{c_k} , of each thallus were estimated from Equations 1 and 2 and compared with numbers counted directly from micrographs. As can be seen in Table 1, on average, there is only a 7% difference between the estimated and the actual numbers. Likewise, the differences between the actual cell numbers within rings $k - 1$ and k are close to those estimated from Equation 5 (Table 1). That the estimated cell numbers often slightly exceed the actual numbers is not unexpected since the actual cell numbers recorded will be influenced by whether or not all the anticlinal divisions in the initial ring have been accomplished prior to the passage of this ring k to ring $k + 1$. The actual cell numbers and their increase during the last step of thallus growth

Table 1. Mean dimensions ($\mu\text{m} \pm \text{SE}$) of initial cells (a cells) in the outer layer (layer k) of four thalli of *Coleochaete orbicularis*. Cell length (l_r) and breadth (l_c) were measured as described in the Methods. Cell numbers are either actual counts or estimates derived from the mathematical equations on the basis of cell dimensions. Other symbols are as described in the text. Thallus number 1 is illustrated in Fig. 3

Variable	Thallus number			
	1	2	3	4
l_r (μm)	16.20 ± 0.68	16.88 ± 0.88	17.66 ± 0.84	17.45 ± 0.77
l_c (μm)	15.31 ± 0.57	15.42 ± 0.66	15.68 ± 0.57	14.74 ± 0.52
Aspect ratio (l_r/l_c)	1.15 ± 0.08	1.21 ± 0.10	1.22 ± 0.09	1.27 ± 0.08
N_r	9.7 ± 0.2	9.5 ± 0.2	9.4 ± 0.2	9.3 ± 0.2
r_k (μm)	176.0 ± 7.4	183.3 ± 5.9	174.5 ± 9.1	179.1 ± 4.5
N_{c_k} (from Eqns 1 and 2)	64	65	67	69
N_{c_k} (from Eqn 4)	62	63	61	67
N_{c_k} (actual counts)	68	73	70	72
$N_{c_{k-1}}$ (actual counts)	67	64	69	65
$N_{c_k} - N_{c_{k-1}}$ (actual counts)	1	9	1	7
$N_{c_{k+1}} - N_{c_k}$ (from Eqn 5)	6.65	6.88	7.08	7.44

Figure 4. Graphical representation of thallus development in four individuals of *Coleochaete orbicularis*. The graph shows the number of cells within successive rings. The cell ring number is also a measure of distance from the centre of the thallus which is occupied by four founder cells or their daughters (ring 1). A single regression line (unbroken line) was found to fit all four sets of data. This unbroken line, if read from right to left, traces a 'backward look' at the development of any given thallus, from the stage at which it was photographed (with 9–10 rings), back to when it was a one-ringed thallus. The current, most recently completed, ring in all four thalli is ring number 9. Symbols ●, ○, ▼ and ▽ denote data from thalli 1, 2, 3 and 4, respectively. The broken line traces the predicted number of cells in successive rings, given an aspect ratio of 1.213 for the outermost, initial ring. This value is the mean of the aspect ratios for this ring in the four thalli.

thus appear to conform to the mathematical model whereby these numbers are predicted on the basis of the dimensions of the cells found currently in ring k at the thallus perimeter.

The developmental history of the thalli is contained within their cell patterns and can be revealed by counting the number of cells within each of their four cellular sectors. The cell

Figure 5. Estimates of the average aspect ratio of the marginal cells within the four major sectors of the four thalli at different distances from their centres. The line joins the mean value of the aspect ratios at each respective stage of thallus growth.

counts allow an estimate to be made of the frequency of the E/W-directed productions (determining the number of cells within a ring) that have accompanied the S-directed productions (determining the order number of the ring). At the current stage of development, the outermost complete cell ring, k , is the ninth ring from the centre. In each sector, the ninth ring contains approximately 18 cells. Inspection of Fig. 4 shows that the cell layer with half this number of cells is the fifth layer. Thus, the set of anticlinal divisions which doubled the number of cells originally present in the fifth layer coincided with a period when there were four periclinal divisions, each of which generated one new cell layer. The broken line in Fig. 4 traces the relationship between the number of cell rings and the number of cells within a ring predicted on the basis of their having been generated by initial cells holding the average of the aspect ratios given in Table 1 (i.e. a value of 1.213). Predicted numbers compare favourably with those actually observed.

Another backward view of development is gained from estimates of the aspect ratio of the marginal cells during thallus growth. This can be done by rearranging Equation 2, so that aspect ratio is derived as $(360 N_c)/(2\pi \theta N_r)$. The values are shown in Fig. 5; they appear to remain relatively stable over the period of thallus development.

The future development of each thallus can be predicted on the basis of Equations 3 and 4, which are used to update the values of N_c for successive cell rings (Fig. 6). Commencing from

Figure 6. Predicted, forward estimates of cellular development of four thalli *Coleochaete orbicularis*. The predicted number of cells, N_{c_k} , within the outermost cell ring (k) is plotted against the number of descendant cells, N_r , within a radial file. Forward estimates of N_{c_k} are based on the current mean aspect ratio and the number of cells that lie within the current cell layer k (see Table 1) for each thallus. The current, actual value of N_{c_k} is shown at the left-hand end of each line. The numbers 1–4 denote the lines predicted for the four thalli 1–4 whose numerical data are given in Table 1. Values of N_{c_k} have been transformed to $\log_2$ to allow easy assessment of the doubling of cell numbers (each integer corresponds to one doubling).

thalli with nine cell rings, one further doubling of the initial population by E/W-directed productions is predicted to occur concomitantly with six further productions in the S direction. Thus, the number of initials doubles during a period in which the number of cells in the files which they support also doubles.

Vascular cambium of hybrid aspen

In comparison with the situation in *Coleochaete*, the problem of predicting the relative frequencies of anticlinal and periclinal divisions in the cambial initial cells becomes slightly more complex because not only do the derivative cells, p_g , divide, but so do their descendants. The latter cells may participate in up to four rounds of division. Also, within each radial file, cells approximately double their diameter in the radial plane as they leave the meristematic zone and begin to mature. Thus, depending on the number of cambial cells per file, and the number of maturing cells produced per complete set of cell productions on the xylem side of the cambium, the interval between successive periclinal divisions of the initial can be associated with between approximately 30 and 160 μm of radial growth of the xylem (see Table 2).

The dimensions of cells in the cambium of the two types of hybrid aspen trees sampled during June 1999 are recorded in Table 2. Also given are the mean radial and tangential dimensions of the most recently matured xylem fibres. From the point of view of the predictions of the mathematical model, the value of l_r is of particular interest because this parameter contributes to the value of r_k in Equations 3 and 4 and, hence, to the estimates of the current number of cambial initial cells. At the time (June 1999) when these cellular dimensions were estimated, the cambium of the wild-type (T89) trees was maintaining a maximal size of approximately 10 cells per cell file on its xylem-producing side. Therefore, it is of interest to estimate, by means of Equations 3 and 4, the relative frequencies of anticlinal and periclinal divisions involved in xylem

Table 2. Mean dimensions ($\mu\text{m} \pm \text{SE}$) of cambial initial cells (a cells) and of the most recently matured xylem fibre cells in four wild-type (T89) and four transgenic (G1'2'D) hybrid aspen during June 1999. Cell length (l_r) and breadth (l_c) were measured as described in the Methods. The number of cambial cells per row (N_s) includes the initial cell, a , as well as p_s and all its meristematic descendants. Other cell numbers were estimated as described in the text

Variable	T89		G1'2'D	
	a cells	Xylem fibres	a cells	Xylem fibres
l_r (μm)	6.10 ± 0.10	16.21 ± 0.51	5.77 ± 0.17	12.43 ± 0.37
l_c (μm)	15.99 ± 0.37	12.61 ± 0.40	15.98 ± 0.31	12.06 ± 0.42
Aspect ratio (l_r/l_c)	0.31 ± 0.01		0.28 ± 0.01	
N_s	10		5	
r_k (μm)	4088 ± 274		2902 ± 206	
θ°	29.2 ± 1.9		39.2 ± 2.1	
z (μm)	162.1		62.2	
N_{c_k} (extrapolated)	2071 ± 152		1434 ± 90	
N_{c_k} (from Eqn 3)	1606		1140	
$N_{c_k+N_s}$ (from Eqn 6)	1670		1164	
$N_{c_k+N_s} - N_{c_k}$	64		24	

production (Fig. 7) using the observed numbers and sizes of cambial cells and of their xylem fibre derivatives.

It is assumed that the maturation of xylem fibres and the production of new fibre cells by the cambium keep pace with each other. Thus, when each xylem-producing file of the cambium contains 10 cells, 10 mature xylem fibre cells are added to each file following one complete set of S-directed cell productions (i.e. $N_s = 10$). The estimated mean radial dimension (l_r) of a fibre was $16.21 \mu\text{m}$ (Table 2). Hence, each time a p_s cell is produced and its descendants divide, the radius of the xylem (length of the file) increases by $z = (10 \times 16.21) = 162.1 \mu\text{m}$. At the same time, the initial cell is moved from layer k to layer $k + 10$ because of the 10 new cells produced centripetally. Similar incremental radial increases of xylem radius were also at the basis of Nägeli's calculations (Nägeli 1864; see also Lück *et al.* 1970) of how cell number increased in the radial and tangential directions (in our terminology, these increases are due to S- and E/W-directed cell productions, respectively).

The number of cambial initials was estimated as follows. Sectors with an angle of approx. 30° were inscribed on photomicrographs of cross-sections of a number of T89 hybrid aspen trees (Fig. 8a). The sector widens as the radius increases with the accompaniment of anticlinal divisions within the initial ring (Fig. 7). Substituting the actual number of initial cells counted along an perimetric arc in each sector gives an extrapolated mean total number of initial cells, N_{c_k} , of 2071 ± 152 cells for the stems sampled at this time (June 1999). This value is 29% greater than that estimated from Equation 3 (1606 cells). Naturally, methodological reasons, such as the 30° sector being only a small fraction of the whole cross-section, might partly account for this discrepancy. Moreover, no counts of the total number of initial cells were made by which to judge which of the two estimates is closer to actuality. However, another possibility is that the cambial initials divide more frequently in the anticlinal plane than is required by the geometry of the cross-section of the stem: that is, there is a production of initial cells on the cambial perimeter in excess of that required to accommodate radial growth.

Looking back over past development of the xylem leads to another estimate of the relative frequencies of cambial cell productions in both the S and the E/W directions. Using the already described sectors inscribed on micrographs of the cross-sections from three T89 trees, the

Figure 7. Photomicrographs of two neighbouring regions in a cross-section of a stem of wild-type (T89) hybrid aspen showing secondary vascular cambium, the limits of which are indicated by arrows at the right-hand side, and its xylem (X) and phloem (P) derivatives. The filled arrowheads indicate sites within the cambium where an anticlinal division, probably within an initial cell, has initiated a pair of new cambial cell files. A less certain branching of a cell file is indicated by an open arrowhead; this may indicate where tips of cells from a different level in the cambium have intruded into the present level of the cross-section. The initial cells produce daughter cells in both the N direction (towards the phloem) and the S direction (towards the xylem). The directions of cell production (N and S) are indicated by letters and arrows (those which indicate the radial extent of the cambium). The putative cambial initials are marked by small open circles; p_N and p_S cells lie immediately on either side of the initials. Scale bar = 25 μm .

numbers of fibre cells along each radius of the sector were counted. At various distances along these radii, an arc was drawn and the numbers of fibres lying across the arc were counted. It was assumed that the number of fibres lying across any given arc reflects the number of rows of cambial initials that had formerly occupied this location. Using the most recent growth ring (see Fig. 8a) as a reference for counting cells in the radial direction, and assuming that a cohort of 10 cells was produced per complete set of S-directed cambial cell productions, the data then indicated (Fig. 9) that, in the three trees sampled, 19, 22, and 24 complete sets of S-directed productions accompanied each doubling of the initial population as a result of its E/W-directed productions. These cell production frequencies are close to those derived by extrapolation of the future development of the cellular population, as described in the next paragraph.

Simulation can predict the future development of the cambium on the basis of the data pertaining to its current state (i.e. at the time of fixation). In particular, estimates can be made of the relative frequencies of E/W and S productions in the cambial initial layer of the T89 hybrid aspen stems. Using a value $r_K = 4088$ (Table 2) in Equation 3, the number of cells, N_c , contained in the cambial initial layer is estimated to be 1606. At the next simulation step, using Equation

Figure 8. Cross-sections of stems of (a) wild-type (T89) and (b) transgenic (G1'2'D) hybrid aspen. Inscribed on the micrographs are two radii which define a sector of secondary xylem. The radii terminate at the growth ring between the wood of the first and second years, seen near the foot of each micrograph. Cells were counted along each radius. Arcs were drawn at intervals along the radii (not shown) and the number of xylem fibres (or fibre equivalents) along each arc was counted. Micrographs (a) and (b) have the same magnification. Scale bar = 500 μm .

Figure 9. Fibre cell production within radial files in stems of hybrid aspen. Numbers of secondary xylem fibre cells were counted along the radii and arcs of sectors observed in cross-sections (see Fig. 8). Linear regression lines were fitted, though the actual data points have been omitted for clarity. The three solid and the three broken lines, respectively, refer to data from sectors of three wild-type (T89) trees and three transgenic (G1'2'D) trees (the number against each line identifies the individual tree). Some data from the T89 samples lie beyond the right-hand border of the graph. The lines commence at the first growth ring (value of 0 on the abscissa) which separates first-year from second-year wood. In a comparison of the data from wild-type and transgenic material, regression analysis showed significantly different fits ($P < 0.001$). However, parallel regression lines have been fitted to the data from each type of tree because the slopes displayed by each of these two sets of three parallel lines are within the bounds of the statistical error of the slopes of the individually fitted lines. These data can then be used in conjunction with the mathematical model to predict anticlinal and periclinal division frequencies in the cambial initials.

6, the term $(r_k + z)$ is updated to $(4088 + 162.1) = 4250.1$, and N_{c_k} is re-estimated as 1670. Thus, the difference between the estimated numbers of cambial initials before and after one complete set of S-directed productions by the cambium is $(1670 - 1606) = 64$ cells. Iterations show that, by the time there has been one complete set of 25 S-directed productions, one E/W-directed cell production by each initial has brought about one further doubling of the population of cambial initials.

Sectors were similarly inscribed on photomicrographs of cross-sections of transgenic G1'2'D hybrid aspen trees (Fig. 8b). N_{c_k} was estimated as 1434 ± 90 for the June 1999 samples. However, estimating N_{c_k} by means of Equation 3 gave a value of only 1140 cells. The first estimate of the cambial initial cell number, N_{c_k} , is 26% greater than the second. This difference is rather similar to that found (29%) for the T89 trees and may come about for the same reasons, as suggested above.

Backward-looking estimates for transgenic G1'2'D trees were made similarly to those for the T89 trees. Cohorts of five mature cells were assumed to be produced by each complete set of cell productions from the cambium. Data (Fig. 9) estimated from the micrographs then showed that, in the three trees sampled, 24, 26, and 28 complete sets of S-directed productions occurred concomitantly with one doubling of the number of cambial initials by one E/W-directed production from each initial cell.

In order to predict future S and E/W cell production frequencies from the mathematical model, we commence with values of $r_k = 2902$ (Table 2), $N_s = 5$, and $z = (5 \times 12.43) = 62.15 \mu\text{m}$ (Table 2). Updating the estimates of N_{c_k} by repeated application of Equation 6 shows that the difference in cambial initial cell numbers between layers k and $k + N_s$ is 24 cells.

Therefore, 47 complete sets of S-directed cell productions from all the cambial initial cells would occur concomitantly with one further doubling of the cambial initial cell population by means of one E/W-directed cell production by each initial.

DISCUSSION

A simple arithmetical model which utilizes the dimensions of initial cells predicts the relative frequencies of anticlinal and periclinal divisions by these cells during the growth of the annular or cylindrical cellular systems with which they are associated. Underlying these estimates is the assumption that the initial cells have maintained a constant mean size during the previous period of development. The predictions of the model have been validated using data concerning cell size and cell production gathered from secondary vascular tissues of trees (two types of hybrid aspen) and from thalli of the alga *Coleochaete orbicularis*. The thalli are circular discs, each with a meristematic outer layer of cells from which all differentiated internal thallus cells are produced. In the case of the vascular cambium of hybrid aspen, our model solves the problem originally considered by C. von Nägeli in 1864, of determining how many cambial cells are needed in the circumferential plane to accommodate the numbers of cells produced in the radial plane during the course of xylem (wood) production (Nägeli 1864). Other references to the activity and structure of vascular cambium and its derivatives in both angiosperm and gymnosperm trees have also considered the frequency of anticlinal divisions (Angiosperms – e.g. Evert 1961; Cumbie 1967; Gymnosperms – e.g. Bannan 1957a, 1968; Hejnowicz 1961). However, this frequency has often been expressed in terms of the increase (in mm) of the radius of the xylem (Bannan 1960, 1962). Had the width of the xylem cells as well as the radial extent of the cambium been measured the relative frequencies of anticlinal and periclinal divisions in the cambium could have been estimated.

The method described here enables the anticlinal/periclinal division ratio to be extracted from relatively simple measurements on micrographs of cells or tissue cross-sections. By contrast, the frequencies of anticlinal divisions which have been reported in the literature relating to woody tissue are based on the preparation and subsequent ‘tedious microscopic examination’ of serial tangential sections (Bannan 1960). This more direct, but ‘tedious’, approach enables the location of bifurcations in the radial files of xylem cells to be identified, these bifurcations being evidence of anticlinal divisions in the cambium. Usually, however, it is nearly impossible to be certain whether these divisions occurred in the ring of cambial initials or were in a cambial cell (or cells) on the xylem side of the initials. The high frequency of bifurcations, and the frequency with which one of the two rows subsequently terminates (Bannan 1960), suggest that the anticlinal cell divisions which initiate the temporary files are often not in the initial ring itself but are in one of the cellular derivatives of this ring. The radial extent of the temporary file gives a clue to the whereabouts of the division in relation to the initial cell. If the file is short, the anticlinal division probably occurred in a cell towards the inner margin of the ring of cambial cells, whereas a much longer but nevertheless still temporary file would be consistent with it having been founded by cell p_s . For example, the radial file of 40 tracheids described by Bannan (1968) in a cross-section of Douglas fir could be accounted for by an anticlinal division in cell p_s and the concomitant multiplication of its associated file of, say, six cambial cells, by a further nine periclinal divisions. The two files resulting from an anticlinal division in p_s discontinue when a new p_s cell is produced as an S-directed product of a periclinal division in the initial cell, a , at the head of the files. The new p_s cell then supplants the pair of p_s cells which were formed by

the earlier anticlinal division. A new pair of files is *permanently* established only when the anticlinal division is in the cambial initial ring. Both daughter cells of the initial persist in this ring and continue with file production.

As a result of compiling data from up to 20 species of conifers, Bannan (1957a, 1968) concluded that approximately 93% of anticlinal divisions occur in the cambial initials and establish new xylem files; the remaining 7% of such divisions are not in the initial ring but in one of the derivatives on the xylem side. In the present case, the counts of cells in the files enclosed within sectors of wood (e.g. Fig. 9) might include not only some of the above-mentioned impermanent files, due to anticlinal divisions in cell p_8 , but also the tips of cells growing into the tissue seen in cross-section. Indeed, as was recognized by early researchers into cambial growth (Bailey 1923 and references therein), a portion of the increase in cell number in the initial cell layer of non-storied cambium has nothing directly to do with anticlinal cell division but is the result of the intrusive growth of the tips of the fusiform cells as they lengthen. This intrusive, longitudinal growth of the cells presumably offsets some of the requirements for anticlinal division since it fills up space created within the circumferentially expanding cambial layers. Nevertheless, anticlinal division is eventually necessary in order to increase the number of cells, irrespective of whether or not intrusive growth occurs (in storied cambium it does not occur). Intrusive growth of the maturing fibre cells also brings a similar problem to the exact estimation of their cell numbers within a file.

The net increase in cell files that occurs across the radius of a sector, from its older to its younger end, is likely, nevertheless, to reflect an increase in the number of cells within the cambial initial cell ring. Moreover, the increase in the number of these xylem-fibre files within the sectors shows good correspondence with the rate of increase of initial cells, predicted by the present mathematical model. However, there were some indications of a 26–29% excess of observed initials compared to the numbers estimated by Equation 3. Anticlinal divisions in excess of the numbers required to maintain circular geometry of the cambial initials have already been noticed by Bannan (1950, 1960) and Hejnowicz (1961). The result is that the extra cells are eventually squeezed out from the initial ring. It is interesting to note that the apparent excess of anticlinal divisions in the initial cells would be expected if the cells conformed to a deterministic division programme, such as has been postulated for a similar autoreproductive system by Barlow *et al.* (2001). If, as Bannan states, ‘the production of new fusiform initials in the cambium by pseudotransverse divisions far exceeds the number required to accommodate the cambium to increasing girth’ (Bannan 1960), then the mathematical model outlined here takes on a role beyond that of simply predicting cell numbers; it can highlight deviations from the expected patterns of division and hence challenge the assumption that the division programme is dependent upon geometrical constraints to which the cell can respond. Geometry may be the enabling factor for divisions in *Coleochaete*, but may not be so for higher plants.

The numbers of anticlinal and periclinal divisions derived from the mathematical model are estimates. Attempts to corroborate these numbers were made through analysis of actual tissues. Although cell productions have been assumed to be distributed at random within the ring of initials, there might, nevertheless, be localized regions of high and low cell production depending on the topography of the cambium (cf. Bannan 1957b), and this might contribute to a wavy perimeter of the cambial layer and hence to methodological errors in estimating the actual numbers of cambial initial cells based on the assumption of circularity. However, a wavy perimeter would accommodate even more cells than a circular one, so it is possible for there to have been an even greater frequency of ‘excess’ anticlinal division products than is evident from the estimated values of N_{c_k} in Table 2. The marginal meristem of *Coleochaete orbicularis* can also show evidence of non-uniform cell production in the early stages of thallus development (data not shown), with some sectors being in advance of others; but this may be a feature of the way the cells grow and

creep over their substrate. Another point to emphasize is that, following a division, there are no preferred values to which the dimensions of the initial cells (either algal or wood-forming) are set. What are conserved, both before and after the division of any given cell, are the *mean* aspect ratio and the *mean* cell dimensions that are characteristic of the whole population of initial cells.

With respect to any given initial cell, the aspect ratio would change throughout the inter-divisional period. In the case of a cambial initial or marginal meristem cell, this ratio would change progressively over a number of generations until reset to its initial value by a switch in the direction of cell production. The ratio changes because the initial cells, besides growing radially and producing daughter cells in the S direction, are at the same time growing tangentially. In fact, it may be that cell production in the E/W direction is the result of a 'critical aspect ratio' being achieved which then favours an anticlinal rather than a periclinal division. This point has already been discussed by Barlow (1994) and Barlow & Adam (1989) in the context of anticlinal and periclinal divisions in the cell files of inner cortical tissue of tomato roots. Hence, the relative frequencies of anticlinal and periclinal divisions in the initial cell layer may reflect the number of divisions that need to be accomplished during the time interval in which a 'critical ratio' is attained, thus leading to a switch in the orientation of cell division. Ultimately, therefore, the division frequencies reflect the relative rates of cell growth in tangential and radial planes which, in turn, are dictated by the geometry of a circle. It is also possible that the expected division frequencies might be modulated in response to the interplay of turgor pressures present with the dividing and the differentiating populations of cells, as suggested by Wegner (2000) to account for the characteristic layered cellular structure of shoot apices.

The terminology employed here, using for the most part the notion of E/W-, S- and N-directed cell productions as a complement to the more familiar notion of anticlinal and periclinal divisions (Fig. 1), derives from a study of plant tissue construction where a directional context to cell division was found to be convenient for the simulation of the position of daughter cells in relation to their mother cells (Barlow *et al.* 2001). This terminology is useful also for describing the behaviour of the cambial initials, where the daughter cells of a divided cambial initial have a definite position with respect to the initial layer, i.e. one of the daughters of such a division can be either a phloem or a xylem mother cell (p_N or p_S , respectively). Moreover, this terminology can accommodate the 'handedness' of anticlinal divisions and would correspond to either an E-directed or a W-directed cell production. In this respect, the handedness of division reflects the orientation of the pseudotransverse, anticlinal division walls of the cambial initial cells (Hejnowicz 1961; Hejnowicz & Krawczynszyn 1969), where the new division walls are slanted either to the right or to the left with respect to the longitudinal axis of the mother cell. In the present study, we have used a generalized E/W direction of production because, although handedness was recognized as a possible feature of cambial division in hybrid aspen (and, theoretically, in the marginal meristem of *Coleochaete* also), details of it were not investigated.

In the earlier study of Barlow *et al.* (2001) it was proposed that the direction of cell production was determined according to the states attached to the walls of the dividing cell and that, following such a production, these states altered according to a definite sequence that could be codified as a set of directed production rules. These rules were presented as a graph-L-system (Barlow *et al.* 2001) for the construction of plant tissue. In order to account for regular patterns of newly produced cells, the distribution of wall facets holding different states around an autoreproductive *a*-state cell could be crucial. This type of cell has the maximum number of directions for daughter cell productions (as do the cambial initial cells) and hence has the maximum potential for influencing the ensuing pattern of cellular development. The question arises therefore whether the directions and relative frequencies of cell productions within either the secondary vascular cambium of a tree or the marginal initials of an algal thallus may be specified in a similar deterministic way.

ACKNOWLEDGEMENTS

IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. We are also indebted to Mr Derek Paget whose rolled-up carpets prompted our interest in finding a solution to their length and thence led to our consideration of the cambial cell division problem originally proposed by Carl von Nägeli. The sections of hybrid aspen were expertly prepared in the Institut für Forstbotanik und Baumphysiologie, Albert-Ludwigs Universität, Freiburg, Germany, under the guidance of Prof Dr S. Fink and Dr L. Puech. Stained thalli of *Coleochaete orbicularis* were kindly provided by Dr Linda E. Graham, University of Wisconsin, Madison, USA. Part of the present study was carried out with financial support from the Commission of European Communities.

REFERENCES

- BAILEY IW (1923) The cambium and its derivative tissues IV. The increase in the girth of the cambium. *Am. J. Bot.* **10**, 499.
- BANNAN MW (1950) The frequency of anticlinal divisions in fusiform cambial cells of *Chamaecyparis*. *Am. J. Bot.* **37**, 511.
- BANNAN MW (1957a) The relative frequency of the different types of anticlinal divisions in conifer cambium. *Can. J. Bot.* **35**, 875.
- BANNAN MW (1957b) Girth increase in white cedar stems of irregular form. *Can. J. Bot.* **35**, 425.
- BANNAN MW (1960) Ontogenetic trends in conifer cambium with respect to frequency of anticlinal division and cell length. *Can. J. Bot.* **38**, 795.
- BANNAN MW (1962) Cambial behaviour with reference to cell length and ring width in *Pinus strobus* L. *Can. J. Bot.* **40**, 1057.
- BANNAN MW (1968) Anticlinal divisions and the organization of conifer cambium. *Bot. Gaz.* **129**, 107.
- BARLOW PW (1994) The cell division cycle in relation to root organogenesis. In: Ormrod JC, Francis D, eds. *Molecular and Cell Biology of the Plant Cell Cycle*. Dordrecht: Kluwer Academic Publishers, p. 179.
- BARLOW PW (1997) Stem cells and founder zones in plants, particularly their roots. In: Potten CS, ed. *Stem Cells*. London: Academic Press, p. 29.
- BARLOW PW, ADAM JS (1989) Experimental control of cellular patterns in the cortex of tomato roots. In: Loughman BC, Gasparíková O, Kolek J, eds. *Structural and Functional Aspects of Transport in Roots*. Dordrecht: Kluwer Academic Publishers, p. 21.
- BARLOW PW, LÜCK HB, LÜCK J (2001) Autoreproductive cells and plant meristem construction: the case of the tomato root cap meristem. *Protoplasma* **215**, 50.
- CUMBIE BG (1967) Developmental changes in the vascular cambium in *Leitneria floridana*. *Am. J. Bot.* **54**, 414.
- ESAU K (1965) On the anatomy of woody plants. In: Côté, W, ed. *Cellular Ultrastructure of Woody Plants*. Syracuse: Syracuse University Press, p. 35.
- EVERT RF (1961) Some aspects of cambial development in *Pyrus communis*. *Am. J. Bot.* **48**, 479.
- HEJNOWICZ Z (1961) Anticlinal divisions, intrusive growth, and loss of fusiform initials in nonstoried cambium. *Acta Soc. Bot. Polon.* **30**, 729.
- HEJNOWICZ Z, BRAŃSKI S (1966) Quantitative analysis of cambium growth in *Thuja*. *Acta Soc. Bot. Polon.* **35**, 395.
- HEJNOWICZ Z, KRAWCZYŹYŃ J (1969) Oriented morphogenetic phenomena in cambium of broadleaved trees. *Acta Soc. Bot. Polon.* **38**, 547.
- IQBAL M (1994) Structural and operational specializations of the vascular cambium of seed plants. In: Iqbal, M, ed. *Growth Patterns in Vascular Plants*. Portland, OR: Dioscorides Press, p. 211.
- IQBAL M, GHOUSE AKM (1990) Cambial concepts and organization. In: Iqbal, M, ed. *The Vascular Cambium*. Taunton: Research Studies Press, 1.
- LÜCK HB, SERRE F, VIDAL B, ANS B (1970) A propos d'un modèle de la croissance en épaisseur des arbres. *C. R. Acad. Sci. (Sér. D)* **270**, 1996.
- MACDONALD PDM (1978) Age distributions in the general cell kinetic model. In: Valleron, A-J, Macdonald, PDM, eds. *Biomathematics and Cell Kinetics*. Amsterdam: Elsevier/North. Holland Biomedical Press, p. 3.

- MAHMOOD A (1968) Cell grouping and primary wall generations in the cambial zone, xylem and phloem in *Pinus. Aust. J. Bot.* **16**, 177.
- NÄGELI C (1864) Dickenwachsthum des Stengels und Anordnung des Gefässstränge bei des Sapindaceen. *Beitr. Wiss. Bot.* **4**, 1.
- PRINGSHEIM N (1860) Beiträge zur Morphologie und Systematik der Algen. *Jahrb. Wiss. Bot.* **2**, 1.
- PUECH L, TÜRK S, HODSON J, FINK S (2000) Wood formation in hybrid aspen (*Populus tremula* L. $\times$ *Populus tremuloides* Michx.) grown under different nitrogen regimes. In: Savidge, R., Barnett, J, Napier, R., eds. *Cell and Molecular Biology of Wood Formation*. Oxford: Bios Scientific Publishers, p. 141.
- ROMBERGER JA, HEJNOWICZ Z, HILL JF (1993) *Plant Structure: Function and Development*. Berlin: Springer-Verlag.
- SANIO K (1873) Anatomie der gemeine Kiefer (*Pinus sylvestris*). *Jahrb. Wiss. Bot.* **9**, 50.
- TIMELL TE (1980) Organization and ultrastructure of the dormant cambial zone in compression wood of *Picea abies*. *Wood Sci. Technol.* **14**, 161.
- TUOMINEN H, SITBON F, JACOBSSON C, SANDBERG G, OLSSON O, SUNDBERG B (1995) Altered growth and wood characteristics in transgenic hybrid aspen expressing *Agrobacterium tumefaciens* T-DNA indoleacetic acid-biosynthesis genes. *Plant Physiol.* **109**, 1179.
- WEGNER J (2000) A theoretical approach to the genesis of cell layer arrangements in undifferentiated tissues. *Plant Sci.* **153**, 177.