

**ADJUSTMENT OF STORIED PATTERN OF VASCULAR CAMBIUM  
OF *CARAGANA ARBORESCENS* LAM. BRANCHES**

SABINA MICZAJKA, ALDONA GIZIŃSKA, ADAM MIODEK, ANNA B. WILCZEK<sup>\*</sup>

Department of Biosystematics, University of Opole, Oleska 22, 45-052 Opole, Poland;

<sup>\*</sup> Corresponding author: Anna Wilczek: a.wilczek@vp.pl; Tel. (+48) 774016010.

**ABSTRACT:** Our knowledge on the mechanism that leads to formation of storied structure in vascular cambium is still incomplete. Recent studies have revealed that the occurrence of longitudinal anticlinal divisions is insufficient to explain this process. In this study the structure of 2-year-old and 6-year-old branches of *Caragana arborescens*, thus on the early and late stages of storied structure formation, respectively, have been compared. The aim of this comparison was to reveal the cellular events, occurring on those two stages of storiedness formation.

The analysis was carried out on semi-thin transverse and tangential sections of branches, stained with Schiff reagent and toluidine blue. The dimensions of fusiform initials and of rays, number of cells forming stories, as well as frequency and pattern of anticlinal divisions have been examined. Results indicate a heterogeneous character of the stories. Frequency of anticlinal divisions in 2-year old cambium was more than 5 times higher than in 6-year old cambium. In 2-year old cambium, so in time of rapid formation of regular storiedness, short anticlinal divisions (with the relative length of the dividing wall below 70%) frequently occurred, whereas in regular storied cambium of older branches short anticlinal divisions were sparse. This indicates that long anticlinal divisions are typical for already storied cambia, whereas their participation in storiedness formation should be questioned.

**KEY WORDS:** readjustment, intrusive growth, storied cambium, radial growth

## ***Introduction***

Vascular cambium is a lateral meristem producing a new layer of secondary xylem and phloem each year. In several anatomically distinguishable layers of meristematic cells, initial cells and their derivative xylem and phloem mother cells occur. Initial cells have been categorised into two types: ray initials, which deposit ray cells (radial system of transport), and fusiform initials, which deposit elements of axial system of transport (Larson 1994; Evert 2006). In cambium of gymnosperm plants fusiform initials are scattered randomly across the tangential surface, which has been named the nonstoried structure of cambium, whereas in some angiosperm plants fusiform initials may be arranged in horizontal tiers (storied structure). Double-storied structure is characterised by the rays fitted between stories' borders (Larson 1994; Kojs et al. 2004a,b).

Storied structure allows for rapid rearrangement of cambial initials' pattern, which results in the formation of the interlocked grain in wood, a feature influencing wood properties. Interlocked grain occurs in the highest trees of tropical rain forests, in environment with windless conditions but extremely severe competition for light (Record 1919; Carlquist 1988; Kojs et al. 2003; Iqbal et al. 2005). Trees in tropical rain forests grow in a very peculiar manner. They increase their height rapidly, exhibiting relatively small radial increment (compared to growth of temperate climate trees) and no branch formation. Just after reaching canopy layer, their crown is formed and radial increment intensified. For this type of tree growth, the occurrence of double-storied structure of cambium and interlocked grain in wood are believed to be essential (Iqbal et al. 2005). As most of valuable tropical species are characterised by the interlocked grain and double-storied cambium, understanding mechanisms of formation of these features seems to be of key importance.

Ontogenesis of storied pattern is a type of developmental studies of cambial structure (Jura et al. 2005). Formation of storied pattern was described as a result of longitudinal anticlinal divisions (Cumbie 1984; Ajmal et al. 1986; Carlquist 1988). Stories formed due to anticlinal divisions of one initial would be homogeneous. However, recent studies indicate that such divisions are insufficient for rapid formation of regular storied pattern, and that the nature of stories is heterogeneous – the stories are composed of groups of cells undergoing axial translocation, resulting in the proper adjustment of their position (Kojs et al. 2004a,b; Wilczek 2012). The aim of this study was to compare 2-year-old and 6-year-old vascular cambium in order to identify cell events participating in the formation of regular storied structure.

## ***Material and methods***

On 10<sup>th</sup> July 2013 the internodes have been collected from 2- and 6-year old, straight branches (as the main stem was indistinguishable) of *Caragana arborescens* Lam. shrub (Fabaceae). Samples of young branches have been chosen after preliminary examination of hand-made sections of cambium, revealing that in 1-year-old branches only mosaic of cell packets occurs (hence it has been neglected in further examination), whereas in 2-year-old branches small stories are distinct. The oldest examined branches were 6-year-old and characterised by the regular storied structure of cambium.

Fragments of 2-year-old and 6-year-old branches have been fixed in glycerol and 96% ethanol (1:1) and sectioned into samples including cambial tissue with small amount of secondary phloem and xylem. The dimensions of each sample were approximately 2x2x2 mm or smaller. The collected samples were embedded in Epon-812 (Meek 1976). The series of tangential and transverse sections about 3 µm thick were prepared with ultramicrotome (Włoch et al. 2001). The sections were glued to glass slides with Haupt adhesive (1% gelatine in water with 2% phenol crystals and 15% glycerine), stained with periodic acid Schiff's reagent (Schiff reagent –  $C_{19}H_{18}N_3Cl + H_2SO_3$ ) and toluidine blue ( $C_{15}H_{16}N_3SCl$ ), and mounted in Euparal (Włoch and Połap 1994). The photographs have been obtained using Olympus BX41 microscope and Canon EOS 20D camera.

Length and width of fusiform initials (100 cells for both branch ages separately) were measured from tangential sections, in at least 10 different stories and on at least two different samples, using ocular scale calibrated with stage micrometre scale. The mean and modal values as well as the range confined within minimal and maximal observed values have been calculated for both parameters, and for younger and older cambium. The results have been compared by calculating the standard deviation, and t Student test (p value 0.001). For both branch ages, on the basis of mean length of fusiform initials, the average number of stories within the chosen distance (1000 µm) has been calculated in reference to the main stem axis.

The width of the stories has been determined by the number of fusiform initials (20 stories per each branch age), distinct in tangential sections. The examined stories were chosen from at least 3 different samples, and not at the same level in reference to the main stem axis, to reduce their possible similarity. The homogeneous or heterogeneous nature of stories was not taken into account.

The frequency of anticlinal divisions has been examined (in transverse sections) by counting recent anticlinal divisions per 100 radial rows – for each branch age. As a recent anticlinal division we mean such a division, which is still visible in the cambial zone, therefore reflected in at most 6 layers of cells. This method does not reveal the frequency of anticlinal divisions specific for whole annual increment, but is frequently used to distinguish stems of different age (Kojas 2004b; Wilczek 2012).

The pattern of anticlinal divisions was examined in tangential sections. The length of dividing wall and the length of mother fusiform initial (before the anticlinal division occurrence) have been measured, and the relative length of dividing wall has been calculated, according to equation:  $\text{relative length of dividing wall} = \text{length of dividing wall} * 100\% / \text{length of mother fusiform initial}$ . The type of anticlinal division has been estimated on the basis of relative length of dividing wall: longitudinal – if equal to 70% or more, short (oblique or lateral) – if below 70%.

The pattern of rays has been examined in tangential sections. Width of rays was estimated on the basis of the maximal number of ray cells arranged in a single horizontal line, whereas height – as the number of stories through which the ray was extending, by counting fusiform initials contacting with the ray and belonging to consecutive stories.

## **Results**

### *Dimensions of fusiform initials*

Fusiform initials in cambium of *C. arborescens* are generally short. In younger cambium the mean length was 127,23  $\mu\text{m}$  (tab. 1). In older cambium the mean length of fusiform initials was slightly smaller – 118,15  $\mu\text{m}$  (decrease of 7,14%). The mean length of fusiform initials may be used to calculate the average number of stories occurring in the given tangential section of vascular cambium. For example the tangential section of younger vascular cambium with axial dimension 1000  $\mu\text{m}$  would contain an average 7,86 stories, 127,2  $\mu\text{m}$  each tall, whereas the section of older cambium with the same axial dimension would include 8,46 stories (118,2  $\mu\text{m}$  tall each). It is clear that a change of stories number occurring in a given sample of vascular cambium cannot be attained due to shortening or lengthening of cambial surface and stem. Such change has to be related with stories boardsers` translocation.

In the younger cambium length of fusiform initials was more diversified than in older one, which has been revealed by comparing the difference between the longest and the shortest initial: 141,7  $\mu\text{m}$  and 45,5  $\mu\text{m}$  in younger and older cambium respectively (Tab. 1). Calculated deviation (Tab. 1) and the number of initials occurring in each of eight equal ranges of initials

length (Fig. 1) also confirm that conclusion. In older cambium almost all initials occurred in ranges 100-120  $\mu\text{m}$  and 120-140  $\mu\text{m}$ , whereas in younger one longer initials occurred in all eight ranges.

In examined branches the mean width of cambial fusiform initials increased from 10,5  $\mu\text{m}$  to 11,7  $\mu\text{m}$  along with sample age (Tab. 1). The increase of cells' width was distinct in minimal and maximal values observed, as well as in modal values. The range of observed values was slightly larger in older branch than in younger one, which was also confirmed by calculated standard deviation. Mean tangential area of fusiform initials, calculated on the basis of their mean length and width in younger and older branches, increased slightly from 1335,6  $\mu\text{m}^2$  to 1382,94  $\mu\text{m}^2$  (Tab. 2).

#### *Storied pattern adjustment*

The vascular cambium of *C. arborescens* already displays the storied pattern of fusiform initials in younger (2-year-old) branches, but the borders of the stories are frequently slightly oblique in relation to the transverse plane of the branch. In older (6-year-old) branches the borders of stories are usually perpendicular in relation to the main axis (Fig. 2). Relative increment in cambial circumference between 2-year-old and 6-year-old branches was 67%. Assuming no eliminations of fusiform initials and their constant tangential area (Tab. 2), as well as constant contribution of rays in cambial surface, approximately 7 out of 10 fusiform initials should divide anticlinally. The average number of cells forming stories was 5 in younger branches, and 12,45 in older branches, which means a change of 149% (Tab. 2). According to the change of the mean number of fusiform initials in examined stories, 15 anticlinal divisions would occur in a group of 10 fusiform initials, if the stories were homogeneous. The change of maximal number of fusiform initials forming an average storey was even more significant, equal to 182%. Therefore, the increment of stories' width was much greater than increment of cambial circumference.

The comparison of all examined stories in younger and older cambia revealed increase in number of fusiform initials forming stories. In older cambia several stories formed by just several fusiform initials occurred, but most of the stories had been formed by more than 10 fusiform initials, whereas in younger cambium only one storey exceeded 10 initials (Fig. 3).

#### *Dimensions of rays*

The vascular cambium of *C. arborescens* was characterised by diversified rays: uniseriate, biseriate, and multiseriate (triseriate and tetraseriate). The height of rays also varied, from short to tall, exceeding even 12 stories of fusiform initials (Fig. 4). Rays in younger cambium were

more diversified than in older one. Short rays occurred in the younger cambium (between borders of a single storey). All of these short rays were uniseriate. There also occurred tall rays, reaching even up to 5-6 stories. Tall rays were exclusively bi- or multiseriate (triseriate). In older cambium short rays (1 storey tall) were also exclusively uniseriate, but there occurred uniseriate rays taller than 1 storey (reaching 2 stories). Tall rays (reaching 3 or 4 stories) were multiseriate. There occurred wider, tetraseriate rays (absent in younger cambium), all rather tall, reaching 2-4 stories. Although one extremely tall ray (12 stories) was observed, the general tendency was that the height of rays was lower in older cambium (Fig. 4).

#### *The pattern of anticlinal divisions*

The occurrence of anticlinal divisions has been observed in transverse and tangential sections, in both ages of cambium, although the frequency was higher in younger cambium than in older one (Tab. 3). This change seems to be convergent with the decreasing value of relative increment of cambial circumference. In younger cambium contribution of short anticlinal divisions was high (60%), whereas in older cambium only one short division occurred (5%). It is worth to emphasise that numerous short anticlinal divisions were present in cambium displaying strong tendency to storied pattern. Maintaining and further development of regular storied pattern seems to be undisturbed by those short divisions. The shortening of fusiform initials occurring between 2-year-old and 6-year-old cambia seems to be related with numerous short anticlinal divisions.

### **Discussion**

#### *Formation of storied structure*

In last decades numerous studies have brought into question common explanation of cambial circumference increment and initials' rearrangement. Although most papers and course books indicate an intrusive growth as the main mechanism of increment of cambial circumference (Esau 1965; Larson 1994; Evert 2006), many recent studies have negated this statement (Włoch et al. 2002, 2009, 2013; Kojs et al. 2004a,b; Jura et al. 2006; Karczewska et al. 2009; Wilczek et al. 2011, 2015). In these papers, all cases of intrusive growth, examined in detail on the basis of long series of transverse or tangential sections, showed no increment of cambial circumference, since intrusive growth was always related with complementary elimination of neighbouring initials (Włoch et al. 2009, 2013). This phenomenon has been described by the hypothesis of intrusive growth of a cambial initial occurring between tangential walls of neighbouring initial and its closest derivative (Włoch et al. 2002, 2009, 2013; Kojs et al. 2004 a,b; Jura et al. 2006;

Karczewska et al. 2009; Wilczek et al. 2011, 2015). Occurrence of tension in circumferential and axial direction, and compression in radial direction in vascular cambium was commonly assumed. This assumption has been reconsidered by Kojs and Rusin (2011). Diurnal cycles of consecutive shrinkage and swelling of phloem has been reported by Alm  ras (2006). These cycles have been interpreted as mechanical conditions influencing cambial tissue and described as diurnal strains in plants (Kojs and Rusin 2011), which may be regarded as a new concept of radial increment (W  loch et al. 2013). On the basis of this new concept assumptions, many phenomena occurring in cambial tissue may be explained, such as rearrangement of cambial initials (W  loch et al. 2009, 2013; Wilczek et al. 2011, 2015), symplastic growth (Kojs and Rusin 2011; Wilczek et al. 2015), as well as rapid storied structure formation (Kojs et al. 2004a,b; Wilczek 2012; Wilczek et al. 2015).

Storied structure of cambium was visible in 2-year-old branches of *C. arborescens* (Fig. 1; Tab. 2). In first two-three years of cambial activity rapid formation of storied structure has also been observed by Record (1919), W  loch and colleagues (2002), Wilczek (2012) and Kojs and colleagues (2004 b). Increase of stories' width (interpreted as a higher number of fusiform initials forming stories) occurred when frequency of anticlinal divisions decreased.

Translocation of initials or coordinated translocation of whole groups of initials in axial direction, resulting in alignment of their borders with borders of the neighbouring stories, leads to the formation of heterogeneous stories (W  loch et al. 2002; Kojs et al. 2004a,b; Wilczek 2012). The location of stories' borders has been maintained on two sides of tall, multiseriate rays, similarly to results of Wilczek (2012), which clearly indicates that such stories are heterogeneous, hence it seems impossible that numerous groups of cells could be translocated to the other side of rays in such a short time.

### *Dimensions of fusiform initials*

Storied cambia are characterised by shorter fusiform initials than nonstoried ones, mean range is usually contained between 186  $\mu\text{m}$  to 649  $\mu\text{m}$  (Ghouse et al. 1980) or 138  $\mu\text{m}$  to 535  $\mu\text{m}$  (Larson 1994). In examined species (in older branches) mean length of fusiform initials was 118,2  $\mu\text{m}$  (Tab. 1), which place them amongst the shortest fusiform initials.

Increase of mean length of fusiform initials in nonstoried cambium is commonly known (Esau 1965; Larson 1994), however a slight decrease has been observed during storied pattern formation (Butterfield 1972; Ajmal et al. 1986; Kojs et al. 2004b; Wilczek 2012). In this study length of initials decreased between 2-year-old and 6-year-old cambium (Tab. 1), which seems to be a typical feature in storied structure formation. Because axial dimension of vascular cambium

is constant, shortening of fusiform initials has to be related with intense rearrangement of stories. Although the mean value has decreased only slightly, change between fusiform initials' length in younger and older branches was significant, possibly related with the explicit decrease of range between the maximal and minimal value (Tab. 1), which also corroborates previous results (Kojas et al. 2004b; Wilczek 2012). The diversity of fusiform length decreased (Tab. 1), as well as the number of defined ranges of initials' length (Fig. 3). In younger cambium most of initials were in the third defined range, whereas in older cambium – in the second one, which was also indicated by the decrease of modal value (Tab. 1). These results are also in accordance with the previous data (Kojas et al. 2004b; Wilczek 2012). In younger cambium, there occurred several long initials, reaching 230  $\mu\text{m}$ , so almost twice as long as the mean dimension of fusiform initials. Those cells appear to be similar to the 'nails', described by Włoch and Zagórska-Marek (1982).

#### *Rays rearrangement*

Rearrangement of rays manifested in the increase of rays' width, as well as in general decrease of their height (with exception of one particularly tall ray in older cambium, reaching 12 stories), occurred between younger and older cambium (Fig. 4). The increase of rays' width is plausibly related with the increment of cambial circumference, which is a commonly known feature of cambium with multiseriate rays (Esau 1965). In examined cambium small, uniseriate rays also occurred. They may be the new ones, arising adequately to the increment of cambial circumference, in order to maintain usually constant fusiform area / ray area ratio, which has been described by Braun (1955). The tallest rays were wide – usually more than biseriate. These results corroborate with studies on *Laburnum anagyroides*, although in that species rays were wider and slightly taller (Wilczek 2012).

#### *Pattern of anticlinal divisions*

Anticlinal divisions are commonly described as longitudinal in storied cambia or even as a mechanism of this structure formation (Cumbie 1984; Ajmal et al. 1986; Carlquist 1988), whereas the occurrence of short anticlinal divisions is believed to participate in the formation of nonstoried structure of cambium (Cumbie 1967; Bannan 1964). Indeed, in older branches of examined species, most of anticlinal divisions were longitudinal, with the mean relative length of dividing wall 80%. However, in younger branches 60% of observed anticlinal divisions were short (relative length of dividing wall below 70%), and the mean relative length was only 45% (Tab. 3). These results corroborate with previous data, where in the early phase of storied structure formation numerous short anticlinal divisions have been observed (Kojas et al. 2004b;



Wilczek 2012). It is plausible that short anticlinal divisions not only do not interrupt the formation of storied structure, but even participate in this process. On basis of the relation between the location of dividing wall and mechanical conditions of cells, described e.g. by Lynch and Lintilhac (1997), another hypothesis has been put forward (Włoch et al. 2013). According to this hypothesis the occurrence of short (oblique) anticlinal divisions is only a side effect of a specific pattern of mechanical conditions, i.e. shearing strains generated in cambium. The excess of oblique anticlinal divisions is typical for nonstoried cambia (Evert 1961; Srivastava 1973). It is probable that storied structure of cambium allows for rapid relaxation of shearing strains, hence there was no oblique anticlinal divisions' excess observed (Włoch et al. 2013). Storied structure of cambium allows for rapid rearrangement of initials, thus for an instant relaxation of shearing strains, but nonstoried cambium is unable for such a rapid rearrangement, therefore generated shearing strains are greater (Kojas et al. 2004a,b; Włoch et al. 2009, 2013). In accordance with this new hypothesis, maturation of storied structure would be accompanied by oblique anticlinal divisions' frequency reduction, which has actually been observed in this examination, and also in *Laburnum anagyroides* (Wilczek 2012).

### **Conclusions**

In *C. arborescens* storied pattern of vascular cambium is already present in 2-year-old branches. However, the borders of the stories are slightly oblique in relation to the transverse plane. By the sixth year of cambial activity the borders are usually transversally oriented. Stories are heterogeneous – they are formed due to initials' translocation resulting in stories' borders alignment. Short anticlinal divisions do not disrupt the process of storied structure development as such divisions are frequent in the stage of rapid increase of number of fusiform initials forming stories. Moreover, occurrence of short anticlinal divisions is presumably associated with the storied pattern formation. Longitudinal anticlinal divisions are predominant in older samples, in which storied pattern is regular and axial rearrangement is complete.

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### **Tytuł**

Tworzenie piętrowej struktury kambium w 2-letnich i 6-letnich pędach *Caragana arborescens* Lam.

### **Streszczenie**

Ontogeneza piętrowej struktury kambium jest wciąż słabo poznana. Badania wskazują, że udział podłużnych podziałów antyklinalnych jest niewystarczający. Celem pracy było porównanie struktury kambium z 2-letnich i 6-letnich pędów *Caragana arborescens* i określenie zdarzeń komórkowych zachodzących w trakcie formowania piętrowości. Wykonano serie semi-cienkich przekrojów poprzecznych i stycznych, zabarwionych odczynnikiem Schiff'a i błękitem toluidyny. Stwierdzono, że w trakcie ustalania regularnej piętrowości inicjały wrzecionowate ulegają skróceniu. W dwuletnim kambium obserwowano wysoki udział skośnych podziałów antyklinalnych, których nie obserwowano w starszym kambium.

Tab. 1. The data of fusiform initials' dimensions in 2-year-old and 6-year-old branches of *C. arborescens* (n=100). P value after t Student test.

Age of branches	Length		Width	
	2-year-old	6-year-old	2-year-old	6-year-old
Mean value [ $\mu\text{m}$ ]	127.2	118.2	10.5	11.7
Minimal value [ $\mu\text{m}$ ]	88.6	88.6	6.6	7.1
Maximal value [ $\mu\text{m}$ ]	230.2	13.1	15.2	16.2
Modal value [ $\mu\text{m}$ ]	126.5	118.9	11.1	12.1
SD	23.4	9.0	1.8	2.1
P value	<0.001		<0.001	

Tab. 2. The comparison of number of fusiform initials forming stories in 2-year-old and 6-year-old branches of *C. arborescens* (n=20). P value after t Student test.

Age of branches	2-year-old	6-year-old
Minimal – maximal number of fusiform initials in one storey	1 – 11	3 – 31
Mean number of fusiform initials in one storey	5.0	12.4
Variance	6.31	45.73
P value		<0.001
Relative change in number of fusiform initials in stories [%]		149
Circumference of cambial cylinder [mm]	9.4	15.7
Relative increment of cambial circumference [%]		67
Calculated mean tangential area of fusiform initial [ $\mu\text{m}^2$ ]	1335.6	1382.9

Tab. 3. The relative length and the frequency of anticlinal divisions in 2-year-old and 6-year-old branches of *C. arborescens* (n=100).

Age of branches	2-year-old	6-year-old
Number of anticlinal divisions with relative length below 70%	12 (60%)	1 (5%)
Number of anticlinal divisions with relative length equal to 70 % or longer	8 (40%)	19 (95%)
Frequency of anticlinal divisions [%]	12.5	2.3
Mean relative length of anticlinal divisions [%]	45.0	80.0
The absolute dimension of dividing wall ( $\mu\text{m}$ )	53.88	94.24

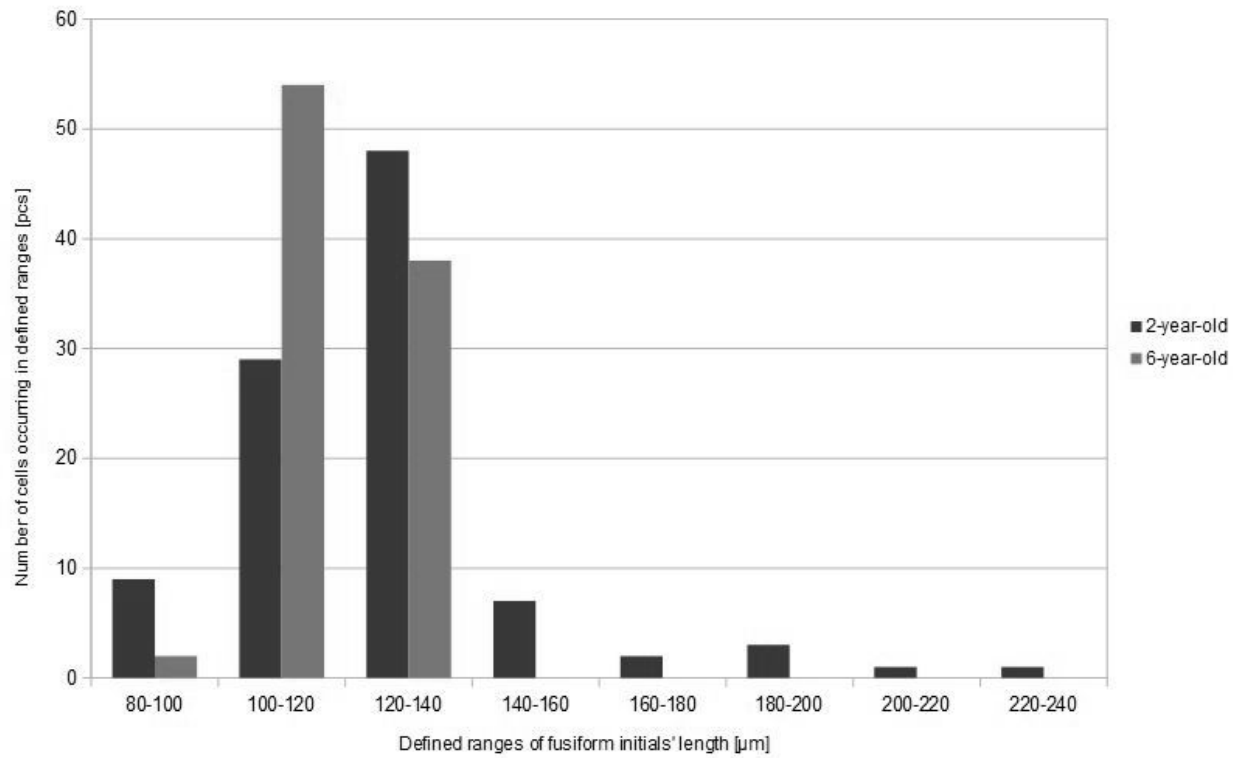


Fig. 1. The comparison of the number of fusiform initials occurring in eight defined ranges of initials' length in 2 and 6 years old branches of *C. arborescens*.

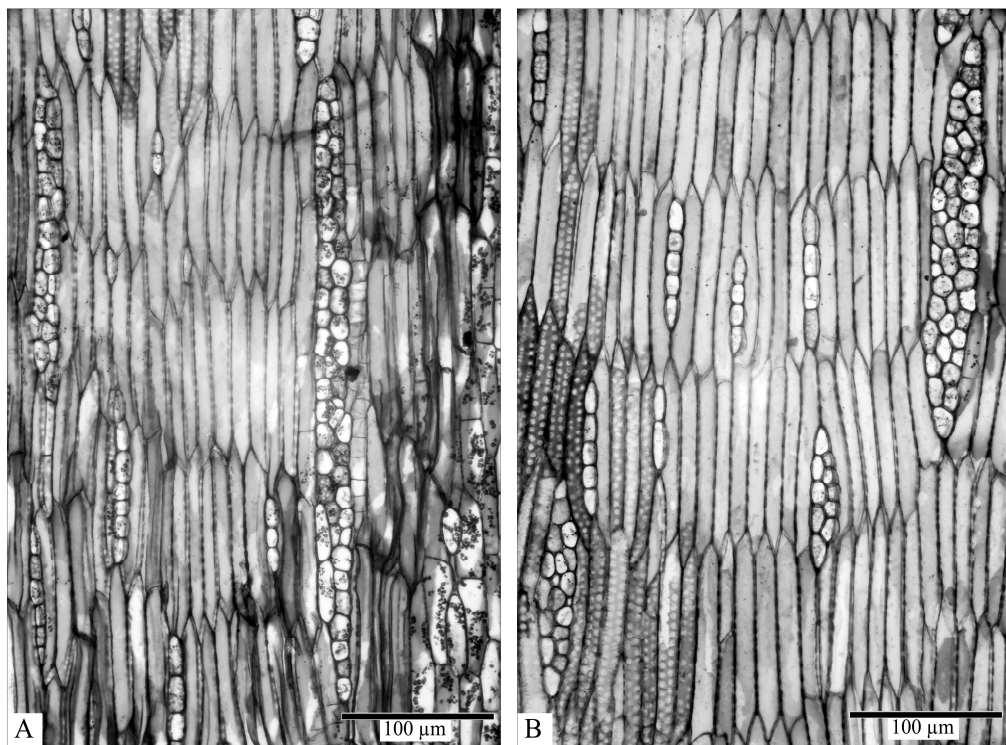


Fig. 2. The storied pattern of fusiform initials presented on tangential sections of *C. arborescens* branches: **A)** 2-year-old, **B)** 6-year-old.

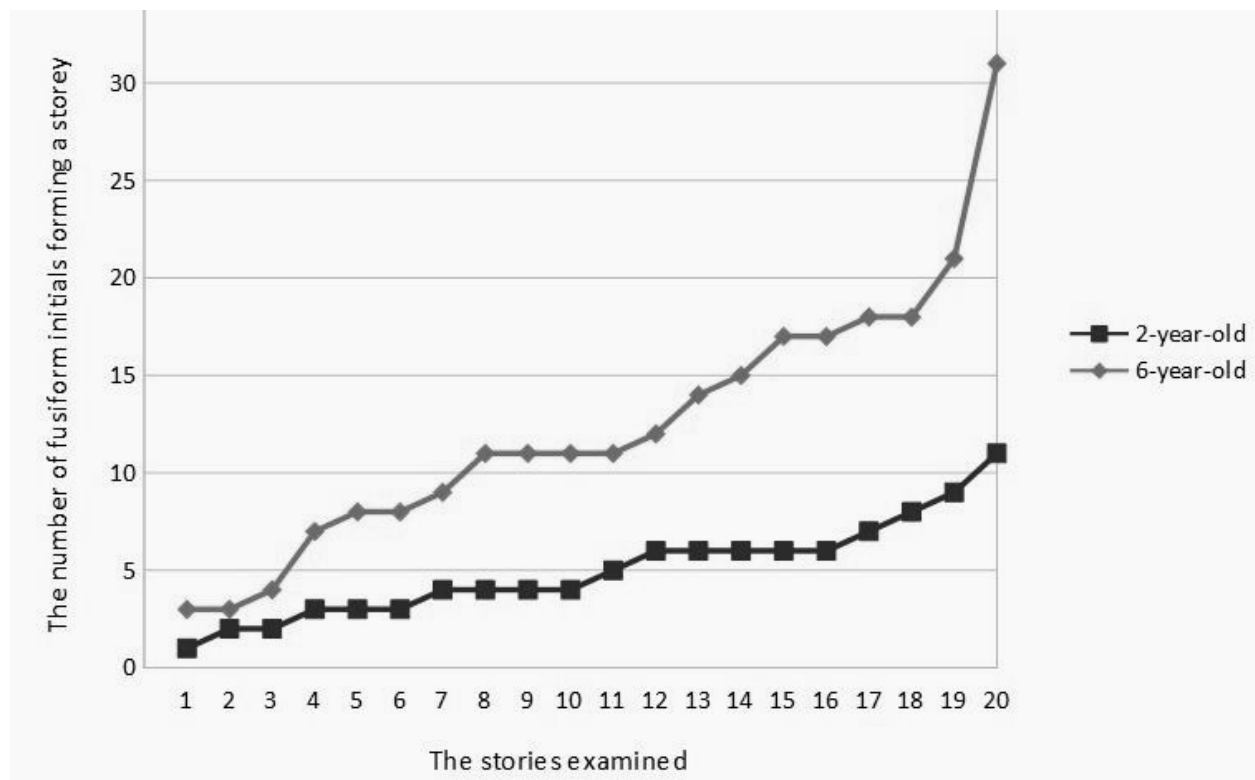


Fig. 3. The comparison of the number of fusiform initials forming stories in 2-year-old and 6-year-old branches of *C. arborescens*. The results have been sorted by increasing number of fusiform initials forming a single storey, for each branch's age separately.

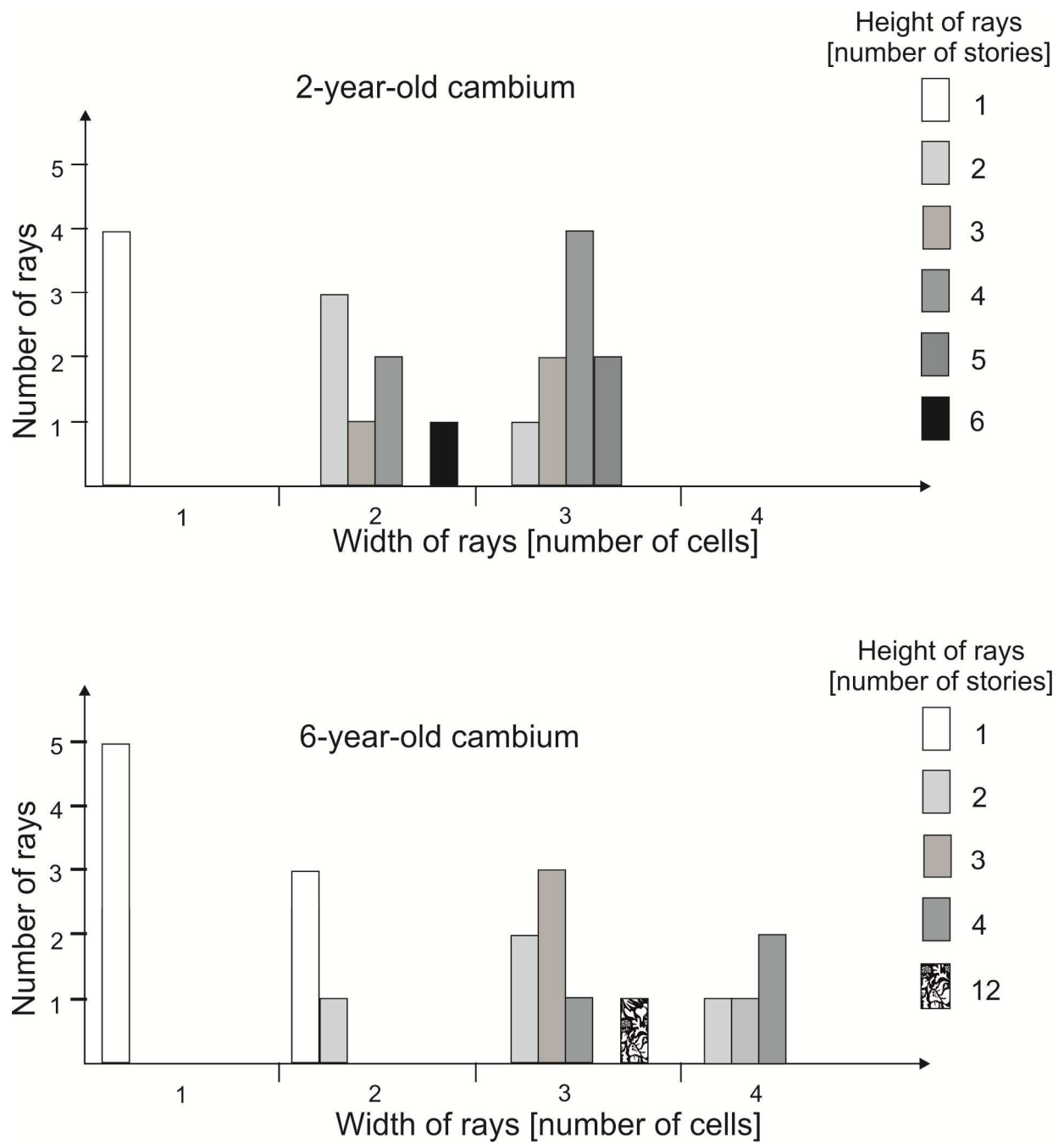


Fig. 4. The pattern of rays in 2-year-old and 6-year-old branches of *C. arborescens*.