RELATED TO PHYLLOTAXIS INTERLOCKED SYSTEMS OF VASCULAR SYMPODIA AND CORTICAL RESIN CANALS IN *ABIES* AND *PICEA* SHOOTS

BEATA ZAGÓRSKA-MAREK, ALICJA BANASIAK

Institute of Botany, Wrocław University
Kanonia St. 6/8, 50-328 Wrocław, Poland
e-mail: beata@biol.uni.wroc.pl

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ABSTRACT

We report on some new aspects of the internal architecture of conifer shoot, proving that it is even more strongly related to phyllotaxis than was initially thought. The number of vascular sympodia in an open system of *Abies* and *Picea* shoots is correlated with the number of cortical resin canals. Both numbers are the consecutive members of the phyllotactic series, which defines the phyllotactic pattern of the shoot. This means that the concentric systems of alternately oriented vascular sympodia and resin canals are interlocked, following the course of superficial opposite parastichies. Such pattern of inner architecture most likely contributes to the mechanical strengthening of the shoot. It also provides a new example of hetero-genetic induction in plant morphogenesis because the development of orderly spaced resin canals is dependent upon earlier differentiation of vascular system. A rapid method of assessing the number of vascular sympodia becomes available by means of counting the number of discrete cortical resin canals visible on the cut surface of the shoot sectioned transversely. Despite being set up on the smaller radius, the number of sympodia is always one step higher in a phyllotactic series than the number of canals.

KEY WORDS: phyllotaxis, vascular sympodia, resin canals, conifer shoot, plant morphogenesis, hetero-genetic induction.

INTRODUCTION

The major principle of morphology of the primary open vascular system in conifers with helical phyllotaxis is that the number of vascular sympodia belongs to the phyllotactic series, which characterizes organography of the shoot (Barthelmess 1935, Sterling 1945, Namboodiri, Beck 1968, Larson 1977). In shoots with the main Fibonacci pattern, the possible numbers of sympodia, called also the vascular parastichies, are: 5, 8, 13, 21, etc. (Fig. 1). Each sympodium in this case is composed of leaf traces that belong to every 5th, 8th, 13th or 21st leaf. In shoots with other patterns of phyllotaxis such as Lucas or main bijugy, the sympodia numbers are correspondingly: 4, 7, 11, 18... or 4, 6, 10, 16...

The selection of the number of sympodia within a given series depends upon how the leaf traces connect with one another. The number is a measure of the anatomic diameter of the shoot (Sinnott 1936; Bucholz 1938). It is high in large, vigorously growing shoots of the leading branches and becomes smaller in higher order shoots from the lower parts of the crown that are often overshadowed and thin. Such primary structure of the shoot vascular pattern is related to the contacts between leaf primordia, which existed in the shoot apical meristem at the time of formation of the particular portion of the shoot. They in turn are established according to the

ratio between the shoot apical meristem circumference and primordia sizes.

Determining the number of vascular sympodia is very important: the whole architecture of the internal structures can be described afterwards, but it is not easy! Hejnowicz (1980) pointed out, that different numbers of sympodia might theoretically serve the same pattern of leaf distribution (Fig. 2). Thus, contrary to some opinions well established in the botanical literature (Kirchoff 1984), we cannot tell for sure from the superficial phyllotactic pattern of the shoot, how many vascular parastichies are present inside. It is especially true in the case of conifer shoots where, as demonstrated elegantly in computer simulations (Cannel, Bowler 1978), the extent of elongation, which in nature is strongly dependent upon the action of environmental factors, affects the pattern of superficial conspicuous parastichies.

Although then, the processes of differentiation in elongating shoot may alter the visible contacts between developing leaves (the lower order parastichies appear with elongation), they do not however change the pattern of vascular connections established earlier. This is an entirely different situation from that, in which the contacts between primordia at the growing shoot apex change ontogenetically. This of course immediately affects the architecture of the primary vascular system in a newly formed portion of the shoot.

Fig. 1. Semi-schematic representation of architecture of the split-open primary vascular system in conifer shoots as it is related to leaf distribution (black dots) and to connections between leaf traces. In all three cases chiral configuration of ontogenetic helix in a Fibonacci phyllotactic pattern is the same (Z), though not the same is the pattern of contact parastichies (from left to right: 2s:3z, 3z:5s and 5s:8z). The sympodia number and orientation (from left to right: 8 Z, 13 S and 21 Z) depend on how leaf traces connect, making a singular vascular sympodium. The number of vascular bundles on a particular level of the shoot, exemplified by the upper edge of each diagram, is higher than the number of sympodia. It is because the departing leaf traces, before entering the leaf, diverge slightly to the left or to the right in the plane of the system.

The presence of a definite number of vascular sympodia means that selection of only one of the two numbers from the opposed parastichy pair (Adler 1974) has been made in a given phase of shoot ontogeny. This, we think, is the only material rationale that can be found behind the Plantefol's theory of foliar helices (Plantefol 1948). The fact, that only one of two sets of alternately oriented parastichies is paralleled by vascular parastichies (sympodia), **makes one of them more important**. With the number of sympodia established, also their orientation becomes fixed (Fig. 1). This choice means that the chiral configuration of primary vascular system architecture in a shoot is automatically determined. This may not be without an effect on biomechanics of the shoot growth.

From Suzuki's monographic study it can be deduced that another shoot pattern, where Fibonacci numbers can be found in conifers, is the pattern of cortical resin canals (Suzuki 1979a, b). The results of our preliminary studies also confirmed that the number of the canals in such species as *Abies* or *Picea* often belongs to the Fibonacci series. Are these two patterns related? Answering this question would require thorough anatomical analysis of the primary structure of coniferous shoots, with special attention paid to the number and mutual spacing of vascular sympodia and resin canals. This was the main aim of our research.

The number of vascular sympodia is difficult to determine, even on the anatomical transverse section, because the departing leaf traces increase the number of vascular bundles, present in the vascular ring at the given level of the shoot (Fig. 1). Another difficulty comes from the fact, that the neighboring bundles often come together so close that it becomes impossible to separate them and recognize whether they represent one only or two functional units of the shoot's anatomical architecture (Fig. 3). Only a very time consuming analysis of very long series of sequential transverse sections may resolve the question. In contrast, resin canals are discrete and quite distant one from another along the shoot circumference. Counting them on one section only does not pose any problem. If the two patterns were related, as already suspected (Zagórska-Marek, Tomlinson 1987), we would receive a relatively easy method of determining the number of vascular sympodia at each level of the shoot.

Fig. 2. Illustration of how the same leaf distribution (Fibonacci) with the same contact parastichy pattern (2s:3z) may be served by more (8 – left case) or less (5 – right case) numerous, Z- or S-oriented sympodia. Only one sympodium, selected arbitrarily, is drawn, to show how leaf traces connect in each case. Base of triangle refers to circumference of the split-open shoot, the other two sides – to contact parastichies.

MATERIAL AND METHODS

The shoots of conifers such as *Abies balsamea* (L.) Mill., *Abies alba* Mill., *Picea abies* (L.) Karsten were collected from the trees growing in the forests of Canada's New Brunswick Province and in Poland's Lower Silesia. The selection has been made with regard to their phyllotaxis and to their anatomical diameter which, as explained earlier, was dependent upon the shoot position in the crown of the tree. All the species were characterized by very distinct pattern of cortical resin canals.

Shoots not fully elongated, approximately 2 cm long, were fixed in acetoalcohol, dehydrated, embedded in paraffin and sectioned with a rotary microtome into series of sequential transverse sections 10 µm thick. Some shoots were sectioned from the bottom upward, the others in opposite direction. On the slides sections were therefore observed, depending upon

Fig. 3. *Abies balsamea* – photographs of transverse sections of two shoots, both with Fibonacci phyllotaxis, but much different in size. The concentric pattern is seen in both shoots made of the inner ring of vascular bundles and the outer ring of conspicuous cortical resin canals. In the right lower shoot the bundles departing to the leaves are flanked each by two resin canals. These usually split producing a derivative pair of canals entering leaf together with the leaf trace. Photographs were taken at different magnifications. The measure of this difference is the size of parenchyma cells.

the series, from the bottom side or from the upper side of the section. Sections were stained with safranin and fast green, dehydrated and closed in Canada balsam. They were analyzed under light microscope, when necessary also in polarized light, to reveal xylem width in the bundles for their better identification. Some images of these sections were prepared with use of camera lucida in a form of drawings, others were created, processed and stored on SGI Workstation INDY (Silicon Graphics, Mountain View, California, U.S.A.), connected to an Olympus BX50 microscope (Olympus Optical CO., LTD, Tokyo, Japan). On serial drawings or printed images the vascular bundles were numbered and identified for every section. It facilitated the subsequent 3D reconstruction of their architecture in the shoots. A similar procedure has been applied to the cortical resin canals.

RESULTS

Phyllotaxis

The type of phyllotactic pattern was determined in each shoot on the basis of contact parastichy pair formula (Zagórska-Marek 1985, 1994). The three most common in conifers helical patterns have been represented in the investigated material. According to their phyllotaxis the shoots were ascribed to three groups: I. main Fibonacci, II. Lucas, and III. main bijugy.

In each group the shoots, depending upon their thickness, could be again divided into three classes: thin, medium and thick. The expression of phyllotaxis (sensu Zagórska-Marek

1994) was different in these classes. Thin Fibonacci shoots had for instance, the expression of phyllotaxis 2:3 or 3:5, medium – 3:5 or 5:8 and thick – 5:8 or 8:13. (Figs 4, 5). As the elongation of the shoots was advanced although not completed, these numbers reflected only approximately the shoot's true anatomic diameter most accurately defined by the number of vascular sympodia and diameter of the vascular ring on the cross-section of the shoot (Fig. 6)

Resin canals

In both *Abies* species as well as in *Picea abies* cortical resin canals form the conspicuous ring outside the vascular cylinder (Fig. 3). They split regularly branching to the leaf. The split takes place simultaneously in two canals flanking the vascular trace departing to the leaf and forms a derivative pair of parallel canals entering this leaf together with the vascular bundle. Sometimes, the resin canal destined to the leaf is formed anew, having in this case no connection with the main cortical canal, which does not split. The initiation of a new canal, however, always takes place in a close vicinity of the canal that would normally split, outside of it.

In all groups and classes of investigated shoots the number of permanent resin canals, those that remain in the ring, always belonged to the phyllotactic series characterizing phyllotaxis of the shoot and was higher in thicker shoots than in thinner ones (Figs 5, 6, 7).

The fixation of that number was so strong, that if one of these canals terminated at a certain level of the shoot, ending blindly in the parenchyma of the cortex, a new one appeared

Fig. 4. *Abies balsamea* – photographs of the surface of four, side-viewed shoots, differing in thickness and not fully elongated. The needles have been removed by breaking them off. Phyllotaxis of all these shoots is the main Fibonacci. Its expressions are given below each photograph.

immediately in exactly the same position. Thus, despite discontinuity in a particular resin canal course, the overall architecture of the system remained unchanged.

The principle that the number of resin canals is the same as one of the numbers of possible parastichies in a particular shoot, together with the fact that between two neighbouring canals the leaf trace departs, means that the resin canals are inclined to the shoot longitudinal axis. They parallel one set of the parastichies thus their orientation must be the same as that of these parastichies.

Vasculature

It has been confirmed in our study that the number of sympodia in conifer shoot with helical phyllotaxis is always the

same as one of the numbers of the opposed parastichy pair. This was true in all the cases of the investigated shoots regardless the type of phyllotactic pattern. In thin shoots the number was smaller than in thick ones (Figs 5, 6, 7). The highest number of sympodia – 55, has been encountered in a leading shoot of *Abies balsamea* characterized by the main Fibonacci pattern. This shoot is shown on the bottom photograph on Fig. 3. The number of permanent resin canals in it is 34. The smallest number of sympodia in the same group was 13. Orientation of sympodia was the same as that of corresponding parastichies on the surface of the shoot.

In all the cases the number of vascular parastichies was one step higher within the phyllotactic series than the number of resin canals (Figs 5, 6, 7, 8). For instance, in the shoot with

Fig. 5. *Picea abies* – drawings made from transverse sections (bottom side up) of three different shoots, all with the main Fibonacci pattern. The opposed parastichy pairs were in these shoots from left to right: 2s:3z, 3s:5z and 5s:8z.. The shoots differ in number of cortical resin canals (Roman numerals) as well as in the numbers of vascular symphodia (Arabic numerals), thus they have different anatomic diameters. Arrows indicate the direction of leaf trace departure. Here and on subsequent Figures all symbols are used in the same way and the bar length is always 0.25 mm. Only permanent canals are shown in all these Figures.

Fig. 6. Drawings made from transverse sections (upper side up) of two different shoots (*Picea abies*– left, *Abies balsamea* – right) both having main bijugate phyllotaxis of the same expression (4s:6z) on the surface but differing in anatomic diameter, which is reflected in different numbers of vascular symphodia: 16 and 26, and of resin canals: 10 and 16.

Fig. 7. *Picea abies* – drawings made from transverse sections (upper side up) of two different shoots, characterized by Lucas phyllotaxis, but having different expression of the pattern: 4z:7s in the left shoot and 7z:11s in the right one. Inside of these two shoots the different numbers of vascular symphodia: 18 and 29, and of resin canals: 11 and 18, are present.

Fig. 8. *Picea abies* – series of drawings made from consecutive transverse sections through the thin shoot sectioned from the bottom upward. Each section is seen bottom side up. The series encompasses one full cycle of reiterative changes in shoot vascular architecture. The cycle is made of two consecutive departures of leaf traces building the same one sympodium, labeled as No. 1 and marked black. The departure of the first leaf trace, making selected sympodium, shown on A, takes place between canals VIII and I. The departure of the next leaf trace, shown on L – between canals VII and VIII. This means that sympodia and canals do not parallel each other longitudinally. The expression of the main Fibonacci phyllotaxis in this shoot was 2s:3z. The numbers of sympodia and resin canals are different: 13 and 8. They represent two consecutive members of the main Fibonacci series. The sympodia and canals parallel the course of superficial opposite parastichies making this way the interlocked pattern. The number of a section in the series is given on the top of each drawing. Further description is the same as on former Figures.

Fig. 9. Diagrammatic reconstruction of the interlocked systems of 10 vascular symphodia and 6 resin canals in conifer shoot with helical bijugate phyllotaxis. Both systems do not interrupt each others continuity. Departing leaf traces (black dots), running perpendicularly to the shoot surface, do not encounter resin canals on their way.

Lucas pattern, in which the number of symphodia was 18, the number of resin canals was 11, in a shoot with bijugate pattern and 16 symphodia the number of resin canals was 10, etc.

In effect, the departure of two successive leaf traces belonging to the same symphodium took place between two different pairs of resin canals. Three consecutive canals, neighboring each other along the shoot circumference, formed these two pairs (Fig. 8). Consequently, between the same pair of canals the traces of neighboring symphodia always departed.

The consecutive numbers from the phyllotactic series refer to alternately oriented parastichies. The succession of these numbers in two, related to the phyllotaxis systems, present in the internal structure of the conifer shoot, means that the orientation of linear vascular symphodia and resin canals in two concentric rings, they form in the shoot, is opposite. This arrangement of resin canals assures their continuity, which does not have to be disrupted by departing leaf traces protruding through the cortex to the surface of the shoot (Fig. 9).

DISCUSSION

The number of permanent resin canals in the stem cortex of *Abies* and *Picea* is correlated precisely with number of vascular symphodia and provides a rapid method of assessment of the latter. Both the numbers belong to the phyllotactic series, they represent the consecutive members of the series and the number of resin canals is always lower, which means that it precedes the other number in the series.

This discovery has three aspects. The first applies to biomechanics of the shoot. The opposite orientation of vascular symphodia and resin canals means strengthening the shoot by two interlocked systems of alternately oriented internal structures. Such pattern resembles the interlocked grain in secondary growth of stems of such trees as *Platanus* or African mahogany *Entandrophragma cylindricum* or the opposite orientation of microfibrils opposite in consecutive layers of the plant cell wall (Zagórska-Marek 1984, 1995). This is worth of pointing out that because in helical phyllotaxis the angles of opposite parastichies inclination to the shoot longitudinal axis are unequal and the same applies to the inclination of two in-

ternal systems discussed. Being of "lower order" than vascular symphodia the resin canals are inclined to the stem axis at a higher angle and in opposite direction than of the symphodia. There is a possibility that due to this fact a vector of possible twisting forces exists in a growing shoot. Of course, there is the question how stiff the system of resin canals is. It seems that there is a certain pressure of resin within the canals: it rapidly exudes from the lumen of the canal, the integrity of which has been destroyed by the cut. This may support the hypothesis that resin canals function also as mechanical units of the shoot.

The second aspect is that a new fascinating example of heterogenetic induction (Lang 1965) in plant morphogenesis has been found. As the pattern of vascular strands develops first in ontogeny, we postulate that this is the one, which induces differentiation in the adjacent cortex, leading to the appearance of the regular pattern of resin canals. From the results that will be published in a separate paper, we know that when the pattern of vascular symphodia changes ontogenetically in a shoot, the number of resin canals adapts to the change. Thus indeed, there is a very strong relationship between these two different systems. It is interesting that the pattern of vascular symphodia does not copy itself exactly in adjacent tissue, which would be quite possible: the ring of resin canals is formed on the bigger radius than the ring of more numerous vascular symphodia. It rather modifies the other tissue differentiation in a way that leads to development of mechanically strengthened shoot.

The third aspect is very practical. It is the possibility of screening a large number of shoots without having to do very time consuming anatomical analysis (serial sectioning). It is especially exciting in light of the fact that even in the fresh cross-sectioned shoots the resin canals become quickly visible and can be counted under binocular. This is facilitated by the pronounced, slowly increasing in size, resin droplets, which quickly appear on the cut surface of the shoot. This method proved to be particularly useful in our further studies on the related to phyllotaxis architecture of the coniferous shoot.

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ZAPLECIONY WZÓR FILOTAKTYCZNY SYMPODIÓW WASKULARNYCH I KANAŁÓW ŻYWICZNYCH W PĘDACH JODŁY I ŚWIERKA

STRESZCZENIE

Architektura wewnętrznych struktur pędu rośliny szpilkowej jest silnie związana z filotaksją pędu. Liczba sympodiów waskularnych oraz liczba korowych kanałów żywicznych są kolejnymi wyrazami ciągu matematycznego odnoszącego się do możliwych połączeń pomiędzy liśćmi (parastych). Oznacza to, że sympodia i kanały, ułożone na przekroju poprzecznym przez pęd w dwóch koncentrycznych pierścieniach, są w przestrzeni trójwymiarowej przeciwnie zorientowane (podobnie jak powierzchniowe parastyche kontaktowe) tworząc wzajemnie zapleciony układ. Przyczynia się to niewątpliwie do znacznego wzmocnienia rosnącego pędu pod względem mechanicznym. Liczba kanałów w ciągu matematycznym jest niższa od liczby sympodiów, mimo że tworzą one pierścień zewnętrzny o większym promieniu niż pierścień waskularny. Oznacza to, że kanały są rozmieszczone w pierścieniu luźniej niż wiązki i jako struktury anatomiczne mają charakter bardziej dyskretny – łatwo je policzyć. Możemy w ten sposób pośrednio uzyskać, dzięki wspomnianej zasadzie, informację o liczbie a więc i o nachyleniu sympodiów. Cechy te, dotyczące architektury pierwotnego systemu waskularnego nie są takie same w pędach o różnej średnicy anatomicznej pomimo identycznego jakościowo wzoru filotaksji. Zależność obu systemów jest nowym, nieznanym dotąd przykładem zjawiska indukcji heterogenicznej w morfogenezie roślin. System waskularny, różnicujący się jako pierwszy pod wierzchołkiem pędu, steruje w nieznanym bliżej sposób różnicowaniem systemu korowych kanałów żywicznych.

SŁOWA KLUCZOWE: filotaksja, sympodia waskularne, kanały żywiczne, szpilkowe, morfogeneza roślin, indukcja heterogeniczna.