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Ontogenesis and structure of phelloid in *Viburnum opulus L.*

MARZENA WACOWSKA, JADWIGA A. TARKOWSKA

Laboratory of Plant Anatomy and Cytology, Warsaw University, Krakowskie Przedmieście 26/28, 00-927 Warszawa, Poland

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Abstract

The ontogenesis and structure of phelloid cells was studied in the cork tissue of *Viburnum opulus* L. Phelloid was found to form as the result of division of various cells of the phellogen zone (from phellogen proper to subepidermal cells). There are quantitative differences in the phelloid which forms characteristic thickenings in its inner tangential walls in one- and may-year-old stems. These thickenings contain pectin compounds, cellulose and lignin but no suberin. Preliminary observations in the electron microscope did not allow identification of phelloid cells earlier than in the period when thickening of their walls begins. The strong contrast staining of protoplasts in these cells is noteworthy.

INTRODUCTION

In the periderm of stems and roots of some gymnosperms and dicotyledonous plants phellem comprises, beside typical cells with suberised walls, also those with a different structure. They may be single or in continuous layers called generally phelloid. The latter may be thin-walled (e.g. *Betula alba*), thickwalled (e.g. *Liriodendron tulipifera*), sclerenchymatous or even of the character of stone cells, with cellulose walls or lignin-saturated ones to various degrees (Metcalfe and Chalk 1950).

Phelloid is rather widespread, it occurs in many species belonging to various families, in cork in various proportions and may be distributed in various ways. The presence of phelloid or its structure (similar type of structure) do not seem to be specific for certain definite systematic plant groups. The role of phelloid is not clear. Thickwalled phelloid is an additional protection for the underlying tissues, and thin-walled phelloid layers (particularly in rhytidome) facilitate the processes of cracking and falling off of older parts of the bark as for in-

stance in *Betula alba* (Mühldorf 1926). It is known that in the discussed type of cells tannins may be accumulated (phlobaphene phelloid), terpene compounds, for instance in *Betula alba* (Hegnauer 1964, Kohlmünzer 1980) or mucilages in some desert plants (Solereder 1908).

In spite of the common appearance of phelloid, detailed data are lacking in the literature on its ontogenesis, structure and function. This problem seems interesting and of more general significance since phelloid, like phellem, and phelloderm is formed as the result of activity of the same phellogen. The question is, what factors cause the differentiation of cells different from the surrounding ones at definite but different sites in various plants.

MATERIAL AND METHODS

The object of study were one-year- and many-year-old stems of *Viburnum opulus* L. (*Caprifoliaceae*).

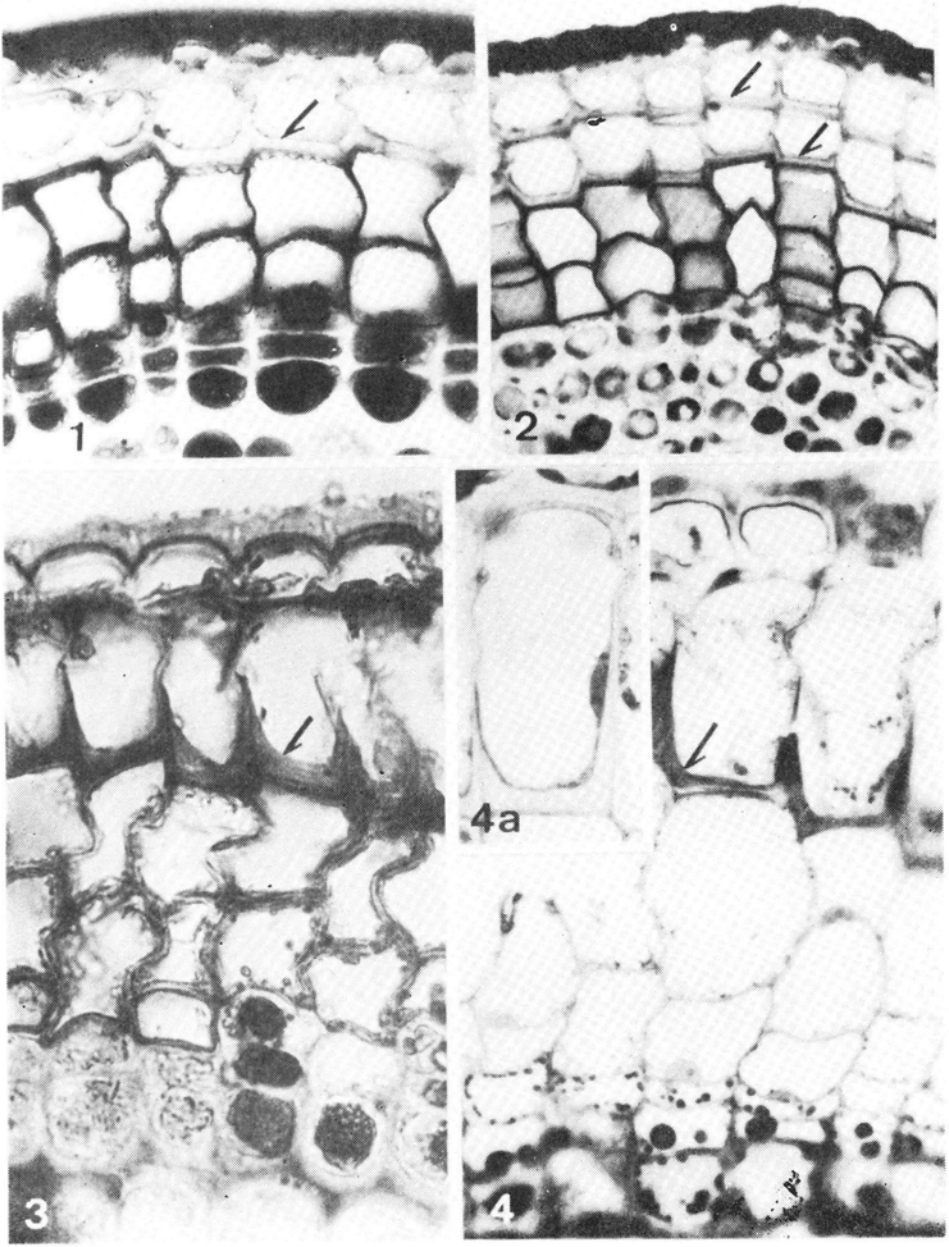
The observations were made in a light microscope (LM) and preliminarily in an electron microscope (EM). For LM examination (freehand and microtome sections in paraffin) fresh material was used or fixed in chromacetoformalin (CrAF 0.5 : 1 : 20). The microtome sections were stained mainly with iron haematoxylin after Heidenhain (Gerlach 1972).

The chemism of the phelloid cell walls was studied with the application of routine specyfic staining and microreactions and also in polarised light. On freehand sections the presence of starch in the periderm cells was tested with Lugol solution and that of tannins (in the reaction with hydrated iron chloride).

The material for semithin and ultrathin sections for the EM was fixed in Karnovsky solution (1965), pH 7.2. Semithin sections 0.25 to 0.50 μm thick were stained with toluidine blue (Williams et al. 1973) at room temperature. Ultrathin sections were cut on an LKB (Sweden) ultramicrotome, contrasted by the method of Reynolds (1963) and inspected in the EM Tesla BS 500.

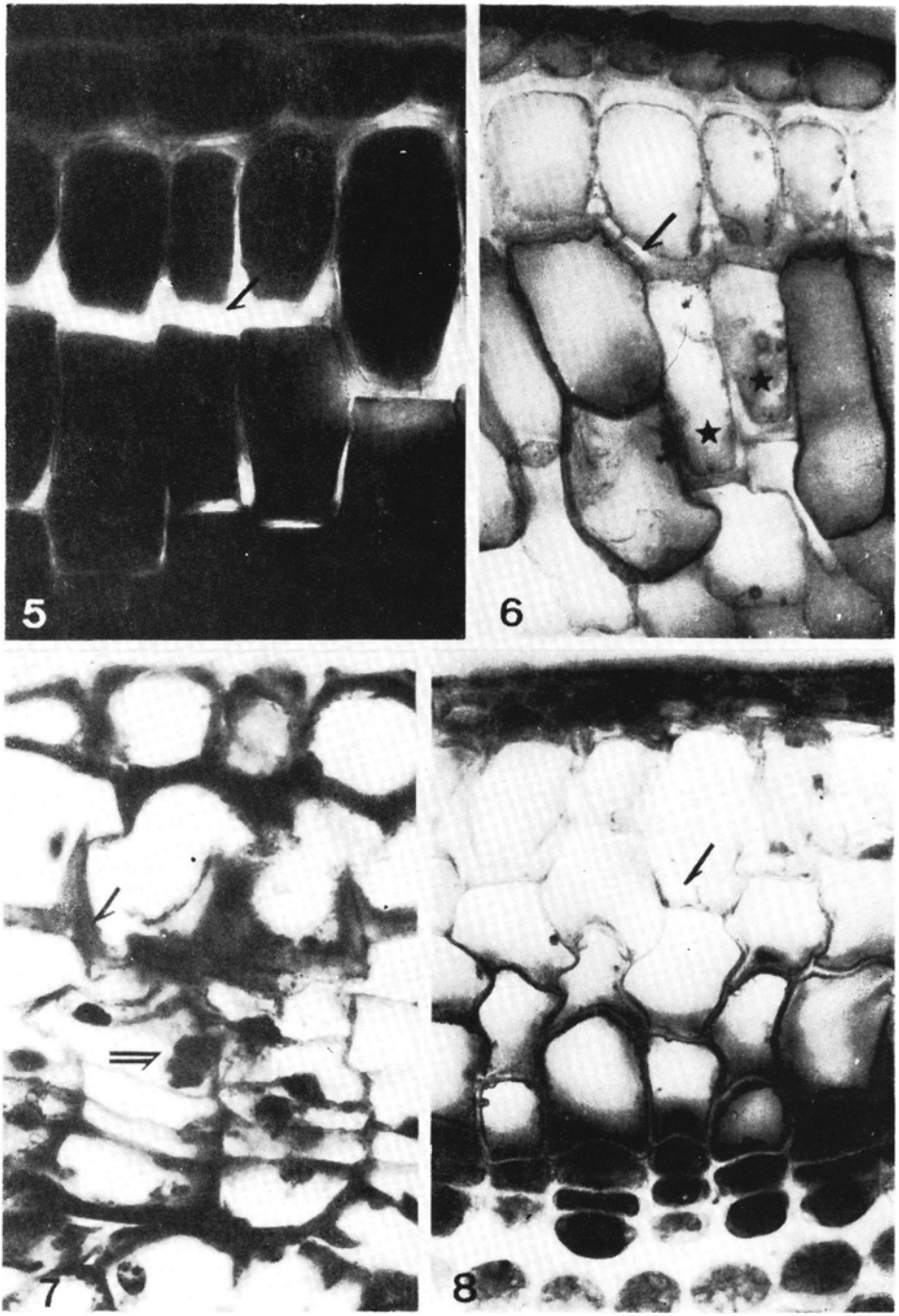
RESULTS

The young one-year-old stem of *Viburnum opulus* is at first covered with epidermis with a thick cuticle and later with peridermis initiated in the subepidermis. The innermost layer of peridermis consists of phelloderm — cells of parenchymatous character with greatly thickened walls bordering on the primary cortex parenchyma (Figs. 1, 2 and 8). There is a single layer of phellogen proper and several layers of



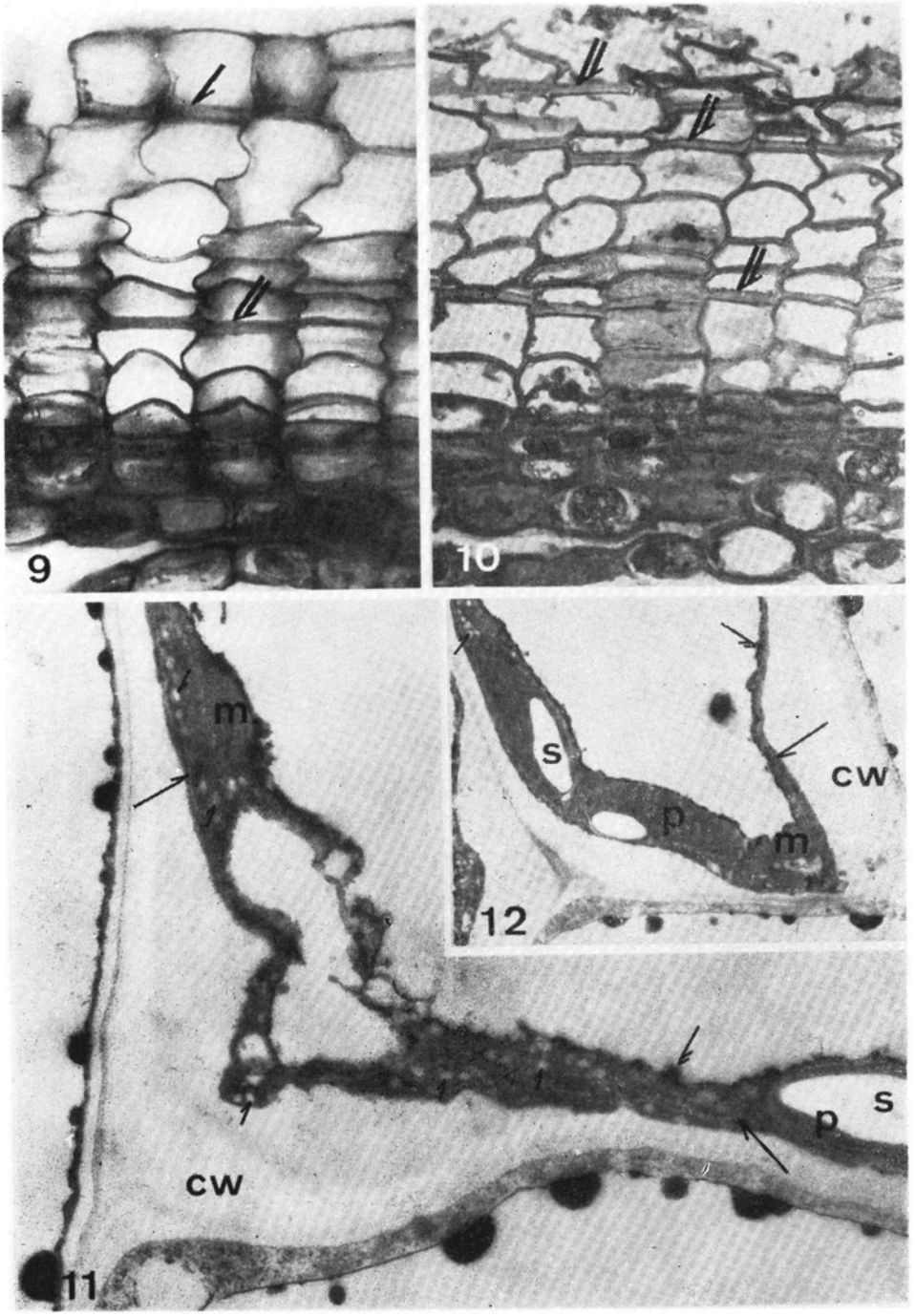
Figs. 1-4. Cross sections through periderm of one-year-old stems. On all micrographs horseshoe-like thickenings of the inner tangential phelloid walls are visible (arrows)

Fig. 1. One-layer phelloid. The innermost periderm layers contain tannin (dark cell interior). CrAF, Sudan III. × 280. Fig. 2. Two-layer phelloid. Fresh material. Sudan III. × 220. Fig. 3. One-layer phelloid. Tannin granules in internal part of periderm. CrAF, fuchsin with picric acid. × 350. Fig. 4. One-layer phelloid. Marked difference in amount and size of tannin droplets in periderm. Thin cytoplasm layer in phelloid cells along wall (detached from cell walls during fixation). × 400. Fig. 4a. Phelloid cell with very thin cytoplasm layer and cell nucleus. Layered structure of thickened cell wall is visible. × 550. Figs. 4. and 4a. Karnovsky, semithin sections, toluidine blue



Figs. 5-8. Cross sections through periderm of one-year stems. Arrows show characteristic horseshoe-like thickenings of inner tangential walls of phelloid cells

Fig. 5. CrAF, polarised light. × 450. Fig. 6. One-year phelloid. Additional phelloid cells (asterisks) in radial periderm cell rows. CrAF, Sudan III. × 320. Fig. 7. Mature one-layer phelloid. Telophase (double arrow) in lower situated cell. CrAF, iron haematoxylin. × 450. Fig. 8. One-layer phelloid. Tannin in the inner periderm layers. CrAF, $FeCl_3 \times 6H_2O$. × 320



Figs. 9, 10. Cross sections through periderm of many-year-old stems

Fig. 9. Two-year stem. Horseshoe-like thickenings of phelloid walls in periderm of first year (single arrow), batten-like in second year (double arrow). CrAF, alum carmine with metyl green. × 300. Fig. 10. Four-year-old stem. Three phelloid layers of successive years with batten-like thickenings (double arrows). CrAF, iron haematoxylin. × 280

Figs. 11, 12. Cross sections through fragments of maturing phelloid cells. p — plastids, s — starch grains, m — mitochondria, cw — thickened cell wall, long arrows — very narrow and long ER cisterns, short arrows — numerous minute vacuoles, arrows with double heads — tannin molecules at boundary between tonoplast and vacuole. Fig. 11 — × 20 000; Fig. 12 — × 10 000

phellem (usually 4-5) outside the phelloderm, the walls of the 1-2 youngest cell layers are not suberised. The outermost part of the phellem (immediately under the epidermis) consists of phelloid with the characteristic thickenings of the tangential walls in the shape of more or less flat angular horseshoes (Figs. 1-3 and 5-8, arrows).

Phelloid is not only of unequal breadth in the particular stems of the same plant, but even on the same level within one stem. It is formed of one (Figs. 1, 3-5, 7 and 8) or two (Fig. 2) cell layers. Deviations from the regular (layered) pattern are not frequent and appear in the occurence of "additional" cells in the deeper cork layer (Fig. 6, asterisks) or absence of phelloid cells in the continuous layer (in two-layer phelloid).

In the successive years of *Viburnum* stem growth in thickness new cork layers arise. At the boundary of annual increments only one phelloid layer differentiated, which, analogously as in the one-year stems is the outermost layer of each annual ring. In many-year stems phelloid persists usually only in the layers of the last three years, while older ones are desquamated. Beginning with the second year phelloid cells are more elongated (flat) in tangential direction to the stem circumference than in the first year and the thickenings resemble flat battens (Figs. 9 and 10, double arrows).

PHELLOID ONTOGENESIS

The young one-year stems in which the secondary protective system is just forming, are most convenient for observation.

The first division of subepidermal cells leads to phelloderm (inner cells) and primary phellogen formation (cells under the epidermis) in which the next periclinal division occurs. The third division, however, was noted both in the central cells, that is phellogen proper, and in the external cells at the epidermal border. The term "phellogen proper" means the cell layer lying directly over the phelloderm, independently from the development of the whole periderm both in one- and many-year-old stems.

Beginning with the formation of periderm rows consisting of at least four cells overlying one another, the subsequent layers are the result of division which may take place in layers from phellogen proper up to the subepidermal cells. The cells may still go on dividing when the characteristic thickenings are already forming in the phelloid (Fig. 7). It should be mentioned that divisions in phellogen proper cells were only sporadically observed.

In view of the fact that the periderm at the end of the first year of vegetation consists usually of six to seven cells and that phelloid lies always on the outermost periphery and since the division of all pri-

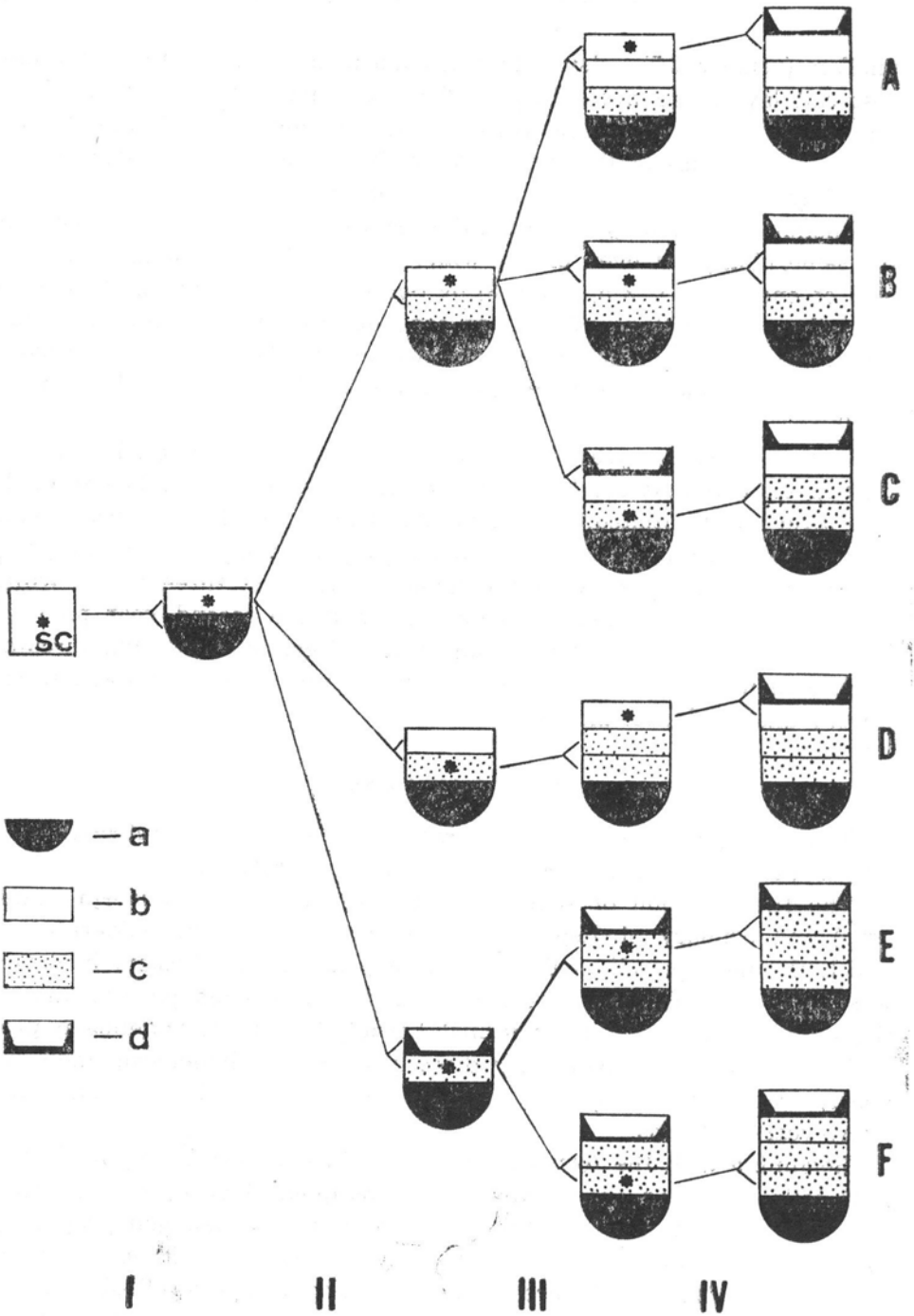


Fig. 13. Variants (A—F) of periderm development in one-year-old stem indicating at what moment of development and as the result of which division phelloid may be formed. I-IV — four successive cell divisions, * — dividing cells, sc — subepidermal cell, a — phelloderm cells, b — subepidermal cells formed after second division and their derivatives, c — central cells formed after second division and their derivatives, d — determined phelloid cells

mary phellogen-derived cells is possible (with the exception of the phelloderm) a diagramme (Fig. 13) was prepared. The diagramme ilustrates observed variants of periderm development. It indicates at what moment of development of secondary covering system and from which cell division phelloid may arise. This diagramme presents only the first four divisions. It is their smallest number observed in the first year of periderm development.

The results from the diagramme in Fig. 13 indicate that there may be three possibilities determining the cells of one-layer phelloid:

1. after the second division if the subsequent divisions do not occur in the cells adjacent to the epidermis — variants E and F;
2. after the third division when it takes place in the outermost cells of the forming periderm, but these cells do not divide any further — variants B and C;
3. after the fourth division occuring under the epidermis, apart from the sites of the earlier ones — variants A and D.

If, however, the mature phelloid is double-layered the division preceding phelloid cell formation gives two equivalent cells which both transform to phelloid, and not to a phelloid and cork cell (cases 2 and 3). The second more internal phelloid layer may form as the result of division of cells under the already determined phelloid (cases 1, 2 and 3).

PHELLOID CELLS STRUCTURE

LM and EM observations indicate that even young phelloid cells with incompletely developed walls have enormous central vacuoles and a narrow layer of cytoplasm with the nucleus (Figs. 4, 4a and 12). There is somewhat more cytoplasm in the cell corners where some few mitochondria (m) and plastids (p) usually with starch grains (s) are grouped (Figs. 11 and 12). Other organelles are difficult to identify, especially as the cytoplasm of phelloid cells has a marked tendency to overcontrasting during staining of ultrathin sections by Reynolds method. Within cytoplasm only numerous minute vacuoles are visible (Figs. 11 and 12, short arrows) and long but very narrow cisterns of endoplasmic reticulum (Figs. 11 and 12, long arrows). A great part of the vacuoles in the phelloderm and phellogen proper cells and those immediately above the latter in one or two phellem layers (with unsuberised walls) are usually filled with tannins (Figs. 1, 3, 4, 8, and 10). In the remaining part of the phellem and in phelloid cells tannins are seen only at the boundary of the cytoplasm and vacuoles. In the phelloid cells tannins are in the form of such minute drops that their presence is only revealed in the EM observations (Figs. 11 and 12, double-head arrows). They do not give any distinct colour reaction with iron salts in the LM (Fig. 8).

Particular attention was devoted to the structure and chemism of the thickenings of the phelloid cell walls. The layered structure of the horseshoe- or batten-shaped secondary thickenings of the inner tangential phelloid cell walls is visible in the LM. The radial walls (or their parts in the case of horseshoe thickenings) remain thin (Figs. 1, 6 and 9). In polarised light the thickenings are strongly luminescent (Fig. 5), more than the cellulose walls of parenchyma cells.

The thickenings of phelloid walls contain pectin substances, cellulose and lignin, but no suberin. At first the young cell walls consist of pectin and cellulose. Lignin appears when the thickenings begin to form and its amount gradually increases with thickening of the walls. Lignification starts and is more intensive at the middle lamella. Lignin in the radial walls is deposited only at the middle lamellae, the further parts of the walls consist of cellulose and pectin. The chemical composition of the cell walls of the remaining part of the periderm is different: the phelloderm, phellogen proper and one to three phellem layers immediately above the latter have cellulose walls, whereas the walls of the outermost (the oldest) phellem layers are suberised and give a distinct reaction for suberin.

DISCUSSION

Observations in the LM and EM indicate that during development of the periderm not only the phellogen proper cells may divide, but also its derivatives. It, therefore seems justified to call all the cells of the radial row of periderm capable of division the phellogen zone not phellogen. This would be an analogy to the cambium zone described by D e s p h a n d e and E v e r t (1970) in *Ulmus americana* and *Tilia americana*. These autors demonstrated that in both the investigated species there are seven to nine rows of overlying cells which do not differ from one another in their submicroscopic structure and each of them preserves the ability to divide.

The presence of a phellogen zone, and not of one layer of meristematic cells causes difficulties in the establishment of the phelloid mother cell and the division determining its formation. It is only known that it is not the last division before the end of periderm development in the given vegetation season. Dividing cells of the phellogen zone was still observed when distinct horseshoe-like thickenings could be seen in phelloid cells (Fig. 7). Phelloid itself may originate from various cells dividing at various stages of periderm development.

In the studied *Viburnum opulus* phelloid appears at the boundary of the annual phellem increments, thus it borders directly on cork proper formed in the previous year. Differences were found in the number

of phelloid cells and the shape of the thickenings in their walls in one- and many-year stems.

In the period of formation of the secondary covering system phelloid has one or two layers and the characteristic thickenings have the shape of angular horseshoes. In subsequent years only one phelloid layer forms with batten-like thickenings. The radial walls, however (whole or their fragments), remain unthickened. The characteristic thickenings of the tangential walls contain pectin compounds, cellulose and lignin. Suberin is not a component of these thickenings.

In the LM the internal stucture of phelloid cells does not differ significantly from that of the neighbouring cells of common origin with cork proper. Neither were significant differences found in the ultrastructure of phelloid and of the cells underlying it immediately. The latter meristematic cells are still capable of division and their ultrastructure does not differ from the already determined cork proper cells. No characteristic morphological structural features have been observed in the determined phelloid cells (in the initial stage of wall thickening) which would allow their identification.

An essential feature, as it seems, distinguishing phelloid cells in the EM is, however, the strong contrasting of their protoplasts. This was noted both in almost mature phelloid cells and in the course of their development when the characteristic wall thickening starts. Protoplasts of mature phelloderm also exhibit a stronger contrast than the neighbouring phellogen proper, although not as strong as phelloid. It would seem that this property of protoplasts is connected with different metabolic processes in the cells and their different physiological state. This may be a starting point for further investigations on the processes of cell differentiation. Results presented in this paper concern only preliminary EM observations, but not comprising cytochemical analyses do not allow final conclusions in this matter.

Acknowledgment

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Ontogeneza i struktura felloidu kaliny koralowej (Viburnum opulus L.)

Streszczenie

Badano ontogenezę i strukturę felloidu w tkance korkowej *Viburnum opulus* L. Biorąc pod uwagę wspólne pochodzenie felloidu i fellemu właściwego podjęto próbę określenia charakterystycznych cech strukturalnych pozwalających na zidentyfikowanie rozwijającego się felloidu lub jego komórek macierzystych.

Wykazano, że w pierwszym roku rozwoju perydermy felloid może być jedno- lub dwuwarstwowy, w następnych latach jest jednowarstwowy, ale zawsze jest on najbardziej zewnętrzną warstwą każdego przyrostu rocznego perydermy. Stwierdzono, że charakterystyczne zgrubienia wewnętrznych ścian stycznych felloidu zbudowane są ze związków pektynowych, celulozy i ligniny, natomiast suberyna nie wchodzi w ich skład. Felloid może powstawać w wyniku podziałów różnych komórek strefy fellogenu (od fellogenu właściwego aż do komórek subepidermalnych). Wstępne obserwacje w EM nie ujawniły cech strukturalnych, które pozwoliłyby na zidentyfikowanie komórek felloidu przed okresem, w którym rozpoczyna się grubienie ich ścian. Zwraca jednak uwagę silniejsze kontrastowanie się protoplastów różnicujących się komórek felloidu, co może mieć istotne znaczenie w dalszych badaniach.