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Contribution to the Exodermis in the Rootlets of *Fraxinus excelsior* L.

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Abstract. The exodermis of ash roots is initiated early in the apical meristem. When fully differentiated, it is composed of alternating "long" and "short" cells measuring approx. 70 × 25 × × 25 μm and 25 × 28 × 25 μm respectively. At a short distance from the apex, the long cells undergo structural and histochemical changes from a "primary" towards a "secondary" stage: an impermeable suberized lamella is formed, cellulose lamellae become impregnated by lignin, and the protoplast dies off. The short cells show a distinctly thickened outer wall ("cap") which is composed exclusively of cellulose, and possess abundant cytoplasm with a large nucleus. In the process of ageing the structure of short cells is not markedly affected. Following the early disintegration of rhizodermis, the exodermis works as the external protective layer of the fragile end-root. Its long cells fulfil the mechanical protection, the short cells serve as passage cells for solutions and gases, during the whole life-span of the rootlet.

A well defined exodermis is a characteristic feature of the primary end-roots of the ash (*Fraxinus excelsior* L.), forming a distinct layer just beneath the rhizodermis. The shape and structure of its cells differ profoundly from the neighbouring rhizodermis and cortex parenchyma. This also suggests a different function of the exodermal layer.

The types and structure of the exodermis were extensively studied by KROEMER (1903). According to his own nomenclature, the subrhizodermal layer is called "the interkutis", a name synonymized with "the hypodermis" and "the exodermis". Kroemer found four different types of exodermis: homogeneous exodermis (Einheitliche Interkutis) with one layer of long cells (1), or with several layers of long cells (2); heterogeneous exodermis with one layer of alternating short and long cells (Kurzzellen Interkutis) (3), or with one layer of alternating short and long cells accompanied by several layers of long cells (Gemischte Interkutis) (4). Kroemer described the characteristic features and development of long cells, distinguishing a primary stage (thin cellulose walls, absence of cutinization, and Casparian strips, protoplast with central vacuole, and small nucleus) and a secondary stage (walls with suberin lamellae, sometimes lignified, protoplast often dead).

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Similarly the structure of short cells was described (definite shape, formation of thickened outer tangential wall, a large amount of cytoplasm with a large nucleus, absence of suberization in cell walls, a sort of lignification of thickened "cap"). In a chapter dealing with the thickened outer wall ("Kappe", "cap") in short cells, Kroemer identified several types of thickening. In underground roots, the "cap" is built internally by the protoplast of the cell itself, the inner layer consists of pure cellulose, the outer layer is lignified. The "cap" occurs just beneath the apical meristem and, therefore, is considered to be a normal product of living cells, and not a feature of ageing or decreased permeability. In a comprehensive list of plant species possessing the exodermis in roots, *Fraxinus excelsior* is classified as a plant with the heterogeneous exodermis (Kurzzellen Interkutis).

GUTTENBERG (1940, p. 88—100) reviewed the data known so far, and divided exodermis in two basic types: the homogeneous exodermis (without short cells) and heterogeneous exodermis (with short cells). The author considers the short cells as "passage cells".

VAN FLEET (1950) compares the anatomy and histology of the exodermis and the endodermis in some roots; using histochemical reactions he proved a marked similarity between both structures concerned.

In a paper on mycorrhizae of the ash, JENÍK and KUBÍKOVÁ (1961) referred to the presence of exodermis in rootlets of *Fraxinus excelsior* without giving many structural details; the role of the short cell in the penetration of fungal hyphae was emphasized.

Concerning the terminology, GUTTENBERG (1940) uses the term "the exodermis" (see also ESAU 1960) introduced by VUILLEMIN (1884, sec. KROEMER 1903), while the older term "the hypodermis" is still used in some recent works (EAMES and MACDANIELS 1947, VAN FLEET 1950). KROEMER'S term "the interkutis" was not accepted. In this paper preference is given to the term "the exodermis".

This paper aims at giving a detailed explanation of the structure and development of exodermis in primary end-roots of the ash (*Fraxinus excelsior* L.) with some observations on environmental effects.

Methods

Observations of the shape and structure of the exodermis were carried out on permanent slides prepared by the usual anatomical techniques and stained by a combination of safranin and aniline blue.

Histochemical reactions on suberin, lignin, callose, pectin and cellulose were done on fixed (FAA) and/or live rootlets cut by the freezing microtome. The procedures given in JENSEN (1962) were followed. For the determination of the polysaccharide components of cell walls, the extraction procedure for the use with the periodic acid-Schiff's reaction was employed (JENSEN 1962).

Results

Several years of study in the root system of *Fraxinus excelsior* confirmed beyond any doubt the constant presence of an exodermis in primary end-roots. The root system of the ash exhibits an atypical heterorhizis (KUBÍKOVÁ 1967), e.g. the brachyrhizae (short roots) are not distinctly shortened, and represent the bulk of the primary distal roots. Therefore, if not stated otherwise, the following observations describe the features of the brachyrhizae.

Anatomy of the exodermis

The exodermis is formed by alternating long and short cells (Kurzzellen-Interkutis KROEMER 1903, Exodermis mit Durchlasszellen GUTTENBERG 1940).

The characteristic shape of long and short cells is shown in Figs 1 to 3. On radial section, the long cells exhibit a rectangular shape, contrary to the short cells, which are wedge-like and narrowed towards the centre of the root. The outer wall of the short cell is markedly thickened (3 to 7 μm), somewhat vaulted, and consisting of several layers. The term "cap" in connection with this structure is fully justified. The short cells appear, on the radial section, to be shifted outwards into the neighbouring rhizodermis. On the cross section, the shape of the long and short cells does not differ very much, though the thickened cap can indicate the presence of a short cell. Very instructive is the tangential section showing regular alternation of long and short cells. Long cells have very little cytoplasm and a large vacuole; most of the older cells are dead; short cells, on the contrary, have a large amount of cytoplasm and a large nucleus well stained by the above mentioned dyes.

In some samples, the dimensions of the long and short cells were measured on the longitudinal sections. The results are given in Tab. 1. Comparison of the numbers show relative stability of the dimensions of short cells; similarly

Table 1

Dimensions of the long and short cells on the longitudinal radial sections (in μm)

	Long cells		Short cells	
	tang. dimen.	radial dimen.	tang. dimen.	radial dimen.
Rootlets sampled in the Vltava Valley in Sept. 1959	75·2 ± 3·29	27·3 ± 0·91	28·3 ± 0·9	28·2 ± 0·9
Rootlets sampled in the Berounka Valley, in Nov. 1959*	64·9	22·7	25·2	28·3
Macrorhizae sampled in Petrovice (okr. Klatovy) in Dec. 1959*	66·1	29·6	28·9	31·5

* Because of scarcity of the material, the standard deviation was not counted

the radial dimensions of long cells are relatively stable. The tangential dimensions of the long cells, on the contrary, fluctuate greatly. According to my observations, this dimension depends to a large degree on the age and the diameter of the root. Closer to the apical meristem, the long cells are shorter and they gradually elongate to optimal length; the short cells, however, do not change much after their differentiation. Similarly, smaller roots have relatively smaller cells than bigger roots.

Histochemical composition of exodermal cell wall

The cell walls of the long and short cells differ profoudly: while the walls of the long cells react possitively with Sudan III and phloroglucinol + HCl, thus showing the presence of suberin and lignin, the walls of the short cells react negatively. The reactions for pectins and callose were negative in both types, neither was the reaction for cellulose with the use of chlorzinc-iodium definite.

To get the precise composition of exodermal cell walls, the extraction procedure was used (JENSEN 1962). This method enables the gradual extraction of pectic substances, hemicellulose and noncellulosic polysaccharides. Cellulose is the last remaining substance. After the extraction of a certain substance, the slides are stained by periodic acid-Schiff's reagent (PAS). The intensity of colouration compared with the control, shows the presence or absence of a certain substance. The method was repeated several times on sections of apical parts of primary end-roots sampled in the Botanical Garden Charles University, Prague, in April 1967. The results are summarized in Tab. 2. and they are shown in Figs. 4 to 7, 8 to 11. From this material, it may be concluded that exodermis contained a great deal of pure cellulose, the "caps" were composed almost entirely of cellulose.

Table 2

Results of the extraction procedure of the polysaccharide cell wall components

Treatment Tissues	Control	Pectins extracted	Hemicelluloses extracted	Noncellul. polysacch. extracted
Rhizodermis	Stained red, attached detritus, numerous root hairs	Red colouration unchanged	Disintegration of cells, only remnants left	Small uncoloured cell remnants
Exodermis	Stained red, "caps" distinct	Red colouration unchanged	Red colouration fading, the structure only little influenced	Light, distinct red colouration of the whole layer, mainly of "caps"
Cortex	Stained red, numerous starch grains, endophytic fungus present	Marked disintegration of middle lamellae, red colouration fading	Red colouration disappearing, starch grains disintegrating, marked structural changes, extraction of endophyte	Great structural changes, whole layer uncoloured

The development of the exodermis

The exodermis differentiates in the apical meristem. Not far behind the root tip, at a distance of about 1 mm, the exodermis is already typically composed of long and short cells. The long cells are in a primary stage — the cellulose walls are without any impregnation. This primary stage lasts a very short time even in an actively growing root, in the spring; at a distance of appr. 10 mm from the apex the suberin lamella is already built; in the next 5 mm portion, the cellulose lamellae get impregnated by lignin. Short cells are not changed by ageing, a more or less thickened "cap" is formed close to the apex. The suberization and/or lignification do not occur. KROEMER (1903) described the lignification of the "caps", a feature which was not observed in any of the ash roots studied in this work.

It is of interest to compare these observations with the development of the endodermis. In the root of the ash, the endodermis cells remain much longer in a primary stage: in an actively growing rootlet during the spring no suberization was found in its length of appr. 55 mm. The endodermis becomes suberized later in the period of slackened growth, which was observed on end-roots sectioned in a summer period.

Another interesting point is the comparison of the development of the exodermis with the vitality period of the rhizodermis. Mostly, its undisturbed function lasts for a very short time; on the sections showing fully differentiated exodermis, the rhizodermis may be found in different stages of disintegration (see also KUBÍKOVÁ 1967).

The outer tangential wall of short exodermal cell — the "cap", deserves special interest. It was described in detail by KROEMER (1903) in many plants. With the ash, the "cap" is built by the protoplast of short cell and the thickening exceeds partially to radial cell walls. Longdated observations showed the different width (3 μm — 7 μm) and different staining with aniline dyes in various samples. Special attention was therefore paid to this phenomenon and the width of the "cap" was measured. An attempt was made to find correlations with the age of the root, the period of the year or the habitat of the sampled tree (dry or wet locality). However, about 40 studied samples from different years and habitats yielded no such correlation. Evidently, thin and thick "caps", differently stained, alternate in one rootlet. It was not, therefore, possible to conclude that environmental conditions or the age of the root influence the thickness of the short cell "cap", and, similarly, that the suction force of the root may be effected in this manner, as suggested by GUTTENBERG (1940). The presence of "caps" appears to be a normal feature of short cells in the roots of the ash, and the variability within a single root overrides the seasonal changes.

The function of the exodermis

The exodermis in the ash works in two basic directions:

1. as a protective mechanical tissue after the disintegration of the rhizodermis,
2. it permits the absorption and secretion of solutions.

The described type of exodermis is adapted to both these function: long suberized and lignified cells with decreased permeability cover the greater part, about 3/4 of the area of the exodermal mantle around the root (see tangential section on Fig. 3) and thus they fulfil the mechanical function of the exodermis; short permeable cells with cellulose "cap" cover only about 1/4 of the exodermal mantle area and form passage cells for water solutions. The situation is very similar to leaf epidermis with stomata. According to my observations, the permeability of short cells does not change substantially with ageing, no stopping or impregnation of these cells in older roots was observed. The primary end-roots, therefore, seem to continue acting as suction roots until their final decay or the formation of periderm after a period of several months or even years. The life span depends on the type of root and on the soil conditions.

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JARMILA KUBÍKOVÁ, Katedra botaniky Karlovy university Praha: **Příspěvek k exodermis v koncových kořenech jasanu (*Fraxinus excelsior* L.).** — Biol. Plant. **10** : 455—461, 1968.

Primární koncové kořeny jasanu se vyznačují přítomností exodermis, ležící na rozhraní rhizodermis a primární kůry. Exodermis se diferencuje v primárním meristému a je složena ze střídajících se dlouhých a krátkých buněk o přibližných rozměrech 70 × 25 × 25 μm, 25 × × 28 × 25 μm. V krátké vzdálenosti od kořenové špičky prodělávají dlouhé buňky strukturní a histochemické změny (přecházejí z primárního na sekundární stadium): vytváří se nepermeabilní suberinová lamela, celulosní lamely jsou impregnovány ligninem, protoplast odumírá. Krátké buňky mají zřetelně zesílenou vnější stěnu („čepičku"), která podle výsledků postupné extrakce buněčných stěn je složena převážně z celulosy, a obsahují mnoho cytoplasmy s velkým barvitelným jádrem. Během stárnutí se struktura krátkých buněk zřetelně nemění. Po odumření rhizodermis, ke kterému dochází brzo po úplné diferenciaci exodermis, přebírá exodermis funkci ochranné vrstvy na povrchu jemného primárního koncového kořene: dlouhé buňky fungují jako mechanická ochranná vrstva, krátké buňky slouží jako propustné buňky pro roztoky a plyny po celou dobu života koncového kořene.

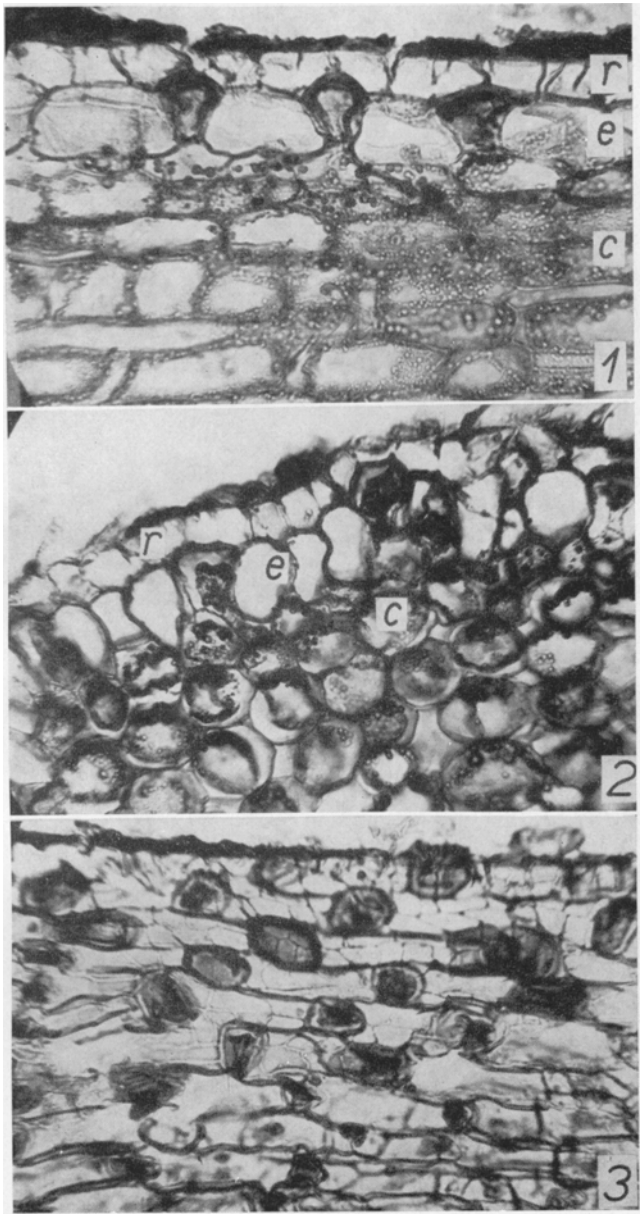

Fig. 1. Radial section of ash rootlet, showing the short and long cells of the exodermis. r — rhizodermis, e — exodermis, c — cortex.

Fig. 2. Cross section of ash rootlet; the "cap" and the presence of cytoplasm distinguish the short cells in the exodermis.

Fig. 3. Tangential section of ash exodermis, showing the regular alternation of short and long cells, and the considerable thickness of short cell wall.

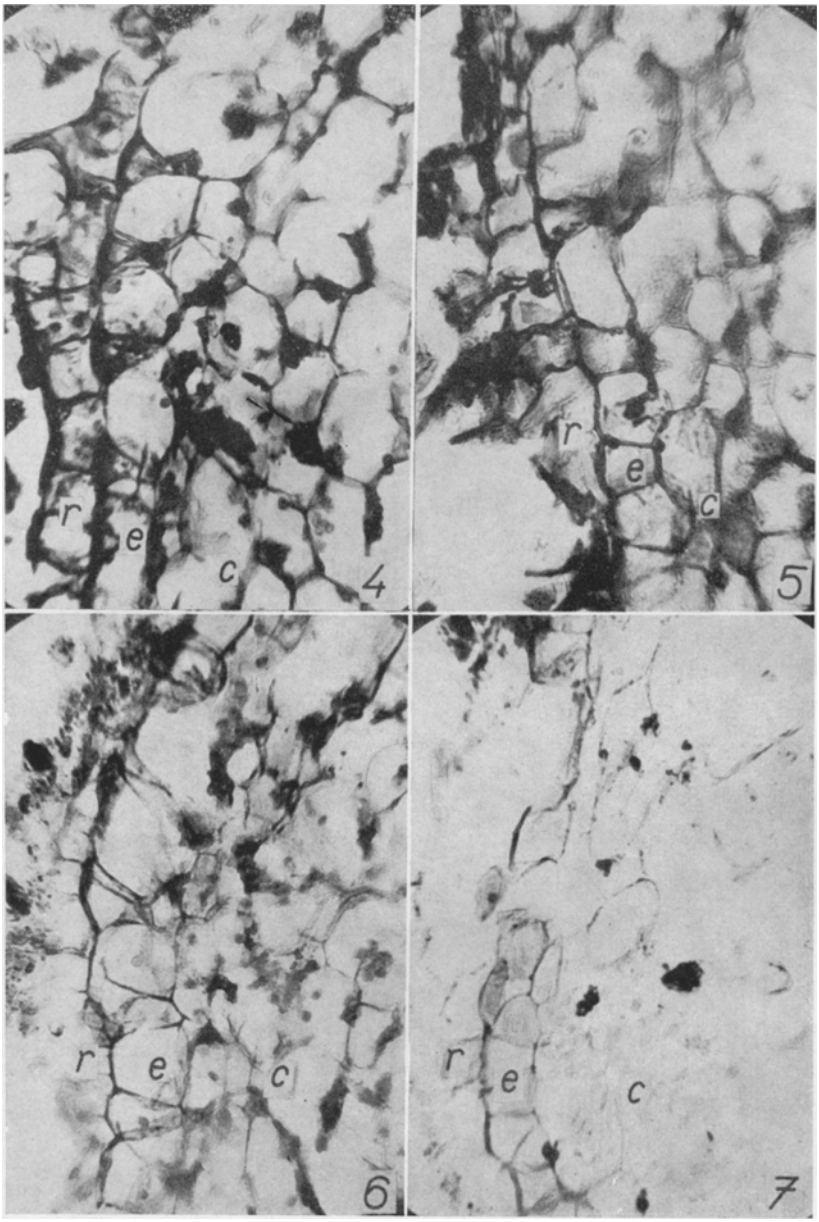

Fig. 4. to 7. Radial longitudinal sections of apical portion of ash end-root, sampled in the Botanical Garden, Praha, in April 1967, showing the results of the extraction procedure of cell wall components.
r — rhizodermis, e — exodermis, c — cortex.
Fig. 4 — control; Fig. 5 — extracted pectins; Fig. 6 — hemicelluloses extracted; Fig. 7 — noncellulosic polysaccharides extracted.

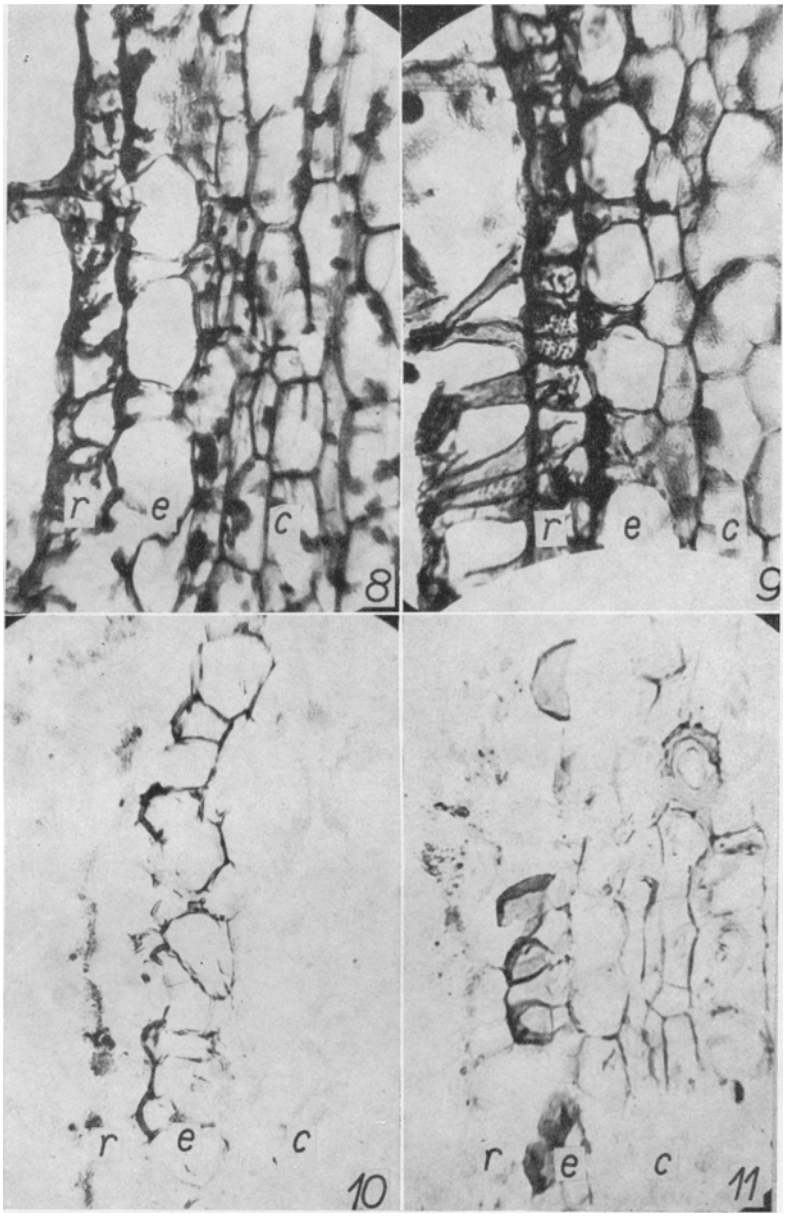

Fig. 8. to 11. Another example of the results of the extraction procedure of cell wall components. For the description see Fig. 4 to 7.

Ярмила Кубикова, Кафедра ботаники, факультет естествознания, Карлов университет, Прага: **К вопросу экзодермис в концевых корнях ясеня (*Fraxinus excelsior* L.)** — Biol. Plant. **10** : 455—461, 1968.

Первичные концевые корни ясеня характеризируются наличием экзодермис, лежащей между ризодермис и первичной корой. Экзодермис дифференцирует в первичной меристеме и состоит из чередующихся длинных и коротких клеток с приблизительными размерами 70 × 25 × 25 μ и 25 × 28 × 25 μ. На небольшом расстоянии от корневой верхушки с длинными клетками происходят структурные и гистохимические изменения (они переходят из первичной стадии во вторичную): образуется непермеабильная суберинова лamелла, целлюлозные ламеллы импрегнируются лигнином, протопласт отмирает. У коротких клеток отчетливо утолщена внешняя стенка («шапочка»), которая по результатам постепенной экстракции клеточных стенок состоит преимущественно из целлюлозы. Короткие клетки содержат много цитоплазмы с большим окрашивающимся ядром. При старении, изменений структуры коротких клеток не видно. После отмирания ризодермис, которое наступает скоро после полной дифференциации экзодермис, функция охранного слоя на поверхности тонкого первичного концевого корня переходит к экзодермис: длинные клетки служат механическим охранным слоем, короткие клетки пропускают растворы и газы в течении остального периода жизни концевого корня.