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CONTRIBUTION TO THE LEAF ANATOMY OF *QUERCUS ILEX* L.

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The leaf anatomy of *Quercus ilex* has been investigated by means of light and scanning electron microscope. The evergreen leaves of this species have marked xeromorphic properties in comparison with the deciduous leaves of *Quercus robur* L., *Quercus cerris* L. etc. Xeromorphy of *Q. ilex* is displayed particularly by a very thick cuticle with cuticular layers and a large number of tufted hairs especially on the lower epidermis. The basis of the tufted hairs is surrounded by epidermis and subepidermis cells, which all have cutin in the walls. These cutin carrying cells represent the pedestal of the hair. The tufted hairs containing a larger cell number sometimes coalesce with the basal hair parts above the level of epidermis and pedestal. However, the uppermost hair parts are free. These hairs protect the leaves from dessication. Moreover, glandular hairs are present on the leaves.

The mesophyll of *Q. ilex* consists only of palisade parenchyma. The leaf veins are connected with other hardy cell elements which are continuous from the upper to the lower epidermis making the leaf very firm. These support walls prevent the thin parenchyma cells from breaking or becoming crumpled during the period of dryness. Some data about the stomata, chemical properties of cell walls and various forms of calcium oxalate crystals are also presented.

Introduction

A good and precise description of the anatomy of evergreen leaf of Q. *ilex* was published by Guttenberg (1907, pp. 417—419). He studied in detail the morphology and explained the function of various leaf structures. Especially the stomata structure of this *Quercus* species was minutely delineated (see Guttenberg 1907, Fig. 1—3).

In the same paper the structure of leaf vascular bundles is taken into consideration. The leaves contain a large number of elongated mechanical cells which fortify the bundles, filling out completely the space from the upper to the lower epidermis.

Im this manner the hard leaf structures are generally constructed according to Tschirch (1881) (Fig. 2A). The hard plates are supported by the upper and the lower epidermis which are also thick and hardy. Such structures are frequent in evergreen leaves of Mediterranean plants. Many other sclerophyllous plants have very similar supporting plates, for instance *Laurus nobilis*. According to Solereder (1899) such arrangement of mechanical cells and bundles is characteristic of some representatives of the group *Cupuliferae*.

Material and Methods

Leaves used for this research were gathered in the plant association *Orno-Quercetum ilicis* H—ć (Horvatić, 1963) on the hill Marjan near Split. The material was investigated by means of light and scanning electron microscope.

Cross sections through leaves were prepared by means of paraffin method and using a Minot's rotation microtome. The sections and tissue fragments were treated with carbon and gold in a vacuum evaporator and then they were micrographed by a JSM-U2 scanning electron microscope.

Leaf morphology and falling off of the leaves

The leaves of *Q. ilex* are very hardy; they have a relatively short petiole and two lineal stipulae which fall off early (Fig. 1B). The blade is 4 to 10 cm long and 2.5 to 4 cm wide. The margin of the blade is integer, serrate or dentate (Fig. 1). The number of dentes per leaf is seldom over 20. Sometimes the dentes are present only on the upper leaf parts. The dentes are ordinarily present on the larger leaves and sometimes they fail to appear on the smaller ones. The dentate leaves are present predominantly on adult trees which are well illuminated. The dentes fail to appear on young trees which grow in the shade.

As this *Quercus* species is evergreen, the leaves remain on the tree several years. According to the data of Jovanović and Ilić-Vukićević (1959) the leaves remain on the tress of *Q. ilex*, on average, two years.

Results

The epidermis

The anatomical investigation were performed mostly on the well illuminaed sun leaves. The upper epidermis had a very thick outer wall which consisted of a cuticle and of cuticular layers. After staining with Sudan III the cuticle and the cuticular layers became red. Only in rare cases a thin layer of cellulose besides the cell lumen remained unstained. Consequently, nearly the whole outer wall of epidermis contained cutin. Many lateral walls of the epidermis contained cutin also in their central parts.

The thickness of cuticle with cuticular layers of outer epidermis was up to 5.7 μm. It can be mentioned that in oaks with deciduous leaves the corresponding cuticle is only 1.4 μm thick. The lower epidermis contained also well developed cuticular layers. The thickness of the cuticle with cuticular layers here was on the average 4 μm.

It is interesting to mention that the cell walls of the epidermis were abundant in lignin. After staining with floroglucin and HCl, cell walls of epidermis, especially the internal walls, were intensively red (cf. (Guttenberg 1907, Linsbauer 1930, p. 79, Esau 1965. p. 157).

Stomata

Guttenberg (1907) designed and described stomata of *Q. ilex* from the above, in cross and longitudinal sections. Moreover he studied the chemical properties of guard cell walls. According to Guttenberg the walls of guard cells are mostly rich in lignin whereas their dorsal walls are built only of cellulose. Guttenberg (1907) has also found that the central parts of the guard cells lumina are narrow, while the end parts are wide. Therefore, it seems that the stomata of *Q. ilex* belong to the »gantelegubovidnyj (Russ.) (Engl. = dumbbell-lip like) type according to the classification by Aneli (1975). The stomata of *Quercus robur, Q. cerris* and *Q. frainetto* also belong to this type (Bačić 1979, 1981).

It is a characteristic of the stomata of *Q. robur, Q. frainetto* and *Q. cerris* that they have a thickening on their exterior guard cell walls (see Fig. 1A and 2—4 of Bačić 1981). These structures were described and micrographed by means of a scanning electron microscope. It must be pointed out that similar thickenings were not present on the stomata of *Q. ilex.*

We should mention that during the study of the stomata of the three cited *Quercus* species, the significance of the structure marked t in Fig. 1 of the paper by Bačić (1981) remained unclear. Today we know that this structure belongs to the stomata and that it represents a part of the inner cell wall of the guard cells. It is present also on the guard cells of *Q. ilex.*

On the ventral walls of *Q. ilex* stomata Guttenberg (1907) detected cuticular outgrowths or pleats which are very probably identical to the lamellae observed by Bačić (1981) in *Q. robur* and other oak species. It is probable that the pleats diminish the transpiration and have an ecological significance.

As it is visible from Fig. 3B, the stomata are surrounded by various numbers of epidermal cells. The presence of these cells shows that the stomata of the evergreen oak belong to the anomocytic type (Metcalfe and Chalk 1950). On the evergreen oak, the number of neighbouring epidermis cells which surround the stomata amounts to 5 to 10. The same number of epidermis cells was established by Bačić on *Q. robur* and other oak species with deciduous leaves.

We have found that the stomata number on leaves of *Q. ilex* varies from 298 to 382 per 1 sq cm and that the average number is 342 per 1 cm sq. This average number is a little larger than those of *Q. cerris* and *Q. robur* and a little smaller than that of *Q. frainetto* (cf. Bačić 1982).

Trichomes

Plant hairs or trichomes are predominatly present on the lower epidermis and they have the function to protect the leaves from too high transpiration. In young leaves of *Q. ilex* the hairs on the under epidermis are so dense that it is not possible to see the surface of the epidermis because the trichomes hinder the view. (Fig. 4A).

The trichomes belong to the type of tufted hairs (Esau 1956). They consist of many cells whose basis is inserted into the epidermis and the other part of hair is free and protrudes out of the epidermis. While the tufted hairs of *Q. robur, Q. frainetto* and *Q. cerris* consist of 2 to 4 cells, the number of cells in tufted hairs of the evergreen oak can be larger and amounts to ten cells. The large hairs soon lose the protoplast and then efficiently protect the leaf from desiccation. Consequently, the tufted hairs are groups of elongated cells whose basal parts coalesce laterally and are inserted in the epidermis, while the upper part of the hair is free. The full grown hairs do not have the living protoplast any more.

The epidermis around the tufted hairs of *Q. ilex* is usually somewhat elevated above the level of the rest of the epidermis. It is probable that this is caused by the activity of the subepidermis. The elevated cells are named pedestal (the German name is »Sockel«) (Fig. 4B, C). The pedestal is cutinised so that after staining with Sudan III it becomes red and can be easily distinguished from the neighbouring cell parts.

Besides the earlier described tufted hair type we have found another type, which is also common in evergreen oak trees. The characteristic of this second type is that the tufted hairs remain, in a part of their length, coalesced above the leaf epidermis, that is above the pedestal. The coalesced part of the hair is called a column. The column can be 10 to 30 μm long (Fig. 4C). From the top of the column numerous separated and long components of the hairs stretch in all directions forming the most efficient part of tufted trichomes.

When we cut across the column with a knife, the section has a regular round form. By means of such sections it is possible to establish the number of cells from which the tufted hairs consist. In Fig. 3C the section through the column of a tufted hair is presented; it consists of nine cells.

The number of tufted hairs on the lower epidermis of *Q. ilex* vary from 94 to 137 per sq. mm and the average number is 115 per sq. mm.

The tufted hairs are present also on the upper epidermis but the number of hairs per sq. mm is smaller than on the lower epidermis.

The hairs fall off from the leaves of *Q. ilex* easily. While the hairs on the young leaves are verv dense, they successively fall off with time, so that a small number of hairs remain on old leaves. The mechanism of falling off of hairs was studied by Keller (1890). According to the data of Netolitzky (1932, 116—119) the trichomes fall off because the cell wall breaks between the cutinized pedestal and non cutinized hair base.

The tufted hairs are also present on the epidermis of the stem. When in the first year of the stem development the epidermis is replaced by the periderm, the tufted hairs become detached from the stem together with other parts of epidermis.

Glandular hairs

On the epidermis of *Q. ilex* glandular hairs were also found. They have a living content and sometimes are pigmented red. The glandular hair contains a filamentous basal part consisting of two to four cells. The enlarged head is round or elongated and has also a living protoplast (Fig. 5A and B). The head consists of four to eight cells.

Mesophyll

The internal parenchyma of the leaf is built exclusively of palisade cells as described precisely by Guttenberg (1907). The walls of these cells are thin and can be stained blue with chlorine-zinc iodine reaction (Braune et al. 1967, p. 289), which shows that the cell walls are made of cellulose. Under the upper epidermis there are two layers of narrow and elongated palisade parenchyma cells ranging from 112 to 154 µm (average 126 µm).

Below the elongated parenchyma cells, in the lower leaf part there are 3 to 4 layers of palisade parenchyma cells, which are also elongated but are two times shorter than the upper parenchyma cells. Among these cells the intercellulars are larger than among the parenchyma cells of the upper part of leaf.

In the parenchyma near the lower epidermis there are many cells with calcium oxalate crystals (druses). These crystals shine intensively in the polarisation microscope between crossed polarisation filters (Fig. 6B and C). Sometimes similar crystals are present also in the central part of the leaf (Fig. 6C), but they are often larger and not so frequent as the crystals near the lower epidermis.

Supporting walls

Even the small vascular bundles of *Q. ilex* leaves are connected with mechanical elements which stretch from the upper to the lower epidermis. This system reinforces the leaf considerably (Tschirch 1881, Guttenberg 1907). According to Guttenberg (1907) such bundle formation prevents the sensitive parenchyma cells from collapsing in the periods of dryness. In the German literature such formations are called »Strebewände« (Guttenberg 1907. Tschirch 1881), but we shall use here the English term 'supporting walls'. The structures are visible in Fig. 2A, where three supporting walls are presented in cross section.

The supporting walls first include the outer epidermis, which is of a robust structure, (Fig. 2A). The internal epidermal cell walls give a faint positive reaction with phloroglucin and hydrochloric acid (Johansen 1940). Then the sclerotic parenchyma cells (s) follow which often contain prismatic crystals of calcium oxalate and have a relatively wide lumen (Fig. 6A, B). Its cell walls give a strong positive reaction with phloroglucin and HCl.

The sclerotic parenchyma leans against a large group of perivascular fibers, which are accumulated in the middle part of the leaf (Fig. 2A f). The fibers have very thick and shining white cell walls and a very small cell lumen. After reaction with phloroglucin and HCl the compound middle lamella of fibers acquired an intensive red colour but the other parts of the cell wall remained unstained.

At the beginning of lower part of the leaf, tracheary elements were situated. There, many tracheids with helical thickenings were present, which gave a clear positive reaction with phloroglucin and HCl.

Following the vascular tissues a small group of white shining perivascular fibers was visible again (f in Fig. 2A). They reacted with phloroglucin and HCl as the fibers of the middle part of leaf did, i.e. only the compound middle lamella reacted positively with red colour.

This group of lower perivascular fibers leans farther below against the sclerotic parenchyma cells, which are similar to the corresponding cells below the upper epidermis. The walls of the sclerotic parenchyma also gave positive reaction with phloroglucin and HCl.

The lower epidermis also has thick and hardy cell walls. The inner cell walls of the lower epidermis gave a strong positive reaction with phloroglucin and HCl, and the outer walls gave a weak reaction with this reagent.

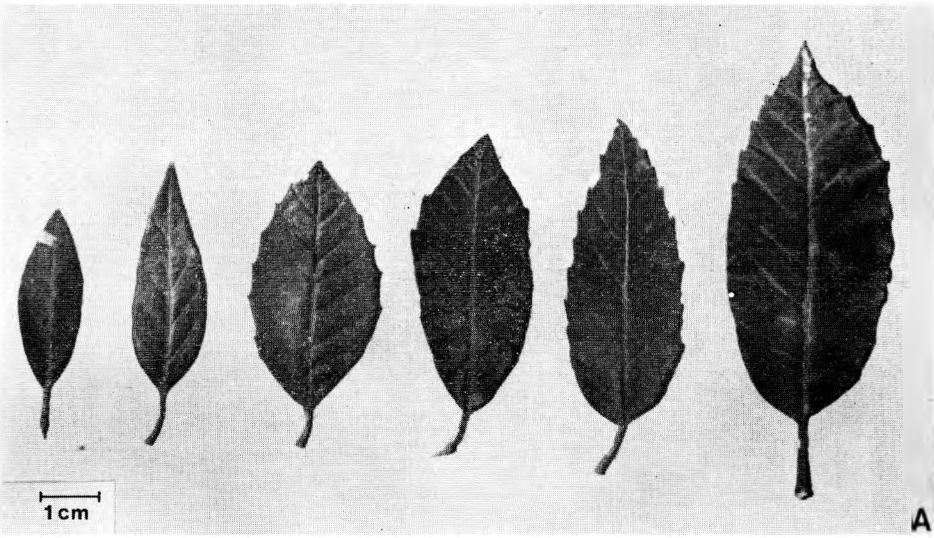
The main vascular bundle

The vascular bundle of the midrib is almost concentric with the internal xylem. In the middle of the bundle there is a sclerotic parenchyma tissue (Fig. 5C s) surrounded by the xylem (Fig. 5C x) which is better developed on the lower part of the bundle. The narrowest xylem elements (protoxylems) are in a direct contact with the sclerotic parenchyma tissue in the centre of the bundle. The sclerotic parenchyma cells and the elements of xylem are strongly lignified and give an intensive red reaction with phloroglucin and HCl.

In the direction of the surface of the leaf, the xylem is followed by phloem. The phloem (ph on Fig. 5C) of lower part of the bundle is better developed and it has a semicircular form while the upper phloem is straight and relatively shorter. Finally, the bundle is surrounded by a ring of perivascular fibers (f in Fig. 5C) which is very thin or completely interrupted on the lateral parts of the bundle. The fibers have very thick cell walls and a narrow cell lumen, and react with phloroglucin and HCl like the other perivascular fibers of the leaf. Round about the bundle there is a common parenchyma tissue (p in Fig. 5C). Further toward the upper and lower leaf epidermis there are some layers of sclerotic parenchyma cells which have lignified cell walls (s in Fig. 5C).

The shade leaves

The structure of shade leaves differs from that of sun leaves. The former are nearly two times thinner, so that their thickness is about 115 μm. The parenchyma of the lower part of the shade leaf resembles spongy parenchyma. Consequently, the shade leaves have a more mesomorphic structure.



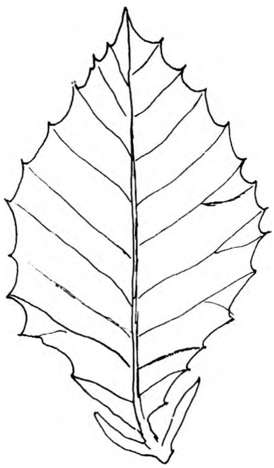


Fig. 1. A. Shape of *Quercus ilex* leaves; B. A leaf with stipulae in natural size.

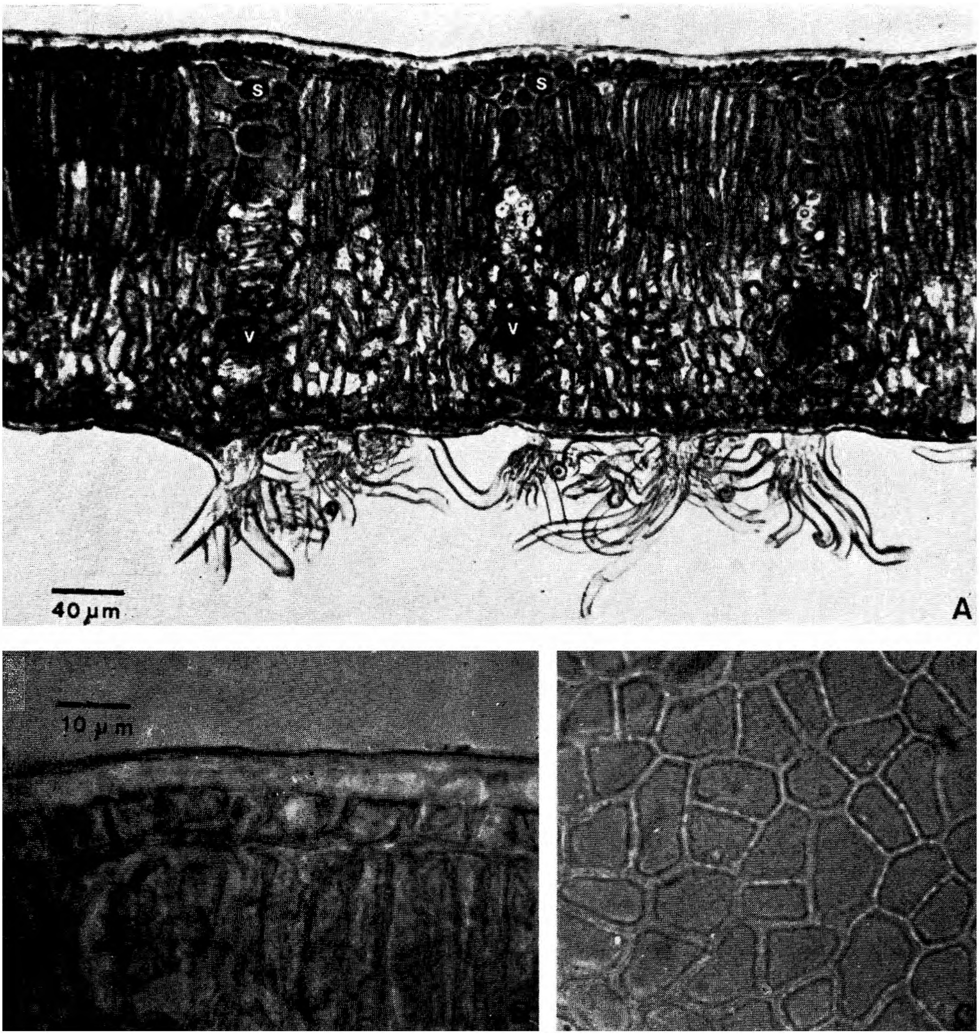


Fig. 2. A. Cross section of a leaf with vascular bundles and tufted hairs on lower epidermis: s sclerotic parenchyma, f perivascular fibres, v vascular tissues; B. Upper part of the leaf with epidermis and palisade parenchyma cells; C. Upper epidermis in face view.

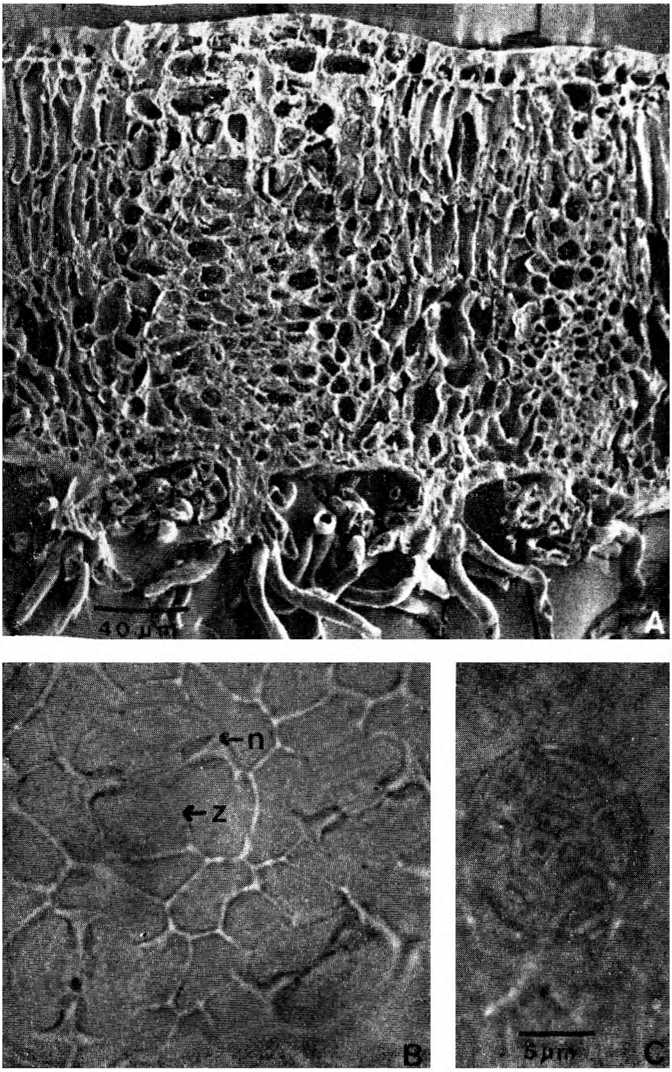


Fig. 3. A. Transverse section of leaf lamina micrographed by a scanning electron microscope. Tufted hairs on the lower epidermis; B. Surface view of lower epidermis with stomata cells: n stomata part where the upper cell wall is thinner; in the other parts the upper wall is thicker (z); C. Transverse section of the column containing nine hair cells. In the background, the lower epidermis is visible.

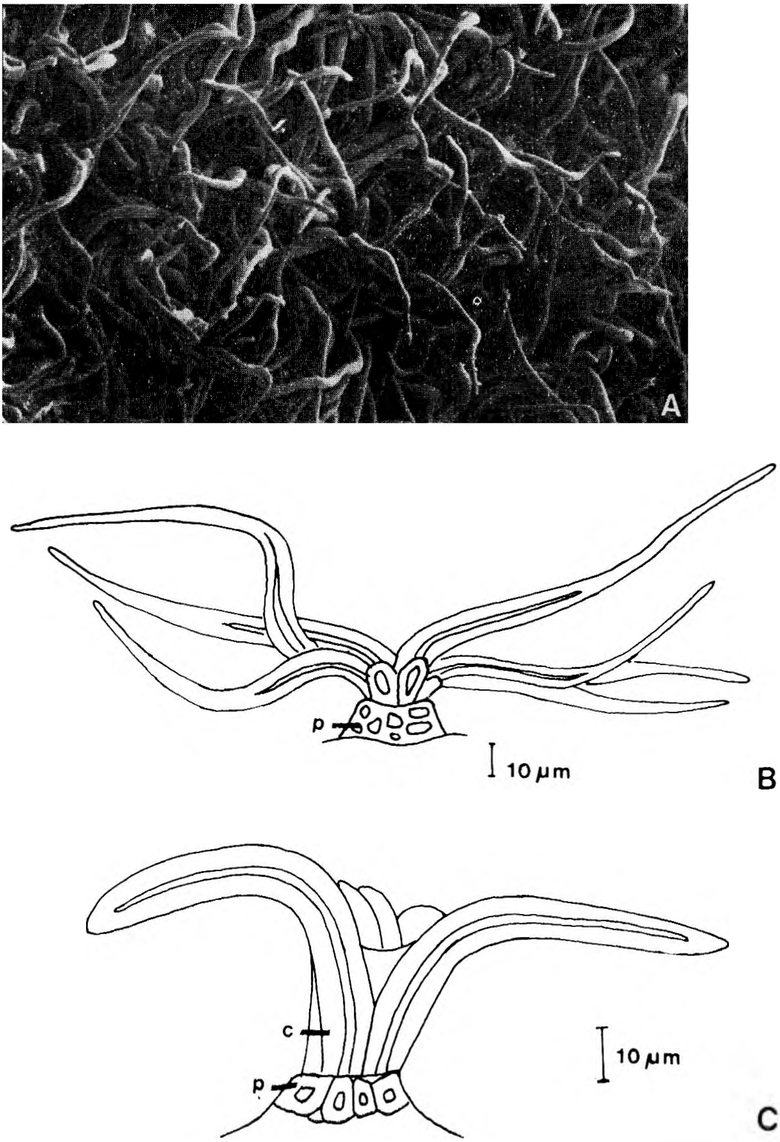


Fig. 4. A. View of the trichomes of lower epidermis in the scanning electron microscope; B. A tufted hair surrounded at the base with pedestal cells (p); C. A tufted hair surrounded with pedestal cells; its basal parts are coalesced forming a column (c).

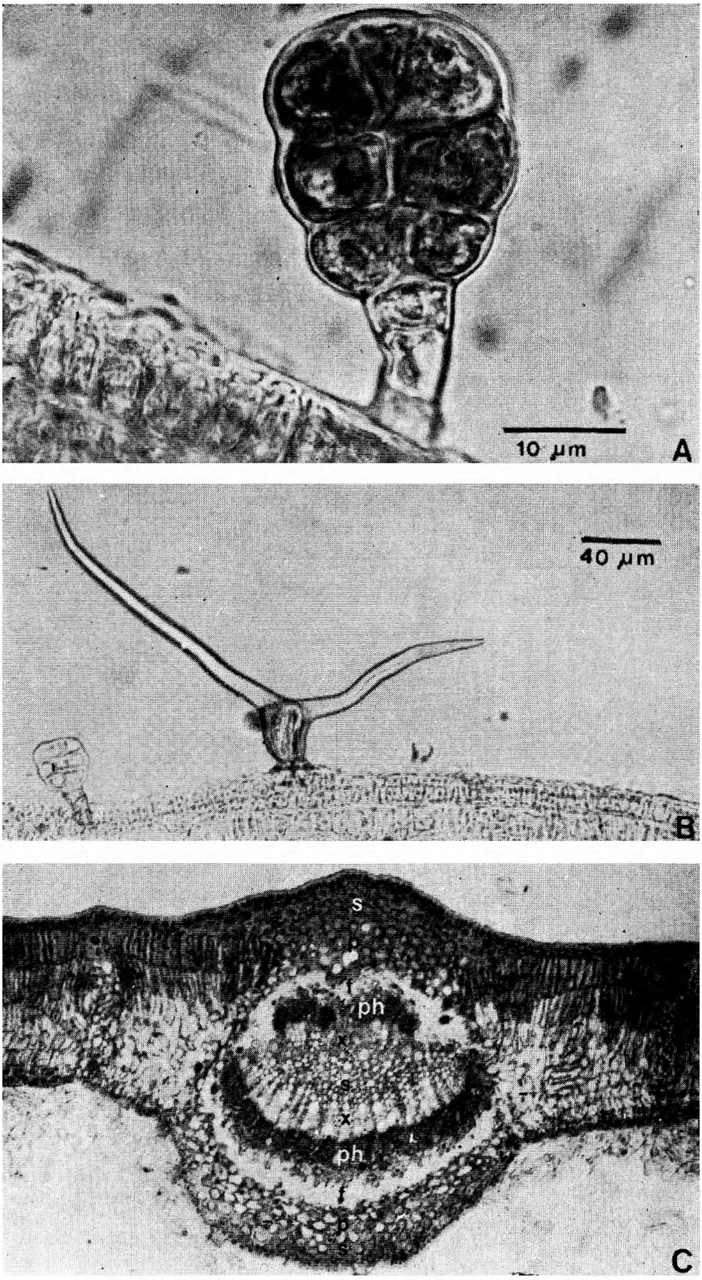


Fig. 5. A. Glandular hair; B. A glandular and a tufted hair; C. Cross section of the leaf midvein: f perivascular fibers, p parenchyma, ph phloem. s sclerotic parenchyma, x xylem.

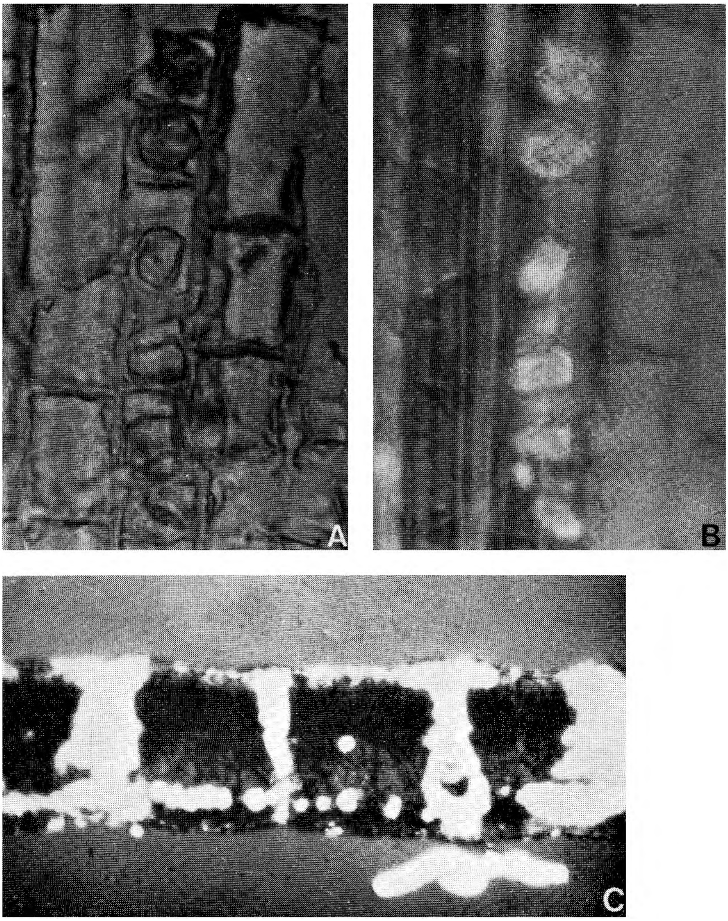


Fig. 6. A and B. Longitudinal sections of the sclerotic parenchyma with enclosed calcium oxalate crystals. A in common light, B in polarized light between crossed filters; C. Cross section of the leaf in the polarized light: four supporting walls, some round druses and one tufted hair shine intensively.

Discussion

While the leaf polymorphismus of *Quercus* species with deciduous leaves is quite pronounced (Bačić 1979), the evergreen leaves of *Q. ilex* are very simple. The cause of this phenomenon is obviously the fact that *Q. ilex* leaves have a smaller surface and are adapted to the intensive aridity in the Mediterranean region during the warm summer months.

There are also some specificities in the leaf anatomy of *Q. ilex*. The outer epidermis of evergreen oak leaf has very thick cuticular layers, about 5.7 µm. According to Baranov (1925) all xerophytes have thick cuticular layers, which exceed 5 µm. In accordance with this definition, *Q. ilex* is also a xerophyte.

The presence of tufted hairs is a characteristic property of the genus *Quercus*. These hairs consist of two to more than ten cells in *Q. ilex*. The cells often laterally coalesce not only with their base, but sometimes also with a further part of the hair, which protrudes above the level of the epidermis forming the column (Fig. 4C). The existence of a column by oak hairs has been noted here for the first time.

We did not find any data in the literature about glandular hairs in the epidermis of evergreen oak leaves, although they were noted in other *Quercus spp.* according to the data by Solereder (1899). Thus, glandular hairs with round or elliptical heads were found on Q. frainetto. According to Lutz (1938) some *Quercus spp.* have glandular hairs containing a handle of 2 to 4 cells and a small head of 4 to 8 cells. These data are in concordance with our results.

Most of the characteristics quoted for evergreen oak leaves, especially thick cuticular layers, straight lateral walls of epidermis cells and well developed palisade parenchyma cells are distinctly xeromorphic characteristics (Zalenskij 1904, Esau 1965 etc.). Other xeromorphic characteristicts of the evergreen oak are: leaf thick more than 200 µm, cuticular layers thick over 5 µm and occurrence of palisade parenchyma in a quantity larger than 50% of the total surface of the leaf cross section (Baranov 1925).

However, some other properties of the evergreen oak can also be considered as xeromorphic. So, for instance, the cuticular pleats (previously named lamellae, cf. Bačić 1981, p. 87) can decrease the transpiration. The cuticular pleats are present also on the stomata of mesomorphic oaks *Q. robur, Q. frainetto* and *Q. cerris*.

The enlarged number of cells in tufted hairs of *Q. ilex* is a xeromorphic property. The cell number in hairs is remarkably larger than in mesomorphic oaks and often amounts to 10 to 12 cells per hair. In mesomorphic oaks, the tufted hairs have only two to four cells. The enlarged number of cells in tufted hairs of evergreen oak is obviously a result of adaptation to the life in dry climate. Although the number of tufted hairs is relatively small, the enlarged number of cells enables them to cover the epidermis completely (Fig. 4A). In this manner the transpiration becomes very low.

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SADRŽAJ

PRILOG ANATOMIJI LISTA ČESVINE (*QUERCUS ILEX* L.)

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U ovom radu istražili smo anatomiju listova česvine s pomoću svjetlosnog i pretražnog elektronskog mikroskopa. Zimzeleni listovi česvine imaju kseromorfna svojstva u odnosu na vrste kojima otpadaju listovi svake godine, kao što su *Quercus robur* i dr. Kseromorfija česvine očituje se naročito vrlo debelom kutikulom i kutikularnim slojevima te velikim brojem čuperkastih dlaka, naročito na donjoj epidermi. Baza tih dlaka okružena je epidermskim i subepidermskim stanicama koje imaju kutiniziranu stijenku i nazivaju se podnožjem (pedestal). Čuperkaste dlake koje se sastoje od većeg broja stanica imaju katkad sraštene bazalne dijelove iznad razine epiderme i podnožja. No njihovi vršni dijelovi su slobodni. Osim tih čuperkastih dlaka na listovima se nalaze još i žljezdane dlake.

Mezofil česvine sastoji se kod listova izloženih sunčanom svjetlu isključivo od palisadnog parenhima. Lisne žile povezane su s čvrstom epidermom mehaničkim stanicama tako da se čvrsti elementi kontinuirano pružaju od gornje do donje epiderme. To znatno učvršćuje list i priječi da se parenhimske stanice zgužvaju ili rastrgnu za vrijeme perioda suše.

Osim toga nalaze se u radu podaci o pučima, kemijskim svojstvima staničnih stijenki i kristalima kalcij-oksalata.

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