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INVESTIGATIONS OF STOMATA OF THREE OAK SPECIES WITH LIGHT AND SCANNING ELECTRON MICROSCOPE

TOMISLAV BAČIĆ

(Teachers' College, Split, Faculty of Philosophy, Zadar)

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Introduction

Among numerous studies which have treated stomata since the second half of the 19th century up to the present days, of special interest are those that have used the characteristic features of stomata to solve the problems of taxonomy. A great number of dicotyledon families have been defined according to the types of stomata (Vesque 1881). On the basis of stomata structure Guyot (1966) has studied philogenetic relations in addition to the taxonomic ones. General information on stomata can be found in the handbooks of Solereder (1899), and Metcalfe and Chalk (1950).

Our investigation deals with the stomata of three species of oaks: *Quercus robur* L., *Q. frainetto* Ten. and *Q. cerris* L. It seems that the stomata of these species have not been studied in detail. Among the relatives of the oak, *Fagus grandifolia* was investigated by Dengler and Mac-Kay (1975), who described some of the characteristic properties of stomata.

Recent studies (Bačić 1979) show that the stomata of the above mentioned oaks have certain structural features by which they can be distinguished. Their guard cells have thinner walls at their ends and a wider lumen, while the walls of the middle parts are markedly thicker. Owing to such a structure Bačić believes that oak stomata belong to the "gantelegubovidnyj" (Russ.) (Germ. = hantel-lippenförmig) type according to the classification by Aneli (1975). According to this classification, the "gangtelegubovidnyj" type is characterized by thin walls at the ends of the guard cell, thick walls of the middle part of the guard cell and a spindle-like form of the aperture. Bačić (1979) considered the stoma of *Q. robur* the closest to the "gantelegubovidnyj" type.

Concerning the relation of the stoma and the neighbouring cells, the stomata of oaks belong to the anomocytic type (cf. Esau, 1965 : 166). Anomocytic stomata are very common in the family of *Ranunculaceae.*

Material and Methods

Oak leaves were collected at a natural habitat in North-East Bosnia at 140 to 180 m above the sea-level, and south-east exposure. The leaves belonged to plants from the association *Quercetum frainetto-cerris* Rudski. The leaves used in examination were fully developed; they were carefully dried and stored in a herbarium.

Fragments taken from the central region of the leaves were used for electron microscopic investigations. The fragments were exposed to carbon and gold vapours in a vacuum evaporator. Only the lower epidermis was photographed, because the leaves were hypostomatic, and thus the stomata situated only at that side. Magnifications of electron microscope figures are 126 to 6320 times. The micrographs were taken with a scanning electron microscope JEOL, JSM — U2.

Results

Structure of stomata

Since Bačić (1979) did not describe the microscopic structures of the stoma, it seems useful to discuss some details of their structure. These details are of importance for the interpretation of electron microscopic figures. Fig. 1 A shows the cross section of a stoma of *Q. robur.* It can be seen that the walls of the central part of the guard cell are markedly thick, with the exception of the dorsal wall which is slightly thinner. A wall joint, i. e. a pit in the outer wall (Fig. 1 A s), appears at the junction of the dorsal wall and the outer wall of the guard cell. The wall joint facilitates the movement of the stoma during the change of the turgor in the guard cells.

The outer wall of the guard cell is not uniformly thickened, so that the part of the outer wall which is nearer to the dorsal wall of the guard cell is considerably thicker. This thickening of the outer wall (Fig. 1 A z) begins at the place where the wall joint is situated, and it extends towards the middle of the stoma along two thirds of the width of the guard cell outer wall. When the stoma is observed from above, the thickening surrounds the stoma forming above the aperture a comparatively shallow area with reduced ventilation (cf. Fig. 2 Bz, C).

A characteristic triangular structure (Fig. 1 A t) can be noticed at the point where the inner wall of the neighbour cell merges with the guard cell. We cannot be quite certain whether the triangular structure actually belongs to the middle part of the guard cell or it is the expanded end part of the guard cell. In the latter case, the triangular structure should not be presented in Fig. 1 A because this figure should contain only the structures of the central part of the stoma.

Fig. 1 A shows the relatively poorly developed cuticular horn (n) at the point where the outer wall of the guard cell joins the ventral wall. The horn represents the cross section through the cuticular ledge. Numerous cuticular lamellae (Fig. 1 A l) can be seen on the surface of the

ventral wall; they are especially noticeable in electron micrographs (Figs. 2 B l, 4 A l). Therefore, we shall discuss the cuticular lamellae, which are certainly a xeromorphic structure, more extensively later on.

Quercus robur

Fig. 2 shows the structure of the lower epidermis of *Quercus robur* leaves, examined in a scanning electron microscope. We shall first consider two stomata which are considerably enlarged so that their structure can be seen in detail (Figs. 2 A, B). The figures show that the stomata are surrounded by a thickening (z) of the outer wall situated on the margin of the stoma. Like other parts of the leaves, the thickenings have numerous laminated and rib-like wax efflorescences. They doubtlessly increase the height of the thickening and thus diminish the ventilation above the stomata and transpiration. The wax efflorescences are more abundant around some stomata (Fig. 2 A) than around others (Fig. 2 B).

Fig. 2 B shows the cuticular ledge (n) in places where the outer wall of the stoma joins the ventral wall. This cuticular ledge appears when in the course of stoma development, the cuticle breaks and forms an aperture between the guard cells. This process can be followed fairly well during the formation of the stomata of *Q. cerris* (Fig. 4). There are no laminated and rib-like wax efflorescences in this species, nor is the thickening much developed; therefore the stages of aperture formation between the guard cells can be easily observed. In Fig. 2 A the aperture is already formed, but the remains of the broken cuticle can still be seen at the border of the outer and the ventral walls.

Cuticular lamellae are seen in the aperture protruding out from the ventral walls of both guard cells. They can be observed in a light microscope (Fig. 1 A l), but are particularly well shown in the micrographs taken with scanning electron microscope (Figs. 2 B; 3 A, B; 4 A). These cuticular lamellae are situated along most of the ventral wall (Fig. 1 Al). Cuticular lamellae certainly reduce the transpiration considerably, and they represent a xeromorphic adaptation.

Fig. 2 C presents the surface structure of the lower epidermis of *Quercus robur*. The height of the thickening on the outer stoma wall is medium and can function as ventilation diminishing space. Fig. 2 d exhibits the structure of the epidermis even at a smaller magnification (126 x). The vein ribs protrude above the surface of the remaining part of the epidermis. The rungs placed lengthwise reinforce the vein ribs.

The same Fig. 2 C shows that the oak stomata are oriented in various directions. The stomata of other oak species described here are also oriented irregularly.

The lower epidermis has tufted hairs. These hairs consist of three or four cells. These cells are joined only at the base, which is planted in the epidermis, while the remaining hairs stick freely out sideways (cf. E s a u 1965 : 168).

Quercus frainetto

This species also has stomata encircled by thickenings of the outer wall, but the thickenings here are somewhat less developed than in *Q. robur*. The laminated and rib-like wax deposits on the thickenings are sometimes less developed (Fig. 3 A) and sometimes are considerable (Fig. 3 B). It becomes obvious that the thickenings of the outer wall are smaller in *Q. frainetto* than in *Q. robur*, if Figs. 3 C and 2 C, which show the lower epidermis of these oak species, are compared.

The cuticular ledge (n) at the border of the outer and ventral walls of the stoma can be seen well in Fig. 3 A, where it has the shape of a regular oval line. An examination of the inside of the aperture shows numerous cuticular lamellae on the surface of the ventral wall. They extend lengthwise, i. e. parallelly with the cuticular ledge, at the beginning of the aperture.

Smaller magnifications show that in *Q. frainetto* there are numerous longitudinal rungs in the area of the vein ribs and some hairs components of which are unicellular and grouped in tufts (Fig. 3 D).

Quercus cerris

The thickenings of the outer wall encircling the stomata of this species are less developed than those of the species *Q. robur* and *Q. frainetto*. Neither are there wax eflorescences on the epidermal surface (Fig. 4 A, B). Therefore, the lower epidermis of *Q. cerris* differs greatly from the two other species discussed above.

The cuticular ledge (n) between the outer and the ventral walls is clearly and well defined, so that even smaller magnifications allow a good view of the light elyptical line. It can often be noticed that the cuticle above the pore is not completely broken. Therefore instead of only one cut, two, three or even four smaller cuts appear along the aperture (Fig. 4 B, C).

In the places where the stomata are fully developed, the ventral wall has a certain number of cuticular lamellae. These lamellae (l) extend more or less continuously and parallelly with the cuticular ledge.

Good pictures of the lower epidermis of *Q. cerris* leaves reveal that the epidermis has a slightly uneven surface, vein ribs with longitudinal rungs and tufted hairs which sometimes contain only two cells (Fig. 4 D).

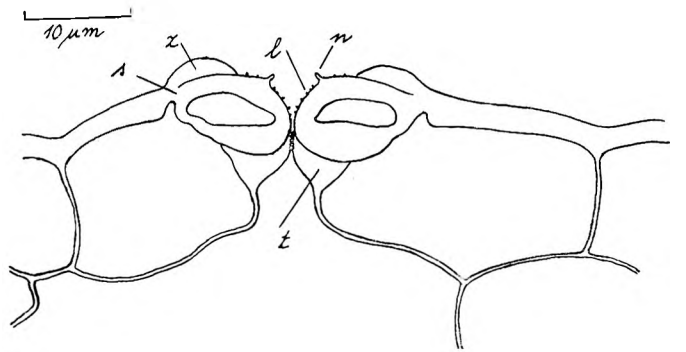
Discussion

According to the data in the literature (J o h a r i 1968—1978) it seems that the stomata of oaks have not been studied till now by electron microscope. As these investigations show, the stomata cells of oaks can also be used for classification purposes within the genus *Quercus*. By means of a scanning electron microscope we have established that the lower leaf epidermis of *Q. robur* and *Q. frainetto* is covered with rib-like or laminated wax efflorescences which are absent from the leaves of *Q. cerris*. This fact justifies the classification of S c h w a r z (1964) who classifies *Q. robur* and *Q. frainetto* in the subgenus *Quercus* L., and *Q. cerris* in the subgenus *Cerris* L.

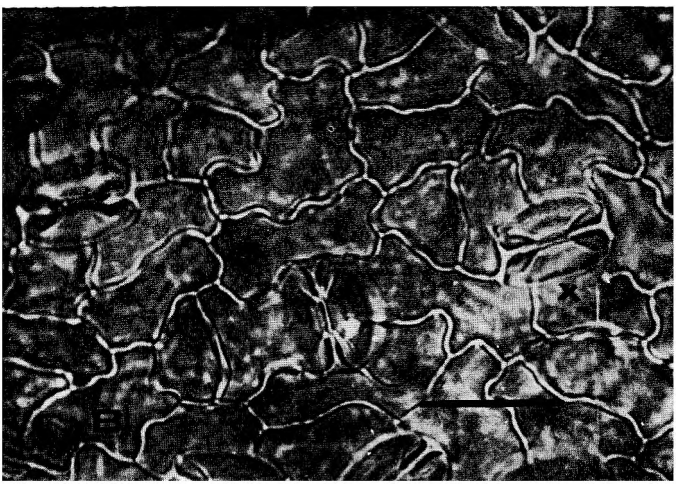
Electron microscopic research shows that interesting cuticular lamellae are present on the ventral walls of guard cells (Fig. 1 A, 2 B, 4 A). The lamellae surely reduce the intensity of transpiration.

However, the light microscopic investigations of guard cells have demonstrated that the walls of their central part are mostly very thick and the walls of their end parts are thin (Fig. 1 B). But a component of the otherwise very thick central wall part is thinner and elastic, and this component is the dorsal wall (Fig. 1 A). The presence of this elastic part indicates that even this central part of the guard cell can be enlarged when the turgor increases (cf. E s a u 1965 : 160).

Moreover, the presence of a wall joint (Fig. 1 A s) indicates that the guard cells are able to move. The wall joints are grooves in the cell walls



A



B

Fig. 1. A. Cross section through stoma of *Quercus robur*. l cuticular lamellae, n cuticular horn in the place where the ventral and outer walls join together, s wall joint in subsidiary cell, t structure in the form of a triangle, z thickening of the outer wall of the guard cell. B. Lower epidermis of *Quercus cerris* in face view. The walls of the middle part of the guard cells are thickened but the walls of the end parts are thin. The best visible stoma is marked x. — Bar in figure B represents 25 µm.

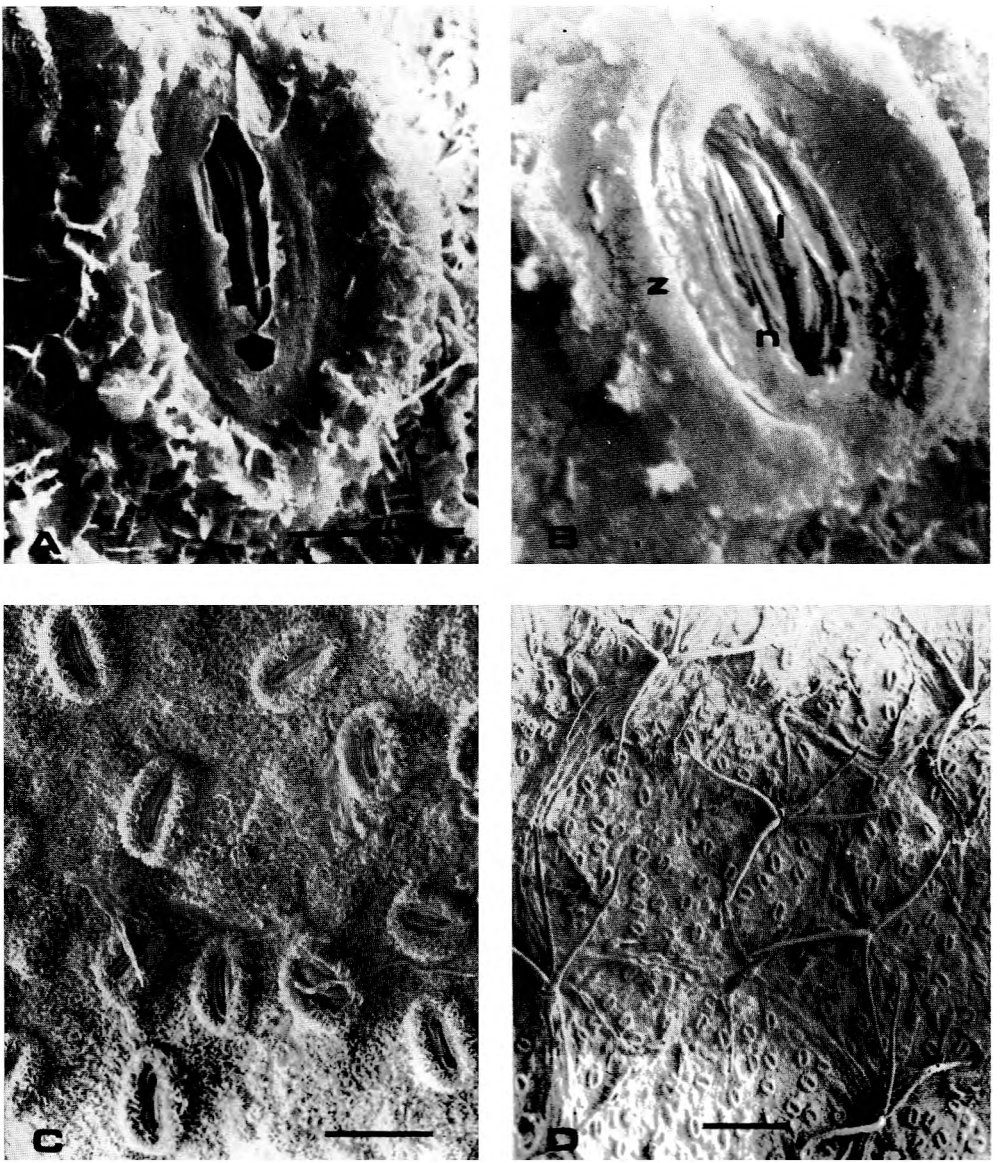


Fig. 2. *Quercus robur*, lower leaf epidermis. A. Stoma with wax efflorescences and horned cuticle above the aperture. B. Stoma with laminated wax efflorescences. The thickening (z) of the outer wall which surrounds the aperture. Cuticular lamellae (l) on the ventral walls. The cuticular ledge (n) between the outer and ventral walls of the stoma. C. A part of epidermis with stomata. The thickenings of the outer walls of the stomata are covered with wax efflorescences. The wax is present also above the ordinary epidermis cells. D. A part of epidermis with tufted hairs and veins. — Bar in figure A also relates to figure B and represents 10 μm. Bar in figure C represents 25 μm and bar in D represents 100 μm.

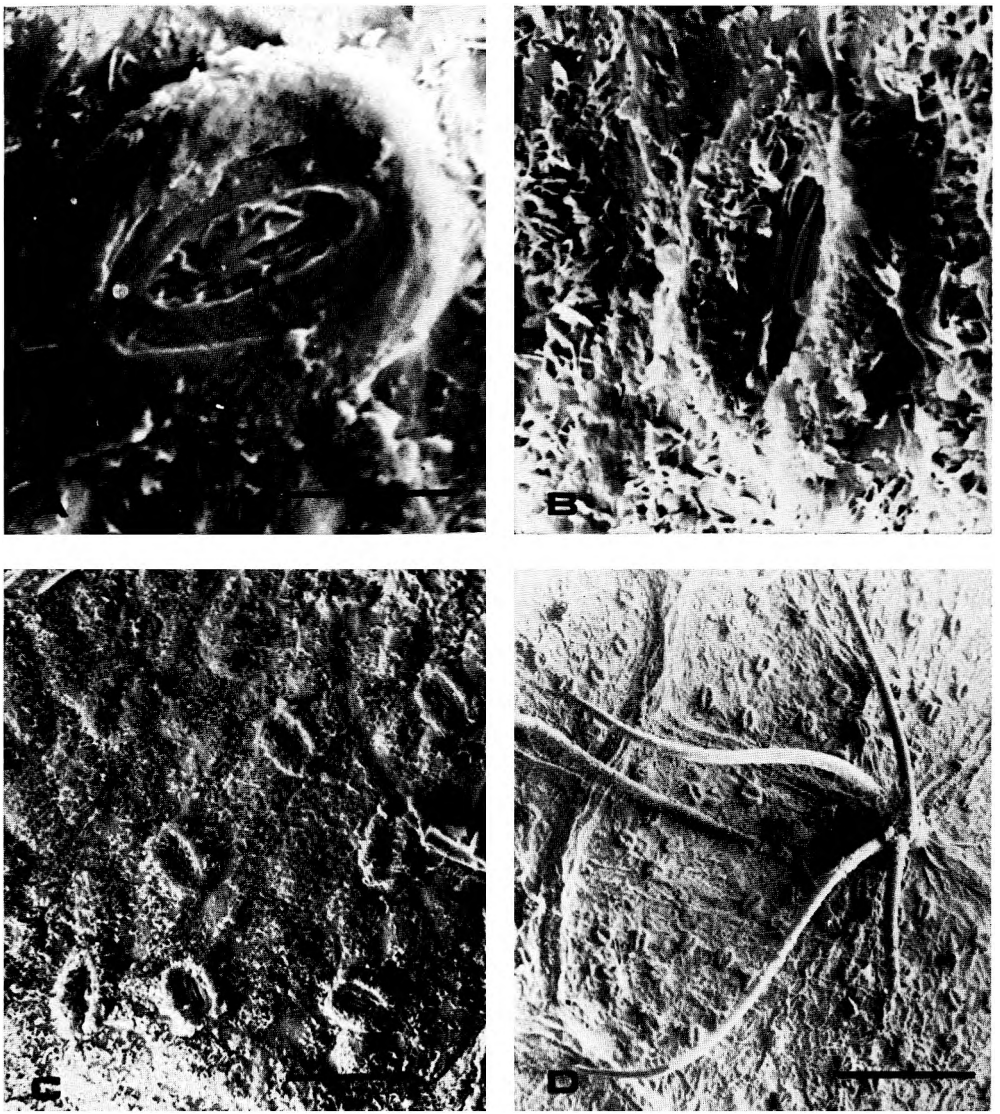


Fig. 3. *Quercus frainetto*, lower leaf epidermis. A. Stoma with a small quantity of wax efflorescences. Cuticular ledge (n) between the outer and ventral walls of the stoma. B. Stoma with wax efflorescences and lamellae on the ventral walls. C. A part of epidermis with stomata. D. Epidermis with hairs and vein ribs. — Bars have the same values as in Figure 2.

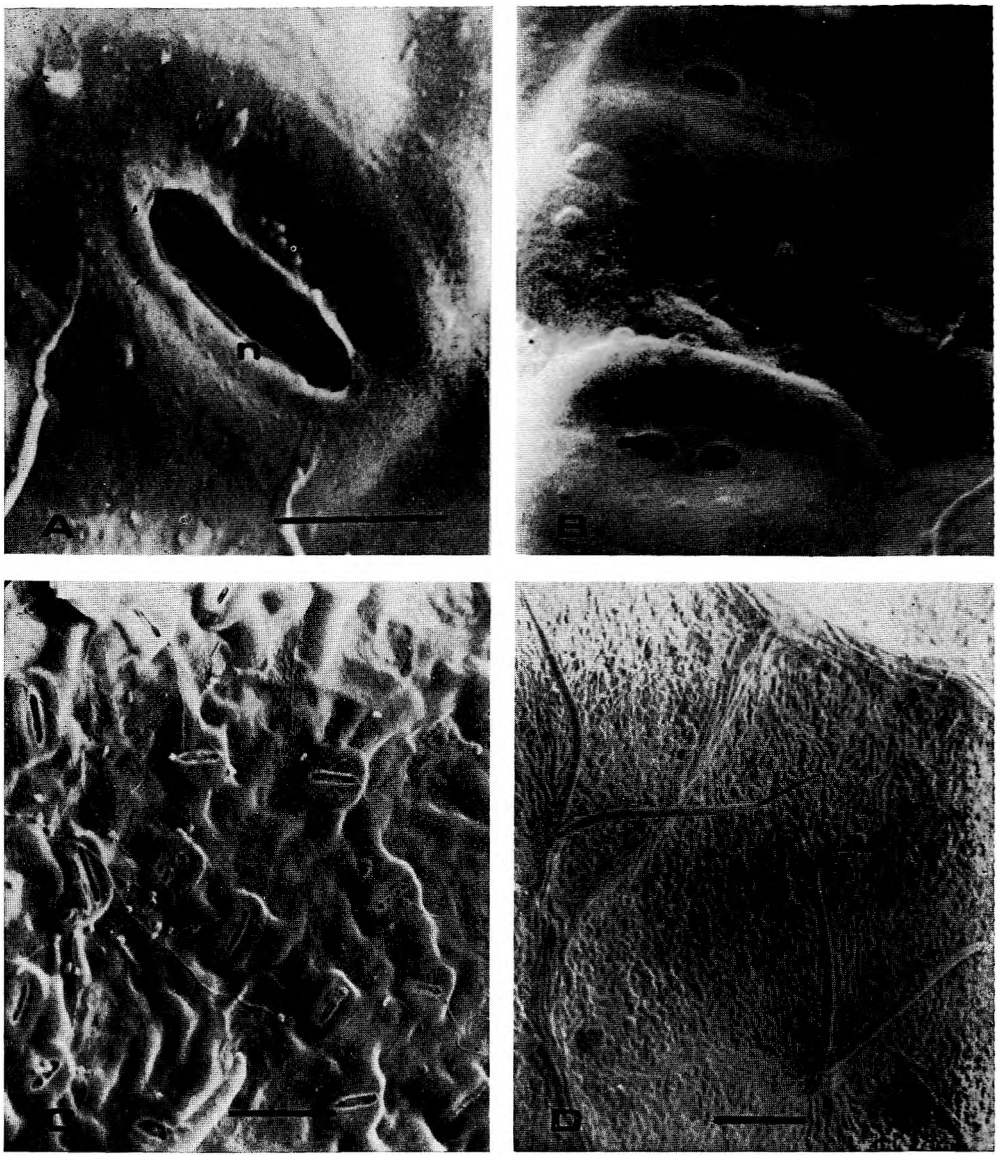


Fig. 4. *Quercus cerris*, lower leaf epidermis. A. Stoma with weakly developed thickening (z) above the outer walls. Cuticular ledges are well visible (n). In the stoma aperture there are many cuticular lamellae (l). B. Two stomata whose apertures are still partially covered with cuticle. C. A part of lower epidermis. D. Lower epidermis with hairs and vein ribs. — Bars have the same values as in Figure 2.

of subsidiary cells in the places where they are connected with the guard cells. The wall joints are always present when the walls of subsidiary cells are very thick (Guttenberg 1952 : 123).

Besides, characteristic thickenings on the outher wall of guard cells have been established; they surround the central part of the stomata almost completely.

It will be useful to investigate anatomically also other oak species.

Summary

The stomata structure of *Quercus robur, Q. frainetto* and *Q. cerris* was investigated by light and scanning electron microscope. With regard to the arrangement of adjacent cells the stomata of oaks belong to the anomocytic type, and with regard to the structure of guard cells to the "gantelegubovidnyj" (= hantel-lippenförmig) type described by Aneli (1975).

It is a characteristic of the guard cells of oaks that its central part has a thick wall and the end parts have thin walls similar to the stomata of *Poaceae*. Although the central wall is thick, its dorsal wall is slightly thinner so that the central part can also be extended when the turgor increases.

On the outer wall of the guard cells there is a thickening which protrudes above the surface of the central part of the stoma so that it can reduce the ventilation above the aperture. The existence of cuticular lamellae on the ventral walls of the guard cells seems to be a xeromorphic property.

The stomata of *Q. robur* and *Q. frainetto* are covered with wax efflorescences, and those of *Q. cerris* are without them. The thickenings on the outer wall of the stomata are higher in *Q. robur* than in *Q. frainetto*.

*

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References

Aneli, N. A., 1975: Atlas epidermi lista. Tbilisi.

Bačić, T., 1979: Morfološke i anatomske karakteristike listova nekih vrsta hrastova iz različitih fitocenoza s područja donjeg Posavlja. Disertacija, Priština.

Dengler, N. G., L. B. MacKay, 1975: The leaf anatomy of beech, *Fagus grandifolia*. Can. J. Bot. *53*, 2202—2211.

Esau, K., 1965: Plant Anatomy. Second Edition. Wiley & Sons, New York — London.

Guttenberg, H., 1952: Lehrbuch der allgemeinen Botanik. 2. Aufl., Akademie-Verlag, Berlin.

Guyot, M., 1966: Les stomates des Ombellifères. Bull. Soc. Bot. Fr. *113*, No. 5—6.

Johari, O. (Ed.): Proceedings of the Scanning Electron Microscopy (1968—1978), SEM Symposia Office, IIT Research Institute, Chicago.

Metcalfe, C. R., L. Chalk, 1950: Anatomy of Dicotyledons. 2 vols. Oxford, Clerendon Press.

Schwarz, O., 1964: *Quercus* L. In: T. G. Tutin et al. (Ed.), Flora Europaea, Vol. I. Cambridge.

Solereder, H., 1899: Systematische Anatomie der Dicotyledonen. Enke Verlag. Stuttgart 1899.

Vesque, J., 1881: De l'anatomie des tissus appliquée a la classification des plantes. Nouv. Archiv. Mus. Hist. Nat., 2. sér., *4*, 1—14.

SAŽETAK

ISTRAŽIVANJE PUČI TRIJU VRSTA HRASTOVA SVJETLOSNIM I RASTERSKIM (SCANNING) ELEKTRONSKIM MIKROSKOPOM

Tomislav Bačić

(Nastavnički studiji u Splitu, Filozofski fakultet u Zadru)

Istražena je građa pući vrsta *Quercus robur* L., *Q. frainetto* Ten. i *Q. cerris* L. iz Jugoslavije. Istraživanja su obavljena svjetlosnim i rasterskim elektronskim mikroskopom. S obzirom na položaj okolnih stanica pući pripadaju anomocitnom tipu, a s obzirom na građu pući »gantelegubovidnom« (= hantel-lippenförmig) tipu prema razdiobi Anelija (1975).

Karakteristično je za pući tih hrastova da su središnji dijelovi zapornica debeli. Iako su središnji dijelovi stijenki većinom debeli, leđna je stijenka tanja i elastična, tako da se taj dio također može proširiti prilikom povećanja turgora. Da se i taj dio može povećati i pomicati, svjedoči brazda (stanjenje), tzv. zglob u vanjskoj stijenci susjedica tik uz zapornice.

Na vanjskoj stijenci zapornica nalaze se zadebljanja koja strše iznad ostale površine pući tako da stvaraju plitak prostor sa smanjenim provjetravanjem. Interesantni su i kutikularni nabori na ventralnim stijenkama zapornica koji su vjerojatno kseromorfna struktura.

Pući vrsta *Q. rubor* i *Q. frainetto* presvučene su djelomično voštanim prevlakama, dok su pući *Q. cerris* bez prevlaka. Zadebljanja na vanjskoj stijenci pući od vrste *Q. robur* jače strše iznad razine epiderme nego od vrste *Q. frainetto*.

Dr Tomislav Bačić
Nastavnički studiji
Teslina ul. 12
YU-58000 Split (Jugoslavija)