

HISTOLOGICAL AND MORPHOLOGICAL COMPARISON OF THE LEAVES OF CYDONIAE

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Abstract

We have carried out leaf-morphological and leaf-histological investigations into *Cydoniae* from Bereczk (B), Constantinople (C), and Leskovác (L).

We have compared the length of leaf-blade, its largest breadth, and the length of leaf-stalk with the morphological characterization of leaves. To the histological investigations we have made, as usual, a preparation by maceration, and cross-sections with celloidin embedding. We have compared our data, which are in every case averages of 100 measurements, with T-test and Wilcoxon's test. On the basis of our results, we can make the following statements.

From among the morphological characteristics of leaves of the three *Cydonia* sorts, there is a significant difference in the length of leaf-stalks, in the breadth of leaf-blades, and in the full length of leaves (petiole + lamina of leaf).

There is a significant difference (0.1) between the cell- and stoma-numbers of the lower-side epidermis. On the other hand, the difference in the epidermis cell-numbers is not even at 5 per cent level between the sorts B and L. The highest is the stoma-index of quince L.

The ratio of participation of the spongy parenchyma is the highest in sort L (1.8) and decreases in the sequence of sorts C (1.25) and B (1.03). We have observed a positive correlation between the size of fruit (average weight) and the relative thickness of the spongy parenchyma, in case of the three investigated sorts.

On the basis of tissue characteristics there is a closer relationship between sorts B and C.

Introduction

Literary survey

As compared with the other fruit sorts, the sort number of quince (*Cydonia oblonga* MILL.) is low even on a world scale.

The number of the registered sorts is, according to BRÓZIK—REGIUS (1957) not more than 7 to 10 in this country. According to our investigations, in Hungary quinces B and C, planted in about 47 per cent each, are the best known. It is difficult to differentiate these, as well as quince L, cultivated in Jugoslavia, on the basis both of their vegetative and generative properties. To the reliable determination of the three sorts the above authors give a direction on a morphological basis. They use from among the vegetative properties the size and form of leaves for characterizing sorts. From among the generative organs, they compare the sorts on the basis of flower and fruit.

The literature, dealing with the quince, is also poor. Apart from the above works, we used, as well, the works by G. KRÜBMAN (1951, 1960), M. MOHÁCSI—A. PORPÁCZY (1954), Á. JESZENSZKY (1966), A. TODOR (1970), J. RAYMAN—P. TOMCSÁNYI (1964), R. RAPAICS (1940), and the statistical publications, listed in References. Histological data are to be found in various comprehensive descriptions. The tissue structure of the stem is dealt with by P. GREGUSS (1945), METCALFE, G. R.—CHALK, L. (1954); the morphology, tissues and chemical composition of the fruit and seed, the stone cells to be found in the fruit by HALMAI—NOVÁK (1963), G. KASSTEN, and U. WEBER (1951), B. HAZSLINSZKY—L. TAKÁCS (1960).

The aim of our work is to characterize the quince sorts from Bereczk, Constantinople, and Leskovác, on the basis of the morphological and histological signs of leaves. We have compared the dimensions of leaf sizes, as well as the quantitative, dimensional data of the tissue elements of the leaf with the average weights, which are characteristic of the sorts of fruit, as well.

Materials and Methods

The investigated sorts of quince are: those from Bereczk, Constantinople, Laskovác (further marked with the letters B, C and L). For the investigation we have used some individuals originated from the same habitat and showing the morphological characteristics of sorts well. The number of the investigated individuals is: from B, K there are 5 each, from L 4. For the morphological comparison of leaves, we have measured 100 full-grown leaves of each sort. These were taken in equal rate from the edge, bottom, middle and inner regions of the foliage.

For comparison the following data were used:

- Length of the leaf-blade: L (distance between points A—B).
- Breadth of the leaf-blade: B (distance between points D—E).
- Length of the leaf-stalk: LS (distance between points B—C).

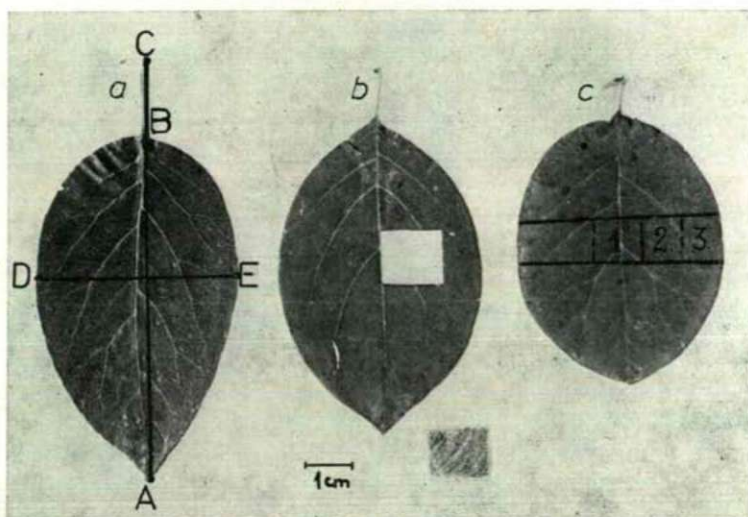


Fig. 1. a) Measuring points used for comparison b) Sampling into the investigation of the epidermis c) Sampling to the cross-section investigation of the leaf.

From the data we have calculated the full length of the leaf (A—C), as well as the ratio of the breadth (D—E) and length (A—B) of the leaf-blade.

The epidermis of the leaf was compared on the basis of the following data: epidermis cell number (E), the number of stomata (S), the length of closing cells (L), the breadth of closing cells (B), the ratio of breadth and length of the closing cells (B/L), stoma-index (I). For investigating the epidermis we have used leaves taken from the region of foliage because, according to our earlier investigations (PATAKY, 1969), with the exception of the B/L, ratio of the closing cells, as well as the length of these, we have found differences in the investigated properties within the same individual.

Sampling for the preparation for maceration (Fig. 1, b) took always place from the identical region of the leaf-blade. This is justified by that some differences can be observed even between the samples taken from different places of the same leaf, in respect of the measurable properties of the epidermis (Mrs. SIMON—MOLNÁROS, 1964; MARÓTI, 1965; GULYÁS, 1961, etc.).

For analysing the mesophyll, we made a cross-section and measured the full thickness of the leaf-blade (in μ): mesophyll + surface and lower-surface epidermis (Fig. 1, c: according to samples 1, 2, 3). Within this, we measured the thickness of the palisade and spongy parenchymas separately (in μ).

The different properties were compared on the basis of 100 survey data, with a double-sample T-test and Wilcoxon's test.

For the leafy-crown shape, characteristic of the sorts, and the fruit average weights, we have used the data of BRÓZIK—REGIUS (1957) MOHÁCSI—PORPÁCZY (1954).

Results and evaluation of the investigations

1) External morphological

Observations:

The sort of longest leaf-stalk and largest leaf-blade is quince B. The measurements of sort L are the smallest, and those of sort C form a transition between both. In respect of the form of leaf, the same can be established (Fig. 2).

In the length of the leaf-stalk, the sorts show a significant difference at 5 per cent level. In the length of the leaf-blade, only sort L differs at 5 per cent significance level from the other two sorts. The breadth of the leaf-blade gives a difference in significance at 5 per cent level, between the single sorts.

We find a difference in significance, similarly at 5 per cent level, in the full length of the leaf in all the three cases, despite that sorts C and B do not show any difference, if we investigate one of the components of the measuring point (the length of the leaf-blade).

The shape of the leaf can be well characterized with the B/L ratio of the leaf-blade. Sort L differs from the two others, at 5 per cent level, in this property. The form of this is namely the most round. For the other two sorts, we have obtained values, which are near each other both in size and shape. (Cf.: Table 1)

Our survey results concerning the external morphology of the leaf support Brózik-Regius's characterization of quince sorts B, C, and L (1957), which can be used well in practice, as well, and according to which, the leaves of quince B are

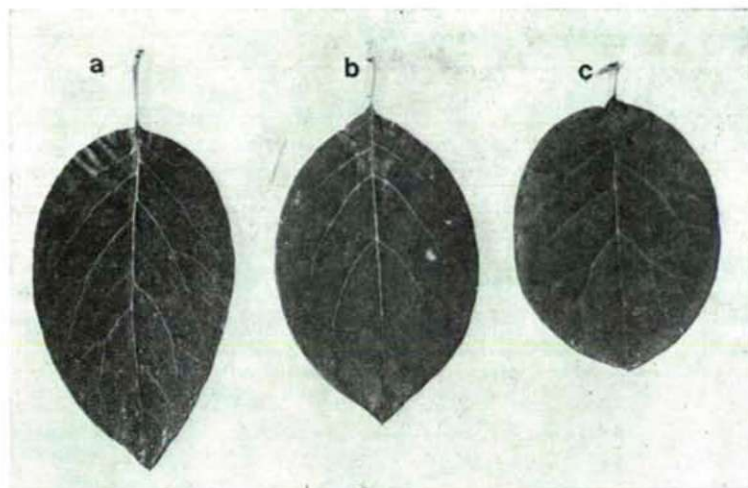


Fig. 2. Leaf forms of the quince sorts from Beretzka (a), Constantinople (b), and Leskovác (c).

very large and the most long-shaped, those of sort L are the most roundish and the smallest ones. Sort C may be put, in size, too, between the two other leaf shapes. As to the leaf-stalk, we have got other results. We have found a continuous transition between the investigated sorts in respect of the length of the leaf-stalk. According to our measurements, quince B has the longest leaf-stalk, then the length of the leaf-stalk decreases in the sequence of sorts C and L.

Table 1. Morphological comparison of the leaves of quince sorts B (Beretzka), C (Constantinople) and L (Leskovác).

Measured property		sorts average	maximum	minimum	sample dispersion	average dispersion
Length of leaf-blade in mm	B	82	110	59	1.06	0.10
	C	79	117	57	0.11	0.16
	L	62	87	46	0.76	0.07
Breadth of leaf-blade in mm	B	57	70	42	0.83	0.08
	C	52	72	38	0.07	0.73
	L	46	60	36	0.49	0.04
Length of leaf-stalk in mm	B	17	25	11	0.29	0.02
	C	12	16	10	0.01	0.14
	L	10	13	7	0.15	0.01
Full length of leaf in mm	B	99	130	73	1.26	0.26
	C	92	131	67	0.12	1.24
	L	72	99	56	0.88	0.08
B/L ratio of the leaf-blade	B	0.69	0.82	0.59	0.07	0.007
	C	0.65	0.84	0.54	0.06	0.006
	L	0.74	0.94	0.61	0.05	0.005
B/L percentage of the leaf-blade	B	69	82	59	7.00	0.70
	C	65	84	54	6.00	0.60
	L	74	94	61	5.00	0.50

As a result of the different environmental effects, the measurements of the leaf (primarily the leaf area or leaf size) strongly change.

For instance, under the influence of the intensity of illumination (VERKERK, 1955; BEAN, 1964), of the daily total energy quantity (NEWTON, 1963), under the influence of geographical latitude, of the height above sea level (BREZHNEV, 1955), etc. We can, therefore, characterize with the obtained data first of all the formal properties of the single sorts and can describe the degrees of the differences between sorts.

2) Histological observations

a) General characterization and histological comparison of the epidermis

It is characteristic of all the three sorts of quince that the leaves are hypostomatic. Stomata placed scattered in the leaf-vein islands are somewhat prominent from the level of epidermis. On the basis of Van Gotthem's classification (1971), the openings of the aspiratory metabolism are of anomocytic type, i.e., the closing cells are not surrounded by side cells. The downiness of the leaves is induced by the intergrown network of the unicellular, long drawn-out covering hair of the thin cell-wall (Plate III picture 1).

The radial wall of the epidermis cells is of wavy course viewed from above. The degree of waviness (its amplitude and denseness) — particularly in sorts B and C — is stronger at the lower surface than at the upper one (Plates II, III.) At the surface epidermis cells, the striation of the epidermis cuticle, which generally follows the longitudinal axis of cells, is characteristic (Plate II, picture 4).

Results of our measuring are summarized in Table 2. (Cf. Table 2)

The size of the surface epidermic cells is the largest in sort B, the smallest in sort C. These differ from each other even at a 0.1 per cent significance level. Sorts B and L however, give not any measurable difference even at 5 per cent level.

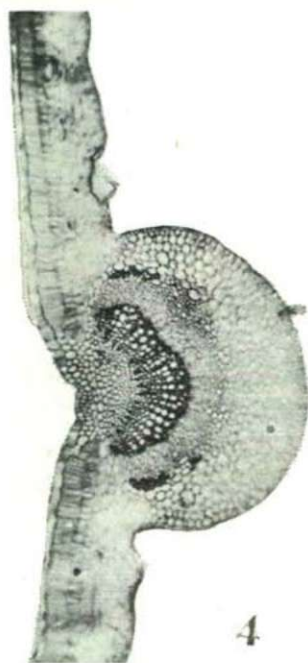
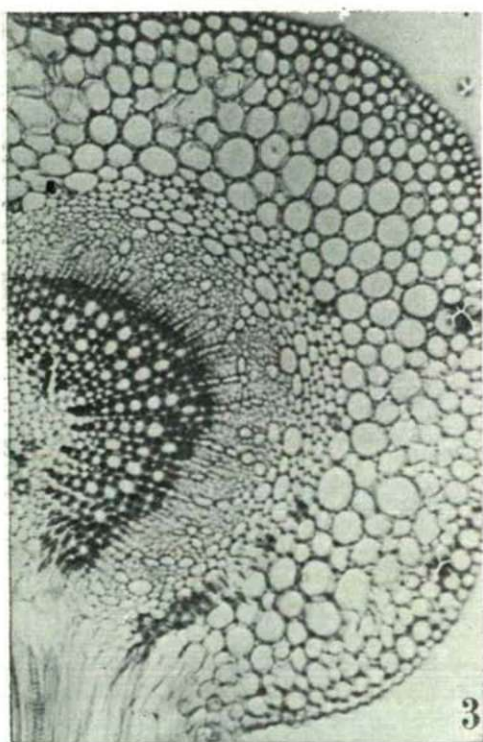
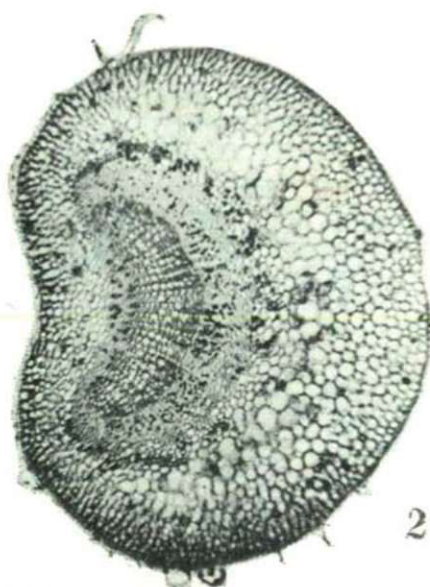
On the basis of the lower surface epidermic cell number, falling on the areal unit, epidermic cells of sort L with the smallest leaf-blade have the largest size. The differences in size are more important at the lower surface because in a joint comparison and in that by pairs (B—C, C—I, L—C) too, the three sorts show a significant 0.1 per cent difference.

We have observed a well-perceptible difference in stoma-number between the three investigated sorts, both compared by pairs and jointly at a 0.1 per cent significance level as well. (Cf. with Table 2)

The stoma index (the ratio of stoma-number and epidermic cell number referred to an areal unit) in the leaves of sort L is strikingly high (13.72), as compared with that of the other two sorts. It is almost double of sort C and differs from sort L, as well, at a 0.1 per cent significance level.

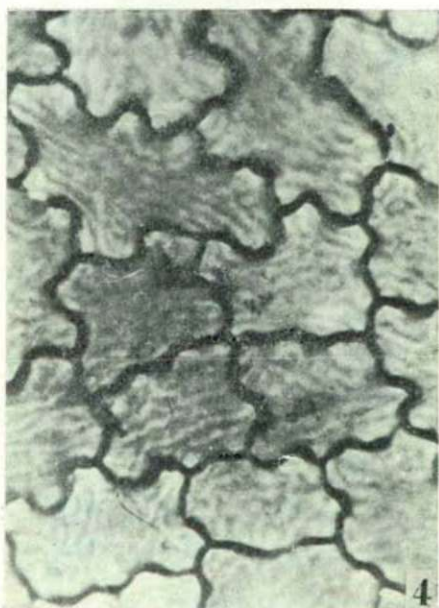
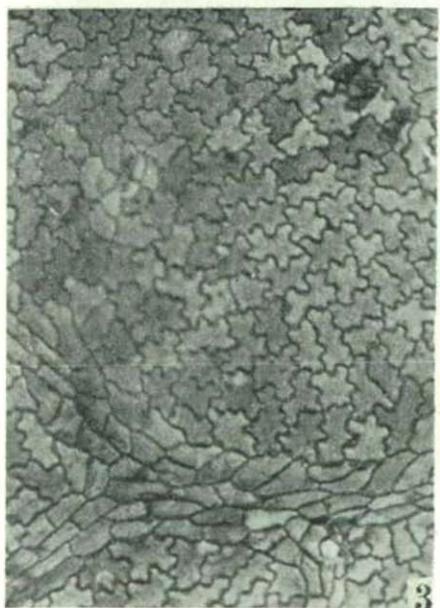
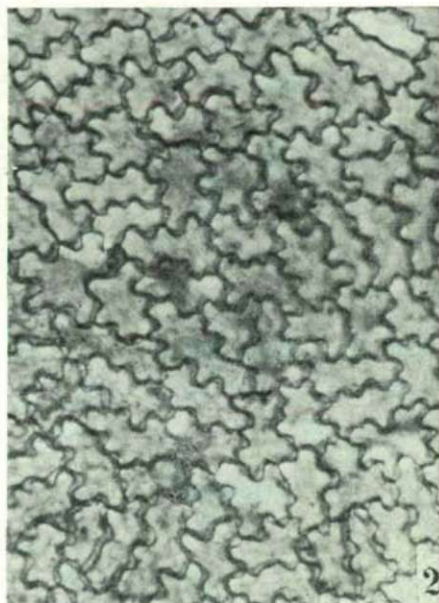
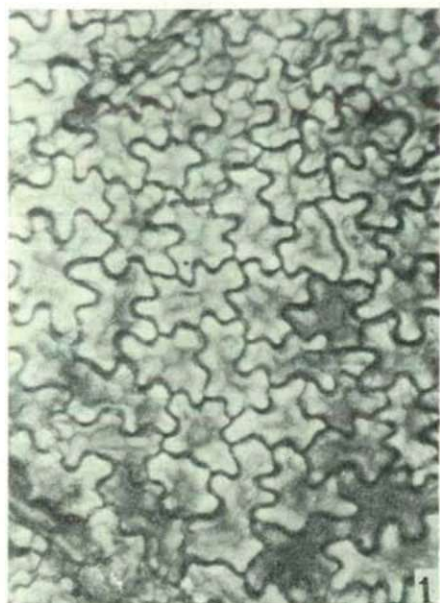
In respect of the length of closing cells, sorts B—C—L are in a decreasing sequence. Between the closing-cell lengths of sorts B and L, a 0.1 per cent significant difference can be established. In breadth, at a 5 per cent level, the two sorts show some difference. Both in length and in breadth, at a 1 per cent level, there is some difference between sorts B—C and C—L.

Plate I



Pictures 1, 4. Leaf cross-section (B), Magnification: $\times 240$ and $\times 49.6$
Picture 2. Leaf-stalk cross-section (B), magn.: $\times 36$
Picture 3. Main vein cross-section (B), magn.: $\times 305$

Plate II



Surface epidermis

Picture 1. Berezck, magn.: $\times 305$

Picture 2. Leskovác, magn.: $\times 198$ quince sorts

Pictures 3, 4. Constantinople, magn.: $\times 198$ and $\times 600$

In the B/L ratio—which this form may be characterized by—there is a 1 per cent significant difference only between sorts B—L and C—L. In respect of the form of closing cells, sorts B—C cannot be separated. On the basis of our measuring, we have found "the most round" stomata at sort L, while those of sorts B and C were more elongated.

Though stoma-number and stoma-index seem to be characteristics, which change less as a result of ecological effects as referred to an areal unit of leaf than the epidermic cell unit; they are yet alone not sufficient in this way either, to delimit some taxonomical categories (species, possibly sorts) (KURSANOV, 1952; Mrs. SIMON—MOLNÁROS, 1964; GULYÁS, 1961; MARÓTI, 1965, 1961; GREGUSS, 1962).

The conservative tissue characteristics are primarily formal properties, e.g. the form of the epidermic cells, the type of stomata, the form of closing cells. (B/L). The joint application of these with other properties, which are less, affected by ecological effects first of all on the basis of the lower surface epidermis, e.g., stoma-index possibly stoma-number, can reliably be applied even in (the identification of sort, if in) these properties the investigated sorts significantly deviate from one another.

(It is interesting to note that BRÓZIK—REGIUS (1957), found the following characteristics of the form of leaves: the form of the leaf of quince B is: broad — oval, the form of the leaf of quince C is: roundish, broad, oval, the form of the leaf of quince L is: very broad, elliptical, sometimes roundish.

It could only be decided on the basis of further investigations whether the forms of leaves and closing cells, the close correlation between their ratios, are accidental or there is a connection between the measured data).

b) General characterization and histological comparison of the mesophyll

The mesophyll of all the three kinds of leaves is of a heterogeneous construction, with a double cell-lined, columnar multi-cell-layer spongy parenchyma (Plate I, picture 1). The spongy parenchyma in the investigated sorts is of a loose structure. The intercellular spaces are much larger than the cell body (Plate III, picture 2). The main vein and the major leaf veins protrude at the side of the lower surface. The collaterally closed vascular bundle of the main vessel is limited kollenchyma, which is hollow towards the side of the lower surface epidermis. The bundle is curved in a crescent form. The phloem is bordered by sclerenchyma bundles, as in the stalk (Plate I, pictures 3, 4).

Our data measured in the leaf cross sections are summarized in Table 3.

Between the investigated three sorts there is — even at 0.1 per cent level — a significant difference in the thickness of leaf-blades. Sort B has the thinnest leaf (131.56 μm). The largest average leaf-thickness was found in sort C (245.33).

There are some differences in the thickness of the palisade parenchyma as well. The difference between sorts C—B and C—L manifests itself at 0.1 per cent significance level as well; but between sorts B—L, only at 1 per cent significance level (Fig. 3).

In respect of thickness of the spongy parenchyma, all the three sorts differ from one another, even at 0.1 per cent level.

If we compare the thickness of the palisade and spongy parenchymas with the full thickness of the leaf, there are differences between the single sorts at a lower

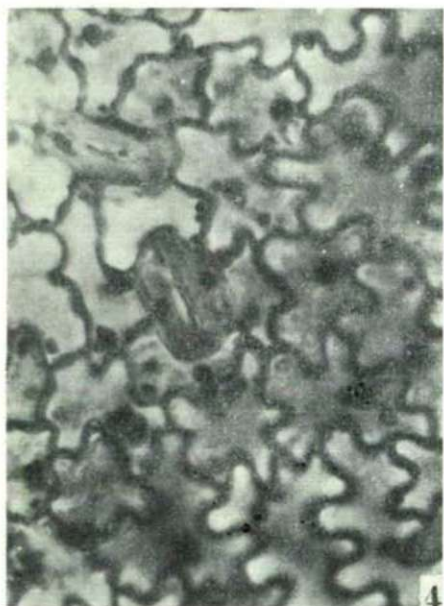
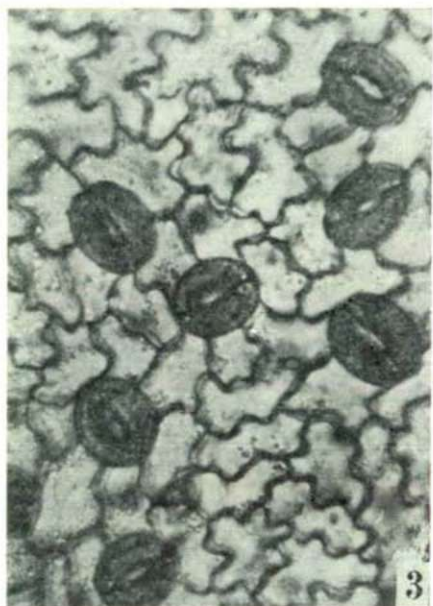
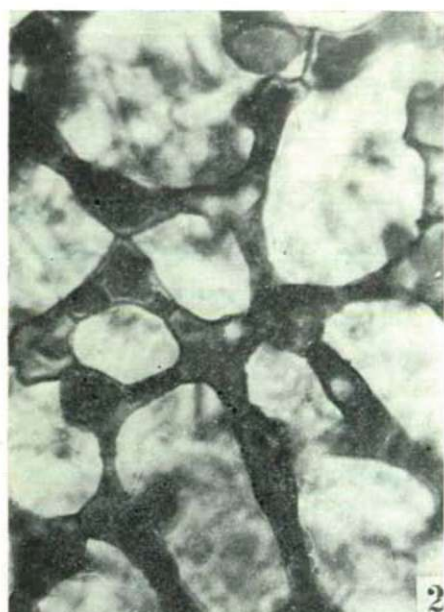
Table 2. Measurement results of the epidermis of quince sorts B (Berezck), C (Constantinople), and L (Leskovác).

Measured properties	Sorts	Average		Maximum		Minimum		Sample dispersion		Average dispersion	
		upper surface	lower surface	upper surface	low. surface	upp. surface	low. surface	upp. surface	low. surface	upp. surface	low. surface
Epidermic cell number/sq. mm	B	953	2803	1184	3360	752	2496	6.44	12.8	0.64	1.28
	C	1001	3064	1200	4000	782	2480	6.08	17.9	0.60	1.79
	L	972	2300	1344	2976	800	2240	6.40	18.3	0.64	1.83
Stoma number/sq. mm	B		268		384		208		2.4		0.24
	C		233		320		144		2.9		0.29
	L		366		512		224		4.4		0.44
Length of closing cells μm	B	27.12		31.25		23.75		1.4		0.14	
	C	22.75		29.15		17.50		1.7		0.17	
	L	21.50		25.00		17.50		1.3		0.13	
Breadth of closing cells μm	B	15.00		19.75		12.50		1.2		0.12	
	C	12.62		16.25		8.75		1.2		0.12	
	L	14.50		17.50		12.50		1.0		0.10	
B/L ratio of the closing cells	B	0.55		0.71		0.45		0.05		0.005	
	C	0.56		0.72		0.41		0.06		0.006	
	L	0.67		0.82		0.57		0.04		0.004	
Stoma index	B	8.72		8.25		7.69		9.90		0.99	
	C	7.07		7.40		4.82		15.00		1.50	
	L	13.72		14.67		9.09		13.90		1.39	

 Table 3. Measurement results of the cross-section of the leaf
 B = Berezck, C = Constantinople, L = Leskovác.

Measured properties	Sorts	Average	Maximum	Minimum	Sample dispersion	Average dispersion
Total thickness of the leaf-blade in μm	B	131.56	148.75	112.50	0.76	0.67
	C	245.33	290.00	212.50	13.46	1.34
	L	160.15	173.75	148.75	4.43	0.44
Thickness of palisade parenchyma in μm	B	54.45	66.25	43.75	3.82	0.38
	C	94.91	123.75	58.75	10.67	1.06
	L	50.28	62.75	45.00	4.40	0.44
Thickness of spongy parenchyma in μm	B	56.35	70.00	40.00	2.41	0.24
	C	126.55	150.00	102.50	9.74	0.97
	L	85.86	96.25	75.00	3.38	0.33
Ratio of the total thickness of the leaf-blade p.p.	B	2.36	2.69	2.09	1.60	0.16
	C	2.63	2.41	2.21	6.40	0.64
	L	3.18	4.02	2.04	4.29	0.42
Ratio of the total thickness of the leaf-blade spongy parenchyma	B	2.32	3.09	2.04	5.92	0.59
	C	1.91	2.25	1.61	1.15	0.11
	L	1.87	2.10	1.67	1.27	0.12
Ratio of spongy par. palisade parenchyma	B	1.03	1.82	0.80	1.62	0.16
	C	1.25	2.30	1.05	1.10	0.11
	L	1.80	2.08	1.50	0.78	0.07

Plate III



Lower surface epidermis

Picture 1. Berezsk, magn.: $\times 63$ quince sorts

Pictures 2, 4. Constantinople, magn.: $\times 600$ and $\times 770$

Picture 3. Leskovác, magn.: $\times 600$

significance level in the ratio of participation of the palisade parenchyma than in that of the spongy parenchyma. It is in the former case at 1 per cent significance level between sorts B—C, L—C and at 5 per cent significance level between B—C.

On the other hand, in the participation ratio of the spongy parenchyma, there is no difference between sorts C—L, even at 5 per cent significance level.

The thickness ratio of the spongy parenchyma and palisade parenchyma (sp.p./p.p.) is different within the leaves the three sorts.

This ratio is the highest for the good of the spongy parenchyma in sort L: 1.8 (53.6 per cent) and differs from that of the other two sorts at 1 per cent significance level. It is in sorts B and C 1.3 (43 per cent), resp. 1.25 (51.5 per cent); these differ from each other only at 5 per cent significance level.

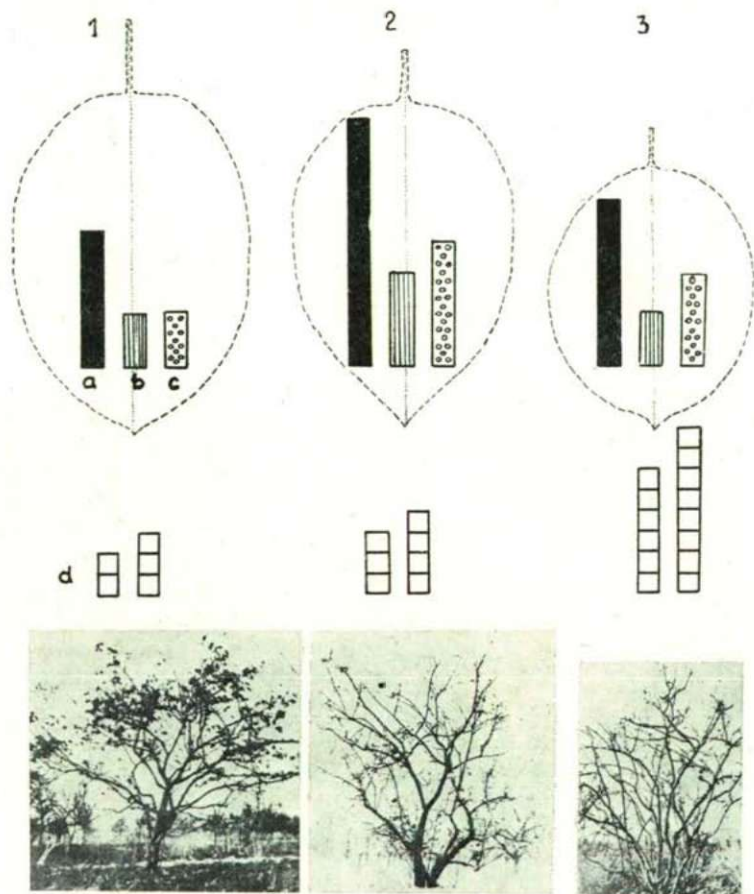


Fig. 3. Sizes, forms of the leaf, fruit, and tree Quince sorts from Bereczk (1), Constantinope (2), and Leskovác (3).

- a) thickness of the leaf-blade μ
- b) thickness of the spongy parenchyma μ
- c) thickness of the palisade parenchyma μ
- d) average weight of fruit, 100 g

Summarizing our results concerning the cross section of the leaf, it is to be established that unambiguously essential (0.1 per cent) differences between the three sorts can be found in absolute value — in the thickness of leafblades and in that of the spongy parenchyma. The designation, used by BRÓZIK—REGIUS (1957): "lighter" for sort B and "of the darkest leaf" for sort C, may originate from the considerable differences in the thickness of lamina, as well. These two data are however, considerably affected by water supply. BREZHNEV (1955) demonstrated, namely, that in the irrigated areas the lamina becomes thicker, the quantity of the spongy-parenchyma cells multiplies.

And not only the effect of water supply can be considered as generally valid to the change in the tissue structure. The geographical latitude, the height above sea level (BREZHNEV 1955), the intensity of light (e.g., GRAHL & WILD, 1973; STARZECKI, 1958; TAKÁCS, 1973), the spectral composition of light (e. g., HORVÁTH, 1975 PATAKY—HORVÁTH, 1978) also induced essential changes in the leaf-blade or, within the leaf, in the absolute thickness of the palisade parenchyma, in the size of the spongy and palisade parenchyma-cells, in the number of the cell-rows of the spongy parenchyma, as well as in the ratio of spongy parenchyma and palisade parenchyma.

The data and ratios, measured in the cross-section of the leaf, can — owing to the considerable changes in them — less be used for characterizing species and sorts.

In respect of ratios, there are no unambiguously essential differences between the investigated sorts. At the same time, we can observe a surprising connection between the increase of the percentage of the palisade parenchyma (L=32.0 per cent, C=38.7 per cent, B=41.5 per cent) and between the formation of the leafy crown of the investigated sorts, the size of trees, characteristics of sorts, as well as between the average fruit weights and the development of the spongy parenchyma (Fig. 3).

Table 4. Values of the double-sample T-test, summarizing the morphological properties of the leaf (Bereczk=B, Constantinople=C, Leskovác=L).

Compared property	Compared sorts	T value	Freedom degree
Length of leaf-blade	B & C	- 1.545	+ 198
	L & B	- 15.134	+ 192
	C & L	+ 12.533	+ 174
Breadth of leaf-blade	B & C	- 9.142	+ 198
	L & B	- 16.630	+ 161
	C & L	+ 6.618	+ 173
Length of leaf-stalk	B & C	- 16.169	+ 143
	L & B	- 21.886	+ 148
	C & L	+ 9.309	+ 198
Full length of leaf	B & C	- 4.282	+ 198
	L & B	- 21.667	+ 198
	C & L	+ 12.787	+ 178
B/L ratio of leaf-blade	B & C	- 10.805	+ 187
	L & B	- 1.712	+ 182
	C & L	- 10.713	+ 198

Table 5. Values of Wilcoxon's test, comparing the epidermes of the three quince sorts (where the dispersion of the three samples agreed at a 95 per cent level, T-value and freedom degree are published).

Compared property	Compared sorts	U-value
Surface-epidermic cell count	B & C	3709.5
	L & B	4667.0
	C & L	3964.0
Lower surface-epidermic cell count	B & C	2221.0
	L & B	864.0
	C & L	T-value: -18.62 Freed. d.: +198
Stoma number	B & C	3038.0
	L & B	1120.0
	C & L	591.5
Length of the closing cells	B & C	503.0
	L & B	61.5
	C & L	3314.0
Breadth of the closing cells	B & C	1478.0
	L & B	4100.5
	C & L	1932.5
B/L ratio of the closing cells	B & C	T-value: -0.7195 Freed. degr. +191
	L & B	376.0
	C & L	698.0
Stoma-index	B & C	791.0
	L & B	1220.0
	C & L	964.0

The question may come up, whether the close correlation between the above data was a matter of a mere chance. STARZECKI (1958, 1962) regards the spongy parenchyma as the fundamental mesophyll tissue of the leaf from the point of view of the main function of the leaf. He attributes to the palisade parenchyma primarily the role of a light-filter. This hypothesis seems to be verified by other histological observations, too, in connection with the change in the intensity of light (e.g., GRAHL—WILD, 1973; TAKÁCS, 1973; PATAKY—HORVÁTH, 1978). But we consider as convincing Maróti's opinion (1976), as well, according to which in the palisade parenchyma the first photochemical system dominates. The first photochemical system, which is independent of the second pigment system, may have a part in the cellulose synthesis, in the growth of cell-walls (in determining the size of tree) (MARÓTI—GÁBOR, 1976).

On the basis of the above hypothesis, there can arise the possibility, too, that the spongy parenchyma may possibly have a more important role in the production of fruit and of the accumulated organic matter than that of the palysade parenchyma.

Table 6. U-values of Wilcoxon's test, comparing the leaf cross-sections of the three sorts.

Compared property	Compared sorts	U-value
Total thickness of the leaf-blade	B & C	2815.50
	L & B	3109.00
	C & L	2200.00
Thickness of the palisade parenchyma	B & C	1120.0
	L & B	951.50
	C & L	2038.00
Thickness of the spongy parenchyma	B & C	579.00
	L & B	397.00
	C & L	503.00
Ratio of the total thickness of leaf-blade/p.p.	B & C	3964.00
	L & B	591.50
	C & L	1020.05
Ratio of the total thickness of leaf-blade/sp.p.	B & C	4660.00
	L & B	3967.00
	C & L	T-value: -18.67 Freed. d.: +198
Ratio of the spongy p./palisade p.	B & C	4050.00
	L & B	791.00
	C & L	864.00

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