

Phenotypic variability of epidermis structure and silicon inclusions in the leaves of *Quercus robur* in the Feofaniya Park

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Abstract

The micromorphology of the leaf epidermis, localization, and silicon content in the epidermal cells of *Quercus robur* leaves growing in the shade and under direct sunlight in the Feofaniya Park (Kyiv, Ukraine) were studied using scanning electron microscopy and laser confocal microscopy. Silicon inclusions were found in the anticlinal and periclinal walls of adaxial epidermal cells, trichomes, guard cells of stomata, and walls of regular epidermal cells on the abaxial leaf surface, the amount of which varied according to the conditions of growth. Natural shading and the intensity of solar irradiation were found affecting the size of leaf blades, the ultrastructure of the leaf epidermis, and changes in the silicon content of oak leaves. Studies have shown that the anticlinal walls of the adaxial epidermis and the trichomes and stomata of the abaxial epidermis of leaves are the main silicon accumulators. The findings suggest that changes in leaf microstructure and silicon content contribute to maintaining optimal water balance in plants and can be regarded as signs of phenotypic plasticity in plants and an adaptive marker depending on the sunlight conditions of oak growth.

Keywords: *Quercus robur*, leaf micromorphology, silicon, laser confocal microscopy, scanning electron microscopy, shade influence

Authors' contributions: Dr. Olena Nedukha sampled and processed the plant material, conducted microscopic studies, described the results, and wrote the text of the article. Dr. Olena Zolotareva – sampled the plant material, took photos of the collected material, discussed the data, and edited the text of the article. Dr. Maksym Netsvetov – selected oak trees for research, provided geographical characteristics of trees, and examined the data.

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Introduction

The study of the structural and functional organization of organs and tissues of ancient

oak groves attracts the attention of many researchers due to the stable mechanisms of resistance against biotic and abiotic stresses that the species have developed (Werker,

2000). The resilience of forest ecosystems to extreme environmental conditions depends mainly on the sensitivity of certain species that form the forest. In Ukraine and Europe, one of these species is the common oak (*Quercus robur* L.). The problem of preserving oak forests in Ukraine is warned by climate changes causing the increased frequency of windstorms and droughts. For oaks and other tree species, the leaf epidermis is a principal protective structure that reacts to climate changes, especially to intensified drought and solar activity. The leaf epidermis is the first barrier between the plant and the environment, protecting the plant from various environmental factors, including soil drought, windstorms, and intense solar radiation (Dietz & Hartung, 1996). Epidermal structures, such as stomata and trichomes, play an essential role in the cell water balance regulation and plant adaptation to extreme environmental conditions.

The first stage of photosynthesis is the capture and absorption of photons by chloroplasts, followed by their utilization and scattering. Plants have adapted to light capture by regulating leaf and chloroplast sizes, the location and orientation of leaves, and modifying their photosynthetic apparatus (Björkman, 1981; Björkman & Powles, 1981; Brugnoli & Björkman, 1992; Mathur et al., 2018). In addition, plants have developed several mechanisms of protection against excessive light, including the formation of trichomes and the formation of wax on the leaf surface (Soares et al., 2012; LoPresti, 2015). The developed wax cover causes the increased light-reflectance of the leaf surface and assures the plant's resistance to intense radiation (Hansen et al., 2007; Custódio et al., 2015).

Investigating the structural and functional organization of oak leaves is important for fundamental and applied botany and forestry because these plants (in particular, their leaves, bark, seeds, and acorns) are actively used in phytochemistry, pharmacology, and agriculture. Due to the presence of phenolic acid, terpenoids, and tannins in oak tissues, which have a positive effect on anti-inflammatory, anti-diabetic, and anti-tumor effects, they can be considered promising candidates for the development of new pharmaceuticals for various infectious diseases (Moon et al., 2013; Taib et al., 2020).

The presence of flavonoid compounds (terpenoids) in leaf trichomes and parenchyma was confirmed for two oak species (*Quercus ilex* L. and *Q. coccifera* L.). Moreover, the content of terpenoids in these two species depended on the leaf growth stage and the illumination level (Liakoura et al., 1999). The aboveground organs and roots of oaks contain, in addition to terpenoids, silicon and are also used in medicine (Aseeva et al., 1985). In agriculture, it was recently proposed to use an extract from young oak leaves as a natural resource of biofertilizer, which actively influences tomatoes' growth and biological activity (Tahir et al., 2022).

Considering the role of silicon in absorbing and reflecting sunlight, we decided to stress the idea that silicon should be present in the epidermis of oak leaves and that its content will vary depending on the intensity of sunlight and shade level. We also suppose that the growth of oaks in conditions of variable light and water supply is possible due to their tolerance related to the peculiarities in leaf morphology, in particular, the ultrastructure of the epidermis. This study aimed to investigate the stable and plastic features of the leaf epidermal structure and silicon content for oak trees of different ages growing in Feofaniya Park (Ukraine), related to both species characteristics and phenotypic plasticity under different light intensities.

Material and methods

The object of the study was the leaves of common oak trees (*Quercus robur*, Fagaceae) growing in Feofaniya Park on the outskirts of Kyiv, Ukraine (50°26' N, 30°34' E). Oak leaves for the study were collected on May 31, 2022. The material was collected from the trees growing in both of the Feofaniya tracts in the territory of Holiivskyi district in the recreational area of Kyiv. Four trees were selected for the study, two growing on the Feofaniya hill and two growing much lower, in the hollow. Tree Nr 1 grow on a hill on the territory of the St. Panteleimon Monastery, in the shade, on the lawn next to other trees. Tree Nr 2 grow on the highest point of Feofaniya hill, in the shade, in a dense forest belt near the road, close to Feofaniyiv lakes. Tree Nr 3 grow between the hill and the depression, in the shade, alongside

Table 1. Basic parameters of investigated *Quercus robur* trees and their habitat.

| Tree number | Approximate age, years | Trunk perimeter at a height of 1.5 m from the ground, cm | Photosynthetic photon fluency rate (PPFR), $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ | | Shadiness at the level of lower branches, % | Elevation, m a.s.l. |
|-------------|------------------------|--|--|-----------|---|---------------------|
| | | | cloudy day | sunny day | | |
| Nr 1 | ~200–250 | 353 | 110 | 1200 | 33.0 | 186 |
| Nr 2 | ~80 | 145 | 75 | 850 | 52.8 | 186 |
| Nr 3 | ~200 | 324 | 80 | 1000 | 45.0 | 154 |
| Nr 4 | ~50–70 | 158 | 120 | 1800 | 0.0 | 128 |

other trees at the edge of Feofaniya Park. Tree Nr 4 grow in the depression alone on the lawn near the lakes of Feofaniya Park without shade, 50 m far from the lakeshore. Trees Nrs 1–3 grow under the shade of other trees (oaks and maples), while tree Nr 4 grow separately on a lawn, without shade from other trees (Table 1).

In a sampling day, the weather was rainy and overcast. Photosynthetic photon fluency rate (PPFR) was measured using the light meter Li-Cor LI-250 (USA). PPFR on the adaxial surface of the leaves of oak trees on the sampling day ranged from 75 to 120 $\mu\text{mol quantum m}^{-2}\cdot\text{s}^{-1}$. The mean daytime PPFR on the next day (a sunny day) ranged from 850 to 1850 $\mu\text{mol quantum m}^{-2}\cdot\text{s}^{-1}$. PPFR data and other measured oak parameters are provided in Table 1.

The lowest branches (at the height of 2–2.5 m above the ground) were taken for the study. From these branches, 12–15 leaves of nearly the same size were selected, photographed and measured. For cytological studies, the middle part of every second (upper) lobe in four leaves was cut out. Leaves from 12–15 plants of each ecotype were used for the microscopic and cytochemical investigations.

The middle part of each second lobe (upper) of the leaf blade was fixed for scanning electron microscopy. The specimens were fixed immediately in the field in the solution of 2% paraformaldehyde and 1% glutaraldehyde (1:1, vol.) in 0.5M phosphate buffer (pH 7.2) for 3 h at $\pm 4^\circ\text{C}$ (in a thermos). Then in the laboratory, the samples were washed in the identical buffer, and dehydrated in a series of alcohols (70%, 80%, and 100% ethanol; twice, every 30 min) according to Talbot & White (2013) protocol. After dehydration, the samples were mounted on aluminum tables, sputtered with carbon and gold, and examined using

JSM 6060 LA scanning electron microscope at 30 kV. To determine cell sizes, 30–40 regular epidermal cells, 30–35 stomata, and 20–25 trichomes were used in three samples from each leaf. Statistical significance of the cell size and the stomata density was determined using Origin 6.1 software, including the standard error ($\pm\text{SE}$), and Student's test ($P \leq 0.05$).

The cytochemical study of the presence and content of silicon with laser confocal microscopy has been conducted following Dabney et al. (2016). The samples of the middle part of the second (upper) lobe of leaf blades (10×20 mm) from four common oak trees were exposed to high temperatures in an oven at 250°C for three to four hours until the samples darkened to gray. The prepared leaf samples were examined using a laser scanning confocal microscope Zeiss LSM5 (Germany) using an excitation wavelength of 480 nm and an emission wavelength of 530 nm, respectively. Four leaves from each tree were taken for the study. The average fluorescence intensity in 30–40 epidermal cells (stomata, trichome, and regular epidermal cells) has been calculated. Values of results expressed at the mean and standard errors using Student's test ($P < 0.05$). The fluorescence intensity of silicon and other chemical elements was measured using the Pascal software.

Results

Micromorphology of *Quercus robur* leaves

The leaves of the four selected trees of the common oak, regardless of the place of growth and regardless of age, were characterized by a similar morphology of leaf blades: leaves are short-petiolate; the shape of the leaf blade is elongated-obovate, narrowed downward,

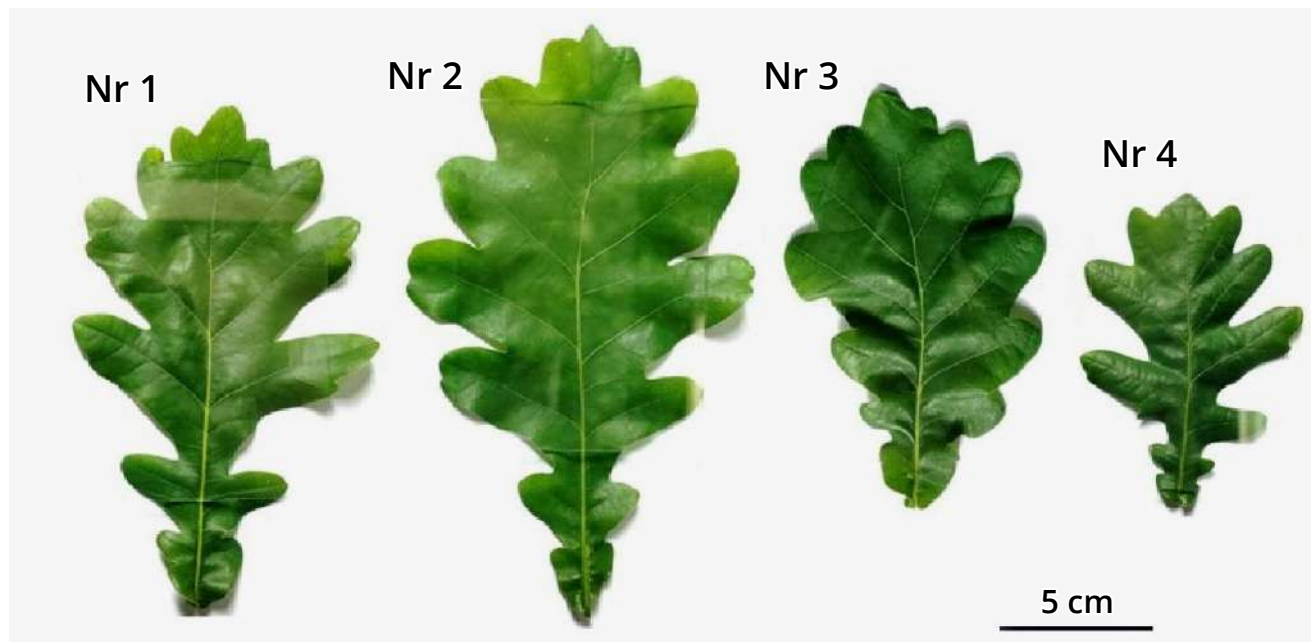


Figure 1. Common view of leaf blades from four *Quercus robur* trees growing in Feofaniya Park, on the outskirts of Kyiv, Ukraine. Trees Nrs 1–3 grow among other trees in the shade; tree Nr 4 grow separately from other trees without shade.

and pinnately lobed (Fig. 1). The leaf blades are blunt, rounded, and have shallow notches between them. Some differences in the leaf size, surface area, and ultrastructure of the epidermis were found.

It was found that the leaves of the first three investigated trees (Nrs 1–3) were quite large and characterized by a significant leaf blade area (Table 2). In contrast, the smallest sizes characterized the leaves of tree Nr 4. The leaf area in tree Nr 4 was 2.5 times smaller than in tree Nr 1, three times smaller than in tree Nr 2, and almost two times than in tree Nr 3. Leaves of all studied *Q. robur* trees, regardless of the place of growth, were hypostomatic.

Scanning electron microscopy

Tree Nr 1

The adaxial surface of leaf blades showed precise contours of the regular epidermal cells and the absence of stomata and trichomes (Fig. 2A). The cells of the upper surface are small, almost square, or rectangular in shape. The anticlinal walls are thick, protruding above the cell surface. The anticlinal and periclinal walls of the regular epidermal cells are covered with solitary lamellar and needle-like waxy structures.

The abaxial epidermis of a leaf is characterized by the presence of trichomes

and stomata, which are irregularly arranged (Fig. 2B, C). The density of stomata is high – 406 ± 31 stomata per 1 mm^2 (Table 2). Stomatal guard cells are covered with lamellar crystalline waxy structures, but the stomatal slit is smooth and free of wax. The surface of the guard cells of the stomata is covered with a continuous layer of needle-like wax structures. The boundaries of the regular epidermal cells are not visible. The density of trichomes is low compared to other studied trees (Table 2). Trichomes are slightly raised, simple, comma-like, with a drop-shaped base and elongated head part.

Tree Nr 2

The adaxial surface of the leaf blade has clear contours of the regular epidermal cells and has no stomata or trichomes (Fig. 2D). The cells of the upper leaf surface are small and similar in shape to those in the tree Nr 1. The cells of the upper surface are almost square or rectangular in shape. The anticlinal walls are as thick as in the tree Nr 1; they protrude above the cell surface. The periclinal walls are depressed. The anticlinal and periclinal walls of regular epidermal cells are covered with solitary waxy structures of various shapes.

The abaxial leaf surface is characterized by an increased density of trichomes and stomata

Table 2. The parameters of sampled *Quercus robur* leaves. **a** denotes significant differences between the parameters in the tree Nr 1 (shaded) from the tree Nr 4 (without shade) ($P \leq 0.05$); **b** denotes significant differences between the parameters in the tree Nr 2 (shaded) from the tree Nr 4 (without shade) ($P \leq 0.05$); **c** denotes significant differences between the parameters in the tree Nr 3 (shaded) from the tree Nr 4 (without shade) ($P \leq 0.05$).

| Parameter | Tree Nr 1 | Tree Nr 2 | Tree Nr 3 | Tree Nr 4 |
|--|-----------|------------|-----------|-----------------------------|
| Leaf size, cm | | | | |
| Long axis | 170 ± 19 | 210 ± 15 | 105 ± 8.9 | 100 ± 7.8 ^{a, b} |
| Short axis (at the level of the second lobe) | 110 ± 11 | 115 ± 10 | 82 ± 7.5 | 52 ± 3.9 ^{a, b, c} |
| Leaf area, cm ² | 104 ± 7.7 | 134 ± 5.7 | 74 ± 15.7 | 38 ± 0.5 ^{a, b, c} |
| Adaxial epidermis | | | | |
| Long axis of regular epidermal cells, μm | 30 ± 1.4 | 32 ± 1.1 | 30 ± 1.1 | 33 ± 1.1 |
| Short axis of regular epidermal cell, μm | 25 ± 1.1 | 25 ± 1.2 | 18 ± 1.6 | 25 ± 1.2 |
| Width of anticlinal walls, μm | 8 ± 0.4 | 8 ± 0.2 | 8 ± 1.7 | 8 ± 0.2 |
| Abaxial epidermis | | | | |
| Stomata | | | | |
| Density, number per 1 mm ² | 406 ± 21 | 434 ± 27 | 470 ± 33 | 483 ± 47 ^{a, b} |
| Long axis of guard cells, μm | 15 ± 1.1 | 25 ± 1.3 | 27 ± 1.1 | 30 ± 1.1 |
| Short axis of guard cells, μm | 7 ± 0.3 | 11 ± 0.5 | 11 ± 0.4 | 11 ± 0.3 |
| Trichomes | | | | |
| Density, number per 1 mm ² | 18 ± 1.9 | 56 ± 3.9 | 22 ± 1.3 | 130 ± 12 ^{a, b, c} |
| Long axis, μm | 70 ± 7.9 | 100 ± 11.0 | 95 ± 10.9 | 90 ± 9.7 ^a |
| Short axis (at the base), μm | 15 ± 1.2 | 19 ± 1.3 | 15 ± 1.3 | 19 ± 1.3 |
| Width of middle elongated part, μm | 7 ± 0.2 | 8 ± 0.5 | 9 ± 0.4 | 8 ± 0.4 |

compared to the tree Nr 1 (Fig. 2E, F; Table 2). Stomata are slightly elongated. The average size of stomata guard cells is represented in Table 2. Guard cells of stomata are covered with needle-like waxy structures, which are about 2 μm long. The stomatal slit is smooth and free of wax. Trichomes are slightly elevated above the regular epidermal cells. Trichomes are simple, unbranched, with a drop-shaped base and a stick-shaped head part. The sizes of trichomes are represented in Table 2. The bases of the trichomes are covered with needle-like crystalline waxy structures (Fig. 2F). The surface of the regular epidermal cells surrounding the stomata is covered with a continuous layer of needle-like waxy structures, due to which the boundaries of the regular epidermal cells are not visible.

Tree Nr 3

The laminar adaxial surface has the same epidermal cells as in previously described

trees, with relatively wide anticlinal walls (Fig. 2G), which protrude above the periclinal walls. Stomata and trichomes are absent on this surface. The epidermal cells of the upper surface are small; most of the cells are rectangular. The walls are covered with a layer of convex, rounded-plated wax structures.

The abaxial epidermis is characterized by the presence of irregularly arranged stomata and trichomes (Fig. 2H, I). The density of stomata and trichomes in the leaves of tree Nr 3 is higher than in trees Nr 1 and Nr 2 (Table 2). The surface of the guard cells of the stomata is covered with thin needle-like waxy crystal structures ranging in size from 1 to 4 μm. The stomata slit is free of crystals. The surface of the regular epidermal cells surrounding the stomata is also covered with crystalline wax structures. The boundaries of the regular epidermal cells between the stomata are not visible. Simple, unbranched trichomes, with a drop-shaped base and a stick-shaped head are

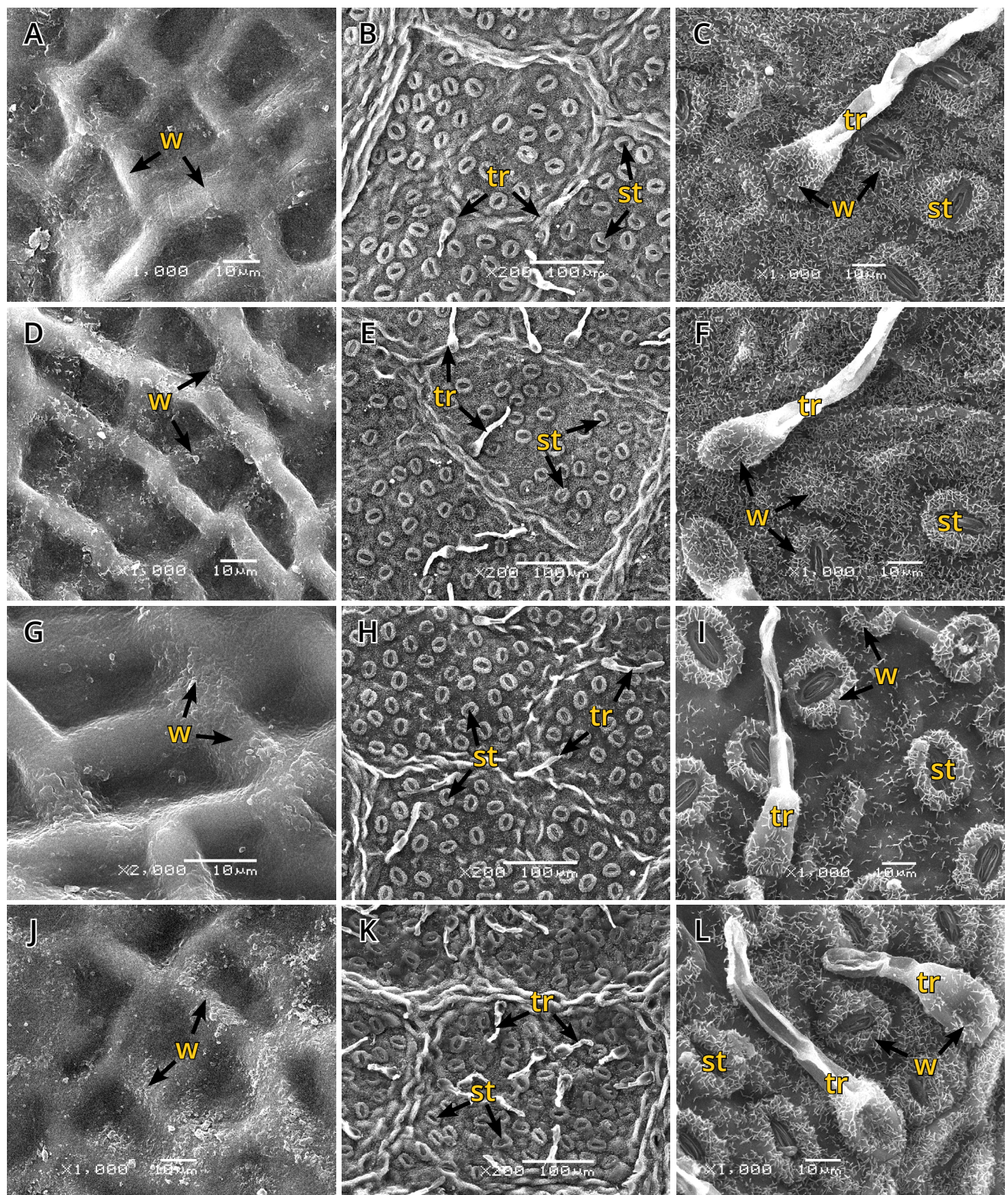


Figure 2. SEM images of the leaf epidermis of four studied *Quercus robur* trees: A-C – tree Nr 1; D-F – tree Nr 2; G-I – tree Nr 3; J-L – tree Nr 4. The adaxial (A, D, G, J) and abaxial (B, C, E, F, H, I, K, L) surfaces of oak leaves. Abbreviations: st – stomata; tr – trichome; w – wax deposits.

located near the cells of protruding veins and sporadically occur close to regular epidermal cells. They are slightly elevated above the epidermal surface. The base of trichomes is covered with needle-like crystalline waxy

structures (Fig. 2I). The sizes of trichomes are represented in Table 2. The surface of the regular epidermal cells surrounding the stomata is covered with a continuous layer of needle-like waxy structures. Due to the

developed cuticular coating, the boundaries between the epidermal cells are not visible.

Tree Nr 4

The upper epidermis has no stomata or trichomes (Fig. 2J). Some cells are characterized by clear contours of the anticlinal walls of the epidermis, while in other cells, the periclinal walls are located at the same level as the anticlinal walls, forming a continuous surface layer. The adaxial epidermal cells are small and similar in shape to those in previously discussed trees. They are almost square or rectangular in shape. The anticlinal walls of adaxial epidermal cells are thick and protrude above the surface. The periclinal walls are depressed. Nearly the entire surface of the anticlinal walls of the epidermis is covered with a layer of small waxy structures of various shapes protruding above the epidermal surface.

The abaxial surface is characterized by an increased density of trichomes and stomata compared to the leaves of other studied trees grown in the shade (Fig. 2K, L; Table 2). Stomata are slightly elongated; the average size of guard cells is somewhat higher than in other studied trees. Guard cells of stomata are covered with needle-like waxy structures; the stomatal slit is smooth and free of wax. Trichomes are located above the regular epidermal cells and along small veins that are slightly raised. The trichomes are simple and unbranched, with a spoon-shaped base and a stick-shaped head. The apical part of the trichomes is slightly curved. Trichomes have needle-like crystalline waxy structures at their bases. The sizes of trichomes are represented in Table 2. The surface of the regular epidermal cells surrounding the stomata is also covered with a continuous layer of needle-like waxy structures, and therefore the boundaries of the regular epidermal cells are not visible.

Localization of silicon inclusions

It is shown that silicon inclusions fluoresce brightly in green in the epidermal cells in the leaves of investigated *Q. robur* plants (Fig. 3A–C).

Tree Nr 1

In the adaxial epidermis, silicon inclusions fluorescence was detected in the periclinal

and anticlinal walls of regular epidermal cells and on the surface of small veins. Silicon inclusions are densely spaced in the anticlinal walls, forming almost continuous layers. In the periclinal walls, individual large silicon inclusions were visible (Fig. 3B), or only a thin layer of amorphous silicon inclusions was present, which was visible at low magnifications (Fig. 3A). The intensity profile of silicon in the anticlinal and periclinal walls differed (Table 3). In the abaxial epidermis of tree Nr 1 leaves, silicon fluorescence was detected in the guard cells of stomata, in the cells surrounding the stomata, and at the base of trichomes (Fig. 3C). The intensity of the silicon fluorescence profile was different in these cells and differed from that of the upper epidermis cells (Table 3; Fig. 3D–G).

Tree Nr 2

In the leaves of oak Nr 2, which grew in the shade at the highest site in Feofaniya, green silicon fluorescence was detected in the epidermal cells of the upper and lower epidermis (Fig. 3H, I). Silicon fluorescence was detected in the periclinal and anticlinal walls of epidermal cells on the adaxial leaf surface. In the abaxial epidermis, silicon fluorescence was detected in stomata, cells around stomata, and trichomes. The intensity of the silicon profile depended on the leaf surface and the cell type (Table 3). The highest intensity of the profile was on the adaxial surface, in the anticlinal walls of the epidermal cells. The highest profile intensity was found on the abaxial surface at the base of trichomes and the anticlinal walls of the regular epidermal cells. It was much lower in the periclinal walls of regular epidermal cells and guard cells of stomata (Table 3).

Tree Nr 3

Green silicon fluorescence in the epidermal cells of the upper (Fig. 3J) and lower epidermis, similar to the two previous specimens, has been detected. Silicon fluorescence was detected in the periclinal and anticlinal walls of adaxial epidermal cells. In the abaxial epidermis, silicon fluorescence was detected in stomata, stomatal surrounding cells, and at the base of trichomes. The intensity of the silicon profile differed in the studied cells of the upper and lower epidermal leaf surfaces (Table 3). The high intensity of the profile was

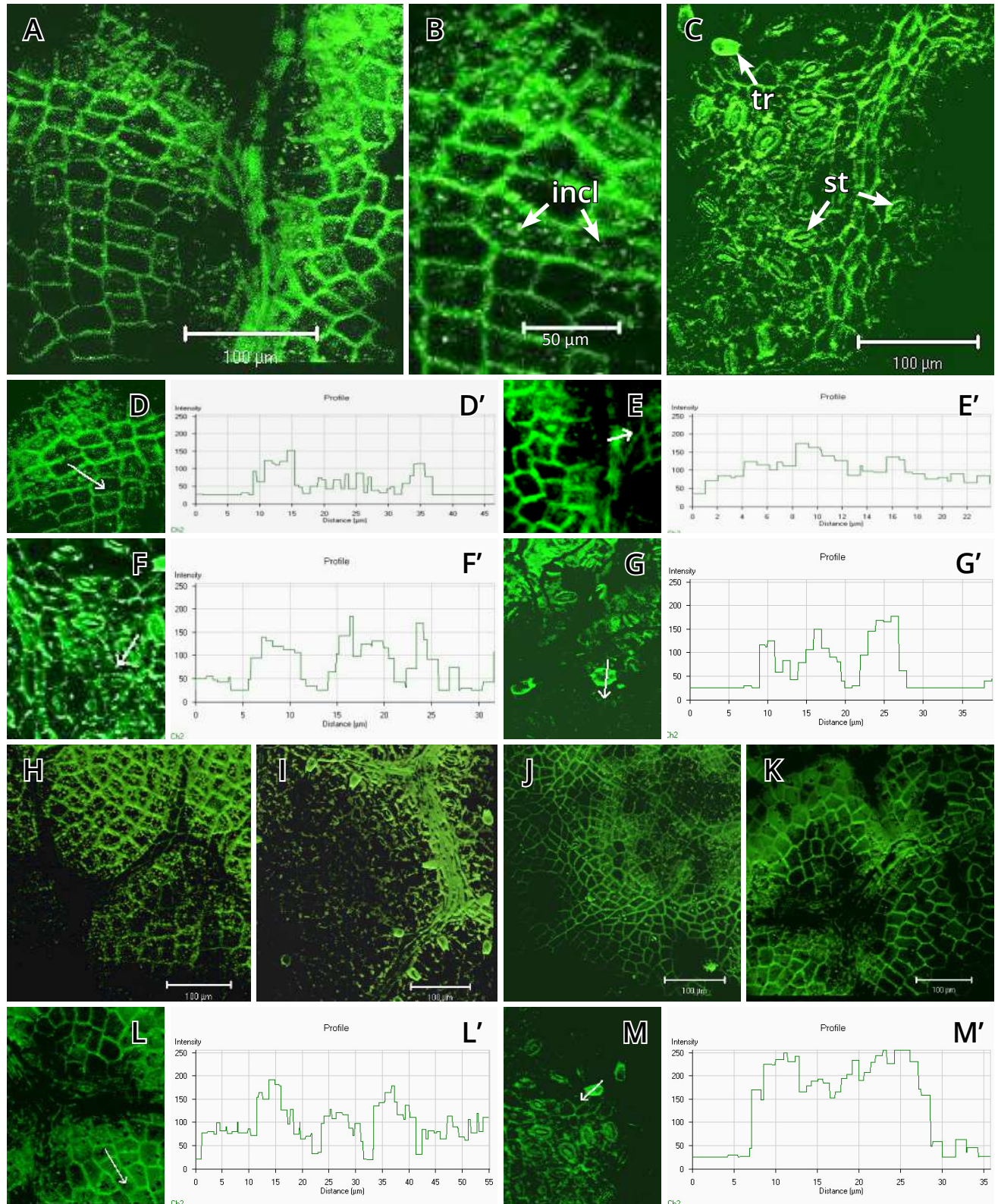


Figure 3. Fluorescence of silicon inclusions visualized with confocal laser scanning microscopy for the adaxial (A, B, D, E, H, J, K, L) and abaxial (C, F, G, I, M) leaf surfaces in *Quercus robur* trees from Feofaniya, Kyiv. A-J – trees grown in the group, in the shade (trees Nrs 1–3); K–M – a tree grown separately, in the sun (tree Nr 4). D'–G', L', and M' – histograms of the intensity profile of silicon (green line) resulted from D–G, L, and M, respectively, where the scanned direction is shown as a white arrow. Abbreviations: st – stomata; tr – trichome; incl – inclusions of Si in the periclinal wall.

Table 3. The intensity profile of silicon inclusions (in relative units) in cell walls of the leaf epidermis of studied *Quercus robur*. **a** denotes significant differences in the parameters in the tree Nr 1 (shaded) from the tree Nr 4 (without shade) ($P \leq 0.05$); **b** denotes significant differences in the parameters in the tree Nr 2 (shaded) from the tree Nr 4 (without shade) ($P \leq 0.05$); **c** denotes significant differences the parameters in the tree Nr 3 (shaded) from the tree Nr 4 (without shade) ($P \leq 0.05$).

| Source | Tree Nr 1 | Tree Nr 2 | Tree Nr 3 | Tree Nr 4 |
|----------------------------------|-----------|-----------|-----------|-----------------------------|
| Adaxial epidermis | | | | |
| Anticlinal wall of regular cells | 138±11.0 | 143±14.0 | 143±12.0 | 153±11.0 |
| Periclinal wall of regular cells | 62±3.2 | 56±4.0 | 61±7.0 | 82±4.5 |
| Abaxial epidermis | | | | |
| Anticlinal wall of regular cells | 100±12.0 | 100±9.3 | 177±13.0 | 180±13.0 ^{a, b, c} |
| Periclinal wall of regular cells | 45±4.3 | 40±3.5 | 77±5.3 | 170±12.0 ^{a, b, c} |
| Trichomes | 155±13.0 | 130±11.0 | 222±11.0 | 192±10.0 ^{a, b} |
| Stomata | 106±10.0 | 67±5.9 | 152±13.0 | 138±11.0 ^{a, b, c} |

on the adaxial surface in the anticlinal walls of the epidermal cells. The highest profile intensity on the abaxial surface was in the anticlinal walls of the regular epidermal cells and at the base of trichomes.

Tree Nr 4

In the leaves of tree Nr 4 growing under direct sunlight, green silicon fluorescence was detected in the epidermal cells of the upper and lower epidermis (Fig. 3K–M). On the adaxial leaf surface, silicon fluorescence was detected in the periclinal and anticlinal walls of epidermal cells. In the abaxial epidermis, silicon fluorescence was detected in stomata, stomatal surrounding cells, and trichomes. The intensity of the silicon profile depended on the leaf surface and the cell type (Table 3; Fig. 3L, M). The intensity of the silicon profile on the adaxial surface in the anticlinal walls of the epidermal cells was nearly twice higher compared to the periclinal walls. On the abaxial surface, the profile intensity was relatively high in all studied cells and highest in the anticlinal and periclinal walls of the regular epidermal cells and the guard cells of the stomata (Table 3).

Discussion

Leaf micromorphology

The results obtained in this study indicate that the oak growing separately from other trees (without shade) had significantly smaller leaf

sizes and, respectively, leaf areas than the oaks growing in groups and surrounded by other trees (in shade). Obtained results regarding the effect of light on leaf size are consistent with the results of studies conducted on other plant species (Terashima et al., 2006; Wu et al., 2017). Thus, Granier & Tardieu (1999) studied the effect of illumination on the growth of sunflower leaves and demonstrated that cell division and elongation directly depend on the illumination intensity. Granier & Tardieu (1999) proved that the cell division rate and elongation decreased with increased illumination.

The leaf is the most flexible organ responding to environmental conditions (Nevo et al., 2013). Its structure reflects the environmental influence more clearly than the stem and root. Smaller leaf sizes result in less water loss, particularly from the adaxial surface. Such phenomenon has been described for leaves of *Olea europaea* L. trees growing in hot and dry conditions (Bacelar et al., 2004) and grasses (Liu et al., 2016). As shown in succulents and mesophytes, minimizing water loss during drought is achieved by increasing both the density of vascular bundles and stomata (Bolhar-Nordenkamp, 1987; Richardson & Berlyn, 2002), thickening the cuticle and epicuticular wax layer (Leon & Bukovac, 1978; Liakoura et al., 1999; Richardson & Berlyn, 2002). Plants usually respond strongly to shading by producing leaves with a larger area and less mass per unit area (Niinemets & Sack, 2006), which results in

more effective light capture per unit mass. The leaves of trees growing in more sunny areas, in addition to their smaller size, are characterized by increased mechanical strength and impact force (Sanson et al., 2001; Onoda et al., 2008) compared to shaded ones.

In the leaves of the oak trees we studied, stomata are located on the abaxial side (hypostomate leaf type). Using the scanning electron microscopy method, we found both common structural features of stomata and apparent differences in their density. In particular, an oak tree Nr 4, growing openly near a lake in a lowland (without shade), showed increased stomatal density compared to the leaves of other oaks (Nrs 1–3) that grew in semi-shaded conditions. It is known that stomatal density can be influenced by several factors, including changes in plant water balance and soil moisture, as well as the intensity of sunlight (Hetherington & Woodward, 2003). Leaves of plants growing under soil drought conditions usually have smaller and more numerous stomata than leaves developing under well-moisturized conditions (Larcher, 2003). The effects of direct sunlight and shading on stomatal density and function have been described by many researchers (Bolhar-Nordenkamp, 1987; Onwueme & Johnston, 2000; Hansen et al., 2007; Matos et al., 2009). Kardiman & Ræbild (2018) investigated 11 species of trees in tropical forests and showed a certain correlation between the density of stomata in their leaves and the intensity of tree illumination: comparing the leaves of trees growing in full sunlight with the leaves of trees growing in 70% shade. Their assessment of stomatal density and size and working maximum stomatal conductance showed that the anatomical parameters of stomata and gas exchange differed between species. Shading significantly affected the size and density of stomata and the rate of their opening. The researchers suggest that the change in stomatal micromorphology is, to some extent, due to the biochemistry of stomatal functioning in response to light conditions, in particular, changes in the activity of enzymes responsible for creating and maintaining a high level of osmotic potential (Raven, 2014). Early studies also revealed that soil moisture affects the morphological characteristics of tree stomata; specifically, during drought, plants were characterized by an increase in the

density of stomata, which were characterized by small sizes compared to leaves of plants developing under well-moistured conditions, which had few large stomata (Larcher, 1960, 2003).

Taking into account the above literature data and our data on differences in stomatal density in the four studied oak trees, we can suggest that not only the increased illumination around the unshaded oak tree Nr 4 but also changes in soil moisture, also affects stomatal formation and flexible regulation of leaf water balance in the studied oak samples. High air temperatures can damage pigments, cytochromes, and membrane proteins (Vatnick & Bruce, 2004; Bashir, 2022). Stomatal transpiration may help oaks regulate the temperature on the leaf surface, cooling it and allowing normal physiological functioning. It is the high illumination of plants, including the studied oaks, that promotes optimal photochemical activity and, consequently, activates gas exchange, which is due to the increased density of stomata in trees growing in direct sunlight.

When studying the ultrastructure of leaf samples from four oaks, we found the presence of simple needle-like trichomes with a pointed apex and an expanded spoon-shaped base on the abaxial surface of the leaves. Similar localization of trichomes has been described for many other oaks, particularly *Quercus laevis* Walter and *Q. rubra* L. (Coder, 2010). Simple, unbranched trichomes characterize the leaves of many tree species, including *Quercus* (Hardin, 1976; Llamas et al., 1995; Nicolić et al., 2003; Leandro et al., 2016). We also found a significant difference in the density of trichomes in the studied oaks depending on the growing conditions. The highest density of trichomes was in the leaves of oak Nr 4, which grew in direct sunlight rather than in the trees in the shade. The increase in the density of trichomes in the leaves of tree Nr 4 may be mediated by their function since trichomes protect the leaf from environmental influences.

As a plant defense barrier, trichomes counteract ultraviolet light, irradiation, pathogen attacks, and excessive transpiration, playing a pivotal role in plant development (Werker, 2000) under extreme temperatures and ultraviolet irradiation (Yamasaki & Murakami, 2014). Trichome density can

modulate leaf heat balance and light photon interception and thus affect plant gas exchange. Trichomes can not only reflect but also absorb ultraviolet radiation, reducing the damaging effect of UV-B on photochemical activity and preventing stomatal clogging. This is due to the accumulation of phenolic substances (flavonoids) in the trichomes, which can effectively absorb UV radiation from 250 to 350 nm (Bickford, 2016). The accumulation of phenolic substances in trichomes occurs during the secondary thickening of the leaf epidermal walls. Phenolic substances are transferred to the cell walls of trichomes, where they are diffusely deposited, providing protection against UV-B radiation and acting as optical filters, screening out wavelengths that can damage sensitive tissues. Protection from strong irradiation is also provided by the increased light reflectance of the surface (LoPresti, 2015; Karabourniotis et al., 2020). Considering the above literature data on the functional importance of trichomes in tree leaves, including oaks, and our data, we can assume that the trichomes of the oak samples we studied can also accumulate or synthesize flavonoids. This question remains open and requires further cytochemical investigation. Regarding the changes in the density of trichomes in the leaves of the oaks we studied, we conclude that the trichome density is a typical sign of the structural plasticity of leaves, which changes to optimize the water balance of the plant and the species' resistance to environmental changes (light intensity and/or changes in soil moisture).

Ultrastructural analysis of the epidermis of oak leaves growing in the Kyiv area showed that the abaxial epidermis is characterized by a large number of waxy inclusions that cover both the guard cells of stomata and the walls of regular epidermal cells in a continuous layer. It is known that wax, formed in the periclinal walls of the epidermis, inhibits transpiration and reflects ultraviolet radiation (Kerstiens, 1996). The presence of wax in the epidermal cells of leaves is inherent in many plants, including tree leaves, which are exposed to unfavorable environmental conditions (Leon & Bukovac, 1978; Liakoura et al., 1999; Chaves et al., 2002; Richardson & Berlyn, 2002; Nevo et al., 2013). The wax on the cuticle surface plays an important role in structuring the surface at the subcellular level, as it can form

crystals that function as the main transport barrier for water and small water molecules from the cells, including ions, as well as reduce the absorption of liquids and various molecules from the outside (Barlott et al., 2017). Considering the mentioned literature data and the results of our studies, we can assume that wax inclusions on the abaxial surface of four tested oak specimens are an adaptive feature protecting against UV irradiation and playing a positive role in maintaining the optimal temperature and water balance.

The role of silicon inclusions

Laser confocal microscopy revealed the presence of silicon inclusions in the cell walls of the adaxial and abaxial surfaces of the leaves of four oaks growing on the outskirts of Kyiv, regardless of their place of growth and light intensity. Using the Pascal program, we found an increase in the intensity of the silicon fluorescence profile in the anticlinal and periclinal walls of the abaxial surface, as well as in the trichomes and guard cells of this surface in oaks (Nrs 3 and 4) that grew under high sunlight intensity compared to the same structures of leaves of shaded oaks (trees Nrs 1 and 2).

Silicon inclusions are a natural form of silicon ions in connection with polysaccharides, proteins, or lipids (Müller & Grachev, 2009). In cell walls, silicon is usually bound to polysaccharides and forms siliceous inclusions (Lins et al., 2002; Guerriero et al., 2016; Grašič et al., 2020).

It is known that silicon can improve the light flux characteristics of both expanded and contracted leaves. This chemical element also reduces the heat load due to silica's effective far-infrared heat transfer, which provides a passive mechanism for cooling the leaves during intense insolation (Wang et al., 2005; Ma et al., 2011). Silicon can absorb light in a wide specter (from infrared to ultraviolet), ca. 1017 photons per cm² per second (Mirshafieyan & Guo, 2014; Yahaya et al., 2013). It has been estimated that the ideal absorption and reflection of light by silicon occurs when the thickness of silicon inclusions (or structures) varies from 110 to 140 nm (Hofmeister et al., 2009).

We observed the increase in fluorescence of amorphous silicon inclusions up to 150 nm

in the cell walls of the adaxial epidermis, and trichomes and closing cells of stomata of the abaxial epidermis of oak leaves growing in direct sunlight. It has been established that such silicon structures in the amorphous state (not in the crystalline one) absorb light best (Hofmeister et al., 2009). We hypothesize that the increase in silicon inclusions fluorescence in trichomes and stomatal cells of oaks growing in direct sunlight increases both the absorption and reflection of sunlight by epidermal cells to optimize the functioning of photosynthesis.

Investigations of silicon inclusions are essential for fundamental and applied botany, including plant-related medicine and pharmacology. It is well known that silicophilous plants containing a high silicon concentration are used in medicine and pharmacology. Adding plant silicon to the human diet promotes calcification and the repair of damaged bone tissue, while inorganic silicates do not show such an effect. Among such silicophilous dietary plants are *Inula helenium* L., *Crocus sativus* L., *Gentiana decumbens* L., and *Bidens tripartita* L. (Aseeva et al., 1985). Many medicinal plants (i.e., *Sedum hybridum* L. and *Rhodiola linearifolia* Boriss.) also contain a lot of silicon (Kolesnikov & Gins, 2001). Hence, the use of oak leaves containing phenolic substances and polyphenol-bounded silicon is perspective.

Conclusions

It was found that the growth of common oak (*Quercus robur*) trees under direct sunlight (without shading) leads to the appearance of xeromorphic features: a decrease in the size of leaf blades, an increase in the density of trichomes, stomata, and waxy inclusions, as well as the formation of thickened periclinal walls of epidermal cells on the adaxial surface of the leaves. We consider these features to be signs of phenotypic plasticity in plants, which are adaptive markers of the effects of sunlight.

The laser confocal microscopy showed that direct sunlight (without shading) increased the silicon content in trichomes and guard cells of stomata. We found that the anticlinal walls of the adaxial epidermis, trichomes, and stomata of the abaxial epidermis of leaves are the main silicon accumulators. We assume that the high

silicon content in oak leaves growing in direct sunlight (without shading) causes a decrease in cuticular and stomatal transpiration.

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Фенотипічна мінливість структури епідермісу та кремнієві включення у листках *Quercus robur* у парку “Феофанія”

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За допомогою скануючої електронної мікроскопії та лазерної конфокальної мікроскопії досліджено мікроморфологію листового епідермісу, а також локалізацію та вміст кремнію в епідермальних клітинах листків дерев *Quercus robur*, що зростали у затінку та під прямим сонячним світлом у парку “Феофанія” (Київ, Україна). В антиклінальних і периклінальних стінках клітин адаксіального епідермісу, у трихомах, замикаючих клітинах продихів і стінках звичайних клітин епідермісу абаксіальної поверхні виявлено кремнієві включення, кількість яких варіювала залежно від умов зростання. Виявлено, що природне затінення та інтенсивність сонячного опромінення впливають на розмір листових пластинок, ультраструктуру епідермісу листків та вміст кремнію в листках дубу. Дослідження показали, що антиклінальні стінки адаксіального епідермісу, а також трихоми і продихи абаксіального епідермісу листків є основними накопичувачами кремнію. Отримані дані дозволяють припустити, що зміни мікроструктури листків та вмісту кремнію сприяють підтримці оптимального водного балансу рослин і можуть розглядатися як ознаки фенотипової пластичності, а також як адаптивний маркер залежно від умов сонячного освітлення дуба звичайного.

Ключові слова: *Quercus robur*, мікроморфологія листка, кремній, лазерна конфокальна мікроскопія, скануюча електронна мікроскопія, вплив затінення