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METHODS

Structural Adaptation of the Leaf Mesophyll to Shading

L. A. Ivanova and V. I. P'yankov[†]

Department of Plant Physiology, Faculty of Biology, Ural State University, pr. Lenina 51, Yekaterinburg, 620083 Russia; e-mail: Larissa.Ivanova@usu.ru

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Abstract—Structural characteristics of the mesophyll were studied in five boreal grass species experiencing a wide range of light and water supply conditions. Quantitative indices of the palisade and spongy mesophyll tissues (cell and chloroplast sizes, the number of chloroplasts per cell, the total cell and chloroplast surface area per unit leaf surface area) were determined in leaves of each of the species. The cell surface area and the cell volume in spongy mesophyll were determined with a novel method based on stereological analysis of cell projections. An important role of spongy parenchyma in the photosynthetic apparatus was demonstrated. In leaves of the species studied, the spongy parenchyma constituted about 50% of the total volume and 40% of the total surface area of mesophyll cells. The proportion of the palisade to spongy mesophyll tissues varied with plant species and growth conditions. In a xerophyte *Genista tinctoria*, the total cell volume, cell abundance, and the total surface area of cells and chloroplasts were 30–40% larger in the palisade than in the spongy mesophyll. In contrast, in a shade-loving species *Veronica chamaedris*, the spongy mesophyll was 1.5–2 times more developed than the palisade mesophyll. In mesophyte species grown under high light conditions, the cell abundance and the total cell surface area were 10–20% greater in the palisade mesophyll than in the spongy parenchyma. In shaded habitats, these indices were similar in the palisade and spongy mesophyll or were 10–20% lower in the palisade mesophyll. In mesophytes, CO_2 conductance of the spongy mesophyll accounted for about 50% of the total mesophyll conductance, as calculated from the structural characteristics, with the mesophyll CO_2 conductance increasing with leaf shading.

Key words: adaptation - mesophyll - spongy tissue - palisade tissue - cell morphology - mesophyll conductance - photosynthesis

INTRODUCTION

In most dicotyledonous plants, leaf mesophyll is divided into the palisade and spongy tissues, which differ in their location, cell morphology, and functional activity. In plants with a dorsiventral mesophyll type, the palisade layer is located on the adaxial (upper) side, whereas the spongy tissue is located on the abaxial (lower) side of the leaf, which results in different light conditions for these tissues. The major part of incident photosynthetically active radiation (PAR) is absorbed in the palisade layer, whereas the spongy tissue receives from 10% [1, 2] to 25% [3] of the average PAR level in the palisade tissue, depending on light intensity and mesophyll structure. Selective absorption of blue and red light in the mesophyll results in changing light spectral quality across the leaf thickness [2, 3]. In dorsiventral leaves, the carbon dioxide concentration also changes along the mesophyll profile, generally, decreasing from the substomatal space (on the abaxial side) toward the palisade layer [3, 4]. It seems likely that the differences in the light regime and gas environment should affect functional characteristics of the palisade and spongy tissues, as well as their contribution to the total leaf photosynthesis.

The idea about the different photosynthetic activities and physiological roles of two mesophyll types was suggested long ago. It is generally accepted that the palisade tissue prevails in the mesophyll of dorsiventral leaves and makes a major contribution to their photosynthesis [2, 3, 5, 6]. The spongy tissue was supposed to be of minor importance for CO_2 fixation and perform other functions. It was assumed [2, 6] that the major function of the spongy tissue is to reflect and send back the light that passed the palisade layer.

The contribution of these two tissues to the total leaf photosynthesis is not yet estimated, which may be due to the lack of methods for evaluating the volume of these chlorenchyma tissues within the leaf. There were attempts to analyze light absorption properties [7] and biochemical characteristics [8, 9] of leaf slices after serial sectioning of the mesophyll, but these slices of equal thickness did not match with different types of chlorenchyma tissues. In other works, cells of the palisade and spongy tissues were isolated by abrading cells of the adaxial or abaxial sides of the leaf, and the assimilation rate per unit weight of the palisade and spongy tissue was measured [10]. However, these data could

[†] Deceased.

Abbreviations: ChMI—chloroplast membrane index, i.e., the total area of the outer chloroplast membranes per unit leaf area; CMI—cell membrane index, i.e., the total area of the outer cell membranes per unit of leaf area; CVC—cell volume per chloroplast; PAR—photosynthetically active radiation.

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Table 1. Plant species studied and places of sampling

Species	Mesophyll type	Steppe slope	Upland meadow	Lowland meadow	Pine forest	Bank of a forest stream
Alchemilla vulgaris L.	DV	+	+	+	+	+
Veronica chamaedris L.	DV	*n*	+	+	+	+
Galium mollugo L.	DV	+	+	+	+	*n*
Genista tinctoria L.	IP	+	+	+	+	*n*
Chamaerion angustifolium (L.) Holub.	DV	+	+	+	+	*n*

Note: DV—dorsiventral, IP—isopalisade; *n*—plants of this species were not found in this habitat.

not be used to calculate the contribution of each tissue to the total assimilation. Attempts to determine the photosynthetic rates of palisade and spongy tissues by measuring photosynthesis on different leaf sides [3] also have some disadvantages because, in a high light, CO_2 assimilation occurs in both tissues, whereas, in low light, the photosynthetic rate is underestimated.

Information on the functional potential of two types of leaf mesophyll is particularly important for ecological studies. One approach to estimating the contribution of each tissue to total photosynthesis is to measure the area of assimilating surfaces. To date, the relationship between the structural and functional characteristics of leaf mesophyll was convincingly demonstrated. The total surface area of mesophyll cells represents the area of the internal leaf barrier for CO_2 diffusion. This parameter was expressed as the cell membrane index (CMI, the ratio of the total mesophyll cell surface area to unit leaf area) [11] and was shown to highly correlate with the mesophyll CO_2 conductance and photosynthetic rate [11–15]. Nobel *et al.* used CMI to study plant adaptation to environmental factors (light intensity, temperature, soil moisture content [12, 16], salinity [17, 18]) and characterize CO_2 diffusion in plants with C_3- and C_4-photosynthesis [13]. The CO_2 fixation rate was shown to highly correlate with the mesophyll cell surface area. Thus, the functional activity of leaf mesophyll and the contribution of each tissue can be estimated by measuring their CMI.

The palisade tissue is composed of regularly shaped cells; the surface and CMI of such cells can be easily calculated as the cylinder surface with appropriate corrections [11, 19, 20]. The cells of the spongy tissue are generally approximated by a sphere [14]. However, these cells have irregular shapes. Hence, their surface and volume cannot be calculated using simple geometrical models. The stereological method, suggested by Parkhurst [21], can be used to determine the total surface area of mesophyll cells within the leaf, but it cannot discriminate between the cell surface areas in different tissues.

Here we suggest a new method for calculating the surface and volume of mesophyll cells, which is based on stereological principles and is applicable to cells of any shape. Using this method, we studied the mesophyll structure to determine the relative contribution of the palisade and spongy tissues to the potential functional activity of leaves in plants sampled from contrasting habitats.

MATERIALS AND METHODS

Plant material. The material was sampled near the Biological Station of the Ural University, which is located near the city of Yekaterinburg. Five species of wild plants with dorsiventral or isopalisade types of leaf mesophyll were analyzed (Table 1). Plants were sampled from various habitats, differing in moisture and light conditions: (1) the southern slope of a steppe hillside, an open place (illuminance at noon over 70 klx, low soil moisture (7–10%); (2) an upland meadow, illuminance at noon over 70 klx, low soil moisture content (10–13%); (3) a lowland meadow, moderate illuminance (below 25 klx at clear noon) combined with an adequate soil moisture content (20–25%); (4) light pine forest, low illuminance (below 10 klx at clear noon) and a moderate soil moisture content (20–25%); and (5) thick deciduous forest at the bank of a forest stream, low illuminance (3–5 klx at noon) and excessive soil moisture content (35–40%). The studies were carried out on mature middle-layer leaves taken from 5–10 plants at the flower bud formation–flowering stage.

Measurement of the quantitative indices of leaf mesophyll. Quantitative indices of leaf mesophyll were measured by the Mokronosov method [11]. The number of chloroplasts per cell and cell abundance per unit leaf area were separately determined for the palisade and spongy tissues in samples fixed with 3.5% glutaraldehyde solution in the phosphate buffer (pH 7.0). The number of cells per leaf surface area was counted in a Goryaev cytometer after macerating the tissue in a 20% KOH solution at 80–90°C. The size of chloroplasts was measured from photographs of transversal sections of fresh leaves in an isotonic solution (Tris–HCl–sorbitol buffer, pH 7.4). The photographs were taken with an MFN-11 (LOMO, Russia) photomicrocamera attachment. The chloroplast length (L_{chl}) and width (D_{chl}) were measured by projecting the negatives on a screen.

The chloroplast volume (V_{chl}) and the chloroplast surface area (F_{chl}) were calculated from Cesaro formula for ellipsoid of revolution $V_{chl} = (4/3)\pi ab^2$, $F_{chl} = 4\pi(ab^2)^{2/3}$, where $a = L_{chl}/2$, $b = D_{chl}/2$. The linear dimensions of chlorenchyma cells in leaf tissue macerated in 0.1 N HCl were measured under a light microscope with an eyepiece micrometer. The volume and surface of the palisade mesophyll cells were calculated by two methods: (a) the routine (mesostructure) method, in which a palisade cell is considered as a regular cylinder, and (b) the projection method, in which the cell surface and cell volume were calculated from cell projections.

Calculation of the cell surface area and cell volume by the mesostructure method. This method [11, 20, 22] is used to calculate the volume and surface area of cells with a regular shape. The surface area and the volume of palisade cells were calculated from the formula for a cylinder, corrected with Tselniker coefficient [19] $V_{cell} = \pi r^2 LK$, where L is the cell length, $r = D/2$, D is the cell width, and K is Tsel'niker coefficient, which depends on the cell length to width ratio. The cell surface area was calculated from the formula for cylinder surface $F_{cell} = 2\pi rL + 2\pi r^2$.

Projection method of calculating the surface area and volume of mesophyll cells. This method is based on stereological principles that allow three-dimensional characteristics of arbitrarily shaped bodies to be precisely determined from their two-dimensional images (sections and projections) [23, 24]. Three-dimensional characteristics (volume and surface area) for cells of the palisade and spongy tissues were calculated from their two-dimensional characteristics (the average perimeter and the average area of cell projection). The method for obtaining cell projections and calculating the cell surface area and cell volume is described in detail below.

Calculated indices. The volume and surface area of cells were calculated for each type of tissue, using the mesostructural (for palisade cells) and projection (for palisade and spongy cells) methods. These data were used to calculate several derived indices of leaf mesophyll.

Cell volume per chloroplast (μm^3):

CVC = V_{cell}/n_{chl}, where V_{cell} is the cell volume, μm^3, and n_{chl} is the number of chloroplasts per cell.

The chloroplast volume per cell volume unit (%):

$V_{chl}/V_{cell} = (n_{chl}V_{chl}/V_{cell}) \times 100$, where V_{chl} is the chloroplast volume, μm^3.

Cell membrane index, i.e., the total surface area of the outer cell membranes per unit leaf area (cm^2/cm^2):

CMI = $(N_{cell}S_{cell})/100$, where N_{cell} is the number of cells per 1 cm^2 of leaf surface area, S_{cell} is the cell surface area, μm^2. The chloroplast membrane index (ChMI), i.e., the surface area of the outer membranes of chloroplasts per unit leaf surface area was calculated in a similar manner.

Mesophyll volume in the leaf (%):

$V_{mes}/V_l = N_{cell}V_{cell}/V_l$, where V_l is the leaf volume per 1 cm^2 of the leaf surface.

Leaf mesophyll conductance, g_m. The CO_2 conductance of the palisade and spongy tissues was calculated from the formula [25, 26]:

$$g_m = D(\mathrm{CMI})bL^{-1},$$

where $D = 8 \times 10^{-6}$ cm^2/s is the diffusion coefficient for CO_2 in water [26], b = 0.68 is the solubility coefficient for CO_2 in water at 30°C, and $L = 10^{-4}$ cm is the average distance from the cell surface to the chloroplast [26]. CMI was determined separately for each type of tissue in each plant sample using the invented projection method. Taking into account the loose packing of mesophyll in the leaf (below 25% of the total leaf volume), we considered that CO_2 diffuses through the whole cell surface; hence, no special correction coefficient [26] was needed. The number of replicate measurements of indices varied with the method: the protocol included 20 replicates for counting cells in macerated tissue, 30 replicates for counting the number of chloroplasts per cell, and 30 replicates for measuring cell and chloroplast sizes. With such number of replicates, the standard error did not exceed 5% [22]. The tables give the mean values.

Projection method for measuring the volume and cell surface area of leaf mesophyll. This method is based on stereological principles, which allow three-dimensional characteristics of bodies to be reconstructed from their two-dimensional images [23, 24]. Stereological methods have been used for several decades in metallography, geology, and mineralogy to calculate the absolute and partial volume of particles in rocks and metal admixtures. Stereological methods permit to calculate the volume and surface area of cell structures in animal tissues [24]. Parkhust [21] used the stereological method in the analysis of transversal sections of leaves to assess the internal surface area of the leaf and the relative volume of tissues. One disadvantage of these studies was that the volume and surface of individual cells could not be determined.

Stereological methods are only applicable to randomly distributed internal structures. Therefore, the high degree of plant tissue anisotropy hampers the application of these methods for analysis of leaf sections. In addition, the application of classical stereological methods for biological tissues requires special equipment such as microtomes and scanners.

We developed a method for measuring the cell surface area and cell volume in the leaf mesophyll, based on cell projections. Our approach combines stereological and morphometric methods applied to individual objects. This approach involves three-dimensional interpretation of planar images obtained as projections of cells from macerated plant tissues. By analyzing a great number of cell projections (at least 30), we calculate the mean cell surface area and the mean cell volume in a sample. This results in a model of an average

cell in a given tissue, which is characterized in terms of the average statistical values of the cell volume and surface area. The method is simple and can be used to calculate the volume and surface area of plant cells of any shape. The method was developed to supplement the method for analyzing the mesostructure of photosynthetic apparatus [11, 22].

Projections of mesophyll cells were obtained with a drawing apparatus RA-4 (Russia) during microscopic examination of a leaf macerated in 0.l N HCl at a magnification of ×200–400. For each tissue type (palisade and spongy mesophyll), 30 projections of randomly selected cells were drawn, and the average area and perimeter of projections were measured. Prior to drawing cell projections, the scale of an eyepiece micrometer was projected on a paper sheet to calibrate the actual sizes of the objects. The area and perimeter of cell projections were calculated using a morphometric measuring grid. The grid represents an array of equidistant perpendicular lines (grid pitch = h). Line intersections and grid nodes are markers for the point-counting method of area measurement, whereas lines are used to count the number of intersections with contours of a figure for perimeter calculation. Such grids were described in [23, 24]. The grid was drawn on a transparent sheet and superimposed on the figure in a random manner. Then, the number of points (grid nodes) fallen into the figure contour (B) and the number of intersections of grid lines with the figure contour (I) were counted. The area of projections was calculated from the equation [23]: $A = Bh^2$, where h is the grid pitch in micrometers, as measured with the scale of eyepiece micrometer projected together with the sampled cells. Perimeter P was calculated by the method of random secant lines from the formula $P = (\pi/4)Ih$ [24]. For cells of the same sample, the coefficient of variation of mean values of A and P was equal to 15–20%; hence, at a given error value of 5%, 30 or more cells should be measured.

Examples of projections of the mesophyll cells from some wild species are shown in Fig. 1. Four cell types were identified, depending on their shape and the presence and size of outgrowths: (1) Elongated, cylinder-like cells without protrusions. Such cells were characteristic of palisade tissues in C_3 species (Figs. 1a–1c) and mesophyll cells in C_4 plants (Fig. 1e). (2) Rounded cells without pronounced protrusions. The length of protrusion did not exceed its width. Such cells were characteristic of chlorenchyma in many C_3 species with homogenous leaf mesophyll, spongy tissues of species with dorsiventral or isolateral mesophyll, chlorenchyma in succulents (Figs. 1a, 1b, 1d), and bundle sheath cells in plants with C_4-photosynthesis (Figs. 1e, 1f). (3) Cells with pronounced protrusions, the length of which significantly exceeds their width (Table 2). These cells were typical of spongy tissues in some species with a dorsiventral type of mesophyll (Fig. 1c). (4) Segmented cells, consisting of several segments, are typical of mesophyll in gramineous plants with C_3- and C_4-photosynthesis (Fig. 1f).

The cell surface area was calculated using the Cauchy formula for convex bodies [27, 28]:

$$S = bA, \quad (1)$$

where S is the surface area of the body, and A is the average area of projections of a certain type of cells. For the first and the second cell types, $b = 4$; for cells with long protrusions ($L/D > 1$), we used a coefficient b accounting for the length to width ratio of protrusions (Table 2).

If the shape and the surface area of an object are known, we can calculate its volume, because the ratio between the surface area and volume depends on the body shape. The body shape is independent of body size and does not change with scale. Hence, it is defined by dimensionless parameters, such as the ratio between the volume, area, and linear parameters of the body [23]. The shape of flat figures is defined in geometry with a two-dimensional form factor that represents the ratio of figure area A to the square of the perimeter P. According to the isoperimetric inequality, the coefficient A/P^2 has the highest value for the circle [27, 28] and is equal to $\pi r^2/2\pi r^2 = 1/4\pi$. For any other plane figure, the following inequality holds

$$A/P^2 \le 1/4\pi. \quad (2)$$

Similarly to the two-dimensional form factor for planar figures, the shape of a three-dimensional body can be characterized by the ratio of the body volume V to its surface area S raised to the power 3/2, which is known as the three-dimensional form factor [23]. Just as with planar figures, in three-dimensional figures, the ratio V^2/S^3 is at highest for a sphere [27], for which it is equal to $(4/3\pi r^3)^2/(4\pi r^2)^3 = 1/36\pi$. Thus, for any three-dimensional body, the following inequality is satisfied

$$V^2/S^3 \le 1/36\pi. \quad (3)$$

According to stereological principles, two- and three-dimensional characteristics of a three-dimensional body are closely related. The three-dimensional form factor of a figure can be determined as the average value of two-dimensional form factors of its projections. To this end, the relation between two-dimensional and three-dimensional factors should be found. This can be achieved by dividing the inequality (3) by (2). Then we obtain the following condition valid for any three-dimensional body:

$$V^2/S^3 : A/P^2 \le 1/9. \quad (4)$$

We shall call this ratio the proportionality coefficient K_n. Calculations of this coefficient for various geometric figures showed that K_n depends on the three-dimensional shape of the body. For example, for a sphere, $K_n = 1/9 \cong 0.11$. Cells in plant leaf chlorenchyma have a nearly spherical surface. The entire set of shapes of leaf mesophyll cells can be described by four types of figures (see description in the text and Fig. 1). We showed that, for most plant cells, K_n varied between 0.11 and 0.08 (Table 3). This coefficient depended on the cell shape and the size of cell protrusions. For

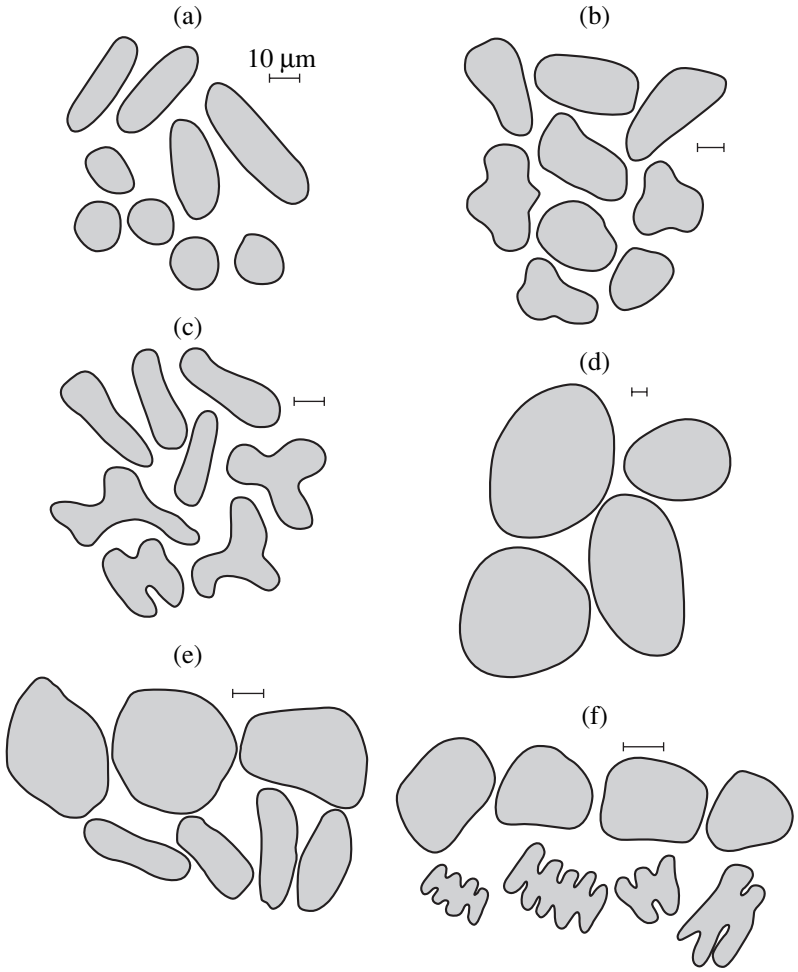


Fig. 1. Cell shape in various types of tissues of some C_3 and C_4 plant species.
(a) *Chamaerion angustifolium* (L.) Holub. (C_3-photosynthesis, dorsiventral type of mesophyll structure; projections represent elongated palisade cells and rounded spongy cells); (b) *Veronica chamaedris* L. (C_3-photosynthesis, dorsiventral type of mesophyll structure, projections represent the palisade cells and spongy cells with short protrusions); (c) *Alchemilla vulgaris* L. (C_3-photosynthesis, dorsiventral type of mesophyll structure, projections represent the palisade cells and spongy cells with long outgrowths); (d) *Sedum purpureum* L. (Schult.) (C_3-photosynthesis, succulent, homogenous type of mesophyll structure); (e) *Amaranthus retroflexus* L. (C_4-photosynthesis, Kranz anatomy, rounded sheath cells, elongated mesophyll cells); (f) *Cleistogenes squarrosa* (Trin.) Kleg. (C_4-photosynthesis, cereal plant, Kranz anatomy, rounded sheath cells, segmented mesophyll cells).

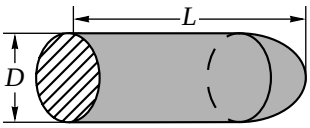
spherical cells, K_n was equal to 0.11. For cylindrical cells (type 1), $K_n$varied between 0.09 and 0.11, depending on the cell length to width ratio. If a cell had protrusions (types 2 and 3), the K_n value varied with the length to width ratio of these protrusions from 0.08 to 0.11. For segmented cells, K_n depended on the number of segments and on their length to width ratio (Table 3). If the differences in cell shape were ignored, the error in the calculated cell volume did not exceed 15%, due to a small variation in K_n. Generally, the length to width ratio for palisade cells did not exceed 5, and a similar ratio for cell outgrowths in complex cells of chlorenchyma rarely exceeded 3.5. This implies that, in palisade and spongy tissues, the error in the cell volume determined with this coefficient does not exceed 9%. Generally, in a given tissue of the same plant species, cells are similar in shape, which allows an average cell model to be built.

Thus, after determining an average value of two-dimensional form factor from the average values of the perimeter and the area of object projections, we can calculate the three-dimensional form factor and, from the cell surface area, calculate cell volume. Using equations (1) and (4) and the values of b and K_n (Tables 2, 3), we can obtain the volume from the formula

$$V = (A^2/P)\sqrt{(b^3K_n)}, \quad (5)$$

where A is the average area and P is the average perimeter of cell projections in a given type of tissue, b = 3.2–4 (Table 2), K_n = 0.08–0.11 (Table 3).

Table 2. Values of coefficient *b*, used for determining the surface area in cells of complicated shape, as a function of the length (*L*) to width (*D*) ratio of their outgrowths

Schematic diagram of an outgrowth in a complex-shape cell							
L/*D*	0.5	0.7	1.0	2.0	3.0	4.0	5.0
Coefficient *b*	4.0	3.7	3.5	3.3	3.2	3.2	3.2

Note: Calculations were performed for the median projection along the long axis of the outgrowth, because it is unlikely that cells could be positioned on the slide with strictly vertical orientation of their outgrowths.

Table 3. Dependence of coefficient K_n on the length to width ratio for cylindrical cells and for cell elements constituting complex cells

Cylindrical cell, *L*—cell length, *D*—cell width		Segmented cell, *L*—segment length, *D*—segment width, *n*—number of segments					Cell with protrusions, *L*—protrusion length, *D*—protrusion width	
L/*D*	K_n	*L*/*D*	K_n, $n > 8$	K_n, $n = 4$–8	K_n, $n = 3$	K_n, $n = 2$	*L*/*D*	K_n
1.5	0.11	1.0	0.11	0.11	0.11	0.11	0.5	0.11
2.0	0.10	1.2	0.10	0.10	0.11	0.11	1.0	0.10
2.5	0.10	1.4	0.09	0.09	0.10	0.10	1.5	0.09
3.0	0.09	1.6	0.09	0.09	0.09	0.10	2.0	0.09
3.5	0.09	1.8	0.07	0.08	0.08	0.09	2.5	0.09
4.0	0.09	2.0	0.07	0.07	0.08	0.09	3.0	0.09
4.5	0.09	2.2	0.06	0.06	0.07	0.08	3.5	0.09
5.0	0.09	2.4	0.06	0.06	0.07	0.08	4.0	0.09
10.0	0.09	2.6	0.06	0.06	0.07	0.08	4.5	0.09
20.0	0.09	3.0	0.06	0.05	0.06	0.07	5.0	0.08

The suggested formulae were verified on plasticine models. All cell models were made of the same plasticine piece, the volume of which was measured seven times by measuring the amount of displaced water. From three to five projections of each model were made on a sheet of paper, their average area and perimeter were calculated, and then the surface area and volume were calculated. Table 4 shows that the cell models differed in the average area and the average perimeter of their projections, as well as in the surface area. However, they had about the same volume, which was close to the volume of plasticine piece of which they had been made.

In the palisade tissue of 32 plant samples, cell volumes calculated from the formula for cylinder with correction for Tselniker coefficient coincided with the volume calculated by the projection method (Fig. 2). The cell volumes determined by different methods showed a positive high correlation ($r = 0.99$). The surface area of palisade cells calculated from the formula for cylinder was always higher than the surface area calculated by the morphometric method (Fig. 3). This difference increased with the cell size and became significant at cell volumes above 10^4 μm^3.

The use of the projection method allowed us to determine an important physiological index of cells in two tissues, i.e., their surface area to volume ratio (*S*/*V*). The surface/volume ratio decreased with the cell size, which was particularly evident for cells of less than 2×10^4 μm^3 in volume (Fig. 4). The *S*/*V* ratio for palisade cells was always higher than that for spongy cells, due to the differences in cell shapes. The form factor was always higher in the palisade than in the spongy cells (Table 5). Thus, at equal cell volumes, the higher the surface/volume ratio, the higher the form factor.

The projection method allowed us to calculate the cell volume and the cell surface area in the spongy tissue and, thus, estimate quantitative indices for chlorenchyma in the whole leaf and compare the functional role of these two tissues in CO_2 assimilation by the leaf.

RESULTS

In the plants studied, the total fraction of leaf chlorenchyma varied between 10 and 24% of the leaf volume (Table 5). This depended on the plant species, the type of mesophyll structure, and growth conditions. Generally, the highest volume of autotrophic tissue was

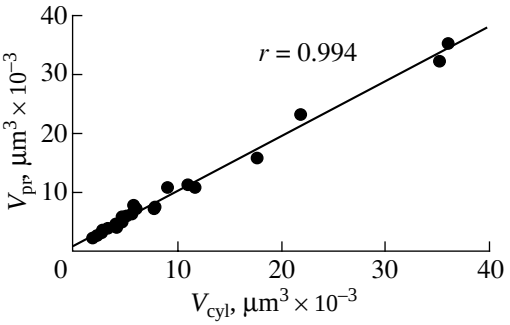


Fig. 2. Volumes of palisade mesophyll cells in 32 plant samples, as calculated from the formula for cylinder with corrections [19] (V_{cyl}) and from projection analysis (V_{pr}). The primary indices were calculated for the same cells.

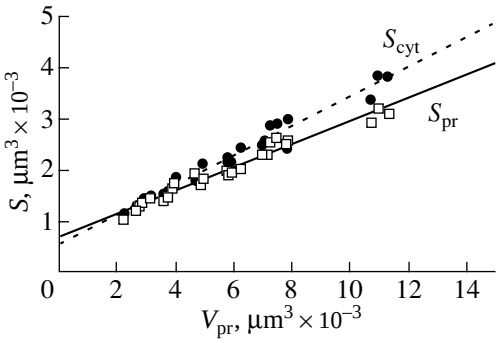


Fig. 3. Relationship between the volume of palisade cells (V_{pr}, projection method) and their surface area (S), as calculated from the formula for cylinder (S_{cyl}) and by the projection method (S_{pr}).

found in meadow plants grown under conditions of high light and optimum moisture supply. The fraction of mesophyll in a leaf volume decreased with shading, this dependence being well pronounced in both the light-requiring species *Genista tinctoria* and in the relatively shade-requiring *Veronica chamaedris.*

All plant species studied showed comparable volume ratios of the palisade and spongy mesophyll. In the shade-requiring species *V. chamaedris*, such parameters as volume fraction, the number of cells, CMI, and ChMI were always higher for the spongy parenchyma than for the palisade tissue (Table 5). In contrast, in a light-requiring xerophyte *G. tinctoria* with an isolateral leaf structure, these indices were always higher in the palisade tissue. Irrespective of the leaf structure and environmental conditions, the quantitative contribution of spongy tissues to the assimilation potential of the leaf was very high in all species studied (Table 5, Fig. 5).

In the spongy tissue, the total surface area of cells and chloroplasts (CMI and ChMI) accounted for at least 40% of the total internal assimilation surface of leaves in the plant species studied (Fig. 5). The number of cells per unit leaf area depended on the type of mesophyll structure, the number of palisade cell layers, and light conditions. The ratio of cell number of the palisade to spongy tissues was at its highest in plants with isolateral leaves (*G. tinctoria*) and in light-requiring plants, such as *Galium mollugo* (Fig. 5, Table 5). The comparison of cenopopulations of plants collected from various habitats revealed a marked decrease in the abundance of palisade cells in a series from the open to shaded habitats. Light conditions affected foremost the abundance of cells in the palisade tissue (Table 5).

Table 4. The volume and surface area of cell models determined by the projection method (n = 7)

Model type	Projection view	Average projection area A, cm^2	Average projection perimeter P, cm	b	K_n	Surface area S, cm^2	Volume V, cm^3
Spherical cell		5.3 ± 0.2	9.1 ± 0.4	4.0	0.11	21.2 ± 0.9	8.2 ± 0.4
Cell with short protrusions		6.2 ± 0.5	12.7 ± 0.4	4.0	0.11	24.8 ± 1.8	8.0 ± 0.9
Short cylindrical cell		5.8 ± 0.1	11.3 ± 0.4	4.0	0.11	23.2 ± 0.5	8.0 ± 0.5
Long cylindrical cell		7.5 ± 0.1	16.3 ± 0.0	4.0	0.09	29.8 ± 0.5	8.2 ± 0.3
Cell with long outgrowths		8.3 ± 0.4	21.0 ± 0.9	3.8	0.09	31.7 ± 1.4	7.4 ± 0.9
Segmented cell, short segments		7.6 ± 0.2	17.4 ± 0.6	4.0	0.09	30.3 ± 0.9	8.0 ± 0.6
Segmented cell, long segments		9.7 ± 0.5	22.1 ± 0.5	3.7	0.08	36.2 ± 1.9	8.5 ± 0.7

Note: The volume of the plasticine, from which the models were made, was equal to 8.3 ± 0.6 cm^3.

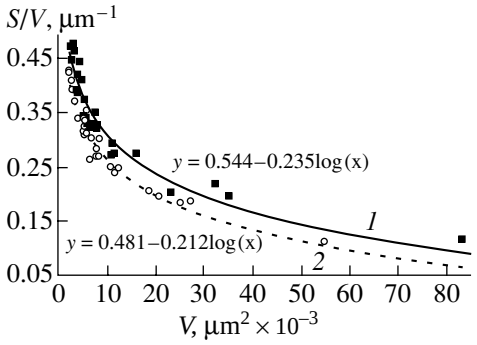


Fig. 4. Dependence of the surface/volume ratio on the cell size in (*1*) palisade and (*2*) spongy mesophyll in 32 plant samples.

Although cells in the spongy and palisade tissue had comparable sizes, the number of chloroplasts was always 5–10% higher in the palisade cells. As a consequence, the cells of palisade parenchyma had a higher plastid concentration, high ChMI, and low values of CVC (cell volume per chloroplast).

In different mesophyll types of plant species examined, the area of the internal assimilation surface, as expressed by CMI and ChMI indices, was mainly determined by cell abundance per unit leaf area (Fig. 5). In all plant species, except *G. tinctoria*, CMI and ChMI values were only slightly higher in the palisade than in the spongy tissue.

Calculation of the mesophyll CO_2 conductance allowed us to compare this index for two types of mesophyll in relation to environmental conditions (Fig. 6). The contribution of different tissues to the total mesophyll CO_2 conductance varied with the light requirements of a species and with environmental conditions. In the light-requiring species *G. tinctoria*, the conductance of the palisade parenchyma constituted about 60% of the total conductance, whereas in *V. chamaedris*, the main part of CO_2 conductance (about 70%) was attributed to the spongy tissue (Fig. 6). Under high light conditions (ecotopes 1 (steppe hillside), 2 (upland meadow), and 3 (lowland meadow)), the palisade CO_2 conductance was significantly higher in most species studied than in spongy tissue. In mesophytes, the highest values of the total conductance of two tissue types were found in plants grown under favorable environmental conditions, i.e., at high light and sufficient soil moistening (ecotope 3, lowland meadow). The values of mesophyll CO_2 conductance were much higher in a xerophyte *G. tinctoria* than in the mesophytes. In this species, the highest conductance values were found in plants grown under high light and water deficit (Fig. 6). Under low light conditions (ecotopes 4 (pine forest) and 5 (the bank of a forest stream)), the total conductance was considerably lower in all species, whereas in mesophytes grown in shaded ecotopes, the conductance was higher in the spongy than in the palisade parenchyma.

DISCUSSION

It is presently known that quantitative characteristics of leaf mesophyll are closely related to the rate of physiological processes. The ratio of the total surface area of mesophyll cells to the leaf area (CMI or A_{mes}/A) positively correlated with CO_2 assimilation rate [12–14, 25]. A close relationship was shown between the internal CO_2 conductance and the total area of the outer membranes of chloroplasts per unit of leaf area (ChMI or A_{chl}/A) [4, 15, 29]. Thus, these indices can be used to assess the photosynthetic activity of leaf and its tissues.

Our data show that, in species differing in the type of leaf mesophyll, the spongy tissue was well developed upon adaptation to various natural light and moisture conditions. This tissue constituted about 50% of the total leaf volume and about 40% of the chlorenchyma surface. This fact reflects the high assimilatory potential of the spongy tissue and its considerable contribution to the total leaf photosynthesis. Under conditions of adequate illumination, the palisade tissue prevailed over the spongy parenchyma in the plants studied. When plants of a particular species grew under forest canopy, the difference between the palisade and spongy mesophyll disappeared and, in some species, reversed. The presence of two types of assimilating tissues in leaves allows plants to acclimate to light conditions. The palisade cells, with their oblong shape and regular arrangement in the leaf, transmit more light than do the cells of spongy tissue, which are irregular in shape and arrangement. The cells in spongy tissues provide for a better light scattering due to multiple light refraction at their surface. Hence, they are better adapted to absorb diffuse light [2].

Our measurements showed that the CO_2 conductance of spongy tissue was comparable to that of the palisade tissue, or even somewhat higher in shaded plants. In xerophytes, the absolute values of the total CO_2 conductance and the contribution of palisade tissue to the total conductance were higher than those in mesophytes, particularly under high light conditions, typical of xerophytes. In mesophytes, the total conductance values were at their highest under favorable moisture conditions and high light intensity. The mesophyll conductance in mesophytes decreased with shading, mainly due to the decreasing conductance of palisade cells.

Our data showed a considerable contribution of spongy tissue to the potential photosynthetic activity of the leaf in the species studied. Based on structural estimates, this contribution is comparable to that of the palisade tissue.

The shape and size of cells also affect the gas exchange rate, because these characteristics affect the ratio of cell surface area to cell volume [30]. According

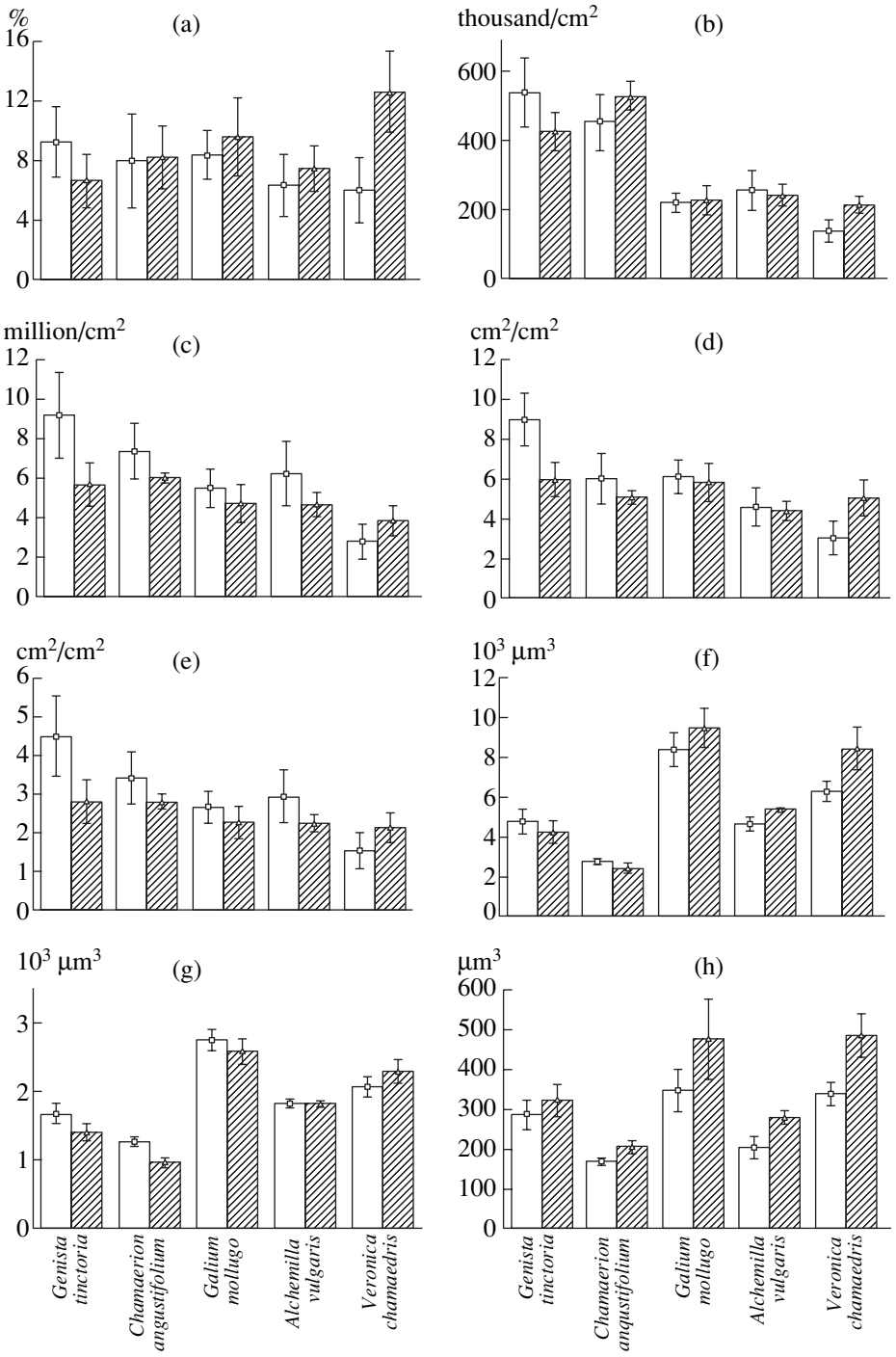


Fig. 5. Comparative characteristics of quantitative indices of the palisade and spongy tissues in plant species studied. Column height corresponds to the mean value for the species of all habitats studied, and bars show the errors of the means. (□—palisade mesophyll, ▨—spongy mesophyll) Plant species are arranged in ascending order of shade requirement: Quantitative indices of mesophyll: (a) mesophyll volume in the leaf, %; (b) number of cells per unit leaf area, thousand/cm^2; (c) no. chloroplasts per unit leaf area, million/cm^2; (d) CMI, cm^2/cm^2; (e) ChMI, cm^2/cm^2; (f) cell volume, thousand μm^3; (g) cell surface area, thousand μm^3; (h) CVC, μm^3.

to Fick's law on the passive diffusion of gases, the time required to elevate the intracellular CO_2 concentration from zero to half of its concentration in the external medium is inversely proportional to *S*/*V* ratio [30]. The smaller the cell, the higher the ratio of cell surface area to cell volume (Fig. 4, Table 5) and the higher the rate of CO_2 diffusion from the internal leaf space to chloroplasts. A negative correlation between the rate of leaf photosynthesis and mesophyll cell size was repeatedly reported in literature [26, 31, 32]. A high negative cor-

Table 5. Average values of characteristics of the palisade (P) and spongy (S) parenchyma in leaves of plants grown in various environments

Species		*Genista tinctoria*				*Veronica chamaedris*				*Chamaerion angustifolium*			
Sampling place		Steppe slope	Upland meadow	Lowland meadow	Pine forest	Upland meadow	Lowland meadow	Pine forest	Bank of a forest stream	Steppe slope	Upland meadow	Lowland meadow	Pine forest
Number of cells, thousand/cm^2	P	802.7	567.0	432.9	344.4	188.3	199.1	81.3	89.8	559.3	575.6	613.7	242.3
	S	531.3	477.4	274.8	418.4	228.3	264.4	211.6	151.3	511.7	492.8	652.7	577.7
Cell volume, thousand μm^3	P	3.7	5.9	6.1	3.8	7.3	71	5.1	6.4	3.1	3.1	3.3	2.3
	S	3.9	5.1	5.0	2.7	8.1	11.5	7.6	8.2	1.9	2.4	2.3	1.9
Cell surface area, thousand μm^2	P	1.3	1.8	1.9	1.4	2.2	2.2	1.6	1.9	1.3	1.3	1.4	1.0
	S	1.3	1.6	1.5	1.0	2.2	2.7	2.1	2.2	0.8	1.0	0.9	0.8
Three-dimensional form factor	P	218	210	222	219	241	254	207	207	289	316	313	235
	S	156	158	163	169	168	162	174	188	151	169	162	156
Surface/volume ratio, μm^{-1}	P	0.38	0.32	0.33	0.37	0.32	0.32	0.34	0.31	0.44	0.44	0.43	0.44
	S	0.36	0.32	0.33	0.40	0.28	0.26	0.29	0.28	0.44	0.42	0.42	0.44
Number of chloroplasts per cell	P	18	19	16	14	22	23	16	15	17	18	15	14
	S	15	15	12	11	23	19	14	15	12	12	10	11
CMI, cm^2/cm^2	P	11.3	10.8	8.5	5	4.3	4.6	1.4	1.8	7.3	7.9	8.8	2.5
	S	7.1	7.7	4.4	4.5	5.1	7.3	4.6	3.0	4.2	4.9	6.3	4.9
ChMI, cm^2/cm^2	P	6.8	5.7	3.2	2.3	2.1	2.6	0.8	0.8	5.2	4.1	4.1	1.6
	S	3.7	3.8	1.5	2.2	2.7	2.9	1.7	1.3	3.4	2.3	3	2.9
CVC, μm^3	P	201	318	379	276	334	313	310	437	184	174	219	163
	S	257	342	418	253	353	610	528	554	158	205	222	174
Volume of chloroplasts in the cell, %	P	14.6	12.4	7.4	12.9	10.1	12.6	12.7	9.2	20.9	13.6	12.2	17.9
	S	11.4	11.5	6.7	14	9.6	6.5	7.4	7.3	24.5	11.5	12	16.7
Mesophyll volume in the leaf, %	P	10.3	11.6	9.1	6.0	8.7	6.7	3.6	5.0	8.4	9.4	12.3	7.3
	S	7.4	8.8	4.9	5.4	12.2	14.6	14.8	8.9	5.3	6.8	9.9	8.9

Table 5. (Contd.)

Species		*Gallium molugo*				*Alchemilla vulgaris*				
Sampling place		Steppe slope	Upland meadow	Lowland meadow	Pine forest	Steppe slope	Upland meadow	Lowland meadow	Pine forest	Bank of a forest stream
Number of cells, thousand/cm^2	P	224.0	217.2	286.9	152.6	317.0	275.0	427.0	140.7	116.7
	S	240.1	223.6	324.6	119.4	273.7	266.0	326.7	172.0	174.0
Cell volume, thousand μm^3	P	11.4	7.8	8.1	8.1	4.2	5.1	4.3	5.1	5.9
	S	11.9	9.9	7.5	8.1	5.2	5.5	5.3	5.1	5.6
Cell surface area, thousand μm^2	P	3.1	2.4	2.5	2.5	1.6	1.8	1.7	1.8	1.9
	S	2.9	2.6	2.0	2.4	1.7	1.7	1.7	1.7	1.9
Three-dimensional form factor	P	277	316	326	282	289	328	352	266	234
	S	186	204	157	233	218	176	185	215	254
Surface/volume ratio, μm^{-1}	P	0.29	0.32	0.32	0.32	0.39	0.39	0.41	0.36	0.33
	S	0.26	0.28	0.28	0.31	0.36	0.32	0.35	0.35	0.36
Number of chloroplasts per cell	P	22	24	29	24	5.1	5.3	7.6	2.6	2.3
	S	16	22	23	24	4.9	4.7	5.6	3.1	3.5
CMI, cm^2/cm^2	P	7.2	5.5	7.6	4.0	3.9	3.3	4.8	1.5	1.3
	S	7.2	6.1	6.8	3.0	2.7	2.3	2.8	1.8	1.7
ChMI, cm^2/cm^2	P	2.7	2.4	3.8	1.8	26	25	26	22	19
	S	2.1	2.3	3.4	1.4	21	18	20	22	17
CVC, μm^3	P	524	327	282	333	162	207	165	233	308
	S	759	443	334	339	253	305	271	236	326
Volume of chloroplasts in the cell, %	P	7.2	9.2	10.1	8.9	19.6	15.3	19.1	13	13.1
	S	4.9	6.8	8.6	8.8	12.6	10.3	11.6	12.8	12.4
Mesophyll volume in the leaf, %	P	10.9	7.3	7.7	7.7	6.2	7.4	9.3	4.6	4.2
	S	12.7	10.3	9.0	6.3	7.2	8.6	9.5	6.0	6.2

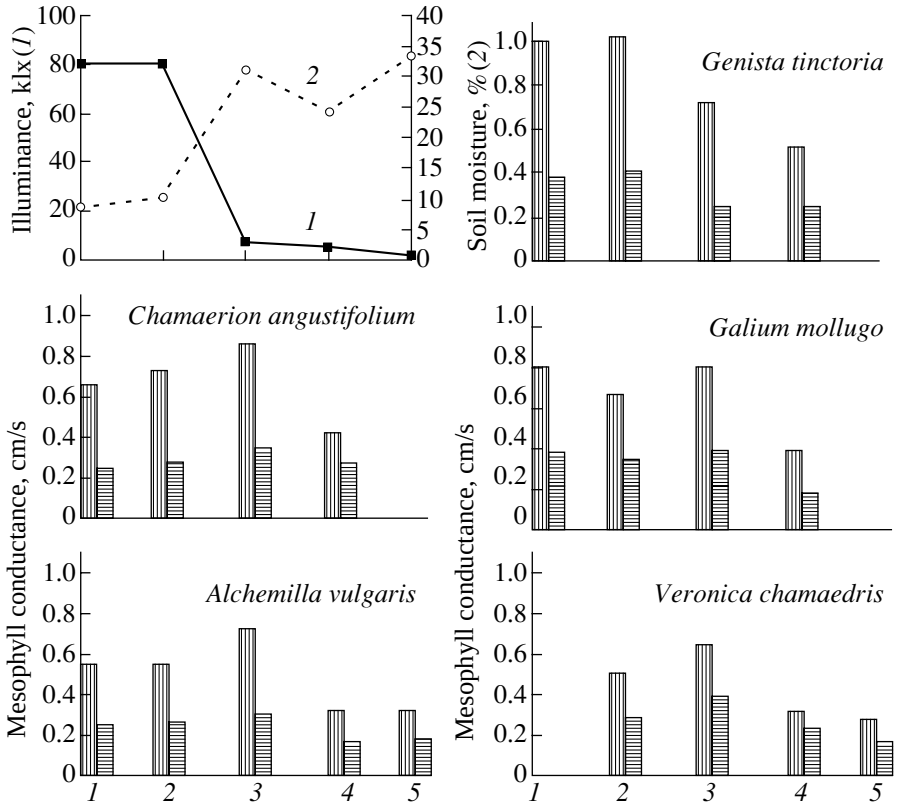


Fig. 6. Mesophyll CO_2 conductance in plant species grown under various environmental conditions.
▥—Total mesophyll conductance (palisade and spongy tissues); ▤ —conductance of spongy tissues. Plant habitats: (*1*) steppe slope; (*2*) highland meadow; (*3*) lowland meadow; (*4*) pine forest; (*5*) bank of a forest stream. The upper left figure—light conditions and soil moisture in the habitats studied; others—mesophyll conductance for each species in the habitats studied.

relation (r = –0.82) between the CO_2 assimilation rate and mesophyll cell size was found in *Lolium* genotypes [31, 32]. Apart from the cell size, the cell shape also affects the surface/volume ratio. Table 5 shows that cells of the same volume may differ in their surface/volume ratio due to various shapes. The higher the form factor, the greater the surface to volume ratio of a cell. On average, the three-dimensional form factor was 1.5 times smaller in cells of spongy tissues than in the palisade cells. In both types of tissues, the form factor of cells depended on environmental conditions (Table 5). Under favorable conditions, this index was at its lowest level in cells of the spongy tissue, whereas, in the palisade cells, it reached the peak. The introduction of the form factor into calculations of cells surface increases the estimated surface area of palisade cells and lowers the relative surface area of spongy cells. As a result, three major structural indices, i.e., the number of cells per leaf area unit, cell size, and the form factor, determine the magnitude of the structural component of CO_2 conductance in the leaf mesophyll (Fig. 6).

In summary, our data indicate the high potential activity and the considerable contribution of the spongy parenchyma cells to the total leaf photosynthesis. According to structural estimates, even in light-requiring plants with the dorsiventral type of mesophyll, the spongy tissue can provide for the conductance of about half of the total CO_2 entering the leaf. The contribution of the spongy parenchyma increases under shading conditions. Thus, the change in the ratio of two types of leaf tissues represents a structural pathway for the photosynthesis acclimation to shading.

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