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# QUANTITATIVE ANALYSIS OF LEAVES OF SUN AND SHADE ECOTYPES OF SOLANUM DULCAMARA L.

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## ABSTRACT

Differences in both surface and inner structure were found in leaves of plants of sun and shade ecotypes of Solanum dulcamara L.: stomatal density of the upper leaf surface, mean number of epidermal cells per leaf, trichome density of the upper leaf surface, volume density of leaf tissues. Leaf area and volume were higher for shade variant.

Key words: leaf anatomy, Solanum dulcamara L., stereology, sun and shade ecotypes.

## INTRODUCTION

Sun and shade plants of one species are known to exhibit immense differences in both physiological performance as well as anatomical structure (rev. by Givnish 1988). Such ecotypical differentiation was described for S.dulcamara, too, but focusing on photosynthetical perfomance not giving the information about structural adaptations to environmental conditions (Gauhl, 1976; Osmond, 1983). The study was performed as a part of more complex research aimed on relationship between life strategy and regenerative capabilities of S.dulcamara. Based on 33hrs-wilting experiment which showed a strong difference in loosing water when leaves were sealed by vaseline, distinctive differences in leaf characteristics were supposed.

## MATERIAL AND METHODS

Leaves of plants of S.dulcamara naturally growing in sun and shade habitats near to Bangor, GB, were studied. The fully developed 7th leaves from the shoot apex sampled from 4 plants per site were quantitatively analysed, leaf area was measured for 10 leaves. Leaf area was estimated by linear integration method (distance between two parallels was chosen as a 1/15 of a mean leaf length for each variant.
Systematic random sampling was applied in order to get leaf segments for measurements (Kubínová, 1993). An oblong net of points determining the segments for sampling was

designed according to the mean leaf width and length in a variant in a such manner that 10 points hit a leaf in average. Since the mean leaf length and width varied for both variants, two nets were designed for sampling, each of them for one variant. A net was randomly applied on the leaf surface, then skipping one hit app. 5 hits indicated segments for inner structure analysis and the others were used for epidermal analysis. Methylmetacrylate imprints were taken from both surfaces to accomplish the epidermal analysis, and wax sections (20 μm thickness) stained by Gentiana violet and Orange G (Němec, 1962), and safranin and fast green (Johansen, 1940) were used for inner structure analysis.

Both types of slides were processed using light microscopy with an application of ocular test systems. Application of frames on the slides was accomplished using an oblong net designed in a such manner that 20 fields per one section or imprint were evaluated in average. The net was actually created using a devise ELTINOR 4 (comp. ROW, Rathenow, Germany) allowing a regular shift of a slide on a stage of microscope under defined steps (shortest possible steps are 50μm in a horizontal direction and 20μm in a vertical direction). Epidermal characteristics (see Fig. 1) were obtained by measurements combining point-counting method with counting particles using unbiased sampling frame (Gundersen, 1977). Volume density of tissues was measured by point counting method. Mean leaf thickness was measured by an ocular ruler on a leaf cross-section under regular steps as a shortest distance between both leaf surfaces. Leaf volume was then obtained as a product of leaf area and a mean leaf thickness.

Results were statistically processed by either two-sample Student t-test (leaf area, leaf volume, epidermal characteristics) or analysis of variance (proportional amount of tissues).

## RESULTS

### *Morphological characteristics*

Leaf area of both ecotypes showed highly significant differences ($P=0.001$); sun: 8.0 (0.8)$cm^2$, shade:17.7 (3.8)$cm^2$ - (standard errors in brackets). Leaf volume was found 98.7 (4.5)$mm^3$ for sun variant, and 367.6 (173.3)$mm^3$ for shade variant. Very high standard deviation for leaf volume of sun variant indicates a high variability of this parameter.

### *Epidermal characteristics*

Epidermal characteristics (Fig. 1) were evaluated on upper and lower leaf surfaces separately, then, if convenient, evaluated for the whole leaf as one unit (Tbl. 1).

Following characteristics were found to be significantly ($P=0.05$) higher for sun variant: stomatal density at the upper surface, and trichome density at the lower surface. Total number of epidermal cells per leaf was the only parameter significantly lower ($P=0.05$) for sun variant. Mean areas of projections of both stomatal cells and epidermal cells were comparable, the latter parameter showed the tendency ($P=0.13$) to be higher for shade variant. Higher number of analysed leaves would prove if the tendency has its significance.

Table 1: Epidermal characteristics for leaves of sun and shade ecotypes of S.dulcamara. Standard errors are given in parentheses. * indicates the significance of difference at 5% level, (*) indicates the significance for only one surface as marked in the line.

| EPIDERMAL CHARACTERISTICS | SUN: Upper | SUN: Lower | SHADE: Upper | SHADE: Lower |
|---|---|---|---|---|
| Stomatal density [$mm^{-2}$] (*) | 14 * (4.3) | 204 (2.0) | 4 * (0.5) | 175 (44.8) |
| Number of epidermal cells per leaf * | 655,417 (3,536) | 931,931 (14,336) | 928,777 (76,902) | 1,324,109 (68,008) |
| Trichome density [$mm^{-2}$] (*) | 25 (3.5) | 35 * (1.9) | 18 (5.8) | 19 * (3.7) |
| Mean area of stoma projection [$\mu m^{-2}$] | 456 (21) | 422 (27) | 534 (54) | 440 (9) |
| Mean area of epidermal cell projection [$\mu m^{-2}$] | 1,179 (69) | 756 (35) | 1,937 (300) | 1,297 (274) |

50 μm :

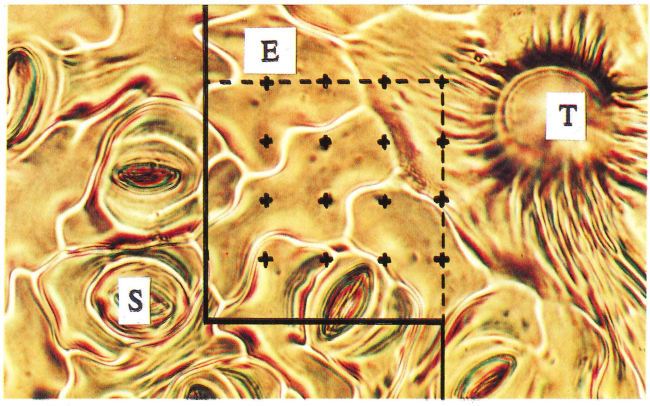


Fig. 1. Microphotograph of epidermal imprint. Lower surface of shade variant. S-stoma, E - epidermal cell, T-trichome. Appliyng the counting frame with the net of points, all types of cells were counted simultaneously with points hitting the different cells.

*Inner structure*

To minimize the overprojection caused by a relatively large thickness of sections for this purpose, oil immersion with oil immersion lense (M=100x) was used for measurements of volume density of leaf tissues (Fig. 2). Volume densities of all tissues proved to be statistically different (P=0.01) with the only exception of vascular tissues (Fig. 3). Significantly higher

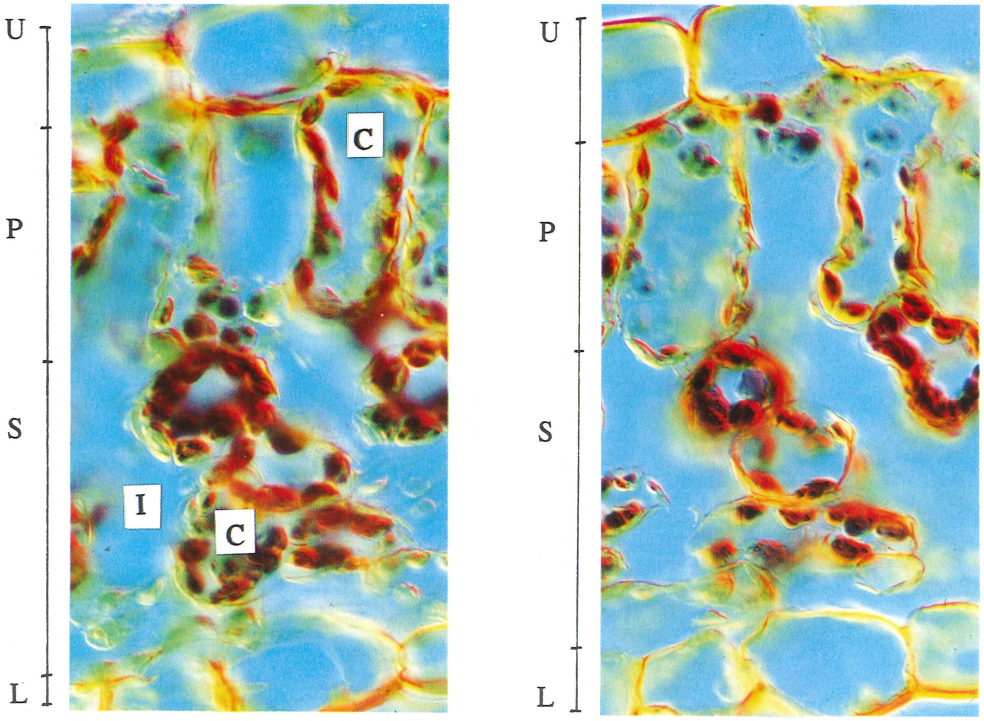


Fig. 2. Microphotographs of leaf cross section. Two optical sections in distance of 14μm inside one physical section. Shade ecotype. Cells with extrusions difficult-to-follow in region of spongy parenchyma. U-upper epidermis, P-palisade parenchyma, S-spongy parenchyma, L-lower epidermis, C-cell, I-intercullar space.

volume densities of both photosynthetical parenchymas were found for sun variant. Lower volume density was detected for both epidermal tissues and intercellular spaces for sun variant. Crystals of Calcium oxalate were relatively abundant for sun variant (2.0%) and only very rarely present for shade variant (0.04%); the last value is expressed in a Fig. 3 as a zero value.

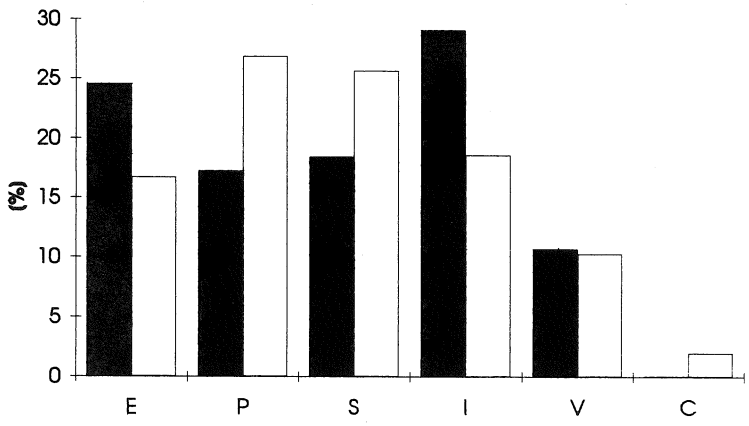


Fig. 3. Different volume density of tissues in leaves of sun (white columns) and shade (black columns) ecotypes expressed as a percentage of total leaf volume. E-epidermis, P-palisade parenchyma, S-spongy parenchyma, I-intercellular spaces, V-vascular tissues, C-crystals of Calcium oxalate.

## DISCUSSION

Results proved some supposed differences of structural adaptations to sun and shade environment. However, not all differences which were supposed from pilot observations were proved by quantitative analysis. For example, stomatal density seemed to be generally higher for shade leaves, but this fact was not proved due to a high variation among individual leaves studied. The differences statistically proved are in accordance with similar studies (rev. by Givnish, 1988).

Interesting results were supposed to be obtained by application of disector principle on cells of leaf photosynthetizing tissues: spongy and palisade parenchyma. Thus, the thickness of 20μm was chosen for cross-sections, the same which proved to be sufficient in a former study on monocotyledonous leaves (Albrechtová and Kubínová, 1991) with isotropic orientation of cells. However, even at this thickness it was not possible to follow extrusions of spongy parenchymatic cells having anisotropic shape in the dicotyledonous leaves and determine exactly in all cases if the profiles of cell sections belong to one cell only or more ones (see Fig. 2). Since no stereological analysis on dicotyledonous leaves has appeared yet, it is hard to tell if it is a general property of dicotyledonous leaves, or only a typical one for leaves of S.dulcamara. To apply successfully disector method it would be necessary to use thicker sections. However, such adjustment could make impossible to use light microscopy anymore.

sections. However, such adjustment could make impossible to use light microscopy anymore. Further attempts are being made in order to find out if it is possible to apply disector principle in the case of dicotyledonous plants at all.

Interesting results (internal-to-surface leaf ratio) from plant physiological point of view could be gained be applying the method of vertical sections. It is indicated from higher proportion of intercellular spaces in shade-grown leaves. This characteristics would be of great information value if accompanied by photosynthetical measurements. Further analysis is supposed to be done.

Hopefully, quantitative anatomical studies will encourage physiological demand in precise structural description of plant anatomical structures.

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