

Seasonal leaf dimorphism in *Potentilla argentea* L. var. *tenuiloba* (Jord.) Sw. (Rosaceae)

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Abstract – A pattern of seasonal changes in the morphological and anatomical leaf traits is reported for *Potentilla argentea* L. var. *tenuiloba* (Jord.) Sw. of temperate-climate areas in central Poland. Leaf area, perimeter, dry mass and lamina thickness were measured in summer and autumn leaves of the same individuals. Dissection index, density and specific leaf area were calculated. Significant differences were obtained between summer and autumn leaves obtained from the same individuals. The shapes of leaves of the *P. argentea* plants varied in the extent of incisions between teeth and the number of teeth on the margins. Fully expanded autumn leaves were larger in weight and area than summer leaves. The autumn leaves had lower leaf mass area and density than the summer leaves. Leaves were covered by considerably more trichomes in summer than in autumn. Anatomical leaf structure also changed with the season. The summer leaves were thick, with a lower number of chloroplasts in the cells of the compact mesophyll. Autumn leaves are thinner, with loose mesophyll. Chloroplasts from the two seasonal types of leaves differ on account of starch grain and plastoglobule content. The large variations in leaf density and thickness recorded here confirm great differences in cell size and amounts of structural tissue within species. Seasonal dimorphism of leaves may result from seasonal drought or from seasonality in leaf production, leaf fall or incoming solar radiation. Within this new context of seasonal leaf dimorphism, *P. argentea* can still be distinguished by the absence of deeply divided leaflets on late-formed leaves. The results confirmed the presence of several morpho-anatomical leaf traits of *P. argentea* that allow the species to adapt to environmental seasonal conditions.

Key words: leaf dimorphism, leaf structure, leaf mass area, *Potentilla argentea*

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Introduction

Plants often have winter and summer leaves that have different morphological and anatomical characteristics, thus they are seasonally dimorphic (ORSHAN 1963, CUNNINGHAM and STRAIN 1969, ARONNE and DE MICCO 2001). Seasonal dimorphism is a flexible ecological strategy, as it comprises very different leaf phenologies and enables plants to tolerate both summer drought and winter cold (PALACIO et al. 2006). Previous studies of leaf morphology and mesophyll structure of seasonally dimorphic species, with two sets of leaves, conducted in Californian coastal shrublands (WESTMAN 1981), the batha of Israel, (ORSHAN 1963) and the phrygana of Greece (MARGARIS 1975, 1977, MARGARIS and VOKOU 1982, CHRISTODOULAKIS 1989, CHRISTODOULAKIS et al. 1990, KYPARISSIS and MANETAS 1993a, b), the coastal maquis of Italy (GRATANI and CRESCENTE 1997, ARONNE and DE MICCO 2001), the matorral of Chile (MONTENEGRO et al. 1979) and the karroid shrublands of the western Cape in South African (WESTMAN 1981) documented the most common features of adaptation of plants to the seasonal climatic changes in Mediterranean ecosystems. Reduced leaf size during the summer months appears to be common to several seasonally dimorphic species (MARGARIS 1975, 1977, SMITH and NOBEL 1977, MONTENEGRO et al. 1979, KYPARISSIS and MANETAS 1993a, KYPARISSIS et al. 1997). Increased leaf pubescence was also associated with summer leaves and it was generally considered a possible adaptation to reduce water loss (SHREVE 1924, WALTER 1973).

Silvery cinquefoil *Potentilla argentea* L. (Rosaceae) is a hemicryptophyte, polycarpic, perennial herb with a persistent main root. It forms rosettes and produces several semi-erect, branched stems that grow up to 30 cm at the study site. The stems and compound palmate basal leaves, each with obovate, five-lobed leaflets, are covered by fine, silvery indumentum, contrasting sharply with the dark green of the adaxial surfaces. The margin of leaf is revolute (i. e. rolled toward the underside of the leaf). All leaves dry at the end of the growing season. This species, common in large parts of Europe, grows in a wide range of habitats. Primarily it is found in open plant communities, including xerothermic and nutrient-poor grassland, or on sands of sparse pine forests. Secondly, it also grows on paved river banks (before they become overgrown by other plants), on roadsides and in other man-made habitats. These plants reproduce by seeds (WOLF 1908, JUZEPCZUK 1941, BALL et al. 1968, GREGOR and GERSTBERGER 2003). In Poland, plants produce flowers and fruits from late April through July (J. Kołodziejek, personal observation). *Potentilla argentea* is the most widespread species of the genus *Potentilla* in Poland. Six varieties have been recognized, namely *argentea*, *decumbens* (Jord.) Focke, *demissa* (Jord.) Lehm., *tenuiloba* and *incanescens* (Op.) Focke, mainly differing in their leaf dissection (SZAFER and PAWŁOWSKI 1955). *P. argentea* has two periods of leaf production which results in the two distinct types of leaves. Leaf initiation typically begins in April, and in dry sites *P. argentea* is completely deciduous during a mid-summer dry period, usually by late July or early August. Autumn leaves develop in late summer, usually in the second half of August, and die in December.

Few studies have discussed the morphological and anatomical characteristics of *P. argentea* leaves and they have mainly been concerned with the indumentum of leaves (e. g., WOLF 1908, SOJÁK 1995, HUBER 1996, STUEFER and HUBER 1998, GREGOR and GERSTBERGER 2003, FAGHIR et al. 2010, 2011, KLINGENBERG et al. 2012). However, little is known about the seasonal changes in leaf morphology and anatomy of *Potentilla argentea*. These seasonal changes in the morphological and anatomical leaf traits have been almost totally overlooked by previous research.

Leaf shape and the indumentum play an important role in the taxonomy of the genus *Potentilla*. The main objective of the study was to explore the seasonal changes in leaf morphology and anatomy in *P. argentea* var. *tenuiloba* (Jord.) Sw.

Materials and methods

Growing location

Seasonal observations of *P. argentea* in the field was made in Napoleonów Forest, 50 km west of Lodz in central Poland (51°92'W, 18°88'N). It was located at the edge of village Niewiesz, at an elevation of approximately 250 m. The local climate is temperate, and the seasons are clearly differentiated. Meteorological data based on 10 years (2000–2010) indicated that the mean annual temperature was 8.8 °C. The average low temperature during winter was –2.5 °C and the average high temperature during summer was 22.4 °C. Annual precipitation (rain and snow) was 587.2 mm, and the frost-free period 271 days.

Population sample for this study includes *P. argentea* individuals that occur in well-drained quartz sand soil. *P. argentea* is commonly associated with *Corynephorus canescens* (L.) P. Beauv., *Rumex acetosella* L., *Erophila verna* (L.) Chevall. *Veronica arvensis* L., *Sedum acre* L., *Spergula morisonii* Boreau, *Herniaria glabra* L., *Thymus serpyllum* L. emend. Fr., *Agrostis capillaris* L., *Artemisia campestris* L., *Trifolium arvense* L., *Dianthus deltoides* L., *Hieracium pilosella* L., *Jasione montana* L., and *Polygonum aviculare* L. This vegetation can be classified as ass. *Spergulo morisonii-Corynephorum canescens* (R. Tx. 1928) ex Czyżewska 1992 (*Sedo-Scleranthetea* Br.-Bl. 1955 em. Th. Müller class). In the sandy soils of the study site, pH-values range between 4.1 and 4.8. Extremely harsh microclimatic conditions, such as soil surface temperatures with high daily amplitudes, along with low pH-values are limiting factors for plant growth (JENTSCH et al. 2002).

Plant material and sampling

Thirty individuals of *P. argentea* were randomly selected and marked in the field along transect, 10–20 m wide, parallel to the margin of the *Pinus sylvestris* forest, in spring 2012. Only well-developed plants were chosen. This procedure increased the probability that there would be a broad sampling of genetic diversity within population. All material was collected from plants growing in unshaded habitats. From each plants two mature rosette leafs from the outer whorl were sampled for biometric measurements at the beginning of July (hereafter referred to as the ‘summer leaf’) and in October (‘autumn leaf’). Leaves without serious herbivore or pathogen damage, as recommended previously (e. g., REICH et al. 1992), were removed with scissors, immediately placed between wet papers, placed into sealed plastic bags and kept in a portable cooler to minimize water loss during transport to the laboratory.

Morphological measurements

After being transported to the laboratory, the leaf samples were placed in water in the dark at 5 °C for 12 h after the petiole was removed under water (GARNIER et al. 2001). This procedure ensured full leaf rehydration. The leaves were then dried with tissue paper to remove surface water.

For each leaf, the surface and perimeter of the whole leaf (leaf of *P. argentea* consists of 5 leaflets) were measured. Leaf outlines were digitized using a digital camera (Nikon, 2048 × 1536 pixels) and analyzed using image-analysis software (SigmaScan Pro 5; SPSS Science, Chicago), which converted the scanned drawing into a bitmap image and determined leaf area (LA, mm²) (one side) and leaf perimeter (LP, mm) of the resulting image. After determination of the area, the leaves were dried for at least 48 h at 80 °C and leaf dry mass (LDM, mg) was determined. Tooth count (TC), the total number of teeth on a whole leaf, was determined visually. All measured parameters are listed in Tab. 1. From the primary data the following variables were derived:

- 1) dissection index (DI) according to KINCAID and SCHNEIDER (1983), McLELLAN (1993, 2000): $DI = \text{perimeter} / [2\sqrt{\text{area} \times \pi}]$,
- 2) leaf mass area (LMA, mg mm⁻²; leaf dry mass per one-sided leaf area), and
- 3) leaf density (LD, mg mm⁻³; leaf dry mass per leaf volume [leaf area × leaf thickness]).

Tab. 1. Morphological and anatomical leaf traits of *Potentilla argentea* var. *tenuiloba* measured in summer and autumn. Means ± SD (n = 30) are shown. F and p-values of a one-way ANOVA are given on the morphology and anatomy of each leaf type. The last column presents the difference between summer and autumn leaves expressed as percentage (abs difference/lowest value). LA – leaf area, LP – leaf perimeter, TC – tooth count, DI – dissection index, LTh – lamina thickness, E_{ad} – adaxial epidermis thickness, PP – palisade parenchyma thickness, PP_c – average area of palisade parenchyma cell profile, PP_{cn} – cell number of palisade parenchyma, SP – spongy parenchyma thickness, SP_c – average area of spongy parenchyma cell profile, SP_{cn} – cell number of spongy parenchyma, E_{ab} – abaxial epidermis thickness, nonglandular hair density on the adaxial [D_{h(ad)}] and abaxial [D_{h(ab)}] leaf surface, LD – leaf density, LDM – leaf dry mass, LMA – leaf mass per area.

Trait	Summer leaves	Autumn leaves	F	p	Variation (%)
Leaf morphology					
LA (mm ²)	159.1 ± 26.9	211.7 ± 34.5	41.75	p < 0.001	33.0
LP (mm)	332.5 ± 25.1	293.1 ± 29.1	21.72	p < 0.001	13.4
TC (teeth/leaf)	23.27 ± 4.22	36.10 ± 4.00	104.6	p < 0.001	55.1
DI	7.48 ± 0.31	5.70 ± 0.40	104.9	p < 0.001	31.2
Leaf anatomy					
LTh (µm)	141.3 ± 14.5	100.2 ± 19.3	287.9	p < 0.01	41.1
E _{ad} (µm)	22.28 ± 1.89	18.65 ± 2.78	115.5	p < 0.01	19.5
PP (µm)	71.02 ± 10.41	47.44 ± 13.05	197.3	p < 0.01	43.7
PP _c (µm ²)	108.1 ± 17.1	167.3 ± 76	6.98	p < 0.05	54.5
PP _{cn} (no./mm ²)	7849 ± 1165	5688 ± 1944	10.42	p < 0.05	37.4
SP (µm)	36.10 ± 8.49	24.25 ± 6.82	116.1	p < 0.01	48.9
SP _c (µm ²)	47.67 ± 6.67	75.01 ± 26.83	10.51	p < 0.01	57.3
SP _{cn} (no./mm ²)	16119 ± 2001	11122 ± 4210	7.70	p < 0.05	44.9
E _{ab} (µm)	11.93 ± 1.39	9.81 ± 1.42	112.7	p < 0.01	21.6
D _{h(ad)} (no./mm ²)	30.57 ± 4.90	16.26 ± 0.88	83.6	p < 0.01	88.0
D _{h(ab)} (no./mm ²)	1477 ± 79	861.7 ± 42.4	349.4	p < 0.01	71.4
LD (µm/mm ³)	13.80 ± 3.13	12.19 ± 2.55	4.45	p < 0.05	13.2
LDM (mg)	15.07 ± 1.91	17.57 ± 1.50	26.36	p < 0.001	16.6
LMA (mg/mm ²)	0.10 ± 0.02	0.08 ± 0.01	6.90	p < 0.05	25.0

Dissection index is sensitive to different aspects of shape. Typically, the dissection index of an entire leaf is slightly larger than 1.0, and more deeply lobed or serrated leaves produce larger values (KINCAID and SCHNEIDER 1983).

To estimate leaf hair density (no. mm⁻²) the middle part of the blade from the central leaflet along the midrib was examined with scanning electron microscope (Tesla BS 340). The leaves were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h at room temperature. After washing in the buffer, the samples were dehydrated in a graded ethanol series, critical point dried with CO₂, placed in gold sputter coater and coated with a very thin layer of gold. Samples were examined at magnification of 300×. The number of non-glandular hairs on the adaxial and abaxial side (in randomly selected area on the edge of the leaf blade) for both leaf types was recorded by counting the total number of hairs in the field of view and dividing this amount by the area of the field of view.

Leaf anatomy and chloroplast ultrastructure

Foliar cross sections were prepared from the central leaflet of both investigated leaf types to measure tissue thickness and analyse their anatomical features under a light microscope (LM) as well as to examine the chloroplast ultrastructure under a transmission electron microscope (TEM). A small portion next to the central vein was cut from the middle of the each freshly harvested mature leaf. Lamina samples were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2, for 2 h at 4 °C. Subsequently they were rinsed with the same buffer and postfixed in 1% osmium tetroxide for 2 h at 4 °C. The material was dehydrated in a graded ethanol series and embedded in Epon-Spur's resin mixture. 1-µm-thick cross-sections were obtained with an ultramicrotome (Ultracut E, Reichert Yung, Germany), then they were stained with toluidine blue and observed under LM (Eclipse 50i, Nikon, Japan). Images were acquired with a camera (Power Shot A 640, Canon, PRC) and processed using Coolview image analysis software (Precoptic Co., Poland). For each image, the following parameters were measured in the leaf lamina between veins: thickness of the lamina (LTh), thickness of the adaxial (E_{ad}) and abaxial (E_{ab}) epidermis, palisade (PP) and spongy parenchyma (SP) layer thickness (30 measurements per each character). Moreover parenchyma cell number per mm² of tissue (PP_{cn} and SP_{cn}) in 10 replicates, cell area of both parenchyma types (PP_c and SP_c) in 100 replicates were estimated and chloroplast number per cell profile in 50 replicates was counted.

The 80-nm-thick leaf cross sections were obtained using the ultramicrotome. Ultrathin sections were placed on formvar-coated nickel grids and stained with a saturated solution of uranyl acetate and subsequently with lead citrate (REYNOLDS 1963). Chloroplasts from palisade and spongy parenchyma were imaged with TEM (JEM 1010, JEOL, Japan) at 80 kV. The average area of chloroplast profile, starch grain and plastoglobule number and their average and total area per chloroplast were estimated using Coolview image analysis software. For each experimental variant 10 chloroplast in palisade and spongy parenchyma were analysed.

All the biometric measurements were conducted on the same plants (individuals) at the same time, so that the results are comparable.

Data analysis

The mean values were calculated for each leaf, and then averaged over two leaves to determine the mean values for each plant. Differences in morphological and anatomical

leaf traits between winter and summer leaves were tested by one-way analysis of variance (ANOVA). The data for all statistical tests were \log_{10} transformed to meet the assumptions of normality and homogeneity of variances implicit in parametric statistical procedures. Correlations amongst leaf traits were analyzed with Pearson's correlation and all reported correlations were significant at an alpha level of $p < 0.05$.

The regression slopes of the relationship between perimeter and square root of area were obtained for the leaves to determine whether there was a deviation from a 1:1 ratio (as defined in HAMMOND 1941, see also LYNN and WALDREN 2001). Pearson's correlation coefficient was used to determine the relationship between the perimeter and square root of area.

The unit of replication for all statistical tests is a plant ($n = 30$ per treatment). All statistical tests were performed using the software package STATISTICA (Statsoft Inc., 2011).

Results

The results demonstrate that the summer leaves of *P. argentea* differ both morphologically and anatomically from the autumn ones.

Leaf morphology

Summer and autumn leaves differed in size (LA, LP) and shape (DI, TC): leaf area (LA) was higher in autumn than in summer leaves. Leaf perimeter also differed significantly for the leaves produced during different seasons ($p < 0.001$): the summer leaves tended to be larger in perimeter (Tab 1; Fig. 1). The relationship between perimeter and square root of area differed between the leaves: the summer leaves had a significantly greater slope than those of autumn (20.34 and 18.08, respectively, $p < 0.0001$) indicating that the perimeter was relatively greater in the former than the latter leaves for a given square root of area (Fig. 2). If so, the leaves produced in summer, having longer perimeter but smaller area, were more deeply toothed than those produced in autumn.

The shapes of both leaf types varied in the extent of the size and number of teeth on the margins. The value of dissection index (DI) was 24% higher in summer. In contrast, the tooth count was 36% higher in autumn leaves (Tab. 1).

Potentilla argentea has two types of leaf hairs, glandular and non-glandular. Both are superficial in origin and a single cell is involved in their formation (unicellular). The glandular hairs are typically spherical. Three forms of non-glandular hairs were observed on the leaf surface: straight, crispate and flexuose hairs. All the forms can be found simultaneously on a single plant. Straight hairs may vary considerably in length from ca. 0.2 mm to a 2 mm; they are adpressed to the surface, crispate hairs are less than a millimeter long, flexuose hairs are flattened, adpressed to the surface, mostly dead and air-filled, especially on the summer leaf surfaces. In both leaf types the adaxial leaf surface was more pubescent than the abaxial, where crispate with flexuose hairs admixed forms dense wooly indumenta (Fig. 3), so that epidermal surface was not visible. Furthermore, the summer leaves were less pubescent than the autumn leaves on the adaxial leaf surface. It was this parameter that varied most markedly (88.0%) among treatments (Tab 1). In the summer leaves the margins of the leaflets were revolute (Figs. 4A–B). Also the veins on the abaxial leaf surface of the summer leaves were covered densely with long straight hairs while on the abaxial surface of the autumn leaves straight hairs were lacking.

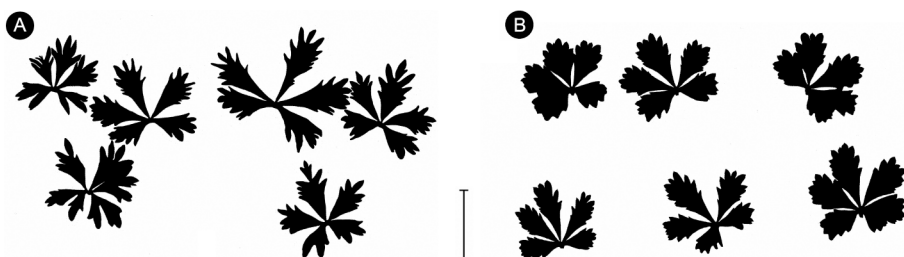


Fig. 1. Silhouettes of representative leaves from 6 plants of *Potentilla argentea* var. *tenuiloba*: (A) summer and (B) autumn leaves (bar = 2 cm).

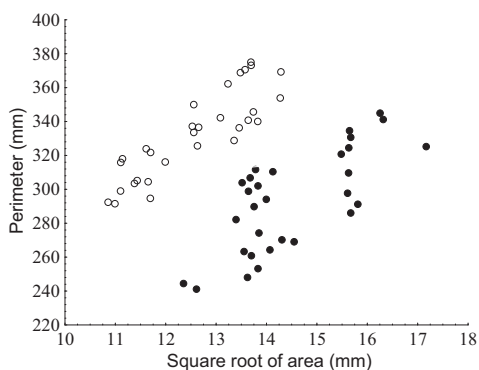


Fig. 2. The relationship between the perimeter and square root of area in summer (open symbols) and autumn leaves (closed symbols) of *Potentilla argentea* var. *tenuiloba*.

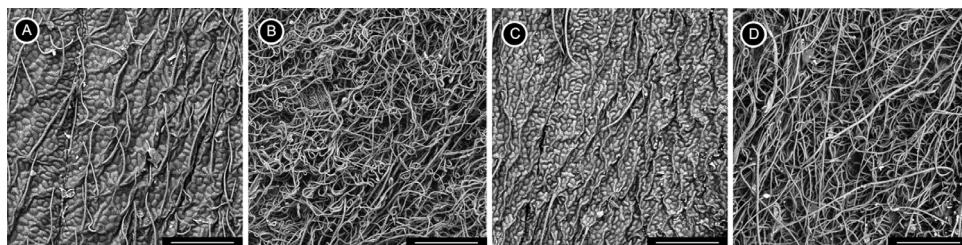


Fig. 3. Scanning electron micrographs of two *Potentilla argentea* var. *tenuiloba* leaf types differing in pubescence: (A) dorsal and (B) ventral view of a summer leaf, (C) dorsal and (D) ventral view of an autumn leaf (bars = 200 μ m).

Leaf anatomy and chloroplast ultrastructure

The anatomical features of leaves also changed with the season. Leaf mass area (LMA) and leaf density (LD) were 20% and 12% higher in summer than in autumn leaves, respectively (Tab 1). LMA was highly correlated with leaf density and, to a lesser extent, thickness, in both (Fig. 5). The thickness of the lamina (LTh) was 41% higher in summer than in autumn leaves (Tab. 1, Figs. 6A–B).

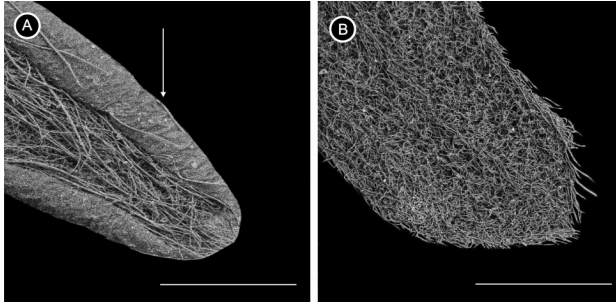


Fig. 4. Scanning electron micrographs of leaf edge of the lower side of (A) summer and (B) autumn leaves of *Potentilla argentea* var. *tenuiloba*. The arrow points at the margin of leaf rolled toward the underside of the leaf (bars = 1 mm).

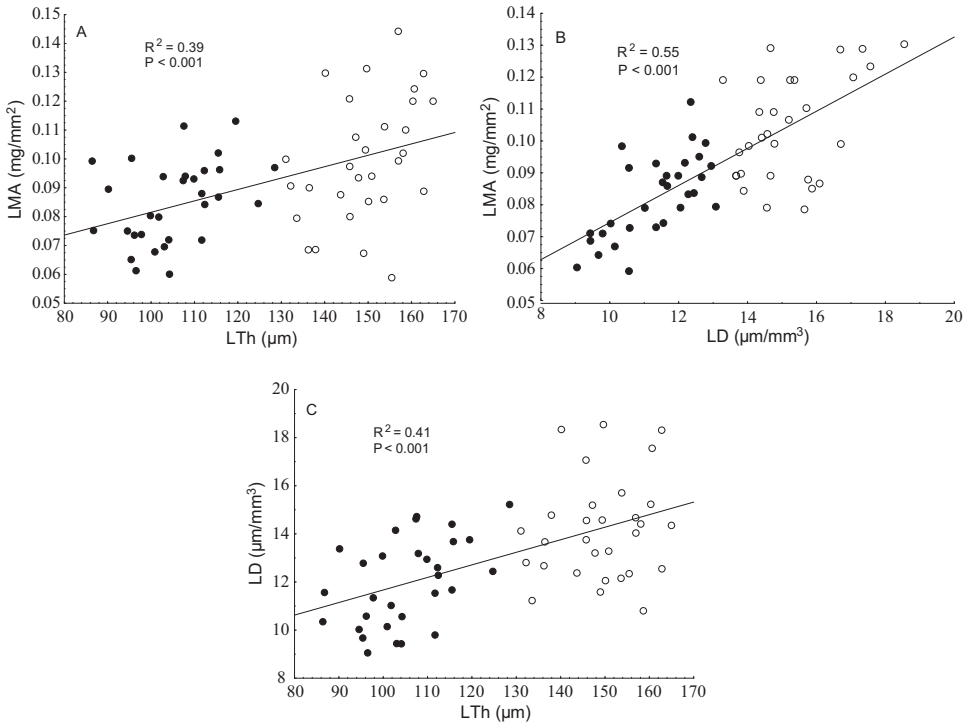


Fig. 5. Regressions between: (A) leaf mass per area (LMA) and lamina thickness (LTh), (B) leaf mass per area (LMA) and leaf density (LD), (C) leaf density (LD) and lamina thickness (LTh). Graphs contains natural trait values, but regression coefficients are expressed as natural logarithms of LD and LTh. Open circles – summer leaves, closed symbols – autumn leaves.

All leaf layers contributed to this increase but mainly parenchyma. While adaxial and abaxial epidermis was thicker in the summer leaves only by about 20%, both palisade and spongy mesophyll thickness increased by 49% (Tab. 1). The parenchyma cells were smaller in the summer leaves and they constituted more compact tissue (Figs. 6A–B). There were

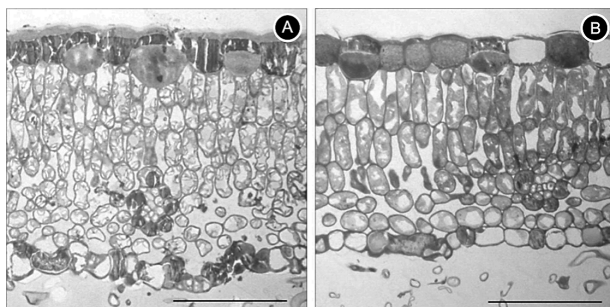


Fig. 6. LM micrographs of semithin cross sections of (A) summer and (B) autumn leaves of *P. argentea* var. *tenuiloba* (bars = 50 μm).

more cells per mm^2 of palisade and spongy mesophyll at the summer leaf cross sections than at autumn ones (Tab. 1). The mesophyll cells in the summer leaves contained fewer chloroplasts but the area of those organelles did not change depending on the season (Tab. 2). However, ultrastructural analyses revealed that chloroplasts from two seasonal types of

Tab 2. Some characteristics of chloroplast ultrastructure in cells of *Potentilla. argentea* var. *tenuiloba* observed in the summer and autumn leaves. Means \pm SD ($n = 30$) are shown. F and p-values of a one-way ANOVA are given on the chloroplast ultrastructure of each leaf type. Chl_N – chloroplast number per cell profile, Chl_A – average area of chloroplast profile, SG_N – starch grain number per chloroplast, SG_A – average area of starch grain, SG_{TA} – total area of starch grains expressed as percentage of chloroplast area, P_N – plastoglobule number per cell profile, P_A – average area of plastoglobule, P_{TA} – total area of plastoglobules expressed as percentage of chloroplast area, ns – not significant ($p > 0.05$).

Chloroplast characteristic	Summer leaves	Autumn leaves	F	p
Palisade parenchyma				
Chl_N (no./cell profile)	5.92 ± 1.02	8.92 ± 1.23	169.55	$p < 0.01$
Chl_A (μm^2)	5.65 ± 2.01	6.27 ± 2.23	1.15	ns
SG_N (no./chloroplast profile)	2.80 ± 0.75	1.60 ± 0.8	13.31	$p < 0.01$
SG_A (μm^2)	1.01 ± 0.47	0.24 ± 0.07	53.58	$p < 0.001$
SG_{TA} (% of chloroplast area)	47.81 ± 12.93	15.87 ± 8.02	28.77	$p < 0.001$
P_N (no./chloroplast profile)	3.00 ± 1.00	6.00 ± 2.40	11.91	$p < 0.05$
P_A (μm^2)	0.13 ± 0.08	0.004 ± 0.002	271.84	$p < 0.001$
P_{TA} (% of chloroplast area)	7.60 ± 3.07	1.52 ± 1.33	192.86	$p < 0.001$
Spongy parenchyma				
Chl_N (no./cell profile)	2.94 ± 1.02	5.06 ± 1.01	87.06	$p < 0.001$
Chl_A (μm^2)	5.91 ± 2.59	4.36 ± 1.00	2.83	ns
SG_N (no./chloroplast profile)	3.30 ± 1.26	2.30 ± 0.64	4.46	$p < 0.05$
SG_A (μm^2)	0.99 ± 0.41	0.24 ± 0.07	69.96	$p < 0.001$
SG_{TA} (% of chloroplast area)	50.16 ± 10.63	17.07 ± 5.91	50.99	$p < 0.001$
P_N (no./chloroplast profile)	3.10 ± 1.22	6.00 ± 2.44	11.58	$p < 0.01$
P_A (μm^2)	0.027 ± 0.01	0.004 ± 0.002	80.87	$p < 0.001$
P_{TA} (% of chloroplast area)	1.67 ± 1.31	0.49 ± 0.12	27.32	$p < 0.001$

leaves differed on account of starch grain and plastoglobule content (Tab. 2, Figs. 7A–D). In the summer leaves, chloroplasts of palisade and spongy mesophyll cells contained more starch grains. The starch grains were almost four times bigger than those in the autumn leaves and occupied about 50% of chloroplast area. The chloroplast starch grains decreased in volume from summer to autumn, when they occupied only a small portion of the chloroplast stroma. In the autumn leaves starch grains filled only 15.87% and 17.07% of the chloroplast area in palisade and spongy mesophyll, respectively (Tab. 2). Plastoglobules were less abundant in chloroplasts of the summer leaves however, especially in palisade parenchyma cells they were much bigger and occupied a greater percentage of chloroplast area. In the summer leaves total area of the plastoglobules (P_{TA}) of palisade parenchyma cell chloroplasts amounted to 7.60% as compared to 1.52% in autumn ones and in spongy parenchyma cell chloroplasts those values were 1.67% and 0.49%, respectively (Tab. 2). Large plastoglobules were less electron-dense than the small ones in the chloroplasts of autumn leaves.

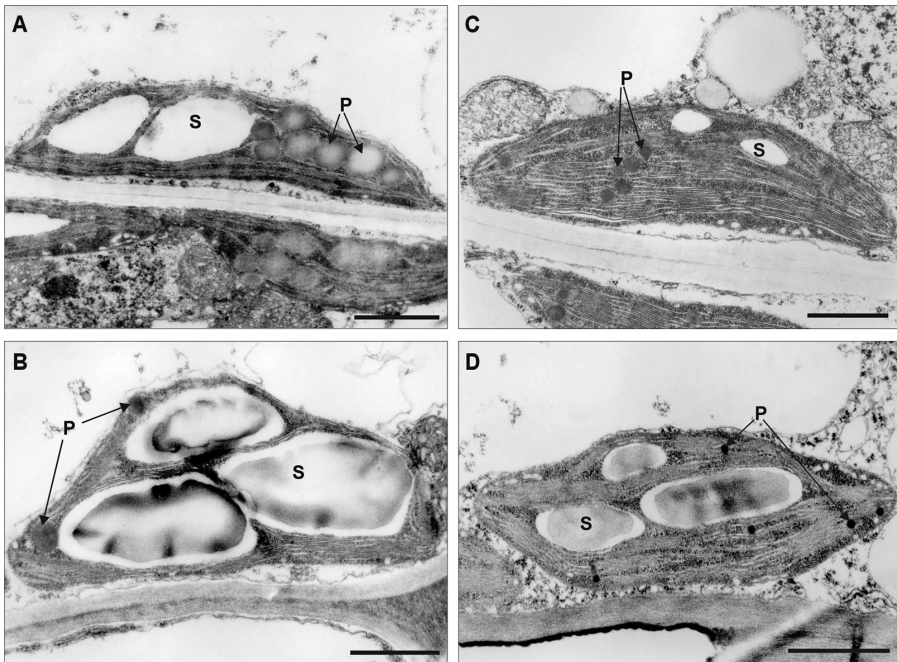


Fig. 7. TEM micrographs of chloroplasts from palisade (A, C) and spongy (B, D) parenchyma cells of summer (A, B) and autumn (C, D) leaves of *P. argentea* var. *tenuiloba*; S – starch grain, P – plastoglobule (bars = 1 μ m).

Discussion

In this paper, we have shown that *P. argentea* exhibits seasonal variations in leaf morphology and anatomy. Several of these differences result from adaptive responses to seasonal change in solar radiation, temperature while others may be due to inter-individual variations (WINN 1996, 1999).

Season appeared to affect the lamina density and thickness. Previous studies showed significant decrease in leaf thickness under low irradiance and a marked increase in leaf density under low soil water availability (SMITH and NOBEL 1977, WITKOWSKI and LAMONT 1991, GROOM and LAMONT 1997, NIINEMETS 2001, CHANDRA et al. 2004). Thicker summer leaves could be an anatomical adaptation to drought stress. ENNAJEH et al. (2010) discovered that the thickness of leaves in *Olea europaea* L. under drought stress increased more significantly in a drought-resistant cultivar than in a sensitive one. Under the conditions of high solar radiation and water deficiency thicker palisade mesophyll could contain larger numbers of CO₂-fixation sites, while a wider spongy mesophyll layer could be responsible for easier diffusion of CO₂. In *P. argentea* LD, LMA as well as PP_{EN} and SP_{EN} parameters were higher in summer than in autumn leaves. That indicates a greater compactness of leaves exposed to higher solar radiation. Similar differences between leaf anatomical traits were observed between winter and summer leaves of *Cistus incanus* L. subsp. *incanus*. A more compact summer leaf structure is regarded as a protective mechanism that diminishes photochemical damage to the photosynthetic apparatus (CATONI et al. 2012).

The reduced leaf thickness in autumn caused by decrease in number of cells was also described by other authors (e.g., HANSON 1917, JACKSON 1967, FEKETE and SZUJKÓ-LACZA 1973, CARPENTER and SMITH 1981, ABRAMS and KUBISKE 1990, ASHTON and BERLYN 1992, P'YANKOV et al. 1999, RÔÇAAS et al. 2001, MOJZES et al. 2005, OGUCHI et al. 2005). Leaf density may also vary due to differences in cell mass or cell wall thickness (CASTRO-DIEZ 2000). Differences in leaf lamina thickness, and epidermal and palisade cell thickness between the two leaf types of *Potentilla* produced similar results to those reported in many other studies for seasonally dimorphic species (ARONNE and DE MICCO 2001, CATONI et al. 2012).

Different kinds of correlations among LMA, LD and LTh reported by different authors (WITKOWSKI and LAMONT 1991, CHOONG et al. 1992) suggest that all the previous mentioned alternatives are possible (CASTRO-DIEZ 2000). In some cases, variations in LMA were due to changes in leaf density or thickness or both (WITKOWSKI and LAMONT 1991). In this study, variations in LMA were the result of variations in both leaf density and thickness.

Seasonally dimorphic species are characterized by a seasonal reduction in their transpiring surface (RISTIC and CASS 1991, KOFIDIS and BOSABALIDIS 2008). Similar trends were detected in the current study. *P. argentea* autumn leaves were thinner and had more LA with respect to the summer leaves. The larger and thinner leaves in autumn are more advantageous for light capture under low light (CARPENTER and SMITH 1981, DE LUCIA et al. 1996, POORTER 1999, WILSON et al. 1999). Moreover, small-sized, thick, summer leaves provide less surface area for the loss of water through transpiration (e.g., RHIZOPOULOU and MITRAKOS, 1990, BAKER-BROSH and PEET 1997, FALSTER and WESTOBY 2003, HUFF et al. 2003). In this way less transpiration is needed for cooling down the leaf in a high-light environment (MOONEY and DUNN 1970, PARKHURST and LOUCKS 1972, ASHTON and BERLYN 1992, POORTER 1999, OGAYA and PEÑUELAS 2006).

In the summer leaves the margins of the leaflets were revolute. Leaf rolling is known to be a typical response to water deficit in numerous species of grasses adapted to drought. The rolling reduces effective leaf area and transpiration, and thus is a potentially useful drought avoidance mechanism in dry areas (KADIOGLU et al. 2012, SAGLAM et al. 2014).

Predominance of leaflets with narrow, deeply incised teeth also helps to counteract summer environmental stresses. More incised teeth were shown to effectively reduce excessive

leaf temperatures and transpiration, as well as the risk of photoinhibition and photodamage (ROY et al. 1999, ROYER and WILF 2006, ROYER et al. 2005, 2008).

The alteration of leaf hair density may also alleviate water and heat stress (PICOTTE et al. 2009). There is a significant difference in the amount of pubescence present on leaves of *P. argentea* at different times during the growing season. The mean leaf hair density in the population of *P. argentea* increased in the summer season during the dry month of July, indicating that fewer hairs are produced during wetter climatic conditions in the autumn. Generally this is in agreement with the results reported by other investigators (KARABOURN-OTIS et al. 1998, ARONNE and DE MICCO 2001, KOFIDIS and BOSABALIDIS 2008, PICOTTE et al. 2009). Increased leaf pubescence density has been considered an adaptive trait in water-limited environments because it reduces water loss, and decreased leaf hair density might increase light reception under low light. Decrease in leaf absorbance is the result of increase in pubescence density and thickness (EHLERINGER and BJÖRKMANN 1978, EHLERINGER and MOONEY 1978, ROTONDI et al. 2003).

The indumentum and leaf shape show considerable variation among different sections or species of the genus *Potentilla* (WOLF 1908). Consequently, distribution of trichome types is of high taxonomic value for *Potentilla* at both infrageneric and interspecific levels (WOLF 1908, SOJÁK 1995, KOŁODZIEJEK 2009, 2010, FAGHIR et al. 2011). *Potentilla argentea* belongs to the *Potentilla* sect. *Terminales* (Döll) Gren. & Godr. [= *P.* sect. *Argenteae* (Th. Wolf) Juz.] (Rosaceae). Here we focused on the study of the shape of leaves and leaf pubescence, which has a long tradition in the classification of *Terminales*. *P. argentea* is closely related to members of sect. *Collinae* (Th. Wolf) Kołodziejek. It resembles members of sect. *Collinae* due to similar indumentum, shape of its leaflets and their dentation. In autumn, *P. argentea* produces flowering rosettes which may have leaves without dense indumentum of crispate hairs on the lower side, partially rolled edges and only slightly incised teeth. Therefore, the differentiation of *P. argentea* from members of *P.* sect. *Collinae* can pose problems. *Potentilla* sect. *Collinae* differs from *P. argentea* by having both leaf surfaces covered with simple straight hairs mixed with imperfectly stellate ones (KOŁODZIEJEK 2008, 2010). Within this new context of seasonal leaf dimorphism, *P. argentea* can be still distinguished by the absence of imperfectly stellate hairs even on early-formed leaves.

Chloroplast ultrastructure was another major difference between summer and autumn hoary cinquefoil leaves. Chloroplasts of the summer leaves contained large amounts of starch and plastoglobules. In *P. argentea*, the relative volume of starch grains decreased from summer to autumn, when starch grains occupied only a small portion of the chloroplast stroma. Accumulation of starch grains in chloroplast through the seasonal gradient up to summer is a common characteristic of Mediterranean plants (MARGARIS 1975, 1977, KYPARISSIS and MANETAS 1993a, KOFIDIS et al. 2003, KOFIDIS and BOSABALIDIS 2008). It seems very likely that the accumulation of starch grains in chloroplasts of light-treated leaf segments resulted from a net accumulation of photosynthate as suggested by MITTELHEUSEN and VAN STEVENINCK (1971). A decrease in starch grain volume from summer to autumn was observed not only in *P. argentea* but also in other plant species such as *Nepeta nuda* L. (KOFIDIS and BOSABALIDIS 2008) and *Euonymus japonicus* Thunb. (LJUBEŠIĆ et al. 2003). The diminishing number of chloroplast starch grains reflected increased respiration rate during photosynthesis under lower radiation at the end of vegetative season.

Increase in the amount of plastoglobules and their enlargement was observed in chloroplasts as the consequence of various unfavorable conditions including drought and high

light resulting in oxidative stress (MANAC'H and KUNTZ 1999, REY et al. 2000). Changes in plastoglobule number and morphology probably reflected an increased demand for antioxidants under stress conditions. Swelling of plastoglobules was probably the effect of tocopherol accumulation, which is known to protect membrane lipids from oxidative damage and to prevent photoinactivation of PSII (KESSLER and VIDI 2007, BRÉHÉLIN and KESSLER 2008).

Dry mass of leaves was significantly larger in summer than in autumn leaves in *P. argentea*. ORSHAN (1963) found that in a Mediterranean climate seasonally dimorphic shrubs reduce their total leaf phytomass by 39–74% from spring to summer, and their transpiration rates per leaf dry weight unit by 50–76%. He suggested that the reduction in transpiration rates was due primarily to reduced availability of soil water.

The results confirmed the presence of several morpho-anatomical leaf traits of *P. argentea*, which allow the species to adapt to environmental seasonal conditions. Within this new context of seasonal leaf dimorphism, *P. argentea* can still be distinguished by the absence of deeply divided leaflets on late-formed leaves.

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