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Morphological and phytochemical adaptations in some species of *Achillea* sect. *Filipendulinae (Compositae)* in Bulgaria

Abstract

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Epidermal (stomata dimension and density) and stem anatomical features (proportions of the various constituent tissues) have been studied for three Bulgarian representatives of *Achillea* sect. *Filipendulinae: A. clypeolata, A. coarctata* and *A. thracica.* Composition in flavonoid compounds of the leaf exudate was analysed for the same species, plus *A. chrysocoma* of *A.* sect. *Santolinoideae.* The results testify of various degrees of xeromorphic adaptation of the taxa studied.

Introduction

Evolutionary trends in Bulgarian *Compositae,* such as transition from one life form to another, or from the mesophytic to the xerophytic condition, are linked to various ecological parameters (Kuzmanov 1991). Among the major limiting factors are moisture deficiency, extreme temperatures, and grazing pressure.

The adaptation of species to moisture deficient habitats bears not only on morphological and structural characteristics (xeromorphism), but also on the presence of epicuticular flavonoid aglycones (Ivančeva & al. 1990). Correlated morphological, anatomical and phytochemical features may thus reflect environmental adaptation in its various aspects. Searching for such correlations has been the goal of our investigations. We have studied three Bulgarian representatives of *Achillea* sect. *Filipendulinae* (DC.) Afan., to which, for phytochemical purposes, we have added *A. chrysocoma* Friv. (*A.* sect. *Santolinoideae* (DC.) Afan.) for comparison.

Materials and methods

Populations of the four species studied, growing mainly in rocky places and dry grassland in S. Bulgaria, have been sampled during the flowering season, as follows (vouchers have been deposited in SOM):

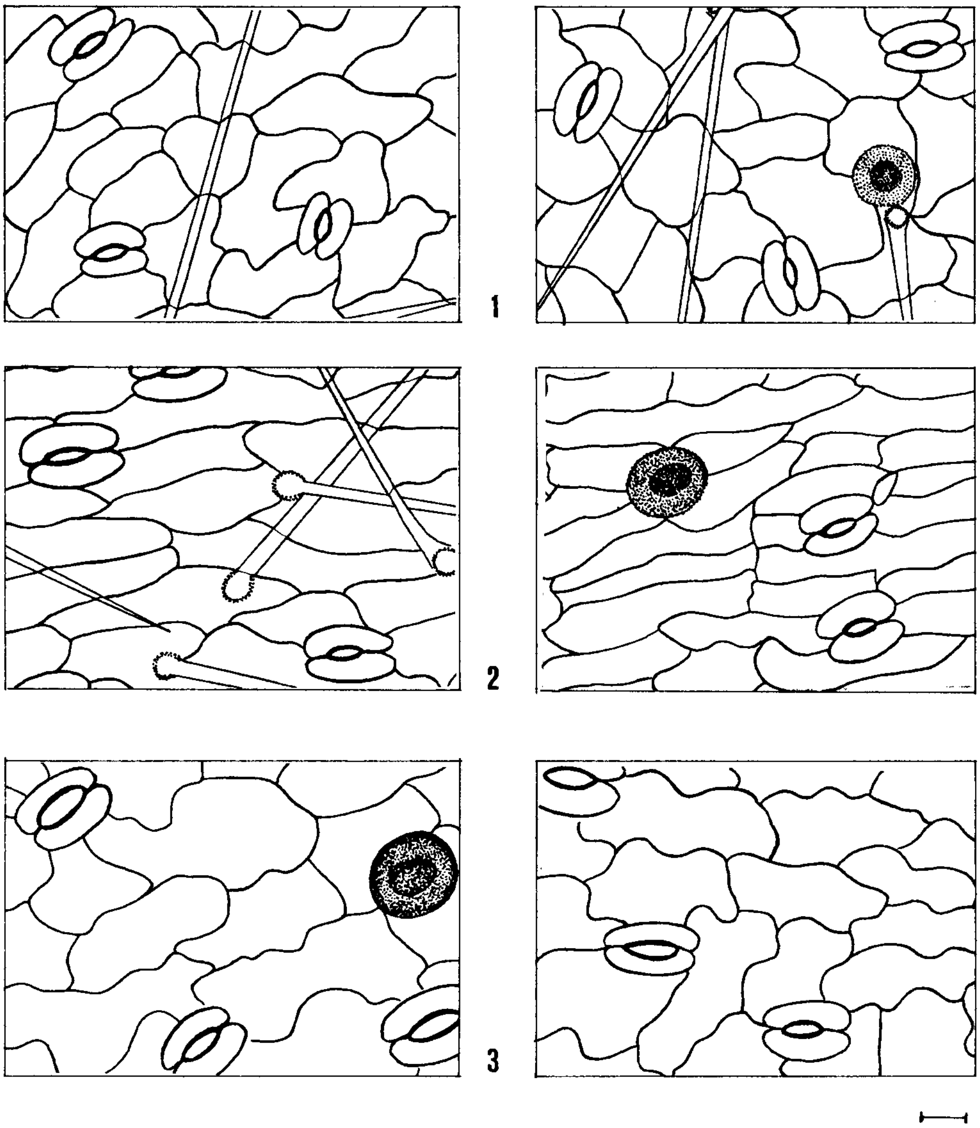

Fig. 1. Epidermis fragments from the upper (left) and lower (right) leaf surface of Bulgarian *Achillea* species. – 1, *A. clypeolata;* 2, *A. coarctata;* 3, *A. thracica.* Scale bar = 10 µm.

Table 1. Features of the epidermis *in Achillea* sect. *Filipendulinae.*

	Species	Mean	Min.	Max.
Stomata length (µm)	*A. thracica*	28.50	23.58	32.58
	A. coarctata	27.69	22.86	31.14
	A. clypeolata	25.86	20.70	31.14
Stomata width (µm)	*A. thracica*	20.53	18.54	24.13
	A. coarctata	18.03	16.20	19.80
	A. clypeolata	15.85	14.40	18.72
Stomata density (mm^{-2})	*A. thracica*	134.70	70.00	175.00
	A. coarctata	139.40	105.00	175.00
	A. clypeolata	253.40	175.00	280.00
Epidermal cell density (mm^{-2})	*A. thracica*	898.30	535.00	1190.00
	A. coarctata	1353.30	1120.00	1540.00
	A. clypeolata	1171.30	945.00	1470.00

- *Achillea clypeolata* Sm.: (1) Golo bărdo, "Orlite", 24 Jul 1987, *Kuzmanov 8633*; (2) Pirin, "Javorovo", 9 Aug 1986, *Kuzmanov 8680*; (3) Sredni Rodopi, "Marciganica", 18 Sep 1986, *Kuzmanov 8697.*
- *Achillea coarctata* Poir.: (1) Struma valley, Kresnensko hance, 28 May 1986, *Kuzmanov 8615*; (2) Zapadni Rodopi, Jundola, 10 Aug 1986, *Kuzmanov 8681.*
- *Achillea thracica* Velen.: Plovdiv district, Manole, 10 Jul 1987, *Kuzmanov 8717.*
- *Achillea chrysocoma:* Pirin, Orlek peak, 2 Aug 1986, *Kuzmanov 8643.*

Stem and leaf structure were studied by the conventional methods of comparative anatomy (Metcalfe & Chalk 1950). Quantitative data (epidermal indexes) were processed by statistical variation analysis.

For the flavonoid aglycone determination, air-dried leaves were washed in acetone and the concentrated solutions (resinous mass) were passed through a Sephadex H-20 column to separate flavonoid aglycones from terpenoids. The flavonoid aglycones were separated using a polyamide column, with toluene, methylethylketone and methanol in different concentrations as eluents. Authentic markers were used for comparison, and UV spectra in methanol and with diagnostic reagents were obtained.

Results and discussion

The leaves of the species studied are isobilateral, with stomata present on both surfaces (Fig. 1). In *Achillea thracica*, the bilaterally arranged palisade cell parenchyma is mostly two-tiered, while in the other species it is single-tiered (Fig. 2). The relative importance of conducting and mechanical tissues, as expressed by an index calculated on the basis of the collenchymal and sclerenchymal layering (not shown), and the degree of cell wall thickness are highest in *A. coarctata.*

Epidermal analysis is, according to many authors, important for taxonomic and ecological purposes. The stomata in all three species are of the anomocytic type (with 4 or 5

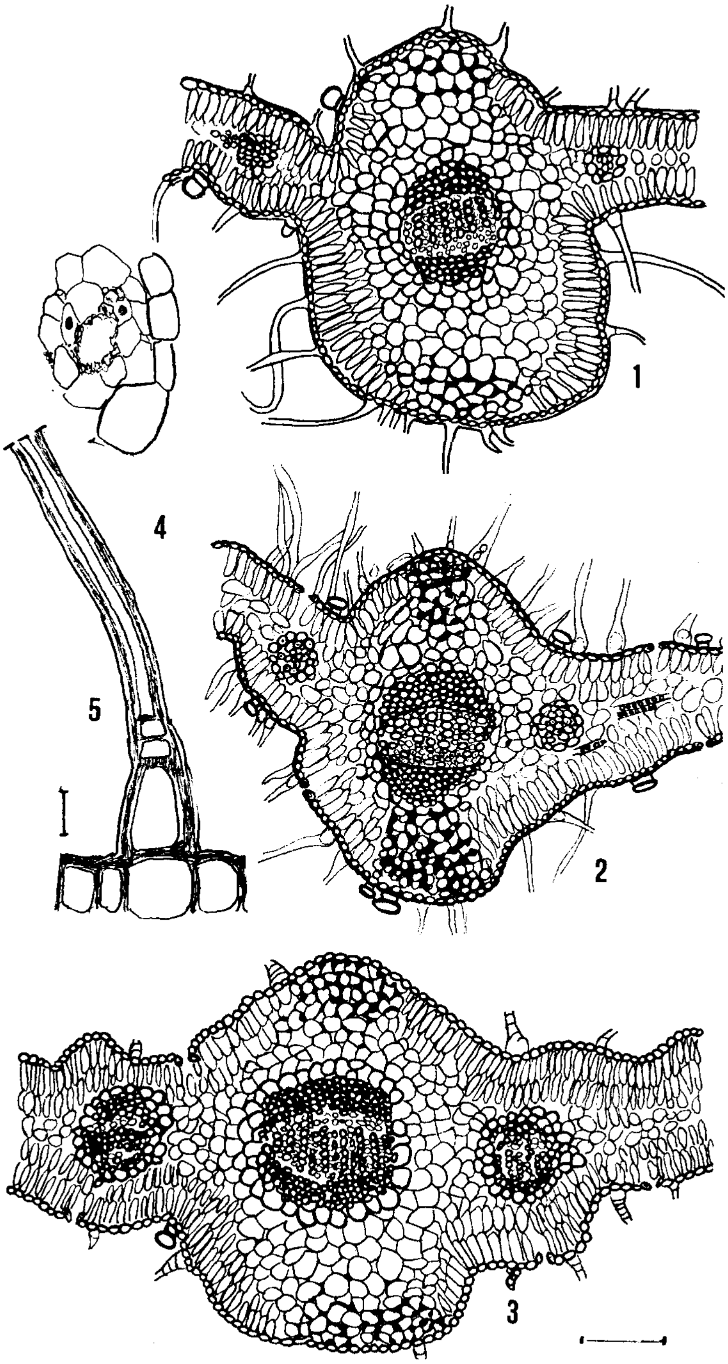

Fig. 2. Leaf structure of Bulgarian *Achillea* species. – 1, *A. clypeolata;* 2, *A. coarctata;* 3, *A. thracica;* 4, secretory canal; 5, non-glandular hair. Scale bar = 1 mm (1-3), 10 µm (4-5).

subsidiary cells, rarely 6 or 3), but their quantitative features vary (length: 20.7-32.58 μm; width: 14.4-24.12 μm; Table 1). An inverse correlation has been established between size and density (number per mm^2) of stomata. The stomata are most dense and smallest in *Achillea clypeolata*, largest and least dense in *A. thracica*. The latter species is also noticeable for having the largest and least numerous normal epidermal cells, whose walls, in surface view, show the most pronounced sinuosities (Fig. 1: 3). All this,

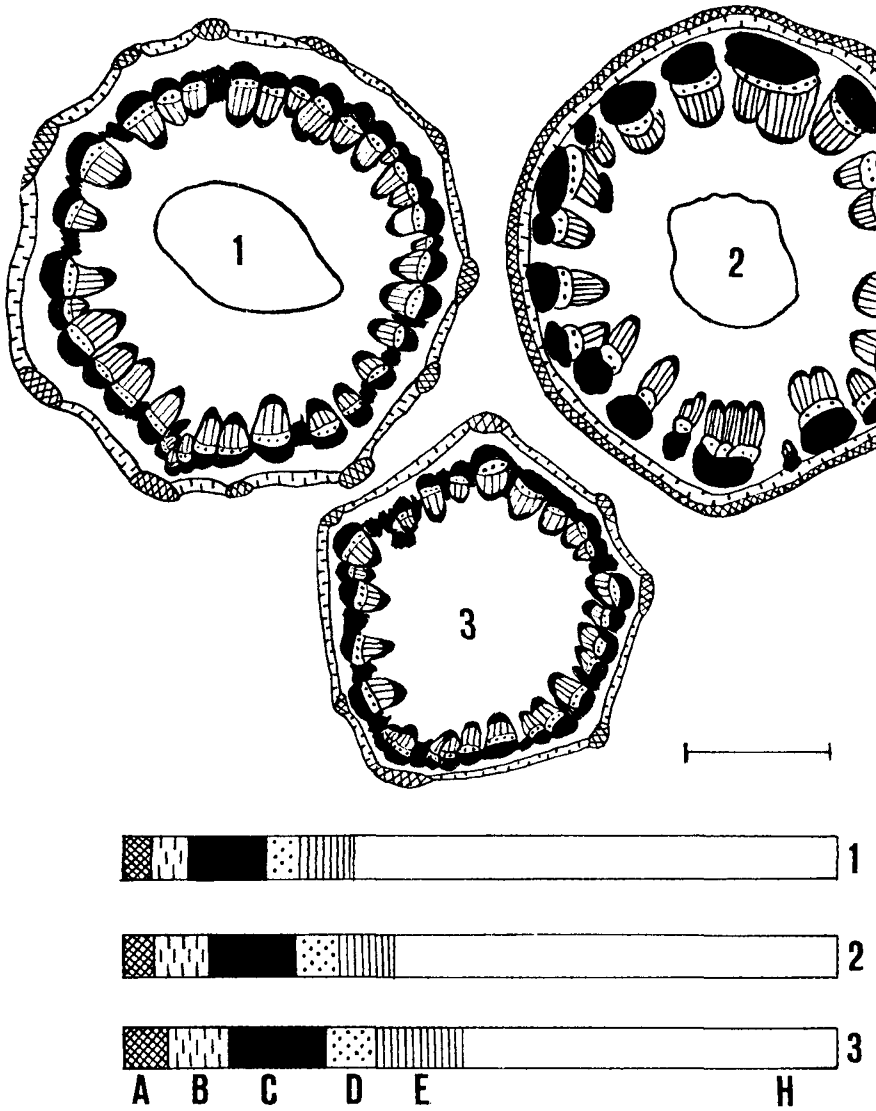

Fig. 3. Stem structure, and proportion between the constituent tissues, in Bulgarian *Achillea* species. - 1, *A. clypeolata;* 2, *A. coarctata;* 3, *A. thracica*. A, epidermis; B, collenchyma; C, sclerenchyma; D, phloem; E, xylem; H, parenchyma. Scale bar = 1 mm.

Table 2. Distribution of aglycones in *Achillea* sect. *Filipendulinae* and *A. chrysocoma*. – ●, large quantity; O, small quantity; +, in traces; –, absent.

Species	Voucher	Compounds (numbered as below) 1	2	3	4	5	6	7	8	9	10	11	12	13
A. coarctata	8615	●	–	–	–	–	–	●	O	O	–	+	+	+
A. coarctata	8681	●	–	–	–	–	–	●	+	–	–	+	–	–
A. clypeolata	8633	●	O	–	–	–	–	O	–	–	O	–	–	–
A. clypeolata	8680	●	O	–	–	–	–	+	–	–	–	–	–	–
A. clypeolata	8687	●	O	–	–	–	–	+	–	–	–	–	–	–
A. thracica	8717	–	–	●	●	●	●	–	●	–	–	+	–	–
A. chrysocoma	8648	–	–	–	–	–	–	–	–	–	–	–	–	●

No.	R	R_1	R_2	R_3	R_4	Compound designation
1	OCH_3	OCH_3	OH	OH	OCH_3	3,6,4'-trimethoxyquercetagetin (centam-reidin)
2	OCH_3	OCH_3	OCH_3	OH	OCH_3	3,6,7,3,4'-pentamethoxyquercetagetin (artemetin)
3	OCH_3	OCH_3	OH	OH	OH	3,6-dimethoxyquercetin
4	OCH_3	H	OCH_3	H	OH	3,7-dimethoxyquercetin
5	OCH_3	H	OH	OH	OH	3-methoxyquercetin
6	OH	H	OCH_3	OH	OH	7-methoxyquercetin
7	OCH_3	OCH_3	H	H	OCH_3	3,6,4'-trimethoxykaempferol (methylbetuletol)
8	OCH_3	H	OCH_3	H	OH	3,7-dimethoxykaempferol (kumataketin)
9	OCH_3	H	OH	H	OCH_3	3,4'-dimethoxykaempferol (ermanin)
10	OCH_3	OCH_3	OCH_3	H	OCH_3	3,6,7,4'-tetramethoxykaempferol
11	H	OCH_3	OH	OH	OH	6-methoxyluteolin
12	H	H	OH	OH	OH	luteolin
13	H	OCH_3	OH	OH	OCH_3	6,4'-dimethoxyscutellarein

added to the fact that *A. thracica* is almost glabrous while the two other species have leaves with a cobwebby hair cover, assigns a relatively low xeromorphic index to *A. thracica* as compared to the other species. Next higher, by the xeromorphic index of epidermis structure, is *A. clypeolata.*

The anatomical structure of the stem is uniform in general terms (Fig. 3). In *Achillea thracica* the hypodermis consists of a complete collenchymal layer, while in the other species the collenchyma is interrupted and alternates with chlorenchyma. The remaining differences between the species are quantitative and boil down to percent correlations between the constituent tissues (chlorenchyma, collenchyma, sclerenchyma, xylem, phloem, and parenchyma of the inner cylinder). These correlations are affected by ecological conditions such as the dynamic and static stress to which the stem may be subjected, which in turn depends on the size and number of stem leaves. A high leaf number may thus explain the comparatively higher sclerenchymal index of the *Achillea thracica* stem (Fig. 3: 3). The percent share in this species of conducting tissues, especially xylem (up to 12.1 %, but only 7-8 % in the other species), is also relatively high. The proportion of parenchyma is inverse: up to 52.2 % in *A. thracica,* 61.3 % in *A. coarctata,* and 69.3 %

in *A. clypeolata*. During ontogenesis, the pith parenchyma is often replaced by a parenchymatic medulla.

Laticiferous tubes and secretory canals are considered an important character in the stem anatomy of the *Compositae* (Metcalfe & Chalk 1950). In some cases they are continuous from the cortex or endodermis through the petiole into the leaf blade, which is exactly what can be observed in the studied species. Bearing in mind that the anatomical and morphological traits of secretory canals and the chemistry of secretions are both adaptively important and taxonomically relevant (Seaman 1982), we have included a phytochemical analysis in our investigation.

The studied species synthesize epicuticular flavonoid aglycones. These are mostly derivatives of quercetin, kaempferol, and luteolin. They are flavones and flavonols, methoxy-substituted at C-3 and C-6, as also observed by others (Valant-Vetschera & Wollenweber 1985).

The chemical structure of the identified flavonoids, and their occurrence in the four species studied, are shown in Table 2. In other *Achillea* species, flavonoids are indicative of a xerophytic ecological type, occurring in species growing in moisture-deficient habitats.

Conclusions

The histological analyses reveal different degrees of xeromorphism and meso-xeromorphism in the studied *Achillea* species. In all of them the leaves have a high palisade index, but they differ in features of the epidermis and stomata, with *A. thracica* showing a considerably lower degree of xeromorphism (absence of indumentum, larger stomata and normal epidermal cells, more markedly sinuous anticlinal cell walls) than the other species, especially *A. clypeolata*. The sclerenchymal index is highest in the leaves of *A. coarctata* and stems of *A. thracica*. Altogether, the studied species show both common and differing combinations of xeromorphic histological traits, as well as differences in the degree of their manifestation. The secretory canals found in all species, and the exuded epicuticular flavonoid aglycones, play an important adaptive role. They protect the plants from UV radiation and correlate with their xerophytic nature.

References

Ivančeva, S., Kuzmanov, B. & Gerasimov, L. 1990: Epikutikularni flavonoidni aglikoni v dva vida na rod *Achillea*. – Farmacija (Sofija) **40:** 20-25.

Kuzmanov, B. A. 1991: Evoljucionni tendencii v bălgarskite sloznocvetni rastenija. – Pp. 7-84 in: Kožuharov, S. I. & Kuzmanov, B. A. (ed.), Evoljucija na cvetnite rastenija i florogenezis, **2.** – Sofija.

Metcalfe, C. & Chalk, L. 1950: Anatomy of the dicotyledons. – Oxford.

Seaman, F. C. 1982: Sesquiterpene lactones as taxonomic characters in the *Asteraceae*. – Bot. Rev. (Lancaster) **48:** 121-595.

Valant-Vetschera, K. & Wollenweber, E. 1985: External accumulation of flavonoid aglycones in *Achillea*. – Pp. 1-6 in: Anonymous (ed.) Proceedings of the International Bioflavonoid Symposium – Szeged.

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