Volatiles from Four *Astragalus* Species: Phenological Changes and their Chemotaxonomical Application

Stefan Platikanov[a,*], Stefan Nikolov[b], Dolja Pavlova[c], Ljuba Evstatieva[a], and Simeon Popov[d]

[a] Institute of Botany, Bulgarian Academy of Sciences, 23 Akad. G. Bonchev Str., 1113 Sofia, Bulgaria. Fax: +359 2 71 90 32. E-mail: stefanpl@bio.bas.bg
[b] Faculty of Pharmacy, Medical University, 2 Dunav Str., 1000 Sofia, Bulgaria
[c] Faculty of Biology, University of Sofia, 8 Dr. Tsankov Blvd., 1164 Sofia, Bulgaria
[d] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 9 Akad. G. Bonchev Str., 1113 Sofia, Bulgaria

* Author for correspondence and reprint requests

Z. Naturforsch. **60 c**, 591–599 (2005); received December 3, 2004/January 26, 2005

This paper shows the changes of the volatile compounds from four *Astragalus* species at three phenological stages: leaf development, flowering and fructification, which might be connected with the plant defense. After GC/MS analyses of *Astragalus glycyphyllos* L., *A. hamosus* L., *A. cicer* L. and *A. spruneri* Boiss., different groups of volatile compounds were found: hydrocarbons, alcohols, aldehydes and ketones, esters, terpenes, chlorinated compounds, etc. Identified volatiles were used for a cluster analysis in order to make chemotaxonomic conclusions for these evolutionary different species.

Key words: Volatiles, Phenological Changes, Chemotaxonomy

Introduction

With its over 2000 species, the genus *Astragalus* is one of the largest angiosperm genera spread in the Boreal region, the Mediterranean, Paleo- and Neotropis (Polhill and Raven, 1981). There are 27 species from the genus *Astragalus* growing in Bulgaria (Andreev *et al.*, 1992). They are classified in 8 different subgenera, according to Gontcharov (1946) and Pavlova (1988).

The subgenus *Phaca* Bunge shows most primitive morphological features – big mesophytes with large and loose blossoms, without or less (limited) hairy, soft and skinny legume. We chose for investigation *Astragalus glycyphyllos* L. from this subgenus.

The subgenus *Hypoglottis* Bunge according to Gontcharov (1946) and Pavlova (1988) appears to be an intermediary link between primitive members from the subgenus *Phaca* and evolutionary more advanced xerophytes from other subgenera. For our investigation, we chose *Astragalus cicer* L. from this subgenus. It possesses both primitive and advanced features.

The subgenus *Cercidothrix* Bunge includes both primitive xeromesophytes and typical xerophytes, has relationships with the earliest differentiated group of species united in the subgenus *Phaca* (Gontcharov, 1946). *Astragalus spruneri* Boiss. is a typical xerophyte with more or less hairy, hard legume and is evolutionary the most advanced among the other three species.

Astragalus hamosus L. from the subgenus *Epiglottis* (Bunge) Willk. is an annual species. *A. hamosus* is among the most highly specified species in the genus *Astragalus*.

For our investigation, we chose these four, in Bulgaria widely distributed, *Astragalus* species.

Four types of metabolites were intensively investigated in the *Astragalus* sp.: phenols, saponins, toxic compounds and polysaccharides. The investigations on the flavonoids from the genus *Astragalus* have been summarized by Krasteva and Nikolov (2000). Data on the saponins found in the genus *Astragalus* and their pharmacological effects have been reviewed recently by Nikolov and Benbassat (1997), Verotta and El-Sabakhy (2001). Three types of toxic principles have been identified: indolizidine alkaloids (Gardner *et al.*, 2001), aliphatic nitro compounds (Johnson *et al.*, 2001) and selenium (Pickering *et al.*, 2003). The polysaccharides take a prominent part in pharmacological effects of *Astragalus* (Smee *et al.*, 1996). A review on the pharmacological and toxicological effects of compounds found in *Astragalus* sp. was published by Rios and Waterman (1997).

0939–5075/2005/0700–0591 $ 06.00

While the chemistry and biological activity of the above-mentioned groups of compounds in the *Astragalus* L. species have been studied in depth, volatile compounds from them have not been investigated. The volatile constituents are of interest, because such compounds often possess a valuable biological activity. They are continually emitted into the atmosphere by plants and take part in the plant-insect relationships. In some cases, they are used in taxonomic and evolutionary studies.

Material and Methods

Plant collection

Samples of the investigated species were collected as shown in Table I.

Isolation and identification of the volatile compounds

Fresh aerial parts of the four *Astragalus* species were collected at three phenological phases: at the stage of leaf development, at the stage of flowering and at the stage of fructification. They have been subjected to distillation-extraction in a Licken-Nickersson apparatus for 4 h. The volatiles were extracted with diethyl ether and analyzed by GC/MS with a Hewlett Packard gas chromatograph 6890 equipped with a Hewlett Packard MS 5973 detector (Hewlett Packard, Palo Alto, CA, USA). A HP5-MS capillary column was used (30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Wilmington, Delaware, USA). Helium was used as a carrier gas and the temperature program was 40 °C to 280 °C at 6 °C/min and a 10 min hold. The ion source was set at 250 °C and the ionization voltage was 70 eV. The GC/MS investigation was based on the interpretation of the mass spectral fragmentation followed by comparisons of the spectra obtained with those of authentic samples. Computer searches in the HP Mass Spectral Library NIST98 (Hewlett Packard) were also applied. In the cases when the spectra of some isomers were very similar and these compounds could not be identified unambiguously, comparisons of the GLC retention times, obtained under the same conditions, were used. When there were no suitable authentic samples and spectra for comparison, no identification was made. Only the unambiguously identified compounds are reported in the Tables.

When GC/MS is used, the size of the peaks is proportional to the corresponding ion currents, which depend on the characteristics of the compound (intensity of the mass spectral fragmentation). For this reason, the results obtained are semiquantitative. When we used GC/MS to compare the chemical composition of different organisms, the deviations are identical and quantitative comparisons can be made.

Cluster analysis

Similarity among the four *Astragalus* species was shown by tree clustering (the joining or tree clustering method uses the dissimilarities or distances between objects when forming the clusters). Differences in volatiles composition among species were computed as "percent disagreement" (distance measuring, treating the presence or absence of each compound as a binary character), with a single linkage, to find "nearest neighbors" across clusters to determine the distances between clusters. We used the program STATISTICA 1999 for Windows (StatSoft, Inc.).

Table I. Collection of the plant material. The voucher specimens were deposited at the Herbarium of the University of Sofia (SO).

	Species/voucher			
	A. glycyphyllos SO 102679	*A. hamosus* SO 102680	*A. cicer* SO 102681	*A. spruneri* SO 102682
Phenological stages				
Leaf development	05.2003	04.2003	05.2003	04.2003
Flowering	06.2002	05.2003	06.2002	05.2003
Fructification	09.2001	09.2001	09.2001	09.2001
Locality	Southern part of Sofia region	Experimental field, Institute of Botany in Sofia	Southern part of Sofia region	Central part of the Western Frontier Mountains

Results and Discussion

GC/MS of the volatiles

The composition of the volatiles from the four investigated *Astragalus* species appeared to be very complex. Alcohols, aldehydes, ketones, acids, esters, ethers, hydrocarbons, terpenes, chlorinated compounds were found and identified. The results obtained are summarized in Tables II, III and IV.

In the composition of the volatiles from the four investigated species, significant differences were found. The same appeared to be true for different phenological phases.

Volatiles variability during different phenological phases

Stage of leaf development (Table II)

The main group of the volatiles at this stage is hydrocarbons, which are concentrated mainly in the evolutionary less advanced species.

The alcohols are concentrated mainly in *A. cicer*, followed by *A. spruneri* and *A. glycyphyllos.* In *A. hamosus*, they are absent. More important are hexenols and hexanols. They are attractants for parasitoids and predators on herbivores (Weissbecker *et al.*, 1999) or are repellents for some insects. 1-Octen-3-ol (mushroom alcohol), which is a strong repellent for some insects (Smart and Blight, 2000) was found only in *A. cicer.* Eugenol, found in *A. cicer*, has been reported to possess antifeedant activity (Hummelbruner and Isman, 2001). Ngoh *et al.* (1998) showed eugenol to possess contact toxicity towards the American cockroach.

The terpenoids are present almost entirely in the evolutionary less advanced *A. glycyphyllos.* Only phytol was found in all samples, but its concentration is much higher in evolutionary less advanced *A. glycyphyllos* and in the highly specialized annual species *A. hamosus.* It should be mentioned that phytol appeared in significant concentrations in stages of first vegetation and flowering. Its esters protect leaves from the loss of water and parasites. Hexahydrofarnesyl acetone is also concentrated mainly in these two species. This compound could have some defensive functions against fungi, because similar methyl ketones with long chains possess such activity.

Esters are present in higher concentrations in evolutionary lower *Astragalus* species. Of special interest are acetates, for which is known (Mattiacci *et al.*, 2001) that they act sometimes as attractants of natural antagonist of the herbivores as the parasitoid *Cotesia glomerata.* Similar acetates, according to Filonow (2002), have a mycoactive effect stimulating the adhesion and germination of conidia of the Gray Mold fungus. Analogously to the esters, the free fatty acids are concentrated mainly in the evolutionary lower species.

Aldehydes and ketones are active participants in the plant-insect relationships. We identified such compounds almost entirely in *A. glycyphyllos.* Nonanal has been shown by Huber and Borden (2001) to have disruptive activity to the response of the Douglas-fir beetle, *Dendroctonus pseudotsugae*, which is a destructive pest of *Pseudotsuga menziesii.* Weissbecker *et al.* (1997) showed that nonanal and decanal are emitted from damaged potato plants by the Colorado potato beetle.

It is known that volatile compounds are among the main participants in the plant-insect interactions. From our research, it is evident that the composition of volatiles at the stage of leaf development in *Astragalus* species strongly depends on the evolutionary level of the investigated species and a bigger number of individual volatile compounds are present in the evolutionary less advanced species.

Stage of flowering

As expected, the composition of the volatiles strongly changes (Table III) at the stage of flowering. Again, the main identified volatiles were hydrocarbons. They were totally absent in the evolutionary most advanced *Astragalus* species at the stage of leaf development, but at the flowering significant amounts of normal straight chain saturated hydrocarbons were obtained in all investigated species. Probably at this stage, there are changes in the composition and functions of the waxes, the ethers disappear and hydrocarbons might take their functions.

One of the most important differences now is the strongly reduced number of alcohols, which have important ecological functions in plants as allelochemicals. Now they disappeared, especially hexenols and octenols. Interestingly, now 2,3-butanediol appears. It was reported as a male pheromone of Melanesian beetle (Rochat *et al.*, 2002). This is an indication that at the stage of flowering there are some changes in the plant-insect interac-

Table II. Composition of the volatile substances (% of the total volatiles) at the stage of leaf development.

Compound	*A. glycyphyllos*	*A. hamosus*	*A. cicer*	*A. spruneri*
Alcohols (total)	*3.4*	*0*	*13.4*	*5*
1-Butanol	–	–	2.1	–
2,3-Butanediol	–	–	0.1	–
1,3-Butanediol	–	–	1.8	–
3-Hexen-1-ol	1.1	–	2.6	3.5
2-Hexen-1-ol	–	–	3.0	–
1-Hexanol	0.1		0.1	–
1-Octen-3-ol	0.1	–	3.6	–
3-Ethyl-4-methylpentan-1-ol	0.1	–	–	–
Benzyl alcohol	0.7	–	–	–
Eugenol	–	–	0.1	–
2-Methoxy-4-vinyl phenol	1.3	–	–	1.5
Aldehydes	*0.2*	*0*	*0*	*0*
Nonanal	0.1	–	–	–
Decanal	0.1	–	–	–
Ketones	*0.7*	*0*	*0*	*0*
3-Methyl-2(2-pentenyl)-2-cyclopenten-1-one	0.7	–	–	–
Acids	*1*	*0.3*	*0*	*0*
Nonanoic acid	–	0.1	–	–
Tetradecanoic acid	0.6	0.1	–	–
Pentadecanoic acid	0.3	–	–	–
Hexadecanoic acid	0.1	0.1	–	–
Esters	*3.9*	*2.1*	*0.1*	*0.3*
3-Hexen-1-ol acetate	1.1	–	–	0.1
2,3-Butanediol diacetate	–	–	0.1	–
Hexadecanoic acid methyl ester	0.1	–	–	0.1
(18:1)-Methyl ester	2.7	–	–	0.1
Glycerol tricaprylate	–	2.1	–	–
Ethers	*0*	*0*	*4.3*	*9.4*
2-Ethoxybutane	–	–	4.1	1.6
1-Ethoxybutane	–	–	0.1	–
1,1-Diethoxyethane	–	–	0.1	7.7
Hydrocarbons	*25.9*	*14.9*	*1.2*	*0*
Heptane	–	0.1	–	–
Heptadecane	0.1	–	–	–
Octadecane	0.1	–	–	–
Nonadecane	0.3	0.1	–	–
Eicosane	0.7	–	–	–
Docosane	2.1	–	–	–
Pentacosane	2.9	–	0.1	–
Hexacosane	2.6	2.1	–	–
Heptacosane	2.9	3.9	1.1	–
Octacosane	2.6	2.1	–	–
Nonacosane	3.3	3.2	–	–
Triacontane	1.7	–	–	–
Dotriacontane	1.1	–	–	–
Hentriacontane	1.6	–	–	–
Docosene	–	3.4	–	–
Squalene	3.9	–	–	–
Aromatic hydrocarbons	*0.1*	*0*	*0*	*0*
Phenanthrene	0.1	–	–	–
Terpenes	*12.1*	*10.1*	*0.1*	*0.9*
Linalool	0.3	–	–	–
2-Terpineol	0.1	–	–	–
Geraniol (Nerol)	2.7	–	–	–
Hexahydrofarnesyl acetone	0.5	0.1	–	–
Phytol	8.5	10.0	0.1	0.9
Others	*0.1*	*0*	*0*	*1.4*
Isobutyl-isothiocyanate	–	–	–	1.3
2,3-Dihydro benzofurane	0.1	–	–	0.1

Table III. Composition of the volatile substances (% of the total volatiles) at the stage of flowering.

Compound	*A. glycyphyllos*	*A. hamosus*	*A. cicer*	*A. spruneri*
Alcohols (total)	*0*	*1.5*	*0.7*	*0.1*
1,3-Butanediol	–	1.0	–	–
2,3-Butanediol	–	0.2	–	–
2-Hydroxy-6,10-dimethyl-5,9-undecadien	–	–	0.1	–
2-Methoxy-3-(2-propenyl) phenol	–	–	0.6	–
2-Phenyl phenol	–	0.3	–	0.1
Aldehydes	*0.2*	*0.7*	*0.2*	*0.1*
Hexanal	–	0.2	–	–
Heptanal	–	–	–	0.1
Nonanal	0.1	0.3	0.1	–
Decanal	0.1	0.2	0.1	–
Acids	*0*	*18.1*	*0*	*6.2*
Octanoic acid	–	0.3	–	–
Butanedioic acid	–	0.2	–	–
Nonanoic acid	–	0.3	–	0.1
Decanoic acid	–	0.2	–	0.1
Dodecanoic acid	–	0.8	–	0.1
Tetradecanoic acid	–	–	–	0.1
Hexadecanoic acid	–	16.3	–	5.8
Ethers	*0*	*0.1*	*0*	*0*
2-Ethoxybutane	–	0.1	–	–
Esters	*3.7*	*0.2*	*0.4*	*0*
Hexanedioic acid ethylhexyl diester	3.7	0.1	–	–
Hexadecanoic acid hexadecyl ester	–	0.1	–	–
Isopropyl myristate	–	–	0.2	–
Hexadecanoic acid methyl ester	–	–	0.2	–
Amines	*0.1*	*0*	*0.1*	*0*
N-Butyl-1-butanamine	0.1	–	0.1	–
Amides	*0*	*0.4*	*0.1*	*0*
N,N-Dibutyl-formamide	–	0.4	0.1	–
Halogenated compounds	*0*	*0*	*0.1*	*0.7*
1,3-Dichloro-2-propanone	–	–	–	0.1
1,3-Dichloro-2-propanol	–	–	0.1	0.6
Hydrocarbons	*21.1*	*26.1*	*44.6*	*22*
Heptane	–	0.3	–	
Cyclotetradecane	1.1	–	–	–
Pentadecane	–	0.3	–	0.1
Hexadecane	0.1	–	0.2	–
Heptadecane	–	–	0.2	–
4,11-Dimethyl pentadecane	–	–	–	0.4
Octadecane	1.2	–	0.3	0.1
Nonadecane	1.4	0.1	0.7	0.7
Eicosane	1.3	1.7	0.1	1.4
Heneicosane	3.1	–	2.1	–
2-Methyl eicosane	–	–	–	1.0
Docosane	3.2	–	2.6	–
Tricosane	–	–	5.8	–
Tetracosane	–	1.9	6.0	–
Pentacosane	4.0	4.2	7.3	2.0
Hexacosane	1.9	2.0	5.0	1.6
Heptacosane	3.8	5.3	6.6	5.5
Octacosane	–	2.2	–	1.8
Nonacosane	–	8.0	–	7.2
Triacontane	–	0.1	–	0.1
Hentriacontane	–	–	–	0.1
Dotriacontane	–	–	3.4	–
Squalene	–	–	4.3	–
Terpenes	*26.1*	*6.3*	*3.2*	*1.4*
Linalool	0.1	–	0.1	–
Hexahydrofarnesyl acetone	–	3.2	0.5	1.4
Phytane	–	–	0.3	–
Phytol	26.0	3.1	2.3	–

tions, which lead to changes of the plant allelochemicals – maybe repellents and parasitoid attractants (hexenols and octenols) are partially exchanged by pheromones. This is in agreement with the increased number and concentrations of the aldehydes (hexanal, heptanal, nonanal and decanal), which besides play a defensive role as repellents (Huber and Borden, 2001), also act as chemical signals after mechanical or herbivore damaging (Weissbecker *et al.*, 2000).

The terpenes appeared at the flowering too – phytol, hexahydrofarnesyl acetone, etc. Of interest is the appearance of linalool, which has been shown (Smart and Blight, 2000) to be an attractant for pollen beetles, *Meligethes* spp.

Another change in the metabolism of *Astragalus* species at this stage is the appearance of low concentrations of in plants rarely found chlorinated compounds. These compounds possess some defensive functions in plants.

Stage of fructification

At the last period of plant development, we observed significant changes in the metabolism of the volatile compounds (Table IV). The most important change in the metabolism at the stage of fructification is the biosynthesis of high concentrations of chlorinated metabolites, mainly chlorinated ethanes, but also other chlorinated hydrocarbons, aldehydes and alcohols. Now they are the main components of the volatile fraction. There are limited data on naturally occurring organochlorine compounds in higher plants. Gribble (1998) reported a limited number of higher plants, where chlorinated compounds have been found. Probably, such compounds possess some defensive functions against fungal and bacterial invasion. One rather speculative explanation can be that in autumn, the main defensive compounds in the *Astragalus* species, saponins, move to the roots and the aboveground parts need a new defense strategy. It might be released by biosynthesis of chlorinated metabolites – a fast process, which does not use very much metabolic energy.

The hydrocarbons appeared to be the second main group of volatile compounds at this stage. They showed some decrease of concentrations. Ethoxy ethers practically disappeared and the hydrocarbons, contrary to the leaf development stage, now predominate in the evolutionary more advanced species *A. cicer* and *A. spruneri.* Probably at the first stage of the vegetation, the waxes in the evolutionary most advanced *Astragalus* species contain significant amounts of ethers, instead of hydrocarbons, but when the biosynthesis of the ethers is inhibited at the later phases of development, hydrocarbons instead of ethers become important wax constituents. At the stage of flowering, the concentrations of ethers suffered a sharp decrease and the hydrocarbons dominated in all investigated species. At fructification and second vegetation, the biosynthesis of the ethers was almost totally inhibited and now hydrocarbons were biosynthesized mainly in the evolutionary most advanced *Astragalus* species. We cannot explain the total absence of hydrocarbons in *A. hamosus* – probably the functions of the waxes are taken now by some other metabolites.

Contrary to the flowering stage, there is now some increase of the concentration of alcohols, but they prevail in *A. glycyphyllos* instead of *A. cicer.* Analogously to the stage of leaf development for the plant-insect relationships important benzyl alcohol and 3-hexen-1-ol appeared to have disruptive activity on attraction of the Douglas-fir beetle, pest of *Pseudotsuga menziesii* (Huber and Borden, 2001). Smart and Blight (2000) reported that benzyl alcohol acts as insect attractant.

Instead of the aliphatic aldehydes, characteristic for the first two stages, now we identified benzaldehyde, which might take the functions of aliphatic aldehydes – another important change we observed in the esters. In the evolutionary less advanced *A. glycyphyllos*, we found methyl dihydrojasmonate, which was demonstrated to play a significant role as an essential regulatory compound for the expression of direct and indirect plant defense (Thaler *et al.*, 2002). Also in the ester fraction, there are some esters, which after hydrolysis liberate antibacterial, antifungal and antiviral acids (Filonow, 2002). There is a very strong decrease in the concentrations of the free fatty acids, which is an indication for the changes of the defensive mechanisms at this period.

Terpenes appeared in low concentrations. Surprisingly, now we did not found phytol, which was the main terpenoid in the previous development phases.

Chemotaxonomy

We used the data from the tables for a cluster analysis of the four species with the program STATISTICA 1999 for Windows.

Table IV. Composition of the volatile substances (% of the total volatiles) at the stage of fructification.

Compound	*A. glycyphyllos*	*A. hamosus*	*A. cicer*	*A. spruneri*
Alcohols (total)	*3.3*	*0*	*0.1*	*0.3*
3-Hexene-1-ol	1.5	–	–	0.1
Benzyl alcohol	1.1	–	–	0.1
4-Hydroxy-4-methyl-2-pentanone	0.7	–	0.1	0.1
Aldehydes	*0.1*	*0*	*0*	*0*
Benzaldehyde	0.1	–	–	–
Acids	*0.1*	*0*	*0*	*0*
Palmitic acid	0.1	–	–	–
Esters	*8.8*	*0.8*	*0*	*0.1*
Methyl dihydrojasmonate	0.1	–	–	–
1-Butanol-3-methoxy benzoate	0.1	–	–	–
Butanoic acid ethyl ester	–	0.7	–	–
Isopropyl myristate	–	–	–	0.1
Acetic acid ethylmethyl ester	8.6	0.1	–	–
Ethers	*0.1*	*0*	*0*	*0*
1,1-Diethoxyethane	0.1	–	–	–
Amides	*0.1*	*0*	*0.1*	*0.1*
N,*N*-Dibutyl-formamide	0.1	–	0.1	0.1
Halogenated compounds	*34.2*	*64.4*	*24.7*	*46.5*
2-Bromo-1,1-dichloroethane	0.1	–	–	–
1,1-Diethoxy-2-chloroethane	0.1	1.6	–	0.8
1,1-Dichloropropane	–	3.0	–	–
1,1,1-Trichloropropane	0.1	9.1	–	–
1,1,2,2-Tetrachloroethane	–	2.8	–	–
Pentachloroethane	3.9	12.8	1.8	3.7
Hexachloroethane	29.9	19.8	21.9	41.3
Trichloroethene	–	4.2	–	–
Tetrachloroethene	0.1	6.0	0.1	0.1
1,2,3-Trichloropropene	–	0.5	–	–
1,2,3,3-Tetrachloro-1-propene	–	1.3	–	–
Trichloroacetaldehyde	–	2.2	0.9	0.6
2,2,2-Trichloroethanol	–	1.1	–	–
Aromatic compounds	*2.1*	*0*	*0*	*0*
Benzyl nitrile	2.1	–	–	–
Hydrocarbons	*10.1*	*0*	*21.6*	*10.2*
Heptadecane	0.1	–	0.1	–
Nonadecane	–	–	–	0.1
Eicosane	1.0	–	2.3	0.6
Heneicosane	1.7	–	4.9	2
Tetracosane	1.5	–	1.8	2.7
Pentacosane	1.4	–	2.3	1.8
Hexacosane	0.9	–	1.9	0.9
Heptacosane	2.1	–	4.1	1.3
Octacosane	0.1	–	1.5	0.8
Nonacosane	1.3	–	2.7	–
Terpenes	*0*	*0*	*0*	*0.1*
Hexahydrofarnesyl acetone	–	–	–	0.1

The tree diagram (see Fig. 1) shows obvious differences for the investigated four species and confirms previous separation into four different subgenera. Two species – *A. cicer* and *A. spruneri* – show some integration between them. They are all perennial species with some more evolutionary development features in xeromesophytes and typical xerophytes – smaller-sized plants, more hairy with skiny and soft legume (*A. cicer*), with woody and hard legumes, distributed in dry habitats (*A. spruneri*) – in

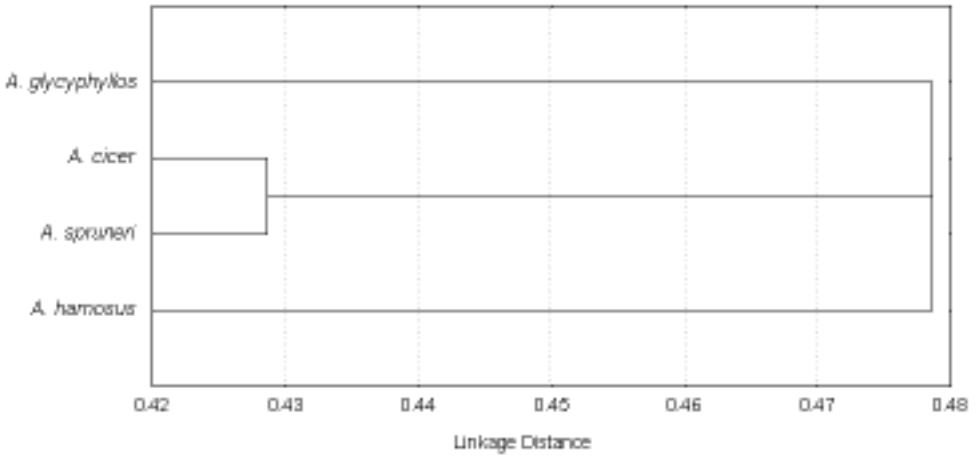

Fig. 1. Cluster diagram of the investigated *Astragalus* species.

contrast to typical primitive mesophytes (*A. glycyphyllos* with its primitive features) – big, glabrous plants, distributed in more or less wet habitats, with lax and big blossoms, skiny and soft legume.

This similarity supports the ideas of Gontcharov (1944) that the evolution in the genus *Astragalus* is of mesophyte type toward xerophytes (*A. glycyphyllos* toward *A. spruneri*). We can relate *A. cicer* to xero-mesophytes because of its macromorphological features (combining both primitive and evolutionary advanced) and preferable habitats (more dry and sunny places).

Annual species are highly specialized in the evolution of the genus *Astragalus. A. hamosus* is separated from the others because of the absence mainly of saturated hydrocarbons during the phase of fructification. It shows high specialization about chlorine compounds in this period. These examples probably are correlated with its annual biological type.

Acknowledgement

The authors are grateful to S. Veleva and D. Antonova for the technical assistance.

Andreev N., Anchev M., Kozucharov S., Markova M., Peev D., and Petrova A. (1992), Field Guide of the Bulgarian Vascular Plants. Nauka I Izkustvo, Sofia, pp. 392–397.

Filonow A. B. (2002), Mycoactive acetate esters from apple fruit stimulate adhesion and germination of conidia of the Gray Mold fungus. J. Agric. Food Chem. **50**, 3137–3142.

Gardner D. R., Molyneux R. J., and Ralphs M. H. (2001), Analysis of swaisonine: extraction methods, detection and measurement in populations of locoweeds (*Oxytropis* spp.). J. Agric. Food Chem. **49**, 4573–4580.

Gontcharov N. (1944), Astragali SSSR. Sovetskaya botanika **6**, 56–62 (in Russian).

Gontcharov N. (1946), Genus *Astragalus* L. In: Flora USSR 12 (Komarov V. L., ed.). Izd. Akad. Nauk SSSR, Moscow & Leningrad, pp. 1–890 (in Russian).

Gribble G. (1998), Naturally occurring organohalogen compounds. Acc. Chem. Res. **31**, 141–152.

Huber D. P. and Borden J. H. (2001), Angiosperm bark volatiles disrupt response of Douglas-fir beetle, *Dendroctonus pseudotsugae*, to attractant-baited traps. J. Chem. Ecol. **27**, 217–233.

Hummelbruner L. A. and Isman M. B. (2001), Acute, subletal, antifeedant and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). J. Agric. Food Chem. **49**, 715–720.

Johnson D. L., Majak W., and Benn M. H. (2001), Excretion of miserotoxin and detoxification of the aglycone by grasshoppers (Orthoptera: Acrididae). Phytochemistry **58**, 739–742.

Krasteva I. and Nikolov S. (2000), Flavonoids from genus *Astragalus* L. Pharmacia **36**, 20–25.

Mattiacci L., Rocca B. A., Scascighini N., D'Alessandro M., Hern A., and Dorn S. (2001), Systemically induced plant volatiles emitted at the time of "Danger". J. Chem. Ecol. **27**, 2233–2252.

Ngoh S. P., Hoo L., Pang F. Y., Huang Y., Kini M. R., and Ho S. H. (1998), Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplanata americana* (L.). Pestic. Sci. **54**, 261–268.

Nikolov S. and Benbassat N. (1997), Triterpenoid saponins and sapogenins from genus *Astragalus* L. Pharmacia **34**, 34–48.
Pavlova D. (1988), Taxonomical Investigation of Representatives of g. *Astragalus* L. in Bulgaria. D. Phil. Thesis, Sofia University (in Bulgarian).
Pickering I. J., Wright C., Bubner B., Ellis D., Persans M. W., Yu E. Y., George G. N., Prince R. C., and Salt D. E. (2003), Chemical form and distribution of selenium and sulfur in the selenium hyperaccumulator *Astragalus bisulcatus*. Plant Physiol. **131**, 1460–1467.
Polhill R. and Raven P. (1981), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew, pp. 357–367.
Rios L. J. and Waterman P. G. (1997), A review of the pharmacology and toxicology of *Astragalus*. Phytoter. Res. **11**, 411–418.
Rochat D., Morin J. P., Kakul T., Beaudoin-Ollivier L., Prior R., Renou M., Malosse I., Stathers T., Embupa S., and Laup S. (2002), Activity of male pheromone of Melanesian rhinoceros beetle *Scapanes australis*. J. Chem. Ecol. **28**, 479–500.
Smart L. E. and Blight M. M. (2000), Response of the pollen beetle, *Meligethes aeneus*, to traps baited with volatiles from oilseed rape, *Brassica napus*. J. Chem. Ecol. **26**, 1051–1064.
Smee D. F., Sidwell R. W., Huffman J. H., Huggins J. W., Kende M., and Verbiscar A. J. (1996), Antiviral activities of tragacanthin polysaccharides on Punta Toro virus infections in mice. Chemotherapy **42**, 286–293.
Thaler J. S., Farag M. A., Paré P. W., and Dicke M. (2002), Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. Ecol. Lett. **5**, 764.
Verotta L. and El-Sebakhy N. A. (2001), Cycloartane and oleanane saponins from *Astragalus* sp. In: Studies in Natural Products Chemistry, Vol. 25 (Atta-ur-Rahman, ed.). Elsevier Science Publishers, Amsterdam, pp. 179–234.
Weissbecker B., Schutz S., Klein A., and Hummel H. E. (1997), Analysis of volatiles emitted by potato plants by means of a Colorado beetle electroantennographic detector. Talanta **44**, 2217–2224.
Weissbecker B., Van Loon J. J., and Dicke M. (1999), Electroantennogram responses of a predator, *Perillus bioculatus*, and its prey, *Leptinotarsa decemlineata*, to plant volatiles. J. Chem. Ecol. **25**, 2313–2325.
Weissbecker B., Van Loon J. J., Posthumus M. A., Bouwmeester H. J., and Dicke M. (2000), Identification of volatile potato sesquiterpenoids and their olfactory detection by the two-spotted stinkbug, *Perillus bioculatus*. J. Chem. Ecol. **26**, 1433–1445.