Pollen analysis of honey from the region of three villages in West Bulgaria

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Abstract The results of pollen analysis of honey samples collected in two successive years (2001–2002) from the region of three villages in West Bulgaria (Novi Han, Pobit Kamuk and Vakarel) are discussed. The paper provides information on the wild and cultivated nectariferous plants that are important for the bees in this region.

Key words: honey, nectariferous plants, pollen analysis

Introduction

The aim of this study is to establish the botanical origin of honey in the region of three villages in West Bulgaria: Novi Han, Pobit Kamuk and Vakarel and to obtain information on the nectariferous plants important for the bees. Pollen analyses were carried out in two successive years (2001–2002) and the main plants visited by honey bees were identified. Pollen analysis of honey samples is the best method to determine the plants used by bees as a source of nectar and pollen (Louveaux & al. 1978; Ricciardelli D'Albore 1998).

There have been few pollen analyses of honey from different regions of Bulgaria: Bozilova & Anchev (1969), Bozilova & Chan (1976), Petkova (1984), Lazarova & Bozilova (2001, 2002), Atanassova & Kondova (2004).

Material and methods

In the present work we studied 20 beehives from three villages. Twenty samples of honey were collected during the spring and summer of 2001 (14 samples from Novi Han, three from Pobit Kamuk and three from Vakarel village). In 2002, another 20 samples of honey were collected from the same hives, or 40 samples of honey have been analysed palynologicaly for the two years.

The samples of honey for pollen analysis were prepared following the methods for non-acetolysed honeys recommended by the International Commission for Bee Botany (Louveaux & al. 1978) and according to the Bulgarian State Standard (BSS) (1990). The frequency of each pollen type in the samples is expressed as percentage of the total pollen sum (P), which includes pollen grains only from entomophilous plants (Louveaux & al. 1978). The frequency of pollen grains of anemophilous plants is calculated on the basis of P (Tables 1, 2, 3, 4). About 500 pollen grains were counted in each sample. Pollen grains of the melliferous flora in the vicinity of the apiaries were used as reference material.

Identification of the plant species collected in the investigated region has followed Jordanov (1963–1979), Velchev (1982-1989), Kozhuharov (1992, 1995).

Study area

The three investigated locations (Novi Han, Pobit Kamuk and Vakarel villages) are similar with regard to their topography, type of vegetation and type of crops. The beehives in Novi Han and Pobit Kamuk

villages are located at the foothills of Mt Lozenska, in the vicinity of a cultivated area and xerothermic herb communities. The beehives in Vakarel are located at the foothills of the Ihtiman divide of Mt Sredna Gora, in the vicinity of a cultivated area and some wastelands (Fig. 1).

Fig. 1. Area of investigation

The climate in the region under study is mildly continental, with warm summer and cold winter (Velev 2002).

Mt Lozenska is dominated by oak and hornbeam forests (Bondev 2002). Distributed are communities of *Quercus dalechampi* , with single trees of *Fraxinus ornus*, *Q. cerris* , *Crataegus monogyna*, *Cornus mas*, and *Corylus avellana*, as well as forests of *Q. frainetto* with *Q. cerris*, *Q. virgiliana*, *Q. pedunculiflora*, *F. ornus*, *Acer tataricum*, and *A. campestre*. In the surroundings of Novi Han xerothermic herb communities are widespread, with *Dichantium ischaemum*, *Poa bulbosa* and *Chrysopogon gryllus*. There are also meadows dominated by *Festuca pratensis*, *P. sylvicola*, *Alopecurus pratensis*, and *Lolium perenne* (Bondev 1991).

Betula pendula, *Salix alba*, *S. carpea*, *F. ornus*, *Populus nigra, P. tremula*, *Tilia tomentosa, T. cordata*, and *Prunus cerasifera* were distributed in an area of 3–4 km around the beehives in Novi Han. Near the hives, we collected and identified many herb species usually visited by bees:

1. On moister places and medows – *Trifolium campestre*, *T. pratense*, *T. hybridum* , *T. resupinatum*, *T. repens*, *Vicia varia*, *V. grandiflora*, *V. cracca*, *Lathyrus pratensis*, *Melilotus alba*, *M. officinalis*, *Medicago minima*, *Lotus corniculatus*, *Onobrychis gracilis*, *Ononis arvensis*, *Mentha longifolia*, *Glechoma hederacea*, *Ranunculus repens*, *R. acris, Ficaria verna*, *Rorippa prolifera, Rapistrum rugosum, Conium maculatum*, *Solanum dulcamara*, *Bellis perennis*, *Taraxacum officinale*, *Leucanthemum vulgare*, *Ornithogalum umbellatum*, *Saxifraga bulbifera*, *Linaria vulgaris, Rhinanthus minor*, *Galium verum*, and *Malva neglecta*.

2. On dry and stony places, and along the roads: *T. arvense*, *Euphorbia cyparissias*, *Linum austriacum*, *Salvia pratensis*, *Erodium cicutarium*, *Capsella bursa–pastoris, Tragopogon dubius, T. orientalis*, *Erophila verna, Brassica elongata*, *Chamomilla recutita*, *Achillea millefolium*, *A. clypeolata*, *Silene vulgaris*, and *Potentilla argentea*.

3. In open forests and bushes in the mountains we have found *Scilla bifolia*, *Muscari botryoides*, *Allium ursinum*, *Hypericum perforatum*, *Fragaria vesca*, *Potentilla micrantha*, *Genista tinctoria*, *Lathyrus vernus*, *Helleborus odorus*, *Anemone ranunculoides*, *A. nemorosa*, *Clematis vitalba*, *Pulmonaria officinalis*, *Chelidonium majus*, *Campanula sparsa*, *Mellisa officinalis*, *Alliaria petiolata*, and *Veronica chamaedrys*.

4. In the cultivated or inhabited lands and along the roads – *Centaurea cyanus*, *Consolida regalis, Anthemis arvensis*, *Papaver rhoeas*, *Convolvulus arvensis*, *Ranunculus arvensis*, *Cichorium inthybus*, *Stellaria media, Lamium purpureum*, *Solanum nigrum*, *Datura stramonium, Cardaria draba,* and other species. The major cultivated plants in the area were *Triticum, Hordeum, Zea, Brassica rapa* var. *oleifera,* and *Helianthus annuus.* There were also gardens dominated by trees of family *Rosaceae.*

According to Bondev (1991), xeromesophytic forests dominate the Ihtiman divide of Mt Sredna Gora, composed of *Carpinus betulus*, *Q. dalechampii*, *Q. frainetto*, and occasionally of *Q. cerris*. Communities of *Fagus sylvatica* are also distributed in humid places, usually on the northern slopes. Secondary xerothermic forests of *C. orientalis* have replaced the destroyed oak forests. Herb communities around Vakarel village resembled the herb communities of Novi Han village.

Results

1. Pollen analysis of the honey samples from Novi Han village

Twenty-eight honey samples collected in two successive years (2001–2002) from 14 beehives have been investigated. All samples were of thick liquid honey with delicious aroma. The honey collected in 2001 (samples 1A to 14A) was light-yellow to yellow-green in colour. The samples collected in 2002 (1B to 14B) were dark-yellow, orange or brownish.

Palynological results have shown dominance of pollen types of family *Fabaceae* in all samples collected in 2001. Dominance of the *Lotus*-type (24.2–48.2 %) and high percentages of other *Fabaceae* pollen types in the pollen spectra have been established in samples 1A, 6A, 7A**,** 9A, 10A, 11A, 13A, and 14A. Samples 2A, 8A and 12A were dominated by pollen of *Vicia*-type (26–39.6 %) and in samples 3A, 4A and 5A *Lathyrus*-type prevailed (17.7–44.2 %). The important secondary pollen types in the pollen spectra were *C. cyanus, Mentha*-type, *Brassicaceae*, and *Apiaceae* (Tables 1, 2). According to BSS (1990), only sample 10A was of unifloral honey. All 14 samples (Tables 3, 4) collected in 2002 were dominated by pollen of *Brassica*-type (20.2–39,8 %). The most important secondary pollen types were *C. cyanus, Taraxacum*-type, *Cirsium/Carduus-type,* and *Menhta*-type. All samples were of polyfloral honey.

Table1. Pollen content (in %) of the honey samples from Novi Han village (1A to 10A) (2001)

Sample N / Pollen taxa	1A %	2A %	3A %	4A %	5A %	6A %	7A %	8A %	9A %	10A %
Lotus type	29.6	18.1	8.6	15.0	2.1	31.9	32.8	8.4	25.5	48.2
Lathyrus type	13.9	0.4	37.8	17.7	44.2	0.9		9.8		
Vicia type		26.0	0.1	16.8		5.3	4.0	28.5	15.6	0.5
Trifolium type				0.7	5.3	7.9		11.6	6.3	0.7
Centaurea cyanus	7.1	11.4	8.0	4.8	5.2	17.2	11.6	15.6	5.4	2.5
Brassicaceae	6.9	4.4	10.0	4.6	4.2	2.0	10.2	5.5	20.7	3.5
Apiaceae	11.1	12.4	6.1	7.6	4.8	3.7	9.4	4.5	11.7	16.2
Mentha type	7.7	1.6	9.3		13.2		4.4	3.4	1.9	10.0
Rosaceae	5.4	0.6	7.0	7.2	8.7	3.5	0.2	0.5	1.6	0.7
Fabaceae	10.0	3.3	2.3	2.4	0.3	5.2				
Taraxacum type	3.3	2.4		2.2	3.3	0.1	1.8			1.1
Cirsium/Carduus type		1.8	2.8			5.0			2.7	
Filipendula							10.2	2.5	0.9	4.9
Ranunculaceae	0.3			1.5				2.8	4.3	3.3
Achillea type	0.7			3.2		0.1	9.0			0.1
Crataegus type				1.3				3.0		2.1
Helianthus annuus				5.3	0.5					
Erodium type	0.3			1.0			0.2			0.5
Tilia			1.9			2.4	0.2			0.1
Geum type			0.7		2.1	4.4		0.1		
Centaurea jacea type						4.0				
Scrophulariaceae					0.3			1.7		
Viola			0.3					0.1	0.1	
Campanula						0.3	2.0			
Lamiaceae		0.3			0.1				1.6	
Rumex				0.1					0.1	0.5
Salix			0.5		1.3		2.0			
Linum	0.1						0.2			0.1
Total pollen sum (P)	530	541	557	576	513	538	500	557	555	510
Pinus	0.1			0.3			1.0			1.5
Betula				0.1	0.1			0.1		
Plantago	0.9	0.3	0.8		2.3	3.3				0.7
Poaceae		0.1	0.1			0.5	0.2		0.1	
Chenopodiaceae							0.2		0.1	
Varia	1.0	1.2		0.5		1.3		0.8	0.7	1.5

2. Pollen analysis of the honey samples from Pobit Kamuk village

All samples were of thick liquid honey with delicious aroma. The colour of the honey collected in 2001 (samples 1′A, 2′A and 3′A) was light-yellow to dark-orange and in 2002 (samples 1′B, 2′B and 3′B) dark-orange.

Palynological results have shown dominance of the *Mentha*-type, (23.1 %), *C. cyanus* (16.9 %) and *Lotus*-type (16 %) in the pollen spectra of the samples collected in 2001 (Table 2). *C. cyanus* (15.5–18.5 %) predominated in all samples collected in 2002 (Table 4). All samples were of polyfloral honey.

3. Pollen analysis of the honey samples from Vakarel village

All samples were of thick liquid honey with delicious aroma. The colour of the honey collected in 2001 (samples 1″A, 2″A and 3″A) was light-yellow and in 2002 (samples 1″B, 2″B and 3″B) dark-yellow to orange.

The pollen analysis established dominance of the *Vicia*-type (29.3 %), *Mentha*-type (18.9) and *Lathyrus*-type (21.4 %) in the samples collected in 2001. Important secondary pollen types in the pollen spectra were *C. cyanus* and *Brassicaaceae* (Table 2). *C. cyanus* and *Mentha*-type predominated in the samples collected in 2002 (Table 4). Important secondary pollen types in the pollen spectra were *Rosaceae* and *Apiaceae*. All samples were of polyfloral honey.

Conclusions

The pollen spectra of 40 investigated honey samples from 20 beehives in the villages of Novi Han, Pobit Kamuk and Vakarel in two consecutive years (2001–2002) have led us to some conclusions about the local flora visited by honey bees for nectar and pollen grains. Thirty-seven pollen taxa have been established in the honey samples, 31 of which were important for the honey bees.

Table 2. Pollen content (in %) of the honey samples from Novi Han (11A to 14A), Pobit Kamuk (1′A, 2′A and 3′A) and Vakarel villages (1″A, 2″A and 3″A) (2001)

Sample N / Pollen taxa	11A %	12A %	13A %	14A %	1′A %	2′A %	3′A %	1″A %	2″A %	3″A %
Lotus type	30.8	8.6	33.9	24.2	20.5	11.0	16.0			
Lathyrus type	10.8	25.3	13.9	2.2		12.5	2.5	9.6	6.8	21.4
Vicia type		39.6		2.2			2.3	29.3	14.9	7.6
Trifolium type			3.7	0.3				1.3		0.2
Centaurea cyanus	5.8	1.3	5.6	16.1	6.9	16.9	15.2	10.7	14.1	18.8
Brassicaceae	6.3	2.8	7.0	12.8	9.7	6.0	8.2	14.0	5.5	10.6
Apiaceae	3.0	1.1	6.5	8.6	12.8	9.0	10.7		8.7	4.8
Mentha type	12.0	0.3	4.9	11.8	23.1	14.4	11.9	3.8	18.9	
Rosaceae	1.7	4.7					5.4	7.0	3.5	6.0
Fabaceae		1.1	5.8		2.0	3.0	0.9	1.0	1.7	1.2
Taraxacum type	0.6		0.1	1.3	2.3				1.1	0.2
Cirsium/Carduus type		5.2		3.2		1.8			3.7	
Filipendula	7.0		7.6	9.6		7.5	0.5	0.8	5.3	8.4
Ranunculaceae				0.1			4.1		3.3	
Achillea type			5.1		6.2		7.2	3.1	7.1	8.2
Crataegus type				1.5		7.8	4.8	0.1		6.2
Helianthus annuus	3.0		1.7	2.5						
Erodium type	0.3		0.3		1.2	3.1	3.1	0.1	2.3	0.4
Tilia	1.5		0.3	0.8						
Geum type					2.1	0.5	0.1			
Centaurea jacea type			0.3			0.3			0.1	
Scrophulariaceae		6.6			2.2			0.1		
Viola	0.1	0.1		0.1	2.2	2.6	2.5	0.1		0.4
Campanula			0.1		3.4			6.3		
Lamiaceae	14.0				1.1					0.4
Rumex					0.3					0.2
Salix		0.1	0.1			0.3	0.3	0.1		
Linum			0.1		0.1					
Pollen sum (P)	583	529	565	590	545	532	512	596	501	500
Pinus		1.1			0.1				0.3	0.2
Betula				0.1	0.1	0.7				0.2
Plantago			1.2		2.0	0.5		1.6		2.2
Poaceae							1.5	0.5		0.6
Urtica	1.0				1.4			0.3	0.1	
Chenopodiaceae									0.3	
Varia	1.3	1.3	1.2	1.8	1.4	1.1	1.9	1.1	1.3	1.8

The results for 2001 have shown as most important nectariferous plants for the three villages the representatives of family *Fabaceae (Lotus, Vicia, Lathyrus, Trifolium,* etc.), *Lamiaceae* (*Mentha, Salvia, Thymus,* etc.), *Brassicaceae*, and *Apiaceae*, distributed mainly in the meadows and pastures. Honey samples from different regions in Bulgaria (Haskovo, Svilengrad, Aitos, Smolyan) also showed dominance of the pollen taxa of family *Fabaceae* (Lazarova & Bozilova 2002). Many species of family *Fabaceae (Trifolium, Medicago, Melilotus,* etc.) have a secondary period of flowering in late autumn or in summer, which is very important for supplying the honey bees with nectar and pollen.

Some honey samples were dominated by the pollen grains of *C. cyanus,* while in others this was important secondary pollen. We have found out that *C. cyanus,* which is widely distributed in wastelands, offers essential bee forage for this region, especially in the dry summer periods when Bluebottle produces high amounts of nectar (Bizev & al. 2003).

The results of Pobit Kamuk and Vakarel villages for 2001 and 2002 were very similar. *C. cyanus* has played a characteristically important role in the honey samples from Pobit Kamuk in 2002.

The honey samples from Novi Han village had different pollen spectra in the two investigated years. In 2002 they were dominated by pollen of the *Brassica*-type. *B. rapa* var. *oleifera* was planted near the beehives and it is a very good nectariferous plant, often visited by honey bees for nectar (Petkov 1973; Bizev & al. 2003). Pollen grains of *H. annuus* were common in the honey samples, especially of 2002, because of the widely cultivated sunflower in the area. This species is also a very good source of nectar and pollen for the bees (Petkov 1973)

While interpreting the results of the pollen analysis of honey samples, one should bear in mind that some of the large pollen grains (such as of *Tilia, Erodium, Geranium, Cirsium,* and *Carduus*) could be lost in the sieving process, contrary to the small-size pollen grains (Snodgrass & Erickson 1992; Ricciardelli D'Albore 1998), such as those of the *Lotus* type in our case. Furthermore, flower morphology and the low pollen productivity of nectariferous plants may affect the quantitative participation of pollen types in honey.

Pollen grains of the anemophilous plants of *Pinaceae, Betulaceae, Poaceae,* etc. were very few in the pollen spectra.

Table 3. Pollen content (in %) of the honey samples from Novi Han village (1B to 10B), (2002)

Pollen taxa \ Sample N	1B %	2B %	3B %	4B %	5B %	6B %	7B %	8B %	9B %	10B %
Brassica type	28.3	39.8	20.2	32.5	36.7	28.9	39.0	34.5	35.0	28.2
Centaurea cyanus	11.6	13.4	19.2	10.2	15.1	11.9	10.0	14.6	5.8	9.0
Cirsium/Carduus type	9.5	6.9	13.0	8.3	3.8	13.4	3.1	0.9	2.7	6.4
Rosaceae	9.3	14.6	3.1	5.4	7.3	2.8	4.2	6.0	7.1	11.6
Mentha type	2.8	4.3	9.3	7.3	9.4	6.4	8.3	9.7	12.8	12.5
Apiaceae	7.4	5.0	9.5	6.8	8.8	10.4	4.2	3.5	7.1	5.8
Taraxacum type	12.5	3.5	5.6	2.4	3.3	1.3	9.1	12.1	6.9	1.5
Helianthus annuus	5.8	2.9	4.8	6.6	2.2	7.9		2.5	1.6	2.9
Filipendula	3.1	0.9	3.6	4.3	1.1	3.0	2.1	3.5	5.1	6.0
Ranunculaceae	3.7	0.3		0.9	2.4	1.7		0.3	5.3	1.9
Achillea type		0.7	2.3	3.9			0.1		2.9	1.5
Tilia	2.1			3.5	4.9	8.5	9.9		1.8	0.7
Fabaceae	1.4	3.1		3.0	2.0			6.6		
Geranium type		0.3	2.9		0.1			1.1		
Lotus type				0.1					0.1	7.4
Lathyrus type				0.3		0.1				
Vicia type			0.1		0.1					1.3
Trifolium type			2.1	0.1			6.2	0.3		0.1
Viola		1.3		0.3		0.1		0.5		
Euphorbia			0.3		0.1				0.1	
Campanula			0.1		0.1			0.1	1.2	
Salix	0.5			0.3		0.5		0.1	0.3	
Linum			0.1				0.3		0.1	
Convolvulus				0.1			0.5	0.1		
Pollen sum (P)	565	534	514	528	541	528	515	513	545	510
Pinus		0.3		0.5			0.1			
Betula				0.3		0.1	0.7	0.7		
Plantago	0.1	0.5	1.1		0.5	0.3		0.1	2.2	0.7
Poaceae		0.1		0.5		0.1	0.1			
Varia	1.2	1.1	1.7	0.9	1.1	1.7	1.9	1.5	0.9	1.3

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Table 4. Pollen content (in %) of the honey samples from Novi Han (11B to 14B), Pobit Kamuk (1′B, 2′B and 3′B) and Vakarel villages (1″B, 2″B and 3″B) (2002)

Sample N / Pollen taxa	11B %	12B %	13B %	14B %	1′B %	2′B %	3′B %	1″B %	2″B %	3″B %
Brassica type	34.9	37.3	35.8	25.8	10.1	9.6	6.5	3.2	5.9	3.7
Centaurea cyanus	14.4	14.1	14.5	12.4	18.5	17.3	15.5	15.6	13.0	12.7
Cirsium/Carduus type	8.7	8.6	6.2	5.4	7.8	7.0	9.6	7.9	9.1	7.0
Rosaceae	7.8	2.5	3.5	4.7	14.0	16.1	11.0	13.0	10.2	15.4
Mentha type	7.8	10.4	7.5	9.0	17.1	12.5	15.7	14.0	15.3	15.6
Apiaceae	6.8	10.6	2.5	11.9	8.5	8.6	6.7	13.4	8.1	11.3
Taraxacum type	0.3		6.2	5.8	1.7	0.2	0.6	1.2	0.6	1.3
Helianthus annuus	2.7	2.5	3.0		1.9	1.8	3.0	1.6	1.6	3.1
Filipendula	2.0	1.9	4.4	6.0	2.5	3.2	5.9	4.8	6.5	4.5
Ranunculacee	0.9	3.9	0.5	1.5	2.5	3.6	5.3	5.4	2.2	3.7
Achillea type	3.3	0.1	0.8	2.6	5.4	7.2	4.7	7.9	8.3	9.5
Tilia		4.7	3.0	4.7		0.2		0.4	1.4	
Fabaceae	3.3		2.1	3.7	1.9	2.8	3.6	4.6	5.1	4.8
Erodium type	0.1			0.3	3.7	1.2	2.6	2.4	2.0	2.5
Lotus type	2.4				0.1			0.2		
Lathyrus type			2.3			4.2		0.4		
Vicia type	0.3	1.1	4.4				4.3		5.9	0.3
Trifolium type			0.1			0.2				0.7
Viola	0.5	0.1	0.3		0.1			0.4	2.0	
Euphorbia			0.1	0.3				0.2		
Campanula					0.3			0.1		
Salix				0.3	1.1		1.0	0.1		0.9
Linum		0.3		0.5				0.2		
Convolvulus	0.1		0.1			0.2				
Pollen sum (P)	538	509	558	529	512	496	488	492	490	511
Pinus		0.1	0.1	0.1		0.2			0.2	
Betula		0.1		0.3	0.1		0.4			
Plantago	1.1			1.7	0.3	1.4	1.6	0.8	1.0	0.5
Poaceae	0.5			0.1			0.2			0.1
Varia	1.1	0.7	1.4	1.7	1.1	1.8	1.0	1.8	1.0	1.3

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