DETECTION OF FLORA SPECTRUM THROUGH HONEY MICROSCOPIC ANALYSIS

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Summary

The floral origin of honey is an important factor in honey quality control. Analysis of pollen and honeydew elements in 180 Greek honey samples from different areas of Greece was carried using standard methods of melissopalynology, during two years. Pollen spectra gave consistent results for the same type of honey from different areas. In six unifloral honeys (orange, sunflower, cotton, thyme, fir and pine) 28 pollen types were identified. Individual honeys contain 15-23 pollen types. Effort was made to detect characteristic combination of pollen grains in every group. Cotton and orange honeys are very poor in cotton and orange type pollen, while predominant pollen was found in sunflower and thyme honeys. Fir and pine honeys show a variety of pollen types but none of them is predominant or secondary. Characteristic spectra of seconday pollens were found for some types of honey, and these spectra may be usefl in distinguishing Greek honeys from those produced elsewhere.

Keywords: honey/pollen analysis/Greece

Introduction

Every natural honey contains microscopic particles which give indications for its geographical and botanical origin.

When a bee visits a flower, according to the structure of the flower, she comes into more or less contact with the anthers. Some of the ripe pollen falls into the nectar, sucked up with it into the bee's honey sac and finally can be found in the extracted honey. Apart from the bee, wind and beekeeper can contribute to the transfer of pollen in the honey.

As well as the nectar in honey is characterised by the pollen grains, so honeydew as source of honey, is characterised by algae, fungal spores and moulds. These microscopical particles can be found on the leaves and needles of conifers or can get in the honeydew by rain or wind. The vast majority of them are osmophilous species, although have been identified other categories of micro-organisms (MAURIZIO, 1959). Algae, spores and mycelia are especially important in the honey sediment as „honeydew indicators".

The melissopalynological analysis of honey has proved to be extremely helpfl in combination with other analytical criteria and organoleptical characteristics. It is used in order to identify the geographical origin of the honey, to detect mixtures of honeys from different locations and countries and to give information about the climatic, geographical, botanical and agricultural condition of an area.

Long term observations of flora in a particular area, can reveal changes in flora and also to trace rare plants. However there are studies which appear to classify the geographical origin of the honey with the use of mineral content (FELLER-DEMALSY, 1990/1991), the chemical composition (TAN et al., 1989; SANCHO et al., 1991; SANZ et al., 1995), the identification of flavonoids (SABATIER et al., 1992; TOMAS-BARBERAN et al., 1993; VIT and TOMAS-BARBERAN, 1998), and the aromatic compounds (AGUAR et al., 1991). The content of flavonoids is also used for the determination of the botanical origin of the honey (FERRERES et al., 1994; SOLER et al., 1995; ANDRADE et al., 1997).

The knowledge of the botanical origin of honey is essential for economical reasons too, as it can affect the price of the product in the market.

There are still some problems in order to determine the "correct" botanical and geographical origin of a honey. In recent years honey plants typical of certain countries or regions are now grown in many other different areas or countries. For example eucalyptus species which are endemic of Australia and New Zealand are used the last years in Greece as boulevard trees and eucalyptus type pollen grains are found in Greek honeys. Furthermore, there are several plants poor or rich in pollen grains, and it is impossible to determine from pollen analysis their contribution to the honey. In addition, there are many plants which have not yet investigated their production of pollen. Pollen analysis still can not devine which quantity of honey is represented by a certain number of pollen grains.

The microscopical characteristics of Greek honey have been partly examined by THRASYVOULOU and MANIKIS (1995).

This study describes the variability of microscopical characteristics of six types of monofloral Greek honey and tries to detect pollen combinations characteristics of certain locations in Greece.

Materials and Methods

Sampling

A number of 180 honey samples were collected directly from beekeepers from all over the country among 1996 and 1998. They were stored at laboratory temperature (as an average, 24°C per year) and analysed immediately. The samples were classified according to their organoleptic characteristics and the electrical conductivity. Samples that were doubtful in botanical origin were excluded from the study and we present the results in table I.

Table I

Pollen analysis of blossom and honeydew honeys

Type of pollen	Orange n=17	Helianthus n=17	Cotton n=11	Thymus n=86	Fir n=11	Pine n=39
Apiaceae	-	M(4), IM(7), S(2)	M(1), IM(5), S(1), P(1)	M(9), IM(4)	M(3)	M(4), IM(3), S(1)
Asteraceae	M(10), IM (1)	S(9), P(7)*	M(2), IM(3), S(1)	M(31), IM(16)	M(4)	M(15), IM(5)
Boraginaceae	M(6)	IM(2)	M(1), IM(1)	M(14), IM(23), S(5)	M(1),IM(1)	M(5), IM(2)
Brassicaceae	IM(1), P(16)	M(4), IM(11)	M(1), IM(3), S(3), P(1)	M(7), IM(33), S(38), P(4)	IM(6), S(5)	M(13), IM(9), S(7)
Castanea sativa	S(1)	M(1), IM(1), S(2)	M(1), IM(1), S(2)	M(1), IM(1)	M(2), IM(1), S(1), P(1)	M(2), IM(6), S(4)
Centaurio spp	-	M(1), IM(1)	-	M(1)	-	M(1), IM(1)
Ceratonia siliqua	-	-	-	-	-	M(2), IM(1), S(1)
Citrus spp	M(5)	-	-	M(6), IM(1)	-	M(1)
Erica spp	M(8)	-	M(3), S(1)	M(11), IM(8)	M(4), IM(3)	M(13), IM(4), S(3)
Eucalyptus spp	M(1), S(1), P(1)	-	M(1), IM(1)	M(10), IM(20), S(7), P(2)	M(1), IM(1)	M(2), IM(4), S(2)
Evenus cretica	-	-	-	IM(1)	-	-
Gossypium hirsutum	-	-	M(4), IM(4)	-	-	-
Hedera helix	-	-	-	-	-	M(2)
Hypericaceae	M(1)	IM(3)	IM(1), S(1)	M(26), IM(24)	M(1), IM(1)	M(2), IM(1)
Iridaceae	-	-	-	M(3)	-	M(1)
Lamiaceae	M(2)	M(4), IM(2)	M(3), IM(2)	S(41), P(45)	M(2), IM(1)	M(8), IM(9), S(1)
Liliaceae	M(2), IM(1)	M(3), IM(1)	-	M(22), IM(1)	M(3), IM(1)	M(8), IM(6)
Linaceae	-	M(1)	M(1)	M(4)	-	M(1)
Myrtus communis	IM(1)	M(1)	M(1), IM(1)	M(7), IM(3), S(2)	-	M(4), IM(3), S(2)
Polygonaceae	M(1)	M(1)	M(1), IM(3)	M(1)	M(1)	M(3), IM(5), S(2)
Pyrus/Prunus	IM(1)	M(1)	M(1)	M(10), IM(1)	M(1)	M(1), IM(1)
Rhamnaceae	IM(1)	IM(4)	M(2)	M(6), IM(2)	M(4), IM(1)	M(7), IM(3)
Robinia pseudacacia	-	M(1)	-	M(2)	-	-
*Tamarix*spp	-	-	-	M(1)	-	-
Trifolium spp	IM(13), S(1)	M(2), IM(2), S(11)	M(1), IM(5), S(5)	M(13), IM(42), S(15), P(1)	M(1), IM(9)	M(13), IM(13)
Vicia spp	-	M(2)	M(2)	M(7), IM(3), S(2)	M(1)	M(2), IM(1)
Vitex angus castus	-	-	-	-	-	M(1)

P = Predominant pollen (>45%); S = Secondary pollen (16-45%); IM = Important minor pollen (3-15%); M = Minor pollen (1-3%); Pollen <1% were not identified
** Pollen of* Helianthus annuus

Melissopalynological analysis

The determination of botanical origin was performed as recommended by LOUVEAX et al. (1987). The pollen grains are counted toghether with the corresponding honeydew materials and their relative proportion was established by counting. There were counted about 1000 grains for blossom honeys and about 300 for honeydew honeys. Grains of anemophilous and entomophilous nectarless plants are recorded separately and the honeydew constituents separately from the pollen grains. The results are expressed in percentages. the classes of pollen grains were given as predominant pollen (>45%), secondary pollen (16-45%), important minor pollen (3-15%), and minor polen (1-3%). the HDE/P ratio is also recorded in pine and fir samples. In the present study we did not took into consideration plant species with percentage below 1%. Also we record the anemophylous species but we did not express them as percentages. For melissopalinological analysis we use an OLYMPUS type CH-2 microscope. The plants were detected at the level of family and species.

Results

Table I shows for each honey origin the frequencyof plant source during the melissopalynological analysis. In orange honey 5 out of 17 samples (29.4%) appear to be minor *Citrus* type pollen grains. *Brassicaceae* appeared to be the predominant pollen of 15 samples (94.1%) while *Eucalyptus* sp. appeared only in one sample. Orange hones show 16 different pollen types. Seven samples of sunflower (43.7%) had predominant pollen and the rest (56.3%) had secondary pollen of *Helianthus annuus*. The most common pollen grains, apart from *Helianthus were* those of *Trifolium* sp., *Brassicaceae*, and *Apiaceae*. Pollen analysis show low percentages of cotton pollen grains in cotton honeys. In four samples (36.4%), appear minor pollen of cotton and other four important minor types (36.4%). Predominant thyme type appeared in 45 (52.3%), and in 41 (47.7%) as a secondary one. All the samples were considered as thyme honey, due to their organoleptical characteristics. A range of thyme type pollen grains of 25.4% to 92.2% was recorded. Apart from thyme pollen, *Brassicaceae* pollen is the most commonly found. It appeared in 82 (95.3%) out of 86 samples. In four of them (4.9%), *Brassicaceae* appeared, as being the predominant pollen. Pollen grains of *Trifolium* spp., *Hypericaceae* and *Asteraceae* had also been found in the vast majority of samples. In thyme honey appear 23 different pollen types. In fir honey appear 15 different pollen types, the less of all other monofloral honeys. the HDE/P rate is 2.05, with a range from 0.9 to 5.8. the most frequently pollen grains present are those of *Brassicaceae*, that are found in all the samples. *Castanea sativa* has predominant pollen in one sample and secondary in another, while *Trifolium* sp. appears important as minor pollen in 10 out of 11 samples. The HDE/P of pine honey appeared to have an average of 2.73 with a range of 0.6-10.02. the most common pollen grains are those of *Brassicaceae, Trifolium* sp., *Erica* sp., and *Asteraceae*. There is no predominant pollen in any sample, while in pine honey appear 23 different types of pollen.

Discussion

The large variety of meliferous sources enable Greece to produce characteristic type of honeys. Beekeepers move their hives from one location to the other following the different bloomings. They start in spring with orange and fir honeys and they end in autumn with pine honey. The weather conditions affect not only the production during the year but also the frequency with that certain types of pollen grains appear in the honey. The results of the different years allow us to detect those combinations of pollen which are characteristic of certain categories.

Orange honey is currently produced from the plants of *Citrus* sp. (*C. sinensis, C. aurantium, C. limon*). Citrus type pollen grains are considered as underrepresented pollens (BARTH, 1973; LOUVEAUX et al., 1978). In *Citrus* species anthers yield little pollen or are completely sterile (MAURIYIO, 1975). In orange trees especially many commercial brands are almost completely self sterilising and set fruit parthenocarpically (McGREGOR, 1976).

However there are other studies wich report high percentages of citrus type pollen grains (SERRA BONVEHI et al., 1987; MUNUENA and CARRION, 1994). In orange honey sediment we notice that the vast majority of pollen grains appear to be those of *Olea europeae*. There are samples where *Olea* pollen is almost exclusive, although the organoleptical characteristics are typical of orange honey. Olive tree produce large quantities of pollen but no honey (SIMIDCHIEV, 1980). In Greece, citrus trees are cultivated toghether with olive trees. Both are blooming at the same period in the spring. As olive trees are classified as anemophilous plants, grains of *Olea* are transferred by the wind and frequently found in orange honeys. This combination if typical of all honey samples we examined.

Helianthus honey is produced from the annual plant *Helianthus annuus* that is widely grown as oilseed crop in Northern Greece. Although the pollen grains of sunflower are considered as under-represented (PERSANO et al., 1980/1981; SAWYER, 1988), we found a range of 33.3% to 64.1%. Our results are able to be compared to those that THRASYVOULOU and MANIKIS (1995) report for Greek sunflower honeys and also the those which has been reported in other countries (GOMEZ FERRERAS, 1987; PEREZ ARQUILLUE et al., 1988: GOMEZ FERRERAS, 1989; FELLER-DEMALSY et al., 1989; PEREZ CARBONELL et al., 1994). We notice that in 13 samples (81.25%) we found pollen of *Apiaceae* species. *Helianthus* belong to the crops which needs a lot of water during cultivation. *Apiaceae* plants are grow on irrigation channels at the same period and so bees visit at the same time both plants. Other Greek monofloral honeys are poor as comparet to *Apiaceae* species with the exception of cotton honeys. We believe that this combination together with the presence of *Trifoliul* species is important in order to detect Greek helianthus honeys.

Cotton honey is produced from the plant *Gossypium hirsutum* that is cultivated in Central and Northern Greece. TALPAY (1985) reported that honeydew of cotton contain *Gossypium* pollen only as minor. Cotton is not listed as being under-represented in pollen of that plant origin (LOUVEAUX et al., 1978). the nectar is produced in flowers and also, at five extra floral locations of the plant (CRANE, 1990). Bees appear to be noticeably reluctant to visit cotton blossom although nectar and pollen are present, as the floral nectar is less attractive to them becase of the sugar combination and concentration (McGREGOR, 1976). the pollen is collected by bees only when there is no more attractive pollen in the area (McGREGOR, 1976; WALLER, 1982). Furthermore, according to WALLER (1982) pollen grains are probably too large and spiny and too difficult for bees to pack. Based on pollen analysis is difficult to identify cotton honey. The organoleptical characteristics seem to be crucial for this type of honey. All the samples containing pollen of *Trifolium* sp. and eigh out of eleven (72.75) pollen of *Brassicaceae* and *Apiaceae* which appeared predominant in one sample (9.1%) each. The presence of *Apiaceae* can be explained at the same way as in *Helianthus* honey. In every sample we also detect pollen from *Chenopodiaceae*. *Chenopodiceae* plants, according to LOUVEAUX et al., (1978), are nectarless but more or less entomophilous. The combination of *Apiaceae* and *Chenopodiaceaei* pollen grains may be characteristic to Geek cotton honey. *Chenopodiaceae* pollen is not expressed in percentages in the table as are nectarless plants.

Thymus honey is produced from the plants *Thymus serpyllum, Thymus capitatus* and *Satureja* sp. PEREZ ARQUILLUE et al. (1995) refers that in thyme honey, thyme pollen was present at low levels >15% while DEBBAGH (1988) reports 71.8% thyme pollen but only in one sample. In the vast majority of samples we notice a significant numer of pollen grains from plants of *Cistaceae. Cistaceae* species yield pollen but not nectar. LOUVEAUX and VERGERON (1964) mentioned that Spanish thyme honey often contains pollen of *Cistaceae* giving typical mixtures with *Thymus* sp. It seems that the same is happening as well in Greek thyme honeys.

Greek thyme honeys, as has been investigated in a previous study (DRIMJIAS and KARABOURNIOTI, 1995) present differences according to the geographical area.

Fir honey represents 5% of the annual honey production (SANTAS and BIKOS, 1979). In Greece honeydew is produced from the insects *Physokerames hemicryphus* and *Eulecanium sericeum* that parasite on *Abies alba* (SANTAS, 1983). Fir honeys contain pollen of different plant species. In Greece, beekeepers move their hives from one honey flow to another. Fir honeys follow blossom honeys. Some of the pollen that bees had collected in spring, appeared in fir honey during harvests. Especially the first harvest seems to be the richest in foreign pollen grains.

Pine honey represents 60% of the annual production of Greek honey (SANTAS and BIKOS, 1979). Pine honey is produced by the insect *Marchalina hellenica (Ganadius)* which is parasitic mainly on *Pinus halepensis* (SANTAS and BIKOS, 1979).

The pollen analysis shows a variety of pollen grains. The secretion of pine trees at the end of the summer follows a period in which beekeepers exploit other bloomings. As happens in fir honey the repeated harvests (more than two) gives to pine honey foreign pollen grains (THRASVOULOU and MANIKIS, 1995). THRASYVOULOU and MANIKIS report a lower average for HDE/P of 0.26. This can be explained by the fact that the samples might have been from the first harvest where a lot of pollen grains vanc still be found.

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