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Research Article

Pollen Characterisation of Maltese Honey

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Abstract. In 2004 and 2005, pollen characterisation of 35 samples of honey collected from the islands of Malta and Gozo, was carried out with the aim to identify the botanical origin of honey produced on these islands. Pollen was extracted from the honey samples via centrifugation and identified to pollen type, generic level and where possible, specific level via microscopic analysis. This was done by comparison with available literature and with the aid of prepared reference slides of pollen collected from the plant species commonly present in the Maltese islands. A total of 61 pollen types were identified from 33 families. The best represented families were the Asteraceae, Brassicaceae, Fabaceae and Apiaceae. Thyme (Thymbra capitata (L.) CAV.) pollen was found to be predominant in ten samples, with a percentage frequency that ranged from 10% to 67%. Hedysarum coronarium L. was found to be predominant in five honey samples with percentage frequencies from 48% to 78% while Lotus spp. pollen was found to be predominant in one honey sample with a percentage frequency of 57%. The remaining 14 honey samples possessed pollen spectra which were characterized by a few frequent pollen types that possessed similar percentage frequencies and were thus considered to be multifloral. This is the first work of pollen characterisation of Maltese honey.

Keywords Pollen – Honey – Melissopalynology.

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1 Introduction

There has never been a study on the pollen of honey produced in the Maltese islands. No work has ever been carried out locally in this field and, so very little is known about which flora characterises local honey except from what is known by the beekeepers themselves. Beekeepers to this day, label their honey according to the season during which it is harvested: spring and autumn honey. Spring honey is known to be polyfloral whereas autumn honey is considered by beekeepers to be mainly produced from nectar collected from eucalyptus and carob trees. A summer honey is also produced but this is labelled by beekeepers as wild thyme honey as wild thyme (Thymbra capitata) is the only bee-important flowering plant species that flowers in abundance during the hot summer months. Little else is in flower during this season.

Maltese honey is highly appreciated and sought after locally, especially thyme honey, which fetches a considerably high price. However, little is known about the microscopic and chemical composition of the honey. With entry to the European Union in May 2004, Malta adopted European legislation on the production and marketing of honey. As a result, Maltese honey must conform to the quality standards defined in EU legislation in order to be placed on the market, both locally and abroad.

This study is a preliminary attempt to gain an insight into the botanical composition of Maltese honey and involves the qualitative analysis of the pollen types found in the honey samples. Qualitative pollen analysis permits the calculation of relative pollen frequencies on the basis of the total count of pollen grains and other honey elements, as well as the identification of

the pollen spectrum that characterises honey produced in a specific geographical area. This type of analysis is therefore important in the identification of the types of honey that are produced locally. Its importance also lies in the added value and improvement of Maltese honey in terms of quality, in such a way as to create a solid niche-market for the product abroad. Quality control of honey relies heavily on melissopalynology, not only for the identification of botanical and geographical origin of honey, but also for the determination of fraudulent activities, such as the blending of Maltese honey with honey originating in other countries.

This study, therefore aims to obtain initial qualitative information on the true botanical identity of Maltese honey and to stimulate interest in a field of study relatively unknown locally.

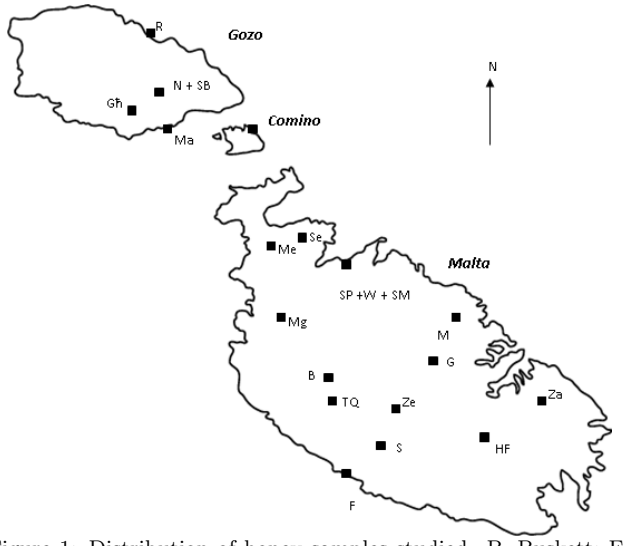

Figure 1: Distribution of honey samples studied. B, Buskett; F, Fawwara; G, Għargħur; Gh, Għajnsielem; HF, ħal Farruġ; M, Magħtab; Ma, Mġarr Gozo; Me, Mellieħa; Mg, Mġarr Malta; N, Nadur; R, Ramla; SB, San Blas; S, Siġġiewi; SE, Selmun; SM, San Martin; SP, St. Paul's; TQ, Ta' Qali; W, Wardija; Za, Zabbar; Ze, Żebbuġ.

2 Methods

Of the 35 honey samples that were collected from beekeepers during 2004 and 2005, a total of 28 samples from 17 localities were collected from Malta, 1 sample from Comino and 6 samples from 4 localities from Gozo (Figure 1). Fifteen samples were harvested by the beekeepers in 2004 while sixteen samples were harvested in 2005. The harvest date of four of the samples was unknown (the sample from Magħtab was not labelled with the harvest date while the beekeepers who provided the sample from Għargħur and Mġarr could not remember whether they had harvested the honey sample in 2003 or 2004).

All the honey samples were collected from the beekeepers except for one sample of honey, which was purchased from a retail outlet as the producer was unreachable. This honey was included in the study as it is one of the main honeys that is found on the local market.

At the time of this study honey samples were collected from most of the commercial beekeepers in Malta and Gozo, which due to the islands' small size was limited. Honey samples were collected from the beekeepers themselves as all except two did not sell their produce through retailers.

For the qualitative analysis, 10g of honey was dissolved in 20ml of distilled water at 40°C, to reduce the viscosity of the honey and enable the extraction of the pollen. The honey solution was poured into a 10ml glass centrifuge tube with a pointed tip and then centrifuged at 2500rpm for ten minutes. A Beckman Coulter Allegra X-22R centrifuge was used to centrifuge the honey samples. Honey samples, which were rich in sugar crystals where subjected to another centrifugation step at 2500rpm for ten minutes by redissolving the pellet in another 20ml of distilled water.

Following centrifugation, the centrifuge tube was tilted at a 45° angle to discard the supernatant, and the last drop was blotted dry with a piece of paper tissue. The pellet was then loosened with a disposable plastic Pasteur pipette (volume 1ml) and the lose sediment was drawn up with the pipette and transferred onto a glass slide. The sediment was spread evenly onto the glass slide over an area of 22×22mm with the aid of the Pasteur pipette as recommended by Von der Ohe et al., (2004). The 22×22mm square was delineated with a marker pen on the underside of the slides. The glass slide was then placed onto a hot plate for a few minutes to allow the sediment to dry. Where possible, two slides were prepared for each honey sample. However, a few of the honey samples were only sufficient to prepare one microscope slide.

While the sediment was left to dry on the glass slide, a small amount of Kaiser's glycerine jelly mountant was transferred to a cover slip of 22×22mm. The cover slip was placed on a hot plate at 40°C to dissolve the mountant (which was kept in the refrigerator and thus solidified). The drop of glycerine jelly was applied to the cover slip in the form of a cross diagonally as suggested in Von der Ohe et al., (2004) to ensure that the pollen grains remained in their drying position when the cover slip was lowered onto the glass slide. The cover slip was lowered onto the sediment slowly so as not to trap any air bubbles and the slides were sealed with clear nail varnish after the mountant had cooled and solidified.

2.1 Preparation of the glycerine jelly mountant

The mountant that was used in this study was Kaiser's Glycerine Jelly which was prepared from gelatine, dis-

tilled water, glycerine and phenol. 40g of gelatine was heated in 210ml of distilled water until the gelatine dissolved. 250ml of glycerine and 1g of phenol were added to the gelatine and the mixture was heated for 15 minutes and stirred until it became smooth.

The glycerine jelly mountant may be coloured by the addition of 0.5 to 1ml of 0.1% (w/v) basic fuchsin solution which stains the pollen grains pink (Von der Ohe et al., 2004). However, this step was not carried out during the preparation of the slides of the honey samples as the stain tends to make the structural features of the pollen grains less visible and it may thus hinder their identification (Ricciardelli D'Albore by personal communication, 2005). The pollen grains were thus left unstained and appeared in different shades of yellow when viewed under the microscope.

2.2 Qualitative Analysis of the Honey Samples

The mounted pollen samples were examined under the microscope at a magnification of ×400. For this analysis a Nikon Eclipse E400 POL microscope with camera attachment was used. The pollen grains were counted in batches of 100, following parallel equidistant lines spaced evenly form one edge of the coverslip to the other.

The percentage frequencies of the pollen types present in the honey can be calculated by counting and identifying between 500 to 1000 pollen grains (Von der Ohe et al., 2004). During this study pollen grains were counted and identified for each honey sample. As microscope slide preparations of the honey samples were observed, the pollen grains were counted in batches of 100 pollen grains. The pollen frequencies of the pollen types identified in each batch were calculated and compared with one another. Results were found to be constant when 700 grains were counted and as a result counting 700 pollen grains was deemed sufficient to obtain reliable pollen frequencies for the pollen types.

Pollen grains were identified to genus and species level only when they could be identified with certainty. Most often this was not possible and they were identified by types. Thus, pollen grains that possessed the characteristics of Cerinthe pollen were classified as Cerinthe type and so on. Wind-pollinated and nectarless species were noted separately. Grains that could not be identified were also counted separately. Pollens grains were not identified because they were not known or else because they were distorted or misshapen. Honeydew elements (fungal hyphae, fungal spores, algae and conidia) were also counted. Complexes of spores and algae were counted as one element.

2.3 Interpretation of Results

The pollen frequencies of the pollen types identified in the honey samples were calculated by dividing the number of pollen grains of a pollen type by the total number of pollen grains counted and multiplying by 100. Pollen frequencies of nectar-producing plants were calculated after the number of pollen grains of nectarless species and wind-pollinated species were subtracted from the total. Once the pollen frequencies were calculated, the pollen types were classified as follows, following Louveaux et al. (1978): "Very frequent" for grains constituting more than 45% of the total; "Frequent" for grains constituting 16-45% of the total; "Rare" for grains constituting 3-15% of the total; "Sporadic" for grains constituting less than 3%. Pollen with a frequency of 1% or less is classified as "present".

Honey samples that were rich in pollen of overrepresented species were recounted excluding the overrepresented pollen type. The botanical origin of the honey was determined by calculating the relative frequencies, excluding the pollen from nectarless species. Honey was considered to be produced mainly from one botanical species when the pollen of this species was predominant in the honey. For overrepresented species, such as Eucalyptus, a minimum threshold of 90% was required (as suggested by Louveaux et al., 1978) to define the honey as belonging mainly to that species, while for underrepresented species, such as Thymbra, a minimum threshold of 10% was considered (as suggested by Terrab et al., 2004).

2.4 Preparation of Reference Slides for the Pollen Library

A pollen library of all the common plant species found in the areas where the honey was produced was compiled as a reference library for the identification of the pollen extracted from the honey samples.

Pollen was taken from the buds of flowers and allowed to open in a contained environment in order to eliminate contamination by pollen in the environment. The procedure that was followed for the preparation of these slides was that of Louveaux et al. (1978). The anthers or whole flowers were washed in a watch glass containing ether. A ring of pollen formed at the edge of the ether solution, the ether was decanted and the pollen was rinsed with fresh ether and allowed to dry. The pollen grains where then transferred onto a microscope slide, warmed at 40°C and mounted in Kaiser's glycerine jelly.

A digital library of the reference material was also compiled with the aid of a Nikon Eclipse E400 POL microscope with camera attachment and a Nikon Coolpix 995 Digital Camera. The photographs of the pollen grains were taken at a magnification of ×400. The pollen

types extracted from the honey samples were identified with the aid of the reference collection of prepared slides the digital photos, as well as photomicrographs and pollen descriptions from literature. The literature used for the identification of the pollen grains was that of Ricciardelli D'Albore, 1998.

2.5 Results

The percentage frequencies of the pollen grains identified in the honey samples, were calculated after subtracting the number of pollen grains of nectarless species and wind-pollinated species from the total number of pollen grains counted.

Table 1: Honey samples studied in 2004/2005, split by locality and harvest period.

	Code	Locality	Harvest Year	Harvest Period
Malta	1	St. Paul's	2005	July
	2	Siġġiewi	2005	Unknown
	3	Mellieħa	2004	July
	4	Wardija	2004	Unknown
	5	Mġarr	Unknown	Spring
	6	Mġarr	2005	Unknown
	8	Wied Musa	2004	Unknown
	10	Buskett	2004	May
	11	Għajnsielem	2004	May
	12	Żebbuġ	Unknown	Autumn
	14	Għargħur	2004	Unknown
	15	Wardija	2004	Spring
	16	Żebbuġ	2005	Spring
	17	Wardija	2004	Unknown
	18	Ta' Qali	2004	Unknown
	19	Mellieħa	2005	Unknown
	20	Mellieħa	2004	Unknown
	22	Selmun	2005	Summer
	23	Zabbar	2004	September
	25	ħal Farruġ	2005	Spring
	27	Fawwara	2005	Unknown
	28	Buskett	2004	May
	30	Mġarr	2005	Autumn
	31	Magħtab	Purchased, undated	Unknown
	32	Mġarr	Unknown	Unknown
	33	San Martin	2004	Spring
	34	Fawwara	2005	July
	35	Fawwara	2005	April
Gozo and Comino	7	Comino	2004	Summer
	9	Nadur	2004	Unknown
	13	Ramla	2005	Unknown
	21	Nadur	2004	August
	24	Mġarr	2005	Unknown
	26	Nadur	2005	August
	29	San Blas	2005	August

The results of the qualitative analysis for 30 of the honey samples studied are shown in table 1. Many authors recommend the identification of 500 – 1200 pollen grains for the determination of reliable pollen frequencies to individual pollen types, expressed with an accuracy of ±1% (Louveaux et al., 1978; Ricciardelli D'Albore, 1997; Von der Ohe et al., 2004). An increased error is obtained for counts up to 500 pollen grains (Moar, 1985). Preliminary observation of the honey samples revealed the presence of few botanical species with prevalent in the honey. Thus, consistent results were obtained with counts of 700 grains. In addition, the pollen grains were counted in batches of 100, and when the results were compared they were found to be in general quite consistent with one another.

A total of 61 pollen types were identified from

33 families. The best represented families are the Asteraceae (Compositae), Brassicaceae (Cruciferae), Fabaceae (Leguminosae) and Apiaceae (Umbelliferae). No particular pollen type was present in all the honey samples studied. The pollen types identified in the honey samples were the following: Hedysarum coronarium, 28 samples (<1 – 64%); Diplotaxis spp., 28 samples (<1 – 23%); Lotus spp., 27 samples (<1 – 57%); Vicia type, 26 samples (<1 – 16%); Papaver type, 26 samples (<1 – 38%); Oxalis pes-caprae L., 26 samples (<1 – 11%); Galactites tomentosa (l.) Moench., 25 samples (<1 – 17%); Daucus type, 24 samples (<1 – 21%); Citrus spp., 23 samples (<1 – 10%); Thymbra capitata, 21 samples (<1 – 65%); Reseda type, 21 samples (<1 – 18%); Eucalyptus spp., 19 samples (<1 – 51%); Borago officinalis L., 18 samples (<1 – 4%); Rhamnus spp., 17 samples (<1 – 29%); Limbarda crithmoides (L.) Dumort., 16 samples (<1 – 3%); Plantago spp. and Acacia spp., 14 samples (<1 – 3%); Glebionis coronaria (L.) Cassini ex Spach., 13 samples (<1 – 1%); Ceratonia siliqua L., 12 samples (<1 – 38%); Euphorbia type and Eriobotrya type, 11 samples (<1 - 5%); Medicago type, 10 samples (<1 – 6%); Erica multiflora L., 10 samples (<1 – 4%); Brassica type, 10 samples (<1 – 1%); Asparagus type, 9 samples (<1 – 10%); Malus type, 9 samples (<1 – 7%); Cercis siliquastrum L., 8 samples (<1 – 4%); Smilax aspera L., 8 samples (<1 – 2%); Apiaceae, 6 samples (<1 – 22%); Trifolium type, 6 samples (<1 – 5%); Cerinthe major L., 6 samples (<1 – 7%); Prunus spp., 6 samples (<1 – 3%); Glebionis type, Carthamus spp., Arecaceae (Palmae), 6 samples (<1 – 1%); Ailanthus type, 5 samples (<1 – 4%); Ecballium elaterium (L.) A.Rich., 5 samples (<1 – 3%); Convolvolus type, Allium type and Cucumis spp., 5 samples (<1 – 1%); Olea europea L., 4 samples (<1 – 2%); Capparis type, 4 sample (<1 – 8%); Poaceae, 4 samples, (<1 – 1%); Cucurbita spp. and Lonicera type, 4 samples (<1%); Smyrnium olusatrum L., 3 samples (<1 – 6%); Matricaria type and Teucrium spp., 3 samples (<1%); Aptenia type, 2 samples (2 – 7%); Nicotiana type, 2 samples (<1%); Carpobrotus type, 1 sample (14%); Ferula type, 1 sample (1%); Hedera type (<1 – 1%); Vitis type, Agave type, Eryngium type, Geranium type, Helianthus type, Echium type, Cupressus spp. and Lavandula type, 1 sample (<1%).

2.6 Pollen Percentage Frequencies of Thymbra capitata and Uniflorality of Honey

Thyme pollen was found to exhibit a pollen percentage frequency of 10% or higher in 10 samples of the 30 that were studied. The percentage frequency in these samples ranged from 10% to 67%. Thyme pollen tends to be underrepresented in honey (Ricciardelli D'Albore, 1998). Terrab et al. (2004) report a percentage frequency of 8% to be sufficient to characterise a thyme honey as unifloral.

Predominance of a pollen type in honey and determination of uniflorality is not easy to determine for species which are underrepresented. In samples no. 4 from Wardija, no. 7 from Selmun and no. 5 from Mġarr, Thyme pollen exhibited percentage frequencies of 67%, 54% and 49% respectively. The other pollen types exhibited percentage frequencies of < 5%, = 10%, and = 13% . Therefore these three honey samples were clearly considered to be characterised by Thyme.

In samples no. 3 (Mellieħa), 8 (Wied Musa) and 23 (Mellieħa), Thyme exhibited percentage frequencies of 33%, 36% and 34% respectively. Sample no. 3 showed Eucalyptus to possess a percentage frequency of 38% whereas sample no. 23 showed Ceratonia siliqua to possess a percentage frequency of 36%. Eucalyptus is a species which is overrepresented in honey (Ricciardelli D'Albore, 1998) and it requires percentage frequencies of up to 90% to characterise a honey, whereas Ceratonia would require a percentage frequency of 45%. These honey samples were thus considered to be characterised by Thyme.

Sample no. 1 from St. Paul's Bay, was difficult to interpret due to the high percentage of unidentified pollen grains in the sample (25%). However, in an extreme event in which all the unidentified pollen grains belonged to one pollen type, this would not have sufficed to characterise the honey, unless the species was underrepresented.

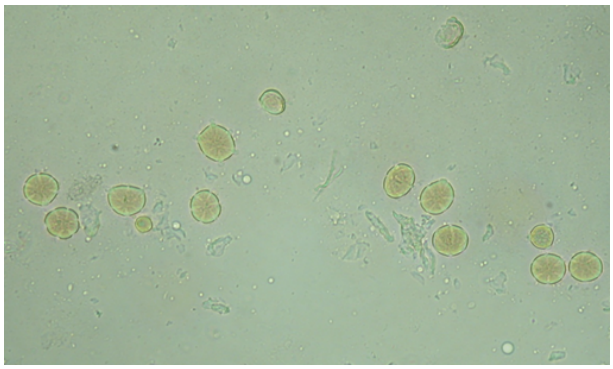

Figure 2: Thyme pollen in honey sample number 1 from St. Paul's Bay.

Honey samples no. 2, 6 and 20 from Siġġiewi, Mġarr and Mellieħa showed Thyme to possess percentage frequencies of 10, 11 and 12% respectively. Further studies would be required to determine whether such honey samples could be considered to be characterised by Thyme when the percentage frequencies of all the other pollen types is low. Lotus in sample 20 possessed a percentage frequency of 32%: a normally represented pollen type which requires a percentage frequency of

45% to characterise a honey. The percentage frequency of unidentified pollen grains in sample no. 6 was rather high (15%) and it was therefore difficult to draw conclusions. Therefore, classification of uniflorality in such cases would require further investigation.

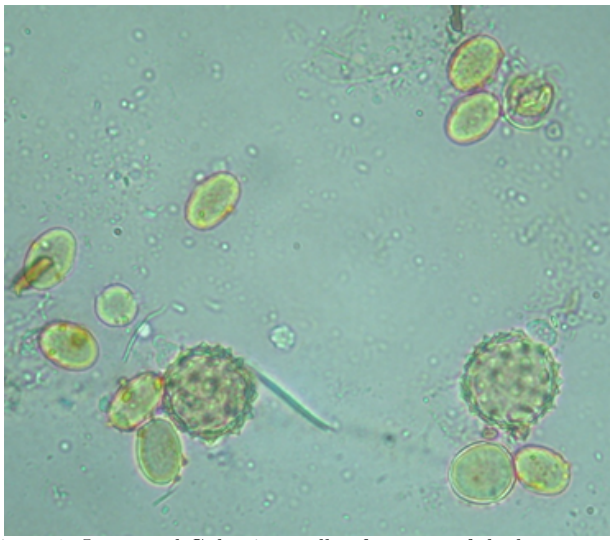

Figure 3: Lotus and Galactites pollen from one of the honey samples studied.

2.7 Pollen Percentage Frequencies of Lotus and Hedysarum and Uniflorality of Honey

Hedysarum and Lotus (Fabaceae) are in full flower in spring and are locally considered to be typical of spring blossom honeys. Hedysarum was found to be predominant in five honey samples (samples 9, 10, 11, 13 and 26). Percentage frequencies ranged from 48% to 78%. This is a very good nectariferous species that is normally represented in honey (Ricciardelli D'Albore, 1998). In all five honey samples the other pollen types exhibited frequencies that were very low and as a result, the pollen spectra were easy to interpret. In addition the percentages of unidentified pollen grains were also low (the highest being 7%) and therefore did not pose any difficulty to the interpretation of the results. All five honey samples were thus considered to be characterised by Hedysarum.

Lotus was found to be predominant in honey sample no. 12, in which this species exhibited a percentage frequency of 57%. Once again the pollen spectrum was easy to interpret as the next most abundant species was Hedysarum with a percentage frequency of 26%. This species requires a frequency of 45% to characterise a honey. All other pollen types present were rare, sporadic or just present. The percentage of unidentified pollen grains was 2%. This honey sample was thus also classified as Lotus honey.

2.8 Multifloral honeys

The remaining 14 honey samples possessed pollen spectra which were characterised by a few frequent pollen types and many pollen types that classified as rare, sporadic or present. The frequent pollen types were: sample 14 (Lotus, 37%); sample 15 (Hedysarum 36%; Lotus, 28%); sample 16 (Lotus, 16%; Hedysarum. 24%); sample 17 (Vicia, 17%); sample 18 (Rhamnus, 29%); sample 19 (Hedysarum, 24%; Lotus, 28%); sample 21 (Hedysarum, 31%; Lotus, 21%); sample 24 (Daucus, 24%; Hedysarum, 20%); sample 25 (Eucalyptus, 25%); sample 28 (Diplotaxis, 15%; Vicia, 17%); sample 29 (Apiaceae and Lotus, 22%; Daucus, 15%); sample 30 (Ceratonia, 41%; Diplotaxis, 25%). All other pollen types in these samples of honey were classified as rare, sporadic or present.

The results showed that no species was predominant in the honey samples and that they were characterised by more than one botanical species. In sample no. 30 which possessed a percentage frequency of 41%, the percentage of unidentified pollen grains in this sample was 9%. However, Ceratonia pollen grains possess a tetracolporate structure that is easy to identify and therefore this value was not considered to hinder the interpretation of the results. All these honey samples were thus considered to be characterised by more than one botanical species and therefore considered to be multifloral in origin.

2.9 Nectarless Species and Wind-Pollinated Species

The nectarless species identified in the honey samples were all present in minor quantities in the honeys that were studied except for Papaver which was present in 26 honey samples and exhibited percentage frequencies of <1% - 38%. This species is nectarless but it is commonly visited by bees for pollen (Ricciardelli D'Albore, 1998). Plantago pollen was identified in 14 honey samples with frequencies of <1 to 3%. This is both a wind-pollinated species (Chambers, 1945) and an insect-pollinated species (Plantago lanceolata has been found to be most effectively pollinated by hoverflies) (Stelleman and Meeuse 1976, Stelleman 1978, 1981) that is visited by bees for pollen. Olea, Vitis, Cupressus, Poaceae and Arecaceae pollen did not occur in frequencies higher than 1% except for Olea which occurred at a frequency of 2% in sample no. 24 from Mġarr.

Pollen from nectarless and wind-pollinated species ends up in honey by contamination processes. Thus, the number of pollen grains from these species is subtracted from the total when the pollen frequencies are calculated, as there is no nectar from these species in the honey.

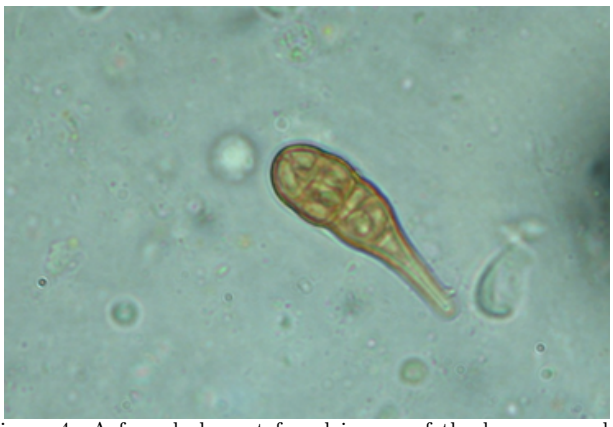

Figure 4: A fungal element found in one of the honey samples studied.

2.10 Honeydew Elements and Honeydew Honey

The number of honeydew elements observed in the honey during the pollen counts was also noted. These included fungal hyphae and fungal spores. Numbers were very low and ranged from none to 29 elements counted in honey sample no. 3 from Mellieħa. Honeydew honeys are produced from the excretions of plant-sucking insects, such as aphids, which occur in large population densities and honeys that are produced from these secretions are characterised by the presence of fungal hyphae and fungal spores of sooty mould. Further studies would be required to assess the potential for honeydew honey production locally.

3 Discussion

The most important finding in this study is without doubt the high percentage frequency of Thyme pollen observed in Maltese thyme honey. In their studies on the characteristics of thyme honeys from Greece, Tsigouri and Passaloglou-Katrali (2000) obtained an average pollen percentage frequency for Thyme of 42% in the honey samples studied and report that the percentage of Thyme pollen in islands can be as high as 85-90%.

The production of honeys with a high percentage of Thyme pollen is possible in the Maltese islands as Thyme has a wide distribution range, being a typical species of Maltese garigue and phrygana: habitats that are very frequent in the Maltese Islands, especially in the north-west of Malta and the island of Comino. In addition, this plant species is in full bloom during the month of June in which little else is in flower. Thus, if a beekeeper monitors closely the flowering period of this species and places the bee hives in an area that is rich in Thyme, the beekeeper may be able to produce a honey which is purely characterised by Thyme, when the honeycombs are removed from the hives as soon as the Thyme flowers begin to fade.

3.1 Pollen Spectra

Pollen spectra comprised 11 to 28 pollen types. The honey samples studied possessed similar spectra in terms of species that occurred in highest abundance except for sample no. 18 from Ta' Qali, which exhibited Rhamnus (29%), Euphorbia (14%), Ceratonia, Citrus and Asparagus as the most abundant pollen types. This honey possessed a spectrum quite unlike the other honey samples studied.

However, the results of the qualitative analysis did not show any honey possessing a particular pollen spectrum that could be traced to a particular geographical area. This is quite understandable as the Maltese islands possess a small surface area and possess a rather homogeneous landscape and honey bees may travel very large distances to locate a food source.

Qualitative analysis not only gives information on the honey spectrum of a honey but can also be employed to observe the variability in the pollen spectrum of a honey from year to year. Honey samples number 10 and 28 were harvested by the same beekeeper from the same location in May 2004 and May 2005 respectively. Sample no. 10 was dominated by Hedysarum (78%) whereas sample no. 28 exhibited Diplotaxis, Galactites, Rhamnus and Vicia as the most abundant pollen types, with frequencies of 11-15%. Therefore, a unifloral Hedysarum honey was harvested in May 2004, while a multifloral honey was harvested in May 2005. This may reflect agricultural practice, since fields may have been planted with Hedysarum one year, but left fallow the next.

Changes in climate from year to year also affect the flowering period and floral abundance and this varies the food availability for the honey bees. As a result, different botanical species are exploited and so different honeys are produced. This highlights the importance of carrying out such microscopic analysis on honey on a yearly basis to determine its botanical origin if the beekeeper is to include this information on the honey label.

3.2 Honey label discrepancies

Beekeepers do have a basic idea of the botanical sources of their honey and some beekeepers label their honey with the plant species they believe to be the floral sources of the honey that they produce. However qualitative analysis of these honey samples revealed that several honey samples were incorrectly labelled. Honey sample no.1 from St. Paul's was harvested in July 2005 and the beekeeper labelled it as spring blossom honey. Analysis revealed this honey to be Thyme honey. Likewise, honey samples no.2 from Siġġiewi and no. 8 from Wied Musa were also labelled by the beekeepers as spring blossom honeys and found to be Thyme honeys. Honey sample no. 30 was labelled as honey

produced from Eucalyptus and Carob. Even though this honey did contain both pollen types (Eucalyptus 51% and Carob 36%), organoleptic analysis and physicchemical analysis of the honey would be required in order to further assess the honey and determine whether it is labelled correctly or not as the percentage frequencies alone are insufficient to precisely determine the botanical origin of this honey.

Both the Codex Alimentarius (2001) standards and the European Honey Directive (Directive 2001/110/EC) standards state that the botanical source of a honey may be reported on the label only if the honey has originated from that source and it possesses "the physicalchemical, organoleptic and microscopic characteristics" that are typical of honey that is produced from that source. Thus, beekeepers must be careful when stating the botanical source of their honey as incorrect labelling may lead to the withdrawal of the honey from the market. The botanical source can only be determined by microscopic analysis of the honey together with sensory and physicochemical analysis. If a beekeeper is to label a honey with the botanical origin, the honey must be subjected to these tests in order to verify the floral sources of the honey and thus its botanical origin.

3.3 Adulteration

Four honey samples studied (nos. 32 – 35) were found to be so poor in pollen grains that 700 grains could not be counted and identified even when the entire sample preparation was observed under the microscope. Due to the small size of these samples, it was not possible to carry out a second extraction in order to obtain a picture of the pollen spectrum of the honey, except for sample no. 34. Still only 328 pollen grains were counted from the two slide preparations. Such low pollen counts were difficult to explain without the aid of quantitative analysis and physicochemical analysis. Unfortunately, quantitative analysis could not be carried out due to the lack of the required apparatus.

Quantitative analysis makes use of a Millipore vacuum filter pump to extract all the pollen grains found in 10g of honey and 500 pollen grains are counted. The number of visual fields which must be observed in order to count 500 pollen grains depends on their density. If the density of the pollen grains is high, fewer fields will be required. The total number of pollen grains in the sample is then calculated using a mathematical formula that takes count of the area of the microscopic field at the magnification used to count the pollen grains and the surface area of the filter paper containing the sediment (Von der Ohe et al., 2004). According to the number of elements (honeydew elements are also counted), honey is placed into five classes. Class I contains less than or equal to 20×10^3 elements and includes honeys with under-represented pollen, Class II contains between 21×10^3 and 100×10^3 elements and includes multifloral honeys, honeydew honeys and mixtures of both, Class III contains between 101×10^3 and 500×10^3 elements and includes unifloral honeys with overrepresented pollen and honeydew honeys, Class IV contains between 501×10^3 and 10^6 elements and includes unifloral honeys with strongly overrepresented pollen and some pressed honeys, Class V includes more than 10^6 pollen grains and includes almost only pressed honey.

The four honey samples, which exhibited an underrepresentation of pollen, could have been fraudulently manipulated by the addition of sugar syrup. This theory, could however, only be confirmed by physicochemical analysis of the honey. Adulteration of honey is the addition of foreign substances to a food product (Sanford, 2003). This is a common practice in which sugar is improperly fed to the honey bees during the honey flow or sweeteners are added to the honey. Sweeteners identified in honey include molasses, corn syrups, maple syrups, sugar cane and sugar beet (Ruoff and Bogdanov, 2004). However, the adulterant that causes greatest concern is the addition of High Sucrose Corn Syrup (HFCS) to honey (Sanford, 2003).

Adulteration of honey with C_4 sugars from the addition of sugar cane or corn syrup may be determined by microscopic analysis in which parenchyma cells, single cells from ring vessels, and epidermal cells that originate from sugar cane stems are counted (Kerkvliet and Meijer, 2000). The presence of a high number of plant cells is a good indication of adulteration by cane sugar. However, the official method for analysing adulteration with cane or corn sugar is the measurement of C_{13} present in the honey, expressed as $\delta^{13}C$, which is measured using an isotope ratio mass spectrometer (IRMS) (Kerkvliet and Meijer, 2000). C_4 plants such as sugar cane absorb more carbon dioxide than C_3 plants, which are the original botanical sources of honey.

Adulteration by the addition of sugar cane and beet is also measured by infrared spectroscopic methods, while addition of high fructose corn syrup is also identified by the analysis for the presence of oligosaccharides, which are not normally present in honey (Ruoff and Bogdanov, 2004).

3.4 Filtration

One honey sample from Magħtab (honey sample no. 31) possessed a high density of diatomaceous sand crystals and an extremely low occurrence of pollen grains. Diatomaceous sand crystals are colourless and semi-transparent when observed under the microscope. They are also irregularly shaped. Their high density in the honey sample prevented the correct identification of the few pollen grains that were present.

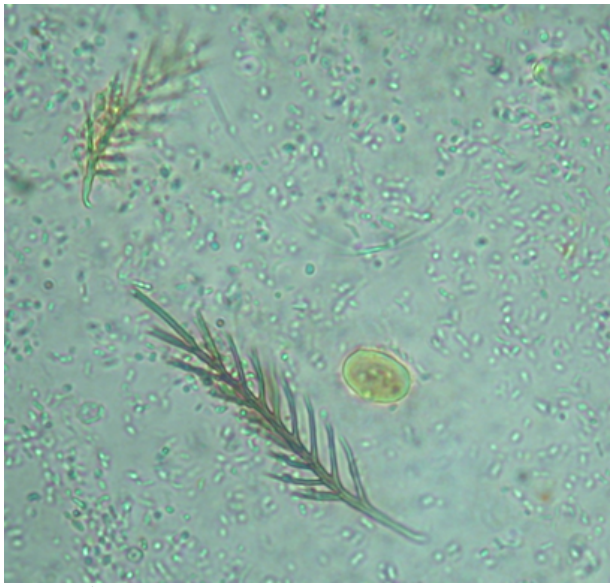

Figure 5: A contaminated honey sample.

Diatomaceous sand is usually employed to filter honey in third countries such as North and South America. The newly revised legislative requirements defined in the Codex Alimentarius, 2001 and the EU Honey Directive (Directive 2001/110/EC) permit the filtration of honey with a mesh size smaller than 0.2 mm with loss of pollen, only if this is unavoidable for the removal of foreign organic and inorganic matter. In any case, honey that has been filtered must be clearly labelled as "filtered" honey if it is to be placed on the market (Ruoff and Bogdanov, 2004). Filtering of honey is not carried out in the Maltese islands and when this honey sample was sent to Professor Giancarlo Ricciardelli D'Albore from the University of Perugia, for a second opinion, he confirmed that the honey most probably was obtained from a Latin American country such as Mexico.

The placement of honey on the market as locally produced honey, even though it was not clearly labelled as honey produced in Malta, is misleading to the consumer and is considered to be a fraudulent practice. It is therefore imperative to carry out routine analyses on honey samples in the Maltese islands to ensure that fraudulent activities are prevented and that the authenticity of these products and their quality is guaranteed to the consumer.

3.5 Hygiene

The poor hygiene of many honey samples was observed during the qualitative analysis(figure 5). Many samples possessed more bee hairs than pollen grains. Several honey samples were also observed to be contaminated by bacteria, such as the honey sample from San Martin.

Beekeepers are currently required to pass a hygiene exam in order to obtain a licence to produce honey. However, more work is required to ensure that proper hygienic standards are maintained by local beekeepers.

4 Conclusion

Botanical characterisation of honey is a vital tool in identifying the floral sources of Maltese honey and the characterisation of them. It is also an extremely useful tool in identifying fraudulent practices in honey production, such as adulteration.

This paper is based on a dissertation submitted by CG to the Department of Biology, Faculty of Science, University of Malta in part fulfilment of the Degree of Master of Science.

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Appendix 1

Results of the qualitative analysis of pollen types in Maltese honey, represented as percentages

Pollen Type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Aizooaceae															
Aptenia type	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Carpobrotus type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amaryllidaceae															
Allium type	-	-	+	+	-	1	-	-	-	-	-	-	-	-	-
Apiaceae															
Daucus type	-	-	-	3	3	2	7	10	3	-	2	1	3	3	+
Eryngium type	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Ferula communis	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Smyrnium type	6	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Apiaceae type	3	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Araliaceae															
Hedera type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arecaceae															
Arecaceae type	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Asparagaceae															
Agave type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asparagus type	-	4	-	-	-	-	-	-	1	-	-	1	-	3	-
Asteraceae															
Carthamus type	-	-	-	-	+	1	-	-	1	-	1	-	-	-	-
Glebionis coronaria	-	-	+	-	+	+	1	+	+	-	-	+	1	+	-
Glebionis type	1	-	-	+	-	+	+	+	+	+	+	-	-	+	-
Galactites type	1	2	1	-	-	9	1	1	10	+	17	1	2	2	-
Helianthus type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limbarda crithmoides	1	2	+	3	-	+	1	+	-	-	+	-	-	+	-
Matricaria type	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
Boraginaceae															
Borago officinalis	-	-	-	-	2	1	-	-	1	-	1	4	+	1	-
Cerinthe type	-	-	-	-	-	-	+	+	-	-	-	1	-	7	-
Echium type	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Brassicaceae															
Brassica type	-	-	-	-	-	+	-	-	+	-	1	+	-	1	+
Diplotaxis type	15	18	-	2	5	3	2	-	4	3	2	1	5	1	3
Capparidaceae															
Capparis type	-	-	-	-	8	-	-	-	+	+	-	-	-	-	-
Caprifoliaceae															
Lonicera type	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Convolvulaceae															
Convolvolus type	-	-	-	-	-	+	1	+	-	-	-	-	+	-	-
Cucurbitaceae															
Cucumis type	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-
Cucurbita type	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-
Ecballium type	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
Cuppressaceae															
Cupressus type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ericaceae															
Erica multiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1
Euphorbiaceae															
Euphorbia type	-	5	-	-	2	+	1	-	-	-	-	-	-	-	-

Pollen Type	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Aizooaceae															
Aptenia type	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-
Carpobrotus type	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-
Amaryllidaceae															
Allium type	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1
Apiaceae															
Daucus type	2	2	-	2	3	2	4	+	21	7	2	2	-	7	1
Eryngium type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ferula communis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Smyrnium type	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Apiaceae type	-	-	-	-	-	1	-	-	3	1	1	-	-	22	-
Araliaceae															
Hedera type	-	-	-	-	-	-	-	-	-	-	-	-	-	1	+
Arecaceae															
Arecaceae type	-	-	-	1	+	-	-	-	-	+	-	-	-	-	-
Asparagaceae															
Agave type	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Asparagus type	-	-	10	1	-	-	+	-	+	5	-	-	-	-	-
Asteraceae															
Carthamus type	-	-	-	-	+	-	1	-	-	-	-	-	-	-	-
Glebionis coronaria	-	-	-	-	-	+	-	-	+	-	+	-	-	-	+
Glebionis type	-	-	-	+	1	+	+	-	+	+	+	-	-	1	+
Galactites type	4	10	-	+	1	6	1	-	6	9	5	3	10	2	+
Helianthus type	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Limbarda crithmoides	-	-	+	+	1	-	+	1	-	-	-	-	-	1	+
Matricaria type	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Boraginaceae															
Borago officinalis	4	+	-	+	+	1	1	-	-	+	1	1	2	-	1
Cerinthe type	1	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Echium type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brassicaceae															
Brassica type	-	1	-	1	-	-	-	-	+	-	-	-	-	1	-
Diplotaxis type	2	5	+	5	2	3	1	+	6	9	3	5	14	6	23
Capparidaceae															
Capparis type	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Caprifoliaceae															
Lonicera type	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Convolvulaceae															
Convolvolus type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Cucurbitaceae															
Cucumis type	-	-	-	1	-	-	-	-	-	-	-	-	-	-	+
Cucurbita type	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ecballium type	3	+	-	-	-	-	+	-	-	-	-	-	-	-	-
Cuppressaceae															
Cupressus type	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Ericaceae															
Erica multiflora	1	1	-	4	-	1	1	-	-	+	+	-	-	-	-
Euphorbiaceae															
Euphorbia type	-	+	14	+	-	1	-	-	-	1	1	-	7	-	1

Pollen Type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Fabaceae															
Acacia type	+	-	+	-	1	+	1	3	-	-	+	-	-	1	-
Ceratonia siliqua	-	7	3	-	-	-	-	-	-	1	-	-	1	-	-
Cercis siliquastrum	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Hedysarum coronarium	+	-	1	2	12	1	10	9	50	64	55	26	39	7	33
Lotus type	2	+	-	3	3	2	6	12	2	-	4	57	10	33	25
Medicago type	+	-	-	-	-	-	-	-	2	+	-	-	-	+	+
Trifolium type	+	-	-	-	-	-	-	-	+	-	-	1	-	-	-
Vicia type	3	+	1	-	3	13	1	2	2	+	1	-	1	7	+
Geraniaceae															
Geranium type	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Lamiaceae															
Lavandula type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Teucrium type	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Thymus capitata	28	10	33	65	49	10	54	33	1	+	-	-	-	3	-
Myrtaceae															
Eucalyptus type	2	23	38	5	-	8	1	-	-	-	+	-	-	6	1
Oleaceae															
Olea type	-	-	-	-	-	-	-	1	-	-	-	-	-	-	+
Oxalidaceae															
Oxalis pes-caprae	1	+	+	1	1	1	-	1	+	+	2	1	+	+	-
Papaveraceae															
Papaver type	1	2	1	2	+	13	-	4	11	18	4	-	23	11	8
Plantaginaceae															
Plantago type	1	-	-	-	-	-	-	3	+	-	-	+	+	-	+
Poaceae															
Poaceae	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-
Resedaceae															
Reseda type	2	6	16	1	-	18	1	4	-	-	-	-	4	2	-
Rosaceae															
Eriobotrya type	5	2	-	5	-	-	-	-	+	1	-	3	2	1	-
Malus type	-	-	-	-	-	-	1	1	-	-	3	-	-	-	-
Prunus type	1	3	-	-	-	-	-	-	-	-	-	-	+	1	-
Rhamnaceae															
Rhamnus type	-	-	-	-	1	-	2	+	-	2	1	-	-	+	5
Rutaceae															
Citrus type	+	-	-	1	-	1	2	-	+	1	2	+	-	+	4
Simaroubacaceae															
Ailanthus type	-	3	-	-	-	1	-	-	-	-	-	-	-	-	4
Smilacaceae															
Smilax type	-	1	-	-	-	-	-	-	-	2	-	+	-	-	-
Solanaceae															
Nicotiana type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vitaceae															
Vitis type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Pollen Type	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Fabaceae															
Acacia type	1	-	-	+	1	-	-	+	-	+	-	-	+	-	-
Ceratonia siliqua	-	-	14	2	+	1	-	35	-	10	+	-	-	-	38
Cercis siliquastrum	-	-	-	+	-	1	1	1	-	1	1	4	-	-	-
Hedysarum coronarium	21	1	-	24	14	19	28	1	18	3	32	7	6	15	7
Lotus type	14	11	-	28	30	13	21	1	12	2	11	3	11	22	2
Medicago type	-	-	-	-	-	2	2	-	-	2	2	-	6	-	-
Trifolium type	5	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Vicia type	11	16	-	3	3	1	+	1	+	3	1	10	15	2	-
Geraniaceae															
Geranium type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lamiaceae															
Lavandula type	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Teucrium type	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
Thymus capitata	-	-	-	5	11	+	9	17	2	5	+	4	-	1	-
Myrtaceae															
Eucalyptus type	3	8	10	8	5	-	4	51	1	22	-	-	-	-	1
Oleaceae															
Olea type	-	-	-	-	-	-	-	-	2	-	-	+	-	-	-
Oxalidaceae															
Oxalis pes-caprae	9	11	+	1	1	3	1	-	1	+	2	-	1	+	+
Papaveraceae															
Papaver type	12	3	-	1	6	38	+	-	5	12	33	17	6	+	7
Plantaginaceae															
Plantago type	-	-	+	-	-	+	-	-	3	+	+	-	+	1	+
Poaceae															
Poaceae	-	-	-	-	1	-	-	+	-	-	-	-	-	-	-
Resedaceae															
Reseda type	2	1	+	4	2	1	6	-	11	3	1	-	-	4	2
Rosaceae															
Eriobotrya type	-	-	-	-	-	-	1	+	-	-	-	-	-	3	-
Malus type	1	-	-	+	+	-	-	-	1	-	-	7	1	-	-
Prunus type	-	-	-	1	-	-	+	-	-	-	-	-	-	-	-
Rhamnaceae															
Rhamnus type	-	5	29	+	-	+	1	-	1	-	+	-	11	1	5
Rutaceae															
Citrus type	1	1	10	1	+	+	1	-	+	1	+	1	-	+	-
Simaroubacaceae															
Ailanthus type	-	-	-	-	-	-	1	-	-	-	-	-	+	-	-
Smilacaceae															
Smilax type	1	-	-	1	-	-	-	-	-	+	-	+	-	+	-
Solanaceae															
Nicotiana type	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
Vitaceae															
Vitis type	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-

+ = values below 1%; - = absence of pollen type in honey sample