Studying the Daily Harvesting of Pollen by *Apis mellifera* (Galicia-NW Spain). Pollen Foraging Pattern from Several Sources

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Abstract: In order to study the behaviour of honey bees in relation to pollen harvesting, and diversity and quantity of pollen from harvested loads, we have studied honey bee visits in different hours of the day and in several months, in four hives located in the South of Galicia (NW of Spain). All hives showed some hourly patterns in common. The honey bee colonies focalised their activity in any pollen sources in different moments of the day [morning –before 12:00 a.m.-(*Ranunculus peltatus*-type, for example); central hours -from 12:00 to 16:00 p.m.; morning and afternoon interval –before 16:00 p.m. (*Cytisus scoparius*-type, *Quercus* spp., for example); and in evening –from 16:00 p.m.-(*Castanea sativa* and *Rubus ulmifolius*-type)]. Sometimes, the collecting pattern of one determined pollen source varies in different hives.

Keywords: Harvesting pollen, daily pattern, honey bees, pollen offer, sources competition, foraging rhythm.

1. INTRODUCTION

The study of pollen harvesting can help to understand the interdependent relationship between bees and regional vegetation. This represents a picture about local flora and environmental conditions of the zone.

Honey bees choose their pollen sources among species present around the hive area and according to several characteristics related to floral biology, shape and corolla colour, presence of nectaries, the relative abundance of each species, etc. The availability of rewards and relative abundance of each species are factors of choice, and the corolla shape and colour, odour, etc. are attraction elements, and they serve to honey bees foraging as guides of specific recognition [1]. This means that floral physiology and honey bee perception will condition the model of bee behaviour [2, 3, 4].

The sugar concentration in nectar determines the reward type on honey bee foraging nectariferous and polliniferous plants. For example, in *Echium plantagineum* L., if the carbohydrates concentration in floral nectar is lower than 40%, bees will prefer to harvest pollen rather than nectar [5]. Meanwhile, if the ambient temperature is sufficiently high enough to cause intense nectar dehydration in flower and a premature dehiscence of anthers, the bee also prefers to harvest pollen rather than nectar.

Inside the bees group, there are polylectic or generalist species (that feed from a wide group of plants) and there are oligolectic or specialist species (that visit only few floral species from one genus or family that are closely related). The honey bee visits high number of species, but they collect important quantities of food from a small group. Some bee species prefer to work on thickly populated floral species (strong bloom in space and in time) but the *Apis mellifera* L. also visits plants with less compact blooms (bloom spaced in time and place) [6].

Honey bees do not forage at random; they do it according to the energetic optimum. Among several flowers belonging to same species, the honey bee prefers those not previously visited by other insects, flowers with anthers filled pollen. They seek to economize harvesting time using as lower energy costs as possible in their displacements [7, 8, 9]. And the foragers of *Apis mellifera* are able to remember the moment of the day at which a particular source is more profitable [8]. Greco *et al*. [10] observed, on a plot of *Asparagus officinali*s L that honey bees start to harvest on one extreme of the plot and from row to row, advancing gradually as reserves are depleted. In *Rhus hirta* Sualwoth (ex *Typhina* L.) plots, these authors also proved that honey bees harvest pollen from masculine flowers in the morning (these masculine flowers hardly produce nectar).

Every moment, foraging cohort of *Apis mellifera* colony is distributed in groups harvesting from different plant species sources (both nectar and pollen collection), in parallel [4, 6]. On the other hand, every forager tends to remain loyal to the food source visited at the beginning of the day [4, 11, 12, 13, 14]. In view of these facts, we wanted to know if it is possible to establish some daily rhythm of harvesting behaviour in relation to the more common pollen sources in the region [1].

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A. mellifera is a very important pollinator agent; maybe because of their dense populations (it is a colonial species, and its importance is also growing, unfortunately, for the disappearance or populations decrease of other pollinator species [15] and because of their behaviour (faithful to source, great learning capacity, flexibility in the handling of different floral architecture and wide season active). These characteristics gives *A. mellifera* a great economic importance (honeybee is the principal pollinator of the crop–natural colonies and colonies introduced artificially are able to increase the quantitative production of crop pollinated by animals, up to 96% [15]-) and they also gives them a great environmental importance (favouring the pollination of wild plants, honeybee helps to keep the zone biodiversity). On the other hand, crops are a great source of food for the bee hive, for a brief period; for the rest of the active season, the bee hive needs nectar and pollen from wild sources for survival. And this gives a great importance to the populations of wild flora and to the relation between wild flora and honey bee (or other pollinators).

Our investigation group had established in several studies, by means of the pollen loads study harvested by *Apis mellifera* in Galicia (NW of Iberian Peninsula), a ranking of species which are common and abundant sources of pollen [16]. We have also obtained data about the influence that corolla colour, floral nectaries presence and density of populations exercise about election of pollen sources [1]. Later, protein content in pollen of different species and its influence about election of pollen sources were also studied [17]. Also, the study of the heterogeneity percentage of pollen loads showed a high fidelity grade of the honey bee to the floral species [18, 19].

Several authors appoint that individual foragers, in one definite date, are very faithful to the source visited previously [14, 20, 21, 22, 23, 24], but little is known about the perseverance of this fidelity to the species throughout the time.

The objective of this research is to answer questions such as: In the view of diversity of available offer, what is the bee behaviour in the harvesting process? Does the visit to different sources vary throughout the day? Does it happen throughout the season? And, are these variations quantitative and/or qualitative? Or, on the contrary, the *Apis mellifera* hives merely forage according to what is available at any time.

2. MATERIALS AND METHODS

The daily rhythm of pollen harvesting was studied, as well as the pollen origin and quantitative important relative of each pollen species, from four hives located in the South of Galicia (NW Spain): Marcón (42^{o}24'52.24'' N, 8^{o}37'3.41'' W; Huso 29, North Hemisphere, x: 531462.01, y: 4695871.31; Pontevedra), Lobios (41^{o}54'4.09''N, 8^{o}5'0.06''W; Huso 29, North Hemisphere, x: 576032.43, y: 4639205.68, Ourense) and the apiary of Viana do Bolo (42^{o}10'49.21''N, 7^{o}6'46.84''W; Huso 29, North Hemisphere, x: 655836.8, y: 4671522.58, Ourense) (Figure **1**). We selected these hives due to their different characteristics: location (Mediterranean –Viana– and Eurosiberian –Marcón in Miñense Subsector and, Lobios in Xuresiano-Queixense Subsector- Biogeographic Regions [25] and; semi urban -Marcón- and natural/rural –Lobios and Viana do Bolo- environments; a single hive for location –Marcón and Lobios- and two hives in one apiary -Viana-) and exploitation type (production for sale –Viana do Bolo-

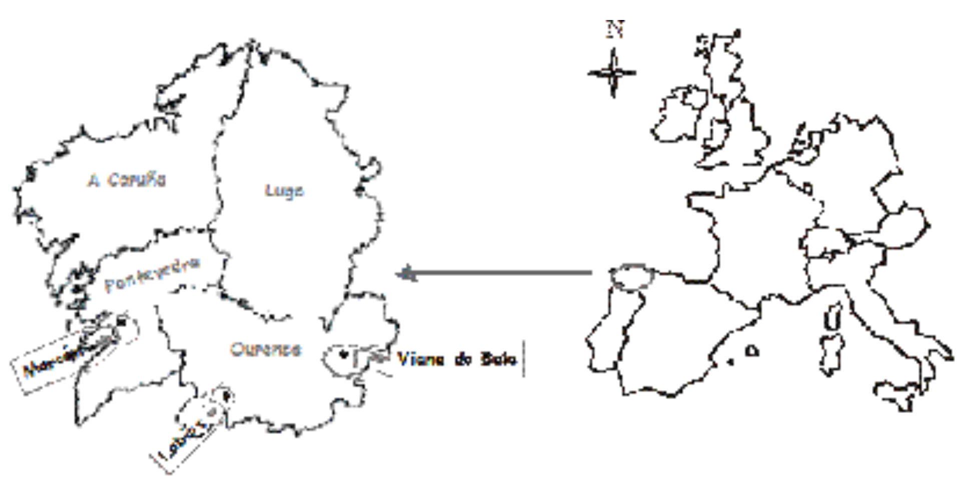

Figure 1: Locations of study beehives.

and for own consumption -Marcón and Lobios-). This allows us to look for constants between bee flora (commons sources of pollen and local variations) and the pollen foraging behaviour of the honey bee. It is also important to point out that pesticides effects in this research, not being non-existent –it is unquestionable its presence in the environment-, has got low intensity (low use of pesticides due to rural abandonment of this area).

Sampling by pollen-traps placed in the hive hole in different hours of the day (consensus was looked for between beekeepers and researchers to take four sample by day: morning, midday, afternoon and evening data; but this was not always possible, because there are variables hours of sampling), during several days and several months. The traps were emptied four times a day, and each quantity obtained made one sample. The pollen was dried on medium temperature (< 45ºC), targeted and kept for later analysis.

One gram of pollen from each sample was taken out for the study. In the elaboration of the microscopy slides, we had used the methodology of pollen analysis proposed by Erdtman [26]. To estimate the relative percentages in each pollen sample, we had used the method proposed by Vergeron [27] –counting and identifying 1200 pollen grains by sample, 400 grains/slide and 4 slide/sample, minimum.

Pollen was identified using the pollen reference collection of the Faculty of Sciences in Ourense and using Palynology identification keys [28, 29, 30, 31]. In this way, the relative importance of each pollen source in each total sample was estimated, and we also estimated the daily rhythm of foraging from each pollen source. This allows us to know the pollen sources visited by honey bees at different intervals, each day, from March to October 2009.

The pollen origin and the quantitative important relative of each pollen species were studied, as well as the daily rhythm of pollen harvesting.

The data obtained from qualitative and quantitative analyses of pollen samples were basis for several statistical treatment.

By χ^2 we tested the significance of evident differences in hour intervals of the day. Differences of sampled quantities inside of hive; and differences in percentages of every species in the curse of the day, for every hive independently.

After these, we analyzed foraging behavior in the curse of the day from pollen sources, for every hive independently, by graphical representations (lines graphs) with the tendency lines.

Table 1: √ **= Value of** χ^2 **is Significance, H₁ Accepted (There are Differences no not Attributable to Chance)**

b: Differences significant about % harvested from each pollen source			
Marcón Hive Dominant Sources		**Lobios Hive Dominant Sources**	
Castanea sativa	√	*Cytisus scoparius*-t.	√
Cultivated *Graminae*	√	*Erica arborea*	√
Cytisus scoparius-t.	√	*Erica umbellata*	√
Echium vulgare-t.	√	*Eucalyptus globulus*	√
Eucalyptus globulus	√	*Halimium alyssoides*	√
Oenothera stricta	√	*Plantago lanceolata*	√
Plantago lanceolata	√	*Quercus pyrenaica*	√
Rubus ulmifolius-t.	√	*Quercus robur*	√
Scandix pect.-ven.-t.	√	*Rubus ulmifolius*-t.	√
Wild *Graminae*	√	*Salix fragilis*	√
		Sedum acre	√
Viana A Hive Dominant Sources		**Viana B Hive Dominant Sources**	
Castanea sativa	√	*Anthemis arvensis*-t.	√
Cytisus scoparius-t.	√	*Castanea sativa*	√
Plantago lanceolata	√	*Consolida ajacis*	√
Quercus pyrenaica	√	Cultivated *Graminae*	√
Ranunculus peltatus	√	*Cytisus scoparius*-t.	√
Raphanus raphan.-t.	√	*Plantago lanceolata*	√
Salix fragilis	√	*Quercus pyrenaica*	√
		Ranunculus peltatus	√
		Raphanus raphan.-t.	√
		Rubus ulmifolius-t.	√
		Salix fragilis	√

a: Differences significant about grams of pollen harvested	
Hives	
Marcón	√
Lobios	√
Viana A	√
Viana B	√

Table 2: Marcón Species that Reach Dominant Percentages (Higher than 45% of the Total Sample) and their Maximum Values. The Shaded Dates Correspond to the Collecting Season of each Pollen Sources

% Pollen Sources Dominant of Marcón Hive	May		June				July				Aug.
	Days		Days				Days				Days
	15	22	5	12	19	29	5	14	23	31	7
Scandix pecten-veneris-t.	50.8^{4}										
Eucalyptus globulus Labill.		100^{1} 59.5^{2}									
Rubus ulmifolius-t.			63.1^{3}		60.2^{3} 55.6^{4}	52.7^{3} 66.1^{4}		60.8^{4}			
Wild *Graminae*			61.8^{1}								
Echium vulgare-t.				56.1^{2} 58.3^{3} 49.1^{4}	68^{1} 52.2^{2}					55.8^{3}	86.2^{4}
Cytisus scoparius-t.						76.4^{2}		85^{2}	56.5^{3}		
Oenothera stricta Ledeb ex Link						100^{1}					
Castanea sativa Mill.							82.8^{2} 76.9^{3} 63.3^{4}				
Plantago lanceolata L.							85.7^{1}	75^{1}			
Cultivated *Graminae*										$66.7^{1,2}$	

Superscripts: 1means sample of 10 hours; 2, 12 hours; 3, 16 hours; and 4, 18 hours harvesting season is marked (—).

Using Excel program, we present, in lines graphs independents for each hive, the relative percentage reached by every pollen taxon in the hour intervals (grey points unit by grey lines), through the sampled days in which this taxon is collected (see the shaded dates in Tables **2, 3, 4** and **5**, of Results) (see the

Table 3: Lobios Species that Reach Dominant Percentages (Higher than 45% of the Total Sample) and their Maximum Values. The Shaded Dates Correspond to the Collecting Season of each Pollen Sources

% Pollen Sources Dominant of Lobios Hive	March	April					May				June		July
	Days	Days					Days				Days		Days
	13	3	10	18	24	29	9	16	23	30	12	20	17
Quercus robur L.		67.1^{2} 57.2^{3}											
Salix fragilis L.	98.2^{2} 83^{4} 74.9^{6}												
Cytisus scoparius-t.			61.9^{2} 60.8^{4}	78.8^{3}		57.3^{4}							
Erica umbellata Loefl. ex L.				63.3^{6}	47.5^{6}								
Erica arborea L.						64^{2}							
Plantago lanceolata L.						45.8^{3}							
Halimium alyssoides (Lam.) K. Koch							$79,2^{2}$ $60,4^{3}$						
Eucalyptus globulus Labill.								67.4^{2}					
Sedum acre L.									47.2^{4}	55.7^{3}			
Quercus pirenaica Willd.												45.9^{2}	
Rubus ulmifolius-t.												80.7^{4} 88.4^{6}	57.3^{2} 93.5^{3} 99.3^{4} 94.1^{6}

Superscripts: 1 means sample of 10 hours; 2, 12 hours; 3, 14 hours; 4, 16 and 5, 18 hours 6, 20 hours harvesting season is marked (—).

Table 4: Viana A Species that Reach Dominant Percentages (Higher than 45% of the Total Sample) and their Maximum Values. The Shaded Dates Correspond to the Collecting Season of each Pollen Sources

% Pollen Sources Dominant of Viana A Hive	April			May		June		July	August		Sept.
	Days			Days		Days		Days	Days		Days
	4	11	18	3	25	16	26	16	4	30	12
Salix fragilis L.	90.2^{3} 84.6^{5} 82.5^{6}										
Raphanus raphanistrum-t.			82.1^{2} 71.4^{3} 64.4^{4} 77.9^{5}		76.8^{4} 84.3^{5}						
Cytisus scoparius-t.				61.9^{4} 49.8^{5}						93.2^{2} 95.2^{4}	95.8^{3} 90^{6}
Quercus pyrenaica Willd.					69.8^{1} 82^{2}		85.7^{1}				
Ranunculus peltatus Schrank						90^{2}					
Castanea sativa Miller							75.3^{4} 91.7^{5}	99^{2} 92^{4} 91^{5}			
Plantago lanceolata L.									83.6^{2}		

Superscripts: 1 means sample of 10 hours; 2, 12 hours; 3, 14 hours; 4, 16 hours; 5, 18 hours 6, 20 hours harvesting season is marked (—).

Table 5: Viana B Species that Reach Dominant Percentages (Higher than 45% of the Total Sample) and their Maximum Values. The Shaded Dates Correspond to the Collecting Season of each Pollen Sources

% Pollen Sources Dominant in Viana B Hive	April			May		June		July		August		Sept.
	Days			Days		Days		Days		Days		Days
	4	11	18	3	25	16	26	16	29	4	30	12
Salix fragilis L.	65.2^{3} 70.4^{5} 67.2^{6}											
Raphanus raphanistrum-t.		63.1^{3} 46^{4}	69.3^{3} 84.6^{4} 81.1^{5}		70.1^{4} 87.1^{5}							
Cytisus scoparius-t.				61.9^{4} 49.8^{5}							92^{2} 98.1^{4} 86.1^{5}	95.6^{3} 98.4^{6}
Quercus pyrenaica Willd.					85.1^{2}							
Anthemis arvensis-t.										46.8^{5}		
Castanea sativa Miller						75.3^{4} 91.7^{5}	88^{4} 83^{5}	98.1^{2} 95^{4} 86.4^{5}				
Ranunculus peltatus Schrank						80^{2}	77^{1}					
Rubus ulmifolius-t.						80.6^{4}			47.9^{4} 97.7^{5}			
Consolida ajacis (L.) Schur.							53.8^{2}					
Plantago lanceolata L.									77.6^{2}			
Cultivated Graminae										61.3^{2}		

Superscripts: 1 means sample of 10 hours; 2, 12 hours; 3, 14 hours; 4, 16 hours; 5, 18 hours 6, 20 hours harvesting season is marked (—).

Figures **4, 5, 6** and **7**, of Discussion and Conclusion). A mathematic expression that allows representing graphically the trend of the experimental results was looked for. For that, the adjustment of the tendency line to the observed results was tested mathematically means of straight, logarithm and polynomial lines; and we obtained that the best adjustment corresponds to lines represented by polynomial functions of variable grade -f $^{P(grade)}$- for each case (2-6) (see thick lines overlapped in the Figures **4, 5, 6** and **7**, of Discussion and Conclusion). It was also added the tendency line in sources with only one day of data. In spite of that, these graphs have not got very much statistical strength; they describe promptly the foraging trend of each pollen source.

3. RESULTS

The treatment and presentation, in a brief and concise form, of the large number of analysed samples (169 samples) is a really complex subject. For this, we decided only present data obtained about Dominant sources (those that reach percentages up 45% of the total sample [32], at last in one sample).

Results show the diversity of plants using as pollen sources by honey bees (187 species included in 54 families –global results can be seen in Doctoral Tesis: "*El análisis del polen corbicular y estudio de factores de la biología floral que condicionan la elección de especies poliníferas*" [33]). Some pollen sources were intensively visited and they contributed with high quantities; others pollen sources contribute with smaller quantities. This suggests that the honey bees choose preferably some species and, on the contrary, they ignore other sources, like it was proved in previous publications [33, 34, 35, 36, 37].

Most pollen sources was identified in species level but, others was identified in pollen type level (artificial category used in melissopalynology that gets together

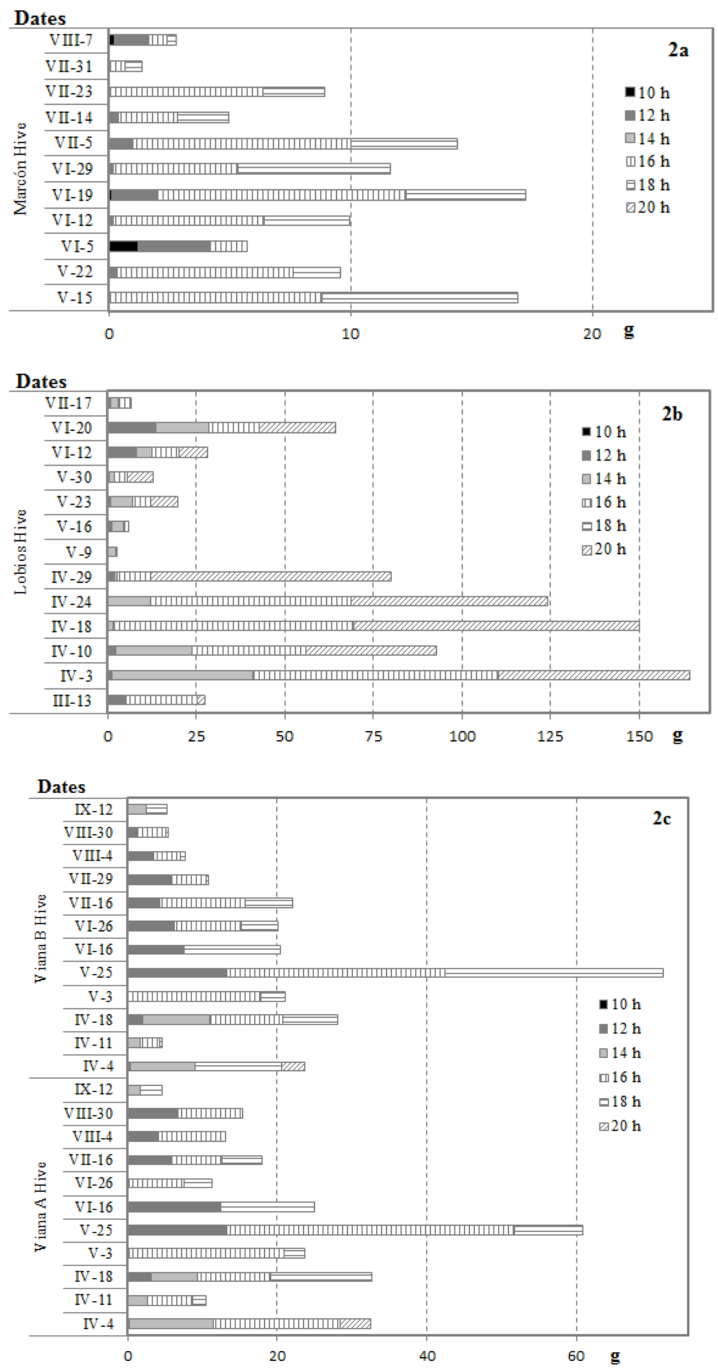

Figure 2a, 2b and 2c: Weight, in grams, of harvested pollen in the different intervals, in all date sampled, in the Marcón, Lobios, Viana A and B hives (different intervals sampled are present by different plots in bars of graphs).

several species with pollen grains very similar and very difficult to distinguish it).

3.1. Quantity of Pollen Foraged

The quantity of pollen collected in the studied hives is highly variable; both to the activity season, as throughout the day and as among colonies. Figure **2** shows weight of each sample (in grams) and variations.

Marcón hive (from May 15 to August 7) harvests lower quantities of pollen. The highest quantities were collected on May 15 and June 19, about 17 g/day (Figure **2a**).

Lobios hive was sampled from March 13 to July 17; the highest quantity of pollen was 80 g harvested at 20:00 on April 18. The quantities harvested in May and July are similar to those collected in Marcon hive (Figure **2b**).

The two hives of Viana were sampled from April 4 to September 12. The highest amount of pollen was harvested in May; the most productive day was May 25, for both colonies, with more than 60 g for Viana A and 71 g for Viana B (Figure **2c**).

Gathering the hour data of all studied hives together, it is possible observe that the 60% of the days present the higher pollen quantities in the central hours of the day (Figure **3**). A similar behaviour was described by Seijo-Coello *et al*. [38] in relation to the kiwi pollination in Galicia. Other authors [4, 39, 40, 41, 42, 43, 44] propose the highest activity during the morning.

It is possible to observe variations of harvested quantities of pollen throughout the day. We used the statistical by the statistical χ^2 to test these variations (Table **1a**).

From results of weight of harvested pollen (in grams), by the statistical χ^2 (calculations made with excel program) we tested the initial hypothesis (H_1) which mains that there are variations in the pollen quantity harvested by foragers with regard to hour intervals that are not attributable to random; in opposite to the null hypothesis (H_0) which mains that *Apis mellifera* foragers harvest pollen throughout the day without variations in the different hourly intervals.

The obtained values are significant in the four hives (α = 0.001). The H_0 is repulsed and the initial hypothesis is accepted as correct.

The results are significant for all the studied hives. The harvested quantities pollen's variations are not attributable to chance.

3.2. Floral Origin of Pollen Foraged

After having analysed quantity of pollen carried by honey bees to hives, we analysed the origin of this crop, the variations and the significance of these variations.

This wide study shows that these four colonies exploit a wide group of pollen sources (187 species belong to 54 families). The importance of each sources (percentage of pollen contribution) is variable (there are 10 taxa Dominant of 52 in Marcón; 11 of 59 in Lobios; 7 of 48 in Viana A and, 11 Dominant taxa of 47 total taxa in Viana B).

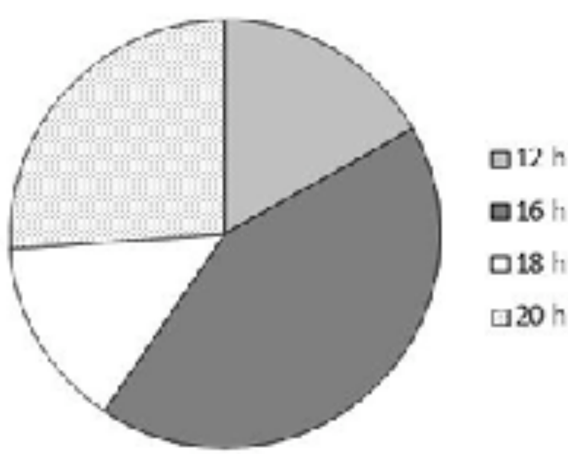

Figure 3: Quantity of harvested pollen (g) in the different intervals –to noon, 16:00 p.m., 18:00 p.m. and till 20:00 p.m.-, in all four hives added together (Marcón does not present samples of 20:00 p.m.; and in Lobios, the 18:00 p.m. and 20:00 p.m. samples are added in a unique 20:00 p.m. sample).

The data obtained show that every pollen source presents a period of foraging by honey bees with peak/s of dominant percentage/s. The breadth of foraging season and the peaks vary according to the source and the hive. (Tables **2** to **5**).

In relation to taxon diversity, there are species common to several hives (for example *Cytisus scoparius*-t. and *Plantago lanceolata* are present in the four hives; *Castanea sativa* and *Rubus ulmifolius*-t. are dominants in three hives) and others turn up occasionally in one hive (for example *Consolida ajacis* or *Oenothera stricta*).

Variations are clear. We used the statistical χ^2 to test if these differences are significant. From data of pollen percentages harvested from each dominant source present in ours samples, we tested the H_1 at species level (*A. mellifera* foragers harvest pollen from each source throughout the day with variations regarding to hour intervals).

Obtained values are significant for all dominant sources, in the four hives ($\alpha = 0.001$). From that, we may accept the initial hypothesis (H_1: there are variations in the pollen harvesting from plant source with regard to hour intervals that are not attributable to chance) as the correct hypothesis and repulsing the null one (H_0: variations attributable to chance) (Table **1b**).

4. DISCUSSION AND CONCLUSION

4.1. Daily Rhythm of Pollen Foraging

According the presents results, some pollen sources are more intensely exploit by *Apis mellifera* than others. This suggests that the honey bees choose preferably some species and, on the contrary, they ignore other sources, like it was proved in previous publications [32, 33, 34, 35, 36].

Moreover it is clear that, in this study, honey bee chooses preferably wild plants.

The differences in the length of foraging season of different sources are clear. Some sources are brief (for example *Oenothera stricta* in Marcón hive, or *Consolida ajacis* in Viana B hive), while others stretch to the total length of this study (for example *Cytisus scoparius*-t). It is important to emphasize the case of pollen type, it clusters blooms in succession and/or overlapped –blooms of similar floral sketch "in tandem or in cascade-. We could observe several peaks separated of *C. scoparius*-t (in four hives) and of *Raphanus raphanistrum*-t (both hives of Viana).

The hives recollected different quantities of pollen in different intervals of day. Recollected quantities are significantly bigger between 12:00–16:00 (central hours of day) as it was tested in this work. (Figures **2** and **3**).

Furthermore, this study proves that different pollen sources are recollected by studied hives in different percentages and at different times of day (Tables **1** and **2** to **5**). In this essay, we studied the tendencies of pollen harvesting by to analyse the data recorded from each source throughout by active season.

Studying the dates of each pollen source, it is possible to observe that each dominant source sketches a daily rhythm of pollen foraging by honey bee hives. From the same plant species, pollen is harvested by honey bee more intensively in the morning, from another floral species in midday, in afternoon and, even during the evening. Also there are particular cases of plants whose harvesting rhythm was not possible to determine, either because its pollen appears in insufficient quantity, or because it is harvested at different times of the day or because the honey bee follows variable patterns when it utilize this source specifically.

For to analyse the foraging behaviour of honey bee hive from each pollen sources dominant, we present the data in line graphs. And with Excel we looked for a mathematic expression that describes the tendency of *Apis mellifera* behaviour when it forages from several pollen sources (according to polynomial functions of variable grade -$f^{P(grade)}$- for each case). Thick lines overlapped in the Figures **4-7** mark the harvesting tendency in each source.

Regarding these graphs, one can appreciate that each species of visited plant seems to show a particular tendency of use by honey bee. It is possible to establish categories of recollecting behaviour.

Results show sources of **morning harvesting** of pollen: *Consolida ajacis*, in Viana B ($f^{P(3)}$) (Figure 7a); *Oenothera stricta*, in Marcón ($f^{P(3)}$) (Figure **4a**); *Ranunculus peltatus*-t., in A and B hives of Viana do Bolo ($f^{P(3)}$) (Figures **6a** and **7b**); wild *Graminae* group, in Marcón ($f^{P(3)}$) (Figure **4b**).

Species with rhythm **of pollen harvesting mainly in central hours** of day (between 12:00 and 16:00) are *Scandix pecten-veneris*-t., in Marcón ($f^{P(5)}$) (Figure **4c**).

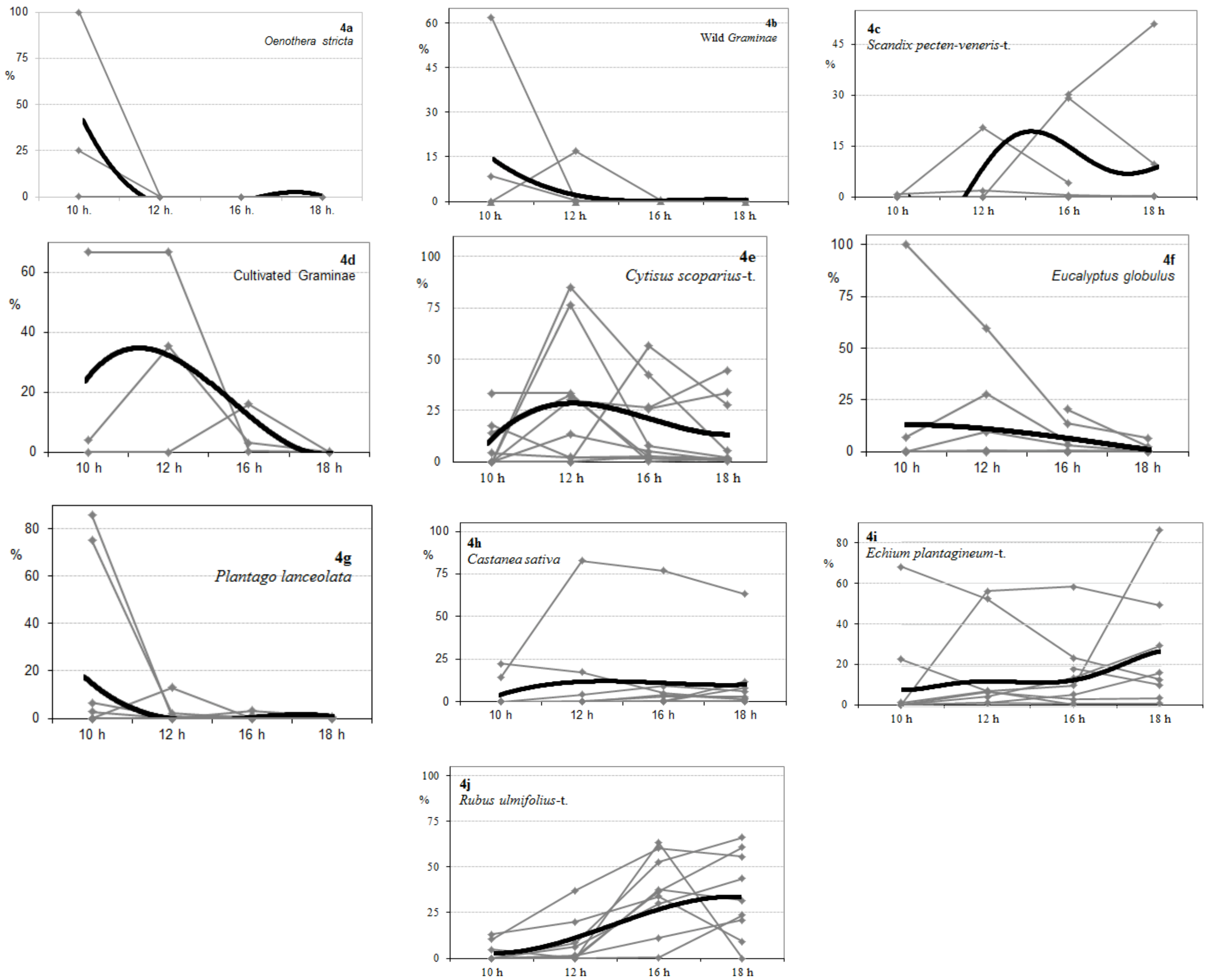

Figure 4: Daily rhythm for pollen sources from **Marcón** hive. Each graphic shows the percentages of pollen contributed in the hourly intervals by each source –grey points and grey lines-, and the tendency of its group –thick black line- (results of samples of shaded dates in Table **2**). Morning Rhythm: *Oenothera stricta* (**4a**) and Wild *Graminae* (**4b**); Central Hours of the day Rhythm: *Scandix pecten-veneris*-t. (**4c**); Afternoon Rhythm: cultivated *Graminae* (**4d**), *Cytisus scoparius*-t. (**4e**), *Eucalyptus globulus* (**4f**), *Plantago lanceolata* (**4g**); Evening Rhythm: *Castanea sativa* (**4h**), *Echium plantagineum*-t. (**4i**) and *Rubus ulmifolius*-t. (**4j**).

At this point, we include an idea important for us, the foraging flexibility of *Apis mellifera*.

The pollen foraging from *Erica arborea* presents a daily rhythm on central hours of the day (f $^{P(3)}$). This is true for all days with *Erica arborea* presence (Figure **5b** –drop graphic of Figure **5a**, rhythm of pollen foraging on central hours of the day), except on April 29. The irruption of *Plantago lanceolata* on this day –April 29- breaks the rhythm foraging causing a slant in the tendency of pollen harvesting from *E. arborea* (Figure **5a** show the global tendency, with the results of all samples).

The sources with **afternoon rhythm** of pollen harvesting, kept it from morning to 18:00 hours (in the same case, even it is possible to observe the valley of pollen collection in central hours of day), were: cultivated *Graminae* group (f $^{P(3)}$) in Marcón (Figure **4d**) and Viana B (Figure **7c**); *Cytisus scoparius*-t. (f $^{P(3,\ 3,\ 5,\ 6)}$) (Figures **4e, 5c, 6b** and **7d**); *Eucalyptus globulus*, in Marcón and Lobios (f $^{P(3)}$) (Figures **4f** and **5d**); *Halimium alyssoides* of Lobios (f $^{P(3)}$) (Figure **5e**); *Plantago lanceolata*, in all colonies studied (f $^{P(3,\ 4,\ 5,\ 4)}$) (Figures **4g, 5f, 6c** and **7e**); *Quercus pyrenaica*, in Lobios (f $^{P(3)}$) (Figure **5g**) and both Viana hives (f $^{P(5)}$) (Figure 6d and 7f); *Quercus robur*, in Lobios (f $^{P(3)}$) (Figure **5h**); and *Raphanus raphanistrum*-t. in Viana hives (f $^{P(6)}$) (Figures **6e** and **7g**).

It is interesting to point out the case of the *Cytisus scoparius*-t. [in the four hives this pollen type shows a tendency represented by a polynomial function of grade five (f $^{P(5)}$)]. Nevertheless, two patterns could be stated. One represented by Marcón and Lobios hives, in which the tendency in recollection is more intensive

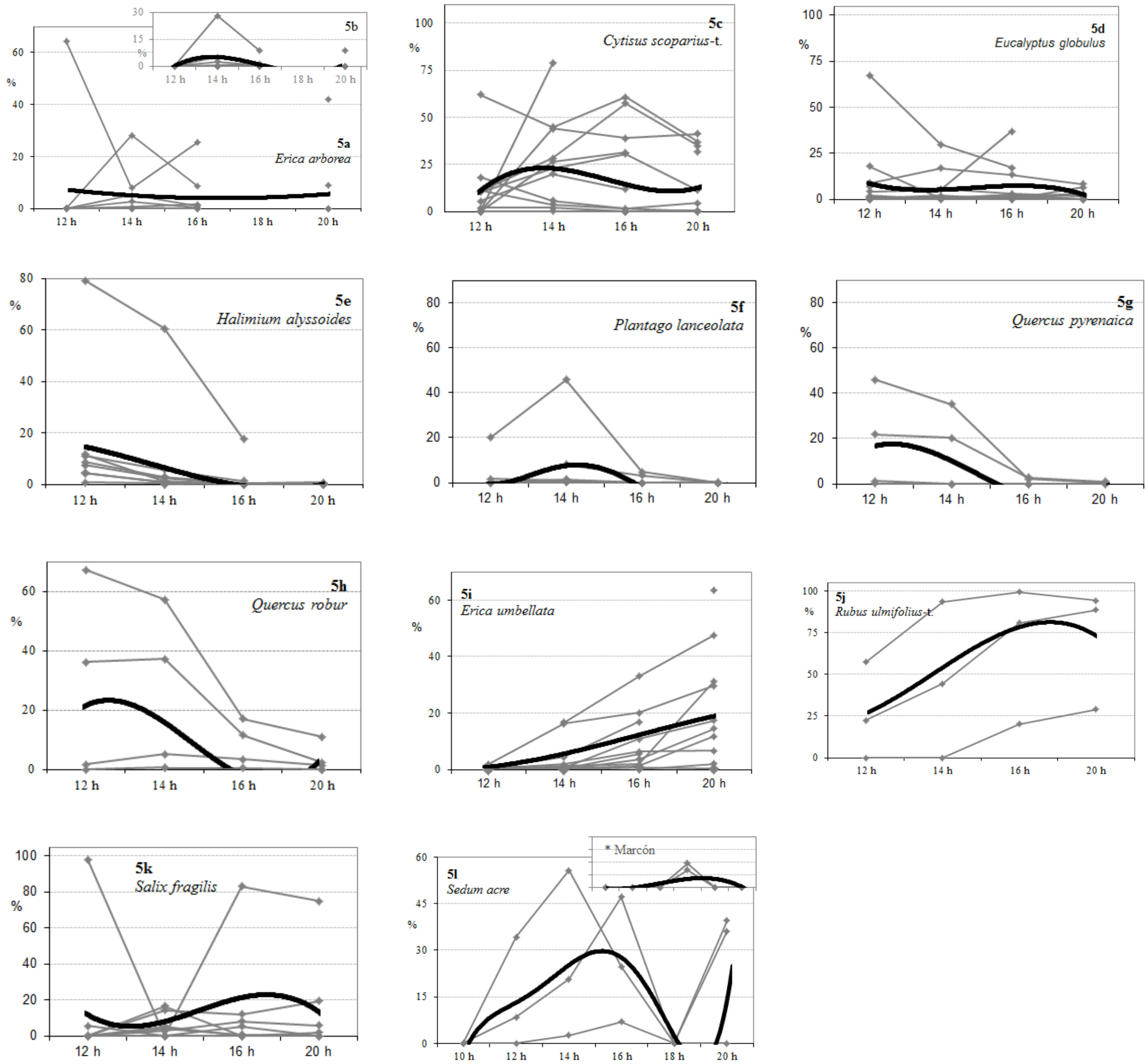

Figure 5: Daily rhythm for pollen sources from **Lobios** hive. Each graphic shows the percentages of pollen contributed in the hourly intervals by each source –grey points and grey lines-, and the tendency of its group –thick black line- (results of samples of shaded dates in Table **3**). Central Hours of the day Rhythm: *Erica arborea* (**5a** and **5b –** rhythm of this source in Marcón hive, like explanatory case was added-); Afternoon Rhythm: *Cytisus scoparius*-t. (**5c**), *Eucalyptus globulus* (**5d**), *Halimium alyssoides* (**5e**), *Plantago lanceolata* (**5f**), *Quercus pyrenaica* (**5g**), *Quercus robur* (**5h**); Evening Rhythm: *Erica umbellata* (**5i**), *Rubus ulmifolius*-t. (**5j**), *Salix fragilis* (**5k**) and *Sedum acre* **–**rhythm of this source in Marcón hive, like explanatory case was added**-** (**5l**).

in the central hours of the day (Figures **4e** and **5e**); and the other one, the pattern observed in Viana do Bolo hives (Figures **6b** and **7d**), in which a increment of pollen foraging takes place between 16:00 to 18:00 hours. This fact could be explained by the different species that belong to pollen type in each biogeographic sector (this pollen type includes species such as: *Adenocarpus complicatus* (L.) J.Gay, *Cytisus multiflorus* (L'Hér.) Sweet, *Cytisus scoparius* (L.) Link, *Cytisus striatus* (Hill) Rothm., *Genista florida* L., *Pterospartum tridentatum* (L.) Willk., *Ulex europaeus* L. and *U. minor* Roth, which blooms happen throughout the active season beehive).

The fact that this pollen type displays an interesting recollecting activity along the day is something worth highlighting.

In the case of *Raphanus raphanistrum*-t, the pattern of maximum harvesting in afternoon is constant for both studied hives (Figures **6e** and **7g**) and in the union of two hives too.

The pollen sources with a **rhythm of foraging in the evening**, the recollection increase during the day, were: *Anthemis arvensis*-t., in B hive of Viana (f $^{P(5)}$) (Figure **7h**); *Castanea sativa* (f $^{P(3)}$), in Marcón (Figure 4h) and Viana (Figures **6f** and **7i**); *Echium vulgare*-t., in

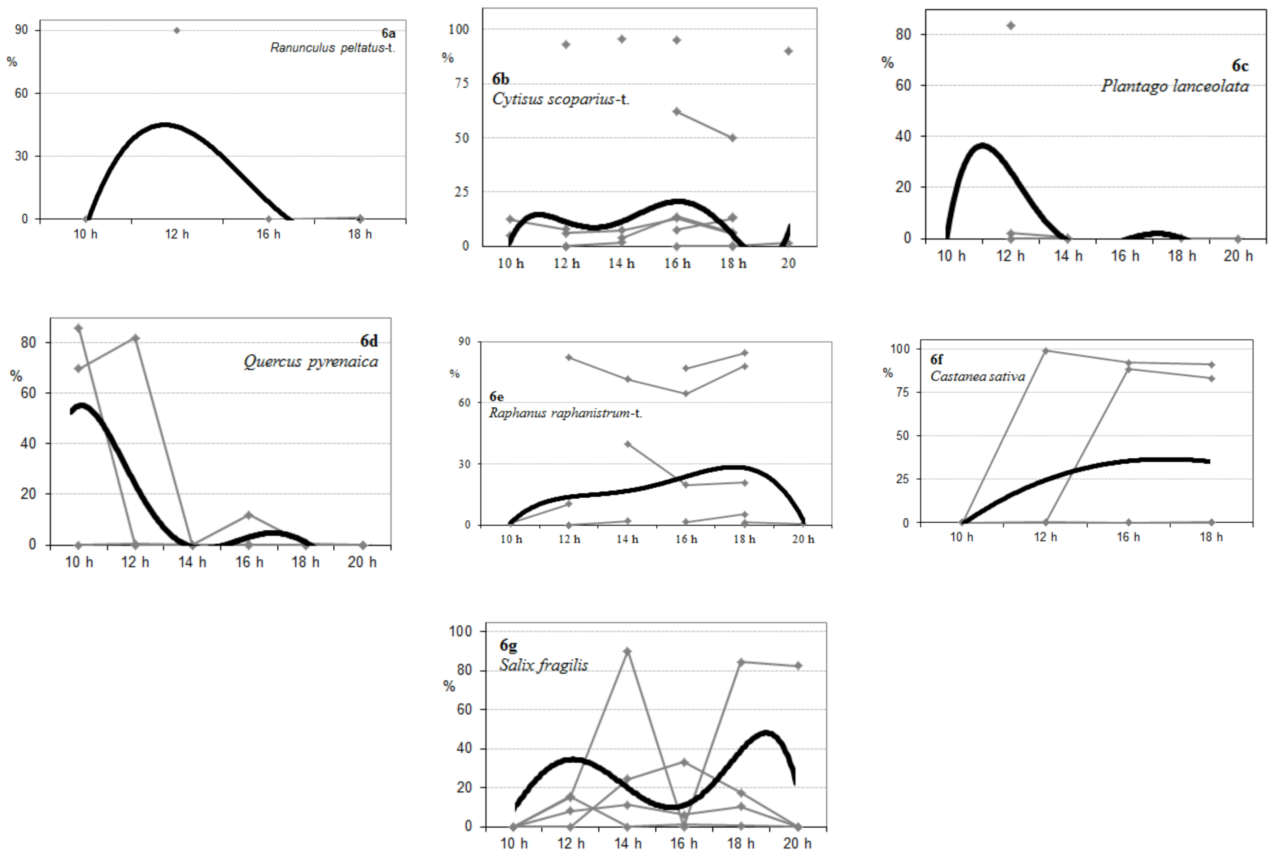

Figure 6: Daily rhythm for pollen sources from **Viana A** hive. Each graphic shows the percentages of pollen contributed in the hourly intervals by each source –grey points and grey lines-, and the tendency of its group –thick black line- (results of samples of shaded dates in Table **4**). Morning Rhythm: *Ranunculus peltatus*-t. (**6a**); Afternoon Rhythm: *Cytisus scoparius*-t. (**6b**), *Plantago lanceolata* (**6c**), *Quercus pyrenaica* (**6d**), *Raphanus raphanistrum*-t. (**6e**); Evening Rhythm: *Castanea sativa* (**6f**) and *Salix fragilis* (**6g**).

Marcón (f $^{P(5)}$) (Figure **4i**); *Erica umbellata*, in Lobios (f $^{P(3)}$) (Figure **5i**); *Rubus ulmifolius*-t., in Marcón hive (Figure **4j** -f $^{P(3)}$-), Lobios (Figure **5j** -f $^{P(3)}$-) and Viana B (Figure **7j** -f $^{P(4)}$-); *Salix fragilis*, in Lobios (Figure **5k** -f $^{P(3)}$-) and both colonies of Viana (Figures **6g** and **7k** -f $^{P(5)}$-) and *Sedum acre* in Lobios (Figure **5l** -f $^{P(5)}$-) and Marcón (drop graphic in Figure **5l** -f $^{P(3)}$-).

Rubus ulmifolius-t., one of the main sources of pollen in the region [45], is foraged during the day (like to *Castanea sativa* and *Cytisus scoparius*-t. pollen) and its percentage increases during the day. The analysis of the tendency in each individual hive, represented by polynomial functions of degrees three and five, indicates an increase along the day (Figures **4j, 5j** and **7j**).

Sedum acre is harvested from 10:00 hours. Its tendency is defined by a polynomial function of degree five (f $^{P(5)}$), however It can be seen a minimum after midday, the maximum takes place at 16:00 hours, and falls and goes up in the 20:00 hours sample. Results of Lobios hive (main pollen in same samples) are those tighter to this rhythm of foreging (Figure **5l**). In Marcón hive, it is harvested in lower quantities, this explains why there are not such rebound in the 20:00 hours sample (exhaustion of the lower supply); its tendency tights to a polynomial function of degree three (drop graphic in Figure **5l**).

Cytisus scoparius-t. and *Plantago lanceolata* pollen sources present the same rhythms of foraging in the four colonies studied. Other taxa, as *Castanea sativa*, *Quercus pyrenaica*, *Salix fragilis* and *Rubus ulmifolius*-t., also show the same rhythms of foraging in three hives. These sources are regarded as sources of high polliniferous value in Galicia [16]. The same taxa collected in dominant percentages in two of the studied hives, show the same pattern in the other ones where these sources are harvested with more modest values -*Eucalyptus globulus*, *Ranunculus pelatus*-t., *Raphanus raphanistrum*-t. and *Sedum acre*- [36].

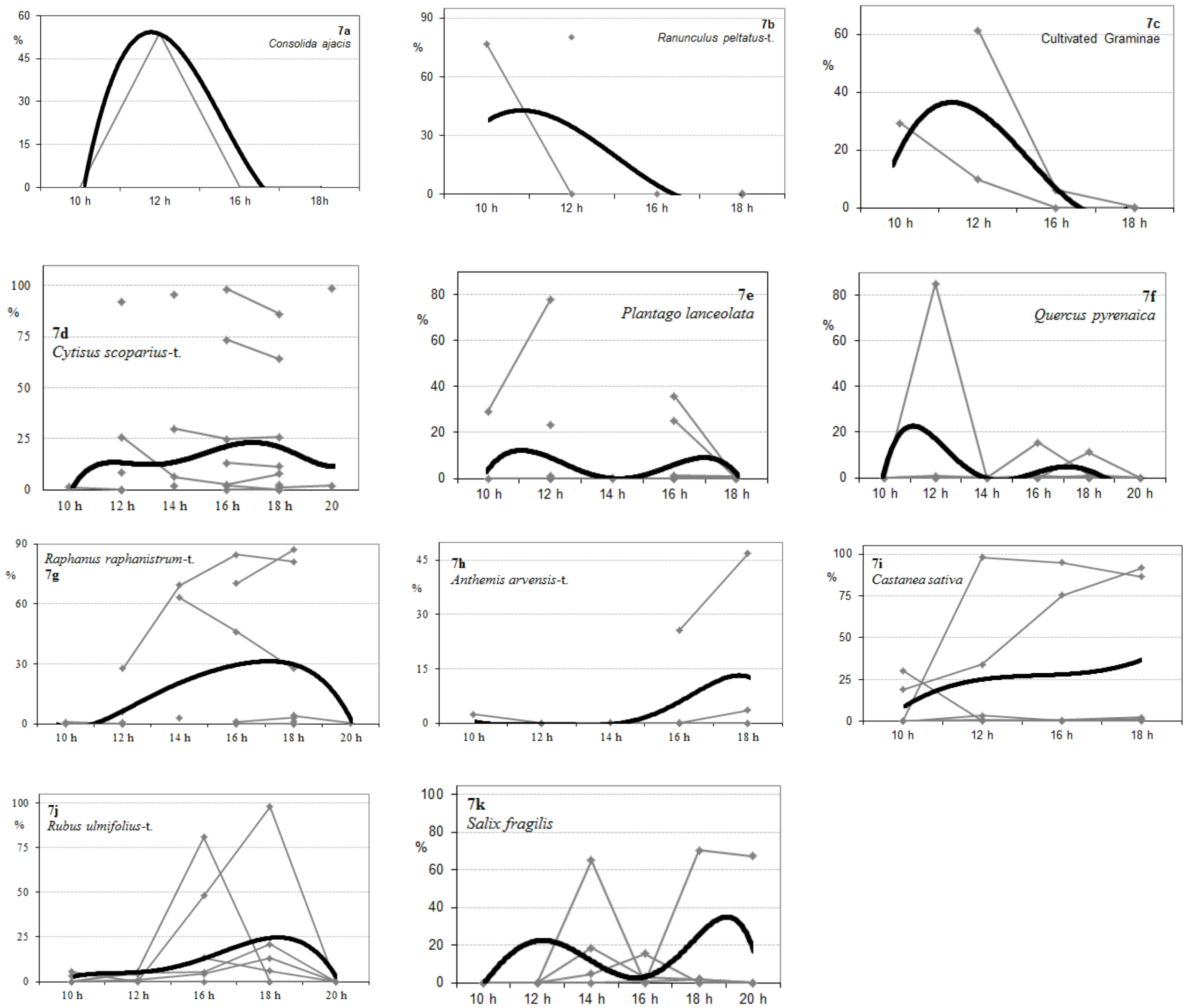

Figure 7: Daily rhythm for pollen sources from **Viana B** hive. Each graphic shows the percentages of pollen contributed in the hourly intervals by each source –grey points and grey lines-, and the tendency of its group –thick black line- (results of samples of shaded dates in Table **5**). Morning Rhythm: *Consolida ajacis* (**7a**), *Ranunculus peltatus*-t. (**7b**); Afternoon Rhythm: cultivated *Graminae* (**7c**), *Cytisus scoparius*-t. (**7d**), *Plantago lanceolata* (**7e**), *Quercus pyrenaica* (**7f**), *Raphanus raphanistrum*-t. (**7g**); Evening Rhythm: *Anthemis arvensis*-t. (**7h**), *Castanea sativa* (**7i**), *Rubus ulmifolius*-t. (**7j**) and *Salix fragilis* (**7k**).

The Viana hives show coincidence with the daily rhythm of pollen foraging from the Lobios colony. The hive from Marcón, on the coast, shows several differences. Comparing the studied colonies it is possible to see noticeable dissimilarities. These dissimilarities can be attributable to light variations of species phenology and to light variances of flora, in different biogeographical sectors. The paper of Dukku, Russom and Domo [46] also point to this idea, they stated that the foraging activity is more directly related with the local offer than with the zone climatology (within the logical range of physiological needs of flight).

On the other hand, despite the high level of parallelism that the Viana hives show, it is possible to appreciate differences in the sources choice. *Consolida ajacis* and *Anthemis arvensis*-t. pollen only appears in colony B but does not in colony A. Undoubtedly, colonies usually choose in each region the same common sources and exceptionally, they also collect other plants which foragers discover or choose in a particular case [36].

The rhythm of foraging behaviour has proven to be more diverse than the one obtained by García-García, Ortíz and Díez-Dapena [23] in an apiary from Huelva (Spain). In the hives studied here, the highest quantity of pollen was harvested in May and June in Marcón and Viana, and in April in Lobios; in Huelva the maximums were produced in March and April. In all cases, the activity is adjusted in the central hours of the day, midday and afternoon; like the present paper, the number of dominant pollen sources are fewer than the pollen sources existing in the hive range.

The daily rhythm of pollen recollection of *Salix fragilis*, *Ranunculus* and *Ericaceae* is similar to one proposed by Sabatini, Giordani and Bottazzi [39]. For *Quercus* pollen, Sabatini, Giordani and Bottazzi [39] propose a daily rhythm of harvesting in the evening and they attribute a morning pattern to *Cistaceae* and *Asteraceae*, taxa not considered in this study due to a low percentage and presence.

CONCLUSION

This research was carried on four *Apis mellifera* colonies located in different biogeographical locations from little wide territory (29,574 Km^2) but very diverse floristically (2,391 species of vascular flora [47]). Therefore, *Apis mellifera* can forage pollen from plants available in the hive's neighborhood, and throughout the day, in different quantities according to species. In whole, the most weight of pollen was collected in central hours of the day (12:00-16:00). However, it does not happen in all species.

It is possible to consider that *Apis mellifera* forage pollen from different sources and in different hours of the day. Profitable sources are visited repeatedly. The biological source imposes a specific rhythm, and many times it is modulated for the equilibrium relation created by the different strategy of advertisement.

Basically, it is possible to propose the same tendencies in forage rhythm for the pollen sources that were observed in this study. So, the highest pollen quantities are foraged in the morning hours, e.g. *Consolida ajacis*, wild *Graminae* group, *Oenothera stricta* and *Ranunculus peltatus*-t. In the central hours, from *Erica arborea* and *Scandix pecten-veneris*-t. In afternoon, cultivated *Graminae*, *Cytisus scoparius*-t., *Eucalyptus globulus*, *Halimium alyssoides*, *Plantago lanceolata*, *Quercus pyrenaica*, *Q. robur* and *Raphanus raphanistrum*-t. Sometimes, the same sources are already exploited in the midday, with a tendency to increase its percentages towards the evening, and mainly in the evening pattern. It is the case of *Anthemis arvensis*-t., *Castanea sativa*, *Echium vulgare*-t., *Erica umbellata*, *Rubus ulmifolius*-t., *Salix fragilis* and *Sedum acre*.

Finally, the honey bee colonies show variances in foraging pattern of the same pollen sources, probably as a result of the dynamic balance between the offers of local flora.

This paper shows/proves that the pollen foraging by *Apis mellifera* presents variations with regard to hour intervals (variations of quantity and quality or floral origin). Because of pollen foragers, at this latitude, work throughout the day, it is logical to think that the flora directs these rhythms of harvesting. But, to know which factor among the ones implicated in this process (character typical of each floral species; competi-tiveness among several offers of pollen; climatic and environmental conditions;…) is the critical factor, more data are needed.

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