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PRELIMINARY STUDY ON EFFECT OF AGRICULTURAL ACTIVITIES IN POLLEN SPECTRUM OF ARGENTINEAN HONEY

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A b s t r a c t

Honey floral origin is determined by the harversting region, but anthropogenic factors as agriculture expansion might modify the environmental flora and consequently honey floral origin. Argentina is one of the most important honey producers worldwide which, since the 1990s, has undergone an important agriculture transformation by the adoption of transgenic crops like soybean (*Glycine max*). However, little is known about the effects of this anthropogenic activity on the floral origin of honey or the statistical tools that could be used to analyse it. The objective of the present study was to evaluate the impact that these environmental modifications have on the pollen spectrum of honey. In order to achieve this, thirty-two samples of blossom honey were collected from three different ecoregions of the Buenos Aires province: Parana Delta and Islands, Espinal, and Pampa, in two different years: 1999 and 2014. The pollen spectrum of honey samples was determined and the data obtained was analysed with multivariate statistical techniques. It could be concluded that the pollen composition of honeys from different ecoregions has significantly changed in the past years because of agriculture expansion and adaptation of transgenic crops (p=0.007). Honey samples harvested in 1999 were characterized by high values of *Helianthus annuus*, while in 2014 an important presence of *Eryngium* sp., *Gleditsia triacanthos*, *Baccharis* type, *Trifolium* sp. and *Glycine max* was observed. The present results show that honey palynological results and multivariate statistical analysis could be used as a preliminary attempt to evaluate environmental modifications.

Keywords: agriculture, floral origin, honey, principal component analysis, redundancy analysis, soybean expansion

INTRODUCTION

Honey botanical and geographical classification, both of economic importance, are correlated. The floral origin is determined by the harvesting region. However, anthropogenic activities as agriculture may modify the pollen spectrum of different honey pollen types and negatively affect the bee population (Rollin et al., 2013). Argentina is one of the major honey exporters, and more than 50% of its honey production is accounted in the Buenos Aires province. Buenos Aires can be divided into different ecoregions, each with distinctive climate conditions, soil and flora (Patrignani, Lupano, & Conforti, 2016). This important apicultural region is also the main agricultural production zone, which during the 1990s suffered an important agriculture transformation by the adoption of transgenic crops and the technology package associated with it. The genetically modified soybeans were introduced in 1996, and since then the production has spiked (Leguizamón, 2014). The expansion of this model has been spread

also in very rich areas with high biodiversity with increasing levels of soybean production (Pengue, 2005). This might negatively affect bee population and honey production.

Previous authors had already associated the biodiversity of an apicultural area with the pollen profile of honey samples (González-Porto et al., 2013), so it could be determined which plants grew in the vicinity of the hives through the study of the honey pollen spectrum (González-Porto et al., 2013). This could be a useful method to evaluate biodiversity modifications associated with anthropogenic activities as agriculture over the years. However, there is limited information about the association of the honey pollen spectrum and the agricultural development and few studies have been focused on the analysis of the pollen profile in areas with particular agricultural characteristics (Alburaki et al., 2017).

Therefore, the objective of this work was to evaluate the changes in the floral precedence of honeys from the ecoregions of Espinal, Pampa, Parana Delta and Islands in the Buenos Aires province harvested in 1999 and 2014. This work proposes the use of two multivariate statistical tools in order to melissopalynologically analyse the environmental modifications.

MATERIAL AND METHODS

Honey collection
Thirty-two samples of blossom honey were collected directly from producers of three ecoregions of the Buenos Aires province: Parana Delta and Islands, Espinal, and Pampa, in two different years: 1999 (14 samples) and 2014 (18 samples). In order to obtain accurate results, all the samples were harvested in summer (December).

Pollen analysis
The pollen of honey samples was analysed out following the method described by Louveaux, Maurizio, & Vorwohl (1978) slightly modified by Fagúndez (2016). The pollen types were identified to species whenever possible, or to genus, tribe or family ranks.

Statistical analysis
Two multivariate statistical tools, Principal Component Analysis (PCA) calculated on the basis of the correlation matrix and Redundancy Analysis (RDA), were utilized with CANOCO 5 software (Plant Research International, Netherlands). Prior to the analysis, data was standardized and centred, so thus each species data had zero mean and unit variance (Braak & Šmilauer, 2012). Furthermore, in an attempt to reduce noise, species with less than ten occurrences were excluded from the multivariate analysis

PCA summarizes the high dimensional space of the data set and orders samples on a two-dimensional plane, while preserving the maximum allowable variance within the data (Braak & Šmilauer, 2012). The first two principal components PC1 (eigenvalue as fraction of the total sum of squares = 0.2973) and PC2 (eigenvalue as fraction of the total sum of squares = 0.1806) were considered statistically significant, based on Kaiser's criterion.

RDA, a constrained linear method, helps to determine the linear relationship between response variables, in this case the species pollen profile, and the matrix of such explanatory variables as geographical origin and harvesting year (Brogna et al., 2017). The null hypothesis associated with the test was that the pollen spectrum of the honeys analysed was unrelated to the explanatory variables, while the alternative hypothesis was that the pollen spectrum responded to the explanatory variables. If the p-value associated with the RDA was lower than 0.05 the null hypothesis was rejected. This analysis was only performed with candidate predictors with an adjusted p-value lower than 0.05. The Variance Inflation Factor (VIF) was checked if the VIF of one explanatory variable was higher than 20, then the variable was correlated with the others and was not included in the analysis.

RESULTS

The two principal components accounted for 48% of the total variance among samples, which seems to indicate poor correlation between the

variables. This probably accounts for the low number of samples used in this preliminary study (only 32), and future determinations should evaluate a higher number of honey samples. The PCA factor loading of species (Fig. 1) summarizes the patterns of the species composition in the honey samples. The position of each sample in the new ordination space can be seen in the diversity diagram (Fig. 2). Associations between samples and their species composition were determined through comparing Fig. 1 with Fig. 2. As presented in Fig. 2, samples from Parana Delta and Islands ecoregion in 1999 are close together, showing important similarities in their floral origin. These similarities could be explained by the presence of such native species as of *Sagittaria montevidensis* and *T. Polygonum hydropiperoide*

(Fig. 1). On the other hand, most of the honey samples from Espinal ecoregion harvested in 1999 were also close together in the upper section of the scatter plot (Fig. 2), which could be explained as the important presence of pollen from *Eucalyptus* sp. (Fig. 1). However, in 2014 the position of honey samples from Espinal was noticeably different (Fig. 2) and could better connected the presence of *Lotus* sp. pollen according to Fig. 1.

Fig. 2 shows that in general samples harvested in 1999 had less species diversity (they present a smaller symbol size in the diversity diagram). Tab. 1 provides the maximum/minimum/average percentage and the standard deviation of the pollen types found in the samples sorted by year of harvesting and location.
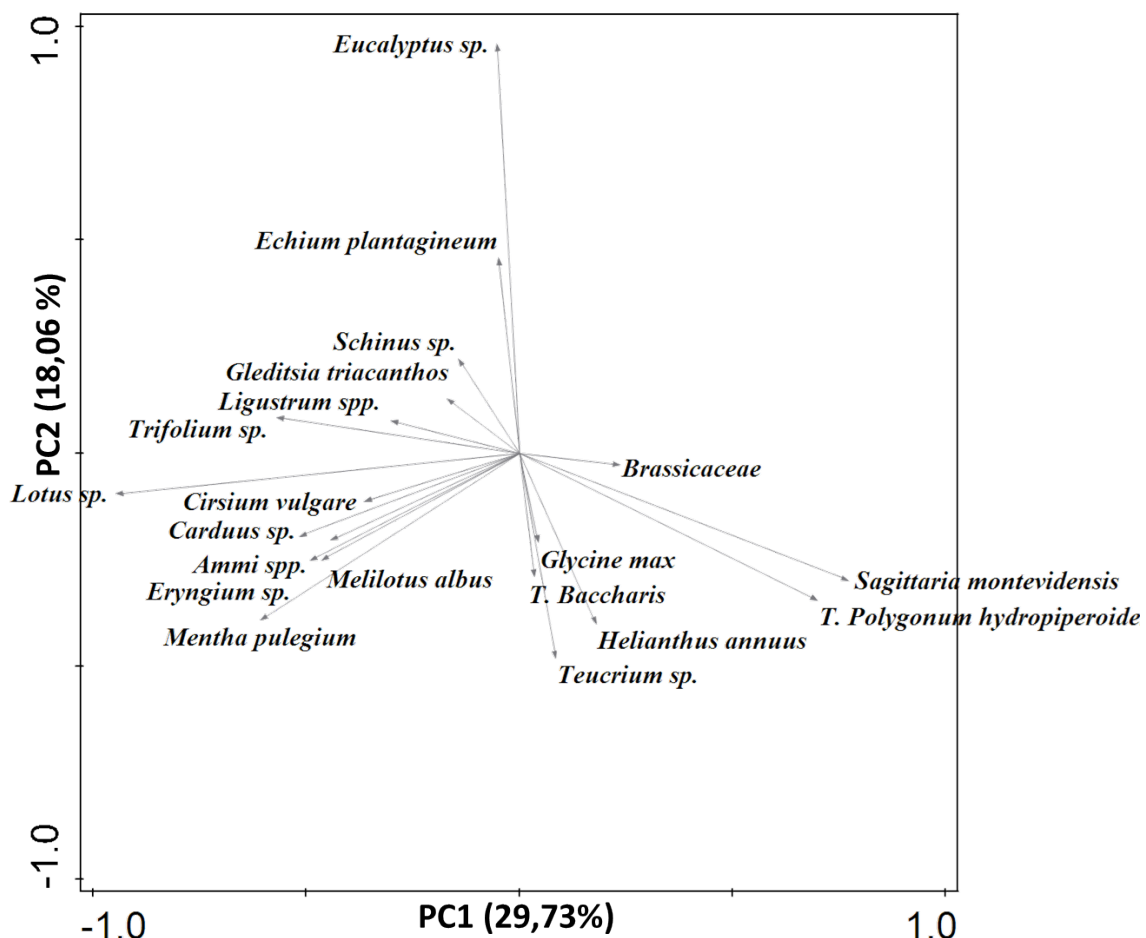

Fig. 1. PCA scatter plot of pollen percentages found in honey samples from different ecoregions harvested in different years (PC1: 29.73 % of variance and PC2: 18.06% of variance). Species are symbolized as arrows, and arrow points indicate the direction of the steepest increase of the values for the corresponding species. The length of the arrow is a measure of the suitability for the species while the angle between arrows indicates the sign of the correlation between the species. A correlation is considered positive when the angle is sharp and negative when the angle exceeds 90 degrees.
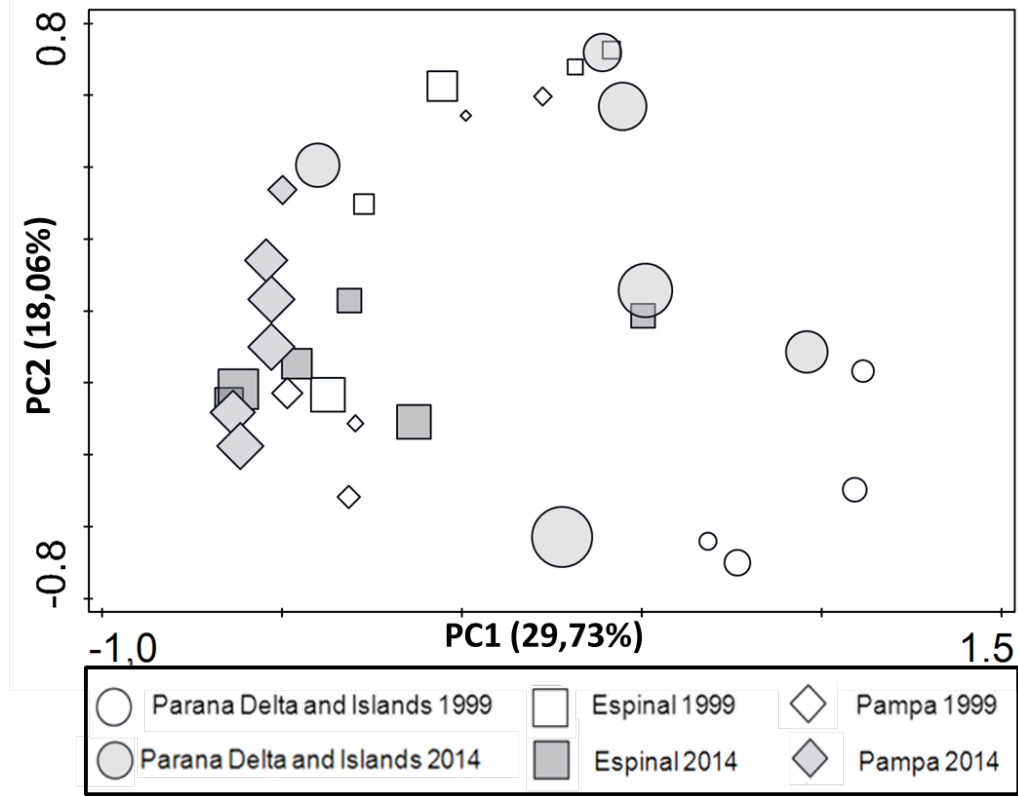
Fig. 2. Diversity plot of the floral origin of honey samples from different geographical regions (Espinal, Pampa, Parana Delta and Islands ecoregions) harvested in 1999 and 2014 (PC1: 29.73 % of variance and PC2: 18.06% of variance). The symbol size reflects the count of species within samples. A big symbol size indicates large species diversity.

Fig. 3. RDA-Biplot of species–explanatory variables (year of harvest and geographical region) of honey samples.

Table 1.
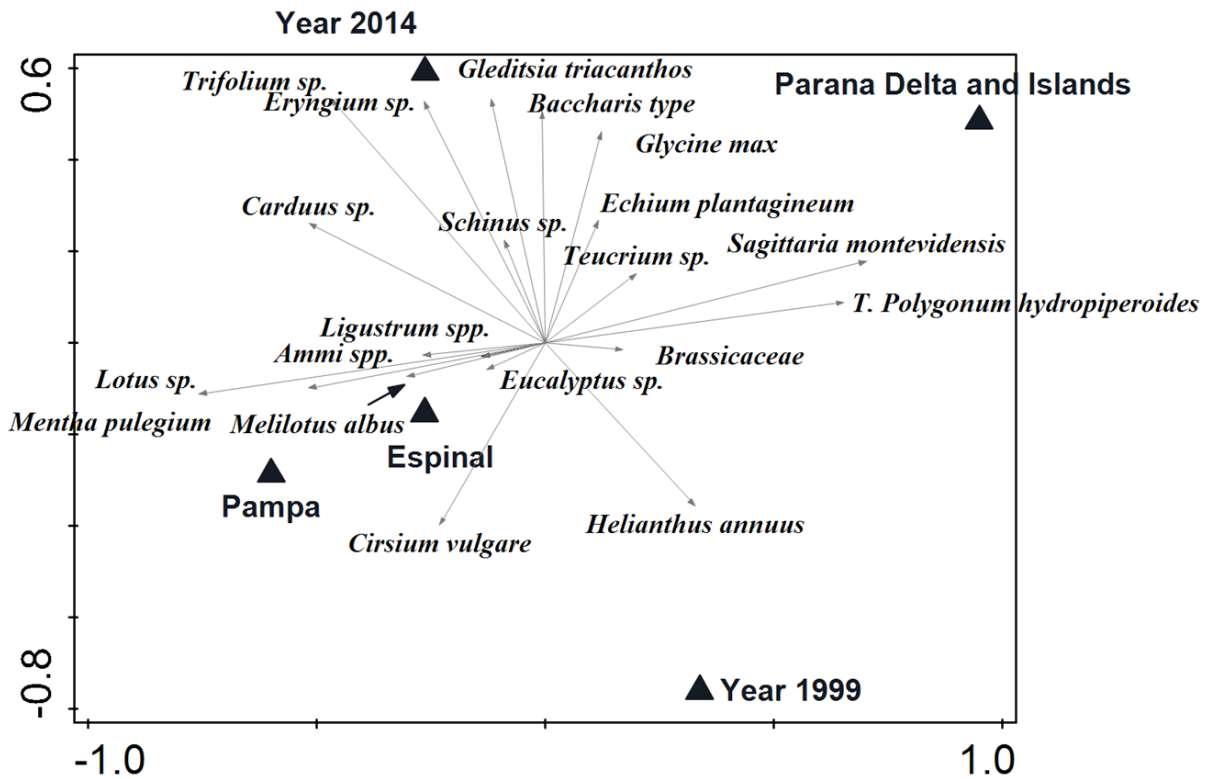Main pollen types found in honey samples from different regions of Argentina (Espinal, Pampa, Parana Delta and Islands) harvested in 1999 and 2014

Botanical Families	Pollen type*	Year 1999 — Espinal					Year 1999 — Pampa					Year 1999 — Parana Delta and Islands					Year 2014 — Espinal					Year 2014 — Pampa					Year 2014 — Parana Delta and Islands				
		n	Min	Max	Mean	SD	n	Min	Max	Mean	SD	n	Min	Max	Mean	SD	n	Min	Max	Mean	SD	n	Min	Max	Mean	SD	n	Min	Max	Mean	SD
Alismataceae	Sagittaria montevidensis	1	-	0.18	0.04	0.08	0	-	-	-	-	4	1.52	48.7	17.4	21.82	1	-	-	-	-	5	-	0.17	0.03	0.07	5	-	29.67	6.31	11.59
Anacardiaceae	Schinus sp.	1	-	0.18	0.04	0.08	2	-	0.20	0.07	0.10	0	-	-	-	-	2	-	0.19	0.03	0.08	4	-	0.70	0.15	0.28	4	-	0.34	0.15	0.13
Apiaceae	Ammi spp.	3	-	1.25	0.37	0.52	2	-	6.72	1.42	2.96	0	-	-	-	-	6	0.18	1.03	0.26	0.40	4	-	2.11	0.84	0.80	4	-	1.88	0.43	0.72
	Eryngium sp.	0	-	-	-	-	0	-	-	-	-	1	-	0.19	0.05	0.10	6	0.36	7.03	2.02	2.61	4	-	7.90	2.12	2.87	4	-	7.00	2.35	3.37
Asteraceae	Carduus sp.	1	-	0.32	0.06	0.14	1	-	0.20	0.04	0.09	0	-	-	-	-	6	0.18	3.54	1.00	1.40	3	-	17.90	5.44	7.06	3	-	0.34	0.12	0.14
	Centaurea sp.	2	-	0.72	0.27	0.37	0	-	-	-	-	0	-	-	-	-	5	-	2.21	0.43	0.89	1	-	3.68	0.89	1.39	1	-	0.19	0.03	0.08
	Cichorium intybus	1	-	0.54	0.11	0.24	0	-	-	-	-	0	-	-	-	-	4	-	0.16	0.05	0.08	4	-	1.39	0.44	0.51	4	-	0.39	0.15	0.14
	Cirsium vulgare	3	-	2.87	0.69	1.23	3	-	1.40	0.51	0.56	0	-	-	-	-	3	-	0.44	0.07	0.18	3	-	0.87	0.29	0.36	0	-	-	-	-
	Helianthus annuus	4	-	7.71	1.66	3.38	5	-	3.34	2.00	1.49	2	-	72.7	34.2	39.9	3	-	0.15	0.02	0.06	3	-	1.57	0.59	0.68	0	-	-	-	-
	Baccharis type	0	-	-	-	-	0	-	-	-	-	4	-	0.73	0.18	0.37	5	-	60.9	14.33	23.7	5	-	1.27	0.45	0.50	5	-	27.98	10.37	13.14
Borraginaceae	Echium plantagineum	5	0.16	3.89	0.96	1.64	2	-	1.64	0.41	0.71	0	-	-	-	-	4	-	0.51	0.12	0.20	5	-	1.45	0.39	0.54	5	-	4.96	1.65	2.15
Brassicaceae	Brassicaceae	5	0.19	3.32	1.19	1.19	3	-	9.25	1.95	4.08	2	-	2.87	1.41	1.44	5	-	3.30	0.58	1.33	5	-	4.55	1.23	1.71	5	-	2.57	1.28	0.86
Fabaceae	T. Adesmia sp.	1	-	0.16	0.03	0.07	1	-	0.90	0.34	0.40	2	-	16.3	4.08	8.15	2	-	40.9	7.07	16.61	2	-	1.91	0.56	0.88	2	-	1.71	0.35	0.68
	Gleditsia triacanthos	0	-	-	-	-	0	-	-	-	-	0	-	-	-	-	4	-	1.41	0.37	0.52	5	-	2.72	0.86	1.15	5	-	3.07	1.01	1.16
	Glycine max	1	-	0.72	0.14	0.32	0	-	-	-	-	0	-	-	-	-	4	-	0.18	0.08	0.08	5	-	2.65	0.94	1.20	5	-	25.63	7.47	11.12
	Lotus sp.	5	0.96	77.0	26.4	31.5	5	4.74	98.32	46.54	40.6	0	-	-	-	-	6	0.31	66.1	39.26	25.06	5	36.86	59.47	49.02	10.14	6	0.17	30.72	6.61	11.97
	Medicago sativa	0	-	-	-	-	0	-	-	-	-	0	-	-	-	-	3	-	0.18	0.03	0.07	5	-	0.57	0.16	0.22	5	-	0.51	0.27	0.18
	Melilotus albus	4	-	34.6	7.65	15.3	4	-	10.42	5.99	2.56	4	-	6.63	2.07	3.14	6	0.91	15.2	5.18	7.22	3	-	9.39	3.97	3.19	3	-	0.90	0.24	0.35
	Trifolium sp.	2	-	0.9	0.21	0.39	3	-	0.60	0.21	0.24	5	-	0.57	0.14	0.28	6	2.63	6.09	2.62	2.48	3	-	28.58	11.30	9.27	5	-	-	14.68	4.01
Lamiaceae	Mentha pulegium	3	-	1.97	0.47	0.84	3	-	37.16	8.53	16.2	1	-	0.18	0.05	0.09	5	-	6.23	2.20	2.33	5	-	5.87	3.25	2.36	0	-	-	-	-
	Teucrium sp.	0	-	-	-	-	2	-	0.20	0.04	0.09	3	-	0.73	0.28	0.35	1	-	0.37	0.14	0.17	3	-	0.53	0.09	0.21	3	-	0.19	0.09	0.10
Myrtaceae	Eucalyptus sp.	5	6.27	96.2	57.2	42.4	5	0.25	90.21	34.8	46.0	5	-	3.89	0.97	1.94	6	1.71	7.58	4.48	2.37	5	-	23.82	10.41	7.93	5	-	74.45	30.61	27.65
Oleaceae	Ligustrum spp.	3	-	1.97	0.80	0.93	2	-	1.34	0.30	0.59	0	-	-	-	-	3	-	9.23	1.63	3.73	3	-	0.95	0.22	0.37	1	-	2.22	0.37	0.91
Polygonaceae	T. Polygonum hydropiperoides	0	-	-	-	-	0	-	-	-	-	4	0.37	35.6	12.43	16.36	0	-	-	-	-	0	-	-	-	-	6	0.10	5.22	1.50	2.12

* Results are expressed in percentage value

n = number of samples in which the pollen type was found; Min = minimum; Max = maximum; Mean = mean value; SD = standard deviation.

The pollen types present in more than 70% of the studied honey samples were *Eucalyptus* sp. (found in 88% of the honey samples), *Lotus* sp. (84% of the honey samples), *Melilotus albus* (72% of the honey samples) and Brassicaceae (72% of the honey samples).

The RDA analysis revealed no significant correlations among the variables as all the factors presented a p-value lower than 0.05 and a VIF<20. The p-value associated to the year of harvesting was 0.007 and this indicates that the pollen composition of honeys from different ecoregions had significantly changed in the past years The RDA biplot is displayed in Fig. 3 (RDA p-value=0.001). According to these results, honey samples harvested in 1999 had high values of *Helianthus annuus,* while in 2014 *Eryngium* sp*., Gleditsia triacanthos, Baccharis* type*, Trifolium* sp*.* and *Glycine max.* Results in Table 1 also indicate a significant increase in the proportion of samples with the *Glycine max* pollen type. In 1999 only 7.1% honey samples contained this pollen type , while in 2014 it was found in 66.7% of the samples .

DISCUSSION

The pollen composition of honey is generally accepted to provide important information about the flora of a region (Caccavari & Fagúndez, 2010). In the present work, the effect of_harvesting time on the pollen profile of honeys was analysed in three different regions of the Buenos Aires province. The Espinal ecoregion is characterized by a warm and humid climate and additionally has been extensively exploited by anthropogenic activity. The Pampa ecoregion has a warm-temperate climate with intense rainfalls during the spring and autumn (Patrignani et al., 2016; Cabrera, 1968). The Parana Delta and Islands has a humid subtropical climate and its flora is characterised by hydrophilic species (Fagúndez, 2016).

As mentioned earlier, similarities were found in the floral origin of samples from Parana Delta and Islands ecoregion in 1999. It could be concluded that native species *Sagittaria montevidensis* and T. *Polygonum hydropiperoide* were the most representative floral origin of these samples. According to Caccavari & Fagúndez (2010) these

pollen types are found very frequently in honey samples from the middle delta of the Parana River.

Furthermore, as explained in the results section, samples harvested in 1999 showed less species diversity than samples harvested in 2014. Recent studies have indicated that anthropogenic activities may influence the availability and diversity of flowering plants and hence, the bees' food sources (Kriesell, Hilpert, & Leonhardt, 2017). Although more samples should have been analysed in order to reach to absolute conclusions, evidence in the present work suggests that the cultivated species provide low nectar amounts forcing bees to explore other resources to increase flower diversity (Louveaux & Vergeron, 1964; Fagúndez, 2016). Bees generally forage within one kilometre of the hive but can fly for more than 14 km if necessary to exploit other plant species. This may increase the diversity of flowers used by a colony (Fagúndez, 2016). Hence, the higher diversity of species found in 2014 may be a bee adaptation to ensure adequate quantity and good quality nectar.

Moreover, higher values of sunflower pollen were found in honey samples harvested in 1999 compared to 2014. These results are in agreement with Pengue (2005) who explained that the land devoted to sunflower (*H. annuus*) production had been significantly reduced in Argentina in the last decade. By contrast, the significant presence of *Eryngium* sp*., G. triacanthos, Baccharis* type*, Trifolium* sp*.* and *G. max* was determined in samples harvested in 2014. The pollinic type *Eryngium* sp*.* is a native species, mostly considered invasive weeds difficult to control. Their dissemination has increased in recent years due to the carriage of the seeds by cattle (Balda, Ressia, & Donselli, 2014). *G. triacanthos* is an exotic species and considered invasive in the Espinal and Pampa ecoregions. To the date, few efforts have been made to control it, and thus its expansion and distribution has increased (Leggieri, 2010). The increased presence of the *Baccharis* type is not completely clear, mainly because this pollen type includes a high number of species and it is extensively distributed in Argentina (Giuliano, 2001). Fagúndez

(2016) also mentioned this pollen type with its high frequency and percentage values in other Argentinean regions.

The increasing amount of *G. max* pollen in honey samples over the past fifteen years could be explained by the significant expansion of soy production. In the last decade, Argentinean fields have lost land devoted to such food crops as maize or sunflower, while soybean production has increased by 126% (Pengue, 2005). Although some authors indicated that soybean flowers offer low-quality nectar and are not particularly attractive for bees, current studies have indicated that some beehives use soybean flowers as the main source of pollen and nectar (Fagúndez & Caccavari 2003; Fagúndez, 2011). These results are in line with our findings that bees can successfully adapt to the presence of soybeans. However, this might modify the price of honey on the market because the quality of this product strongly depends on its floral origin.

The present results indicate that honey pollen profile can successfully reflect environmental modifications because of agricultural activities. Hence, the impact of anthropogenic activities in apicultural regions could be evaluated by a combination of honey melissopalynological analysis and multivariate statistical tools. Evidence in the present work indicates that such anthropogenic factors as agriculture expansion could significantly change the floral precedence of honeys. Moreover, the present work demonstrates that multivariate statistical tools can be used to explore environmental modifications through melissopalynology analysis.

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