



The effect of pollen grain morphology on sugars content of bee-collected pollen: the significance of size and exine ornamentation

MARIA DIMOU , VASILIOS LIOLIOS , MARIA-ANNA RODOPOULOU  &
CHRYSOULA TANANAKI 

Laboratory of Apiculture & Sericulture, Faculty of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

Sugars constitute the main component of bee-collected pollen, which may vary significantly among different pollen taxa according to their botanical origin and the surrounding vegetation. In this study, we investigated the significance of the pollen grain volume and exine sculpture on the sugars content of bee-collected pollen. We analysed the sugar content of 30 monofloral pollen pellets using high-performance liquid chromatography and we measured the volume of the pollen grains. The content of total sugars of pollen pellets was on average 421.0 g kg^{-1} . We found that the volume of pollen grain affects the total content of sugars in bee-collected pollen in a statistically significant way and smaller size pollen grains had comparatively higher sugar values. On the contrary, we found no clear evidence regarding the significance of the exine ornamentation on sugar content. The results revealed that the presence of sugars in bee-collected pollen is a multifactorial issue as several parameters such as the volume of pollen grains, the botanical origin, the flowering season and surrounding vegetation may influence the results.

Keywords: *pollen nutrition, Apis mellifera, pollen grain volume, exine, nectar, pollen loads*

Pollen has been a subject of extensive study not only due to its importance in bee nutrition but also due to its value as a food supplement in human diets (Campos et al. 1997; Denisow & Denisow-Pietrzyk 2016). Bee-collected pollen is characterised by a high concentration of proteins and amino acids as well as reducing sugars, fatty acids, vitamins and minerals (Campos et al. 1997; Villanueva et al. 2002); and it is essential for the survival and reproduction of the bee colonies (Dietz 1978).

The sugar content of bee-collected pollen depends strongly on its botanical origin (Szczęsna 2007; Dominguez-Valhondo et al. 2011; Liolios et al. 2018). Bees themselves also affect the sugar profile and increase the total sugar content of bee-collected pollen when they add nectar or honey to form the pollen loads (Szczęsna et al. 2002; Liolios et al. 2018). When worker bees visit flowers for pollen collection, they get dusted with pollen, and during the grooming process, they add liquid to moisten the

grains and then press them into the form of small pellets, which are stored in their pollen-baskets situated on the hind legs (Thorpe 2000). This liquid consists of nectar when the foraging takes place in plant taxa where both pollen and nectar are harvested, or honey – brought from the hive – when the pollen foraging concerns nectarless plant species (Parker 1926). As the various types of honey or nectar sources differ in their carbohydrate composition, it is apparent that the sugar concentration in bee-collected pollen is influenced by the type of nectar or honey that is available to the bees during the formation of the pellet (Szczęsna et al. 2002; Liolios et al. 2018).

The sugar profile of hand-collected pollen is characterised by high values of sucrose (Heslop-Harrison 1971; Pressman et al. 2012). On the contrary, the main sugars found in bee-collected pollen are the monosaccharides glucose and fructose (Day et al. 1990; Szczęsna 2007; Dominguez-Valhondo et al.

Correspondence: Maria Dimou, Laboratory of Apiculture & Sericulture, Faculty of Agriculture, Aristotle University of Thessaloniki, Thessaloniki 57001, Greece.
E-mail: mdimou@agro.auth.gr

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2011; Liolios et al. 2018). The reduction of sucrose and increase of glucose and fructose in bee-collected pollen could be explained by the activity of the enzyme invertase which is present both in bees' secretions and pollen, and during the addition of nectar for the formation of pollen pellet, it activates and converts the sucrose to the monosaccharides glucose and fructose (Heslop-Harriso 1971; Ejiri et al. 1977; Singh & Knox 1984; Oddo et al. 1999; Pressman et al. 2012).

Previous studies have shown that the structure and the size of the pollen grains may affect the pollen grooming process and collection by the bees (Vaissière & Vinson 1994; Vossler 2015). Considering that the pollen grain morphology may also affect the quantity of nectar or honey added by the bees during the grooming and packaging of the pollen loads, in this study, we investigated the significance of the pollen grain volume and exine sculpture on the sugar content of bee-collected pollen.

Materials and methods

The pollen samples were harvested from the apiary of Apiculture-Sericulture Laboratory of Aristotle University of Thessaloniki, located into the University Farm at the area of eastern Thessaloniki (north Greece). Pollen collection and sugar analysis is described by Liolios et al. (2018). Briefly, the pollen was harvested from pollen traps fitted in the entrance of three beehives, every 1–2 days. The pollen pellets were cleaned of debris, classified according to their colour and kept in glass jars at -21°C until their analysis. Sugar analysis of bee-collected pollen was performed by high-performance liquid chromatography refractive index detector (HPLC-RID) using Agilent Technologies 1200 series system (Agilent Technologies, Santa Clara, CA, USA). Elution was performed using acetonitrile/water (80:20) as the mobile phase, at a flow rate of 1.3 ml min^{-1} . The sugars were separated on a Zorbax column for carbohydrate analysis ($150\text{ mm} \times 4.6\text{ mm}$, particle size $5\text{ }\mu\text{m}$; Agilent Technologies). The column and the RID were maintained at 30°C . The injection volume was $10\text{ }\mu\text{l}$. Furthermore, the HPLC method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. All the results were expressed as grams per kilogram of dry matter of bee pollen (Liolios et al. 2018).

For the microscopic identification of the pollen types, pollen reference slides prepared from plants of the study area were used (Dimou & Thrasyvoulou 2007). In order to estimate the volume of the pollen grains of each taxon, the measurement of 20 grains was applied in polar (p) and equatorial (e) view and

the average of 20 values was calculated. Subsequently, their approximate volume (v) was determined according to mathematical formulae that corresponded to their shape such as sphere ($v = \pi e^3/6$) or ellipse ($v = \pi e^2 p/6$) (Silveira 1991).

Non-parametric Spearman's correlation coefficients were calculated between the sugar content and pollen grain volume. The non-parametric test was preferred since the normality and homogeneity of variance assumptions did not hold in all cases. A T -test was used to compare the sugar content between pollen grains with spine exine sculpturing (i.e. Asteraceae family) and to those without, of similar size. The normality assumption was tested using the Kolmogorov–Smirnov test and the homogeneity of variance was tested using Levene's test. Additionally, the principal component analysis (PCA) was performed to identify variability and to reduce the dimensions of the dataset of the different bee-collected pollen types. The analyses were carried out using the SPSS v.25 (SPSS, Inc., Chicago, IL, USA) and the Minitab v.18 (Minitab, Coventry, UK) software. The significance level of all the statistical tests was set at $\alpha = 0.05$.

Results

In total, 30 pollen types belonging to 24 families that were collected in significant amounts were identified and analysed. The size and volume of pollen loads varied significantly and ranged from 14.2 to $111.4\text{ }\mu\text{m}$ and from 0.7 to $244.8\text{ }\mu\text{m}^3 \times 10^3$ for *Castanea sativa* Mill. and *Pinus halepensis* Mill., respectively. The results of sugar analysis also showed a great variation between the taxa (Table I). The average content of total sugars was 421.0 g kg^{-1} and ranged from 347.1 g kg^{-1} for *Hedera helix* L. to 635.3 g kg^{-1} for *Actinidia chinensis* Planch. bee-collected pollen. The most important sugars in all unifloral bee-collected pollen samples were the monosaccharides fructose and glucose, constituting 94.0% of the total sugar content. The average content of fructose ranged from 155.3 g kg^{-1} to 334.8 g kg^{-1} , while the average content of glucose fluctuated from 135.9 g kg^{-1} to 276.9 g kg^{-1} . Sucrose was not detected in almost half of the samples while bee-collected pollen from *Inula viscosa* had the highest value (Table I).

The effect of the volume of the pollen grains on the total sugar content of pollen pellets showed statistically significant negative correlation ($r = -0.395$, $p < 0.001$, Spearman's rho). Statistically significant negative correlation was also found between the volume of the pollen grains and fructose or glucose content ($r_{\text{fr}} = -0.312$, $p = 0.003$; $r_{\text{gl}} = -0.317$, $p = 0.002$) but not sucrose ($p = 0.081$). The analysis

Table I. Average values of sugars content and volume of pollen grains in 30 monofloral pollen taxa.

Family	Pollen taxon	Season of blooming	Polar view (μm)	Equatorial view	Approximate geometric figure	Volume ($\mu\text{m}^3 \times 10^{-3}$)	Total sugars (g kg^{-1})	Fructose (g kg^{-1})	Glucose (g kg^{-1})	Sucrose (g kg^{-1})
Actinidiaceae	<i>Actinidia chinensis</i>	22	20.2	ellipsoid	$\pi^2\text{p}/6$	4.7	63.53	33.48	27.69	15.3
Fagaceae	<i>Castanea sativa</i>	14.2	9.8	ellipsoid	$\pi^2\text{p}/6$	0.7	44.97	25.61	18.29	4.6
Chenopodiaceae	<i>Chenopodium album</i>	26.4		sphere	$\pi^2/6$	9.6	44.23	21.62	15.89	34.9
Asteraceae	<i>Cichorium intybus</i>	36.9	38.7	ellipsoid	$\pi^2\text{p}/6$	28.9	35.03	20.42	16.85	1.9
Cistaceae	<i>Cistus creticus</i>	41.2		sphere	$\pi^3/6$	36.6	43.56	18.5	15.39	35.5
Convolvulaceae	<i>Convolvulus arvensis</i>	66.4	54.6	ellipsoid	$\pi^2\text{p}/6$	103.6	42.34	19.94	19.11	ND
Cucurbitaceae	<i>Ecballium elaterium</i>	51.4	46.9	ellipsoid	$\pi^2\text{p}/6$	59.2	40.68	22.15	18.6	2.3
Ericaceae	<i>Erica manipuliflora</i>	27.2		sphere	$\pi^3/6$	10.5	43.86	21.2	18.38	37.3
Araliaceae	<i>Hedera helix</i>	28	29.2	ellipsoid	$\pi^2\text{p}/6$	12.5	34.71	19.61	19.58	82.4
Asteraceae	<i>Inula viscosa</i>	33.9	31.8	ellipsoid	$\pi^2\text{p}/6$	17.9	42.96	18.76	15.96	ND
Lamiaceae	<i>Lamium amplexicaule</i>	30.6	25.4	ellipsoid	$\pi^2\text{p}/6$	10.3	37.01	17.46	13.59	ND
Asteraceae	<i>Matricaria chamomilla</i>	25.7	22.4	ellipsoid	$\pi^2\text{p}/6$	6.7	38.45	15.53	19.18	ND
Poaceae	<i>Oryza sativa</i>	42.5	38.8	ellipsoid	$\pi^2\text{p}/6$	33.5	38.33	19.36	16.83	ND
Papaveraceae	<i>Papaver rhoeas</i>	24.8	22.2	ellipsoid	$\pi^2\text{p}/6$	6.4	50.79	23.64	22.65	29
Violaceae	<i>Parthenocissus inserta</i>	36.7	38.8	ellipsoid	$\pi^2\text{p}/6$	28.9	35.77	18.89	16.32	1
Boraginaceae	<i>Phacelia tanacetifolia</i>	22.2	21.6	ellipsoid	$\pi^2\text{p}/6$	5.4	43.28	20.69	21.48	ND
Pinaceae	<i>Pinus halepensis</i>	111.4	64.8	ellipsoid	$\pi^2\text{p}/6$	244.8	44.35	21.67	20.59	6.9
Polygonaceae	<i>Polygonum aviculare</i>	23.8	21.2	ellipsoid	$\pi^2\text{p}/6$	5.6	45.09	24.24	19.69	5
Portulacaceae	<i>Portulaca oleracea</i>	64.2	64.2	ellipsoid	$\pi^2\text{p}/6$	138.5	35.81	17.51	17.26	ND
Ranunculaceae	<i>Ranunculus arvensis</i>	28.5	33.5	ellipsoid	$\pi^2\text{p}/6$	16.7	44.9	23.23	19.49	16.7
Fabaceae	<i>Robinia pseudacacia</i>	27.5	24.5	ellipsoid	$\pi^2\text{p}/6$	8.6	47.59	23.28	21.7	13.7
Rosaceae	<i>Rubus ulmifolius</i>	24.3	21.2	ellipsoid	$\pi^2\text{p}/6$	5.7	42.91	21.22	20.98	ND
Lamiaceae	<i>Salvia verbenaca</i>	42	40	ellipsoid	$\pi^2\text{p}/6$	35.2	36.51	19.66	16.45	ND
Asteraceae	<i>Silybum marianum</i>	41.5	39.3	ellipsoid	$\pi^2\text{p}/6$	33.5	39.67	21.5	17.55	ND
Brassicaceae	<i>Sisymbrium irio</i>	25.5	24.3	ellipsoid	$\pi^2\text{p}/6$	7.9	38.7	19.74	17.59	ND
Asteraceae	<i>Sonchus asper</i>	35.5	40.2	ellipsoid	$\pi^2\text{p}/6$	30	41.88	21.55	18.31	ND
Tamaricaceae	<i>Tamarix parviflora</i>	18.2	17.3	ellipsoid	$\pi^2\text{p}/6$	2.9	41.01	20.32	17.77	7.7
Asteraceae	<i>Taraxacum officinale</i>	30.5	33.4	ellipsoid	$\pi^2\text{p}/6$	17.8	41.62	20.89	18.68	ND
Tiliaceae	<i>Tilia intermedia</i>	38.2	24	ellipsoid	$\pi^2\text{p}/6$	11.5	45.15	20.6	17.74	57.3
Zygophyllaceae	<i>Tribulus terrestris</i>	54.4		sphere	$\pi^3/6$	84.3	38.23	19.99	15.69	12.4

Note: Values representing mean are expressed as grams per kilogram of dry weight. ND, not detected.

regarding the sugar content between pollen grains with spine ornamentation on their exine and those without did not show statistically significant differences ($p_{\min} = 0.145$).

The PCA results indicated that 80.9% of the total variance can be explained by four components (Table II). The first component (PC1) accounted for 30.2% of the total variance and it was positively associated with higher values on total sugars, fructose and glucose. The second component (PC2) accounted for 21.4% of the total variance and it was correlated to pollen grains' polar size and volume. The third component (PC3) explained 16.0% of the total variance and it could be defined by sucrose, exine ornamentation and season (autumn). Lastly, the fourth component (PC4) accounted for 14.3% of the total variance and it was related to seasonal factor. The loading plot confirmed the negative correlation between the volume of pollen grains and the total sugars, fructose and glucose content, as found in the Spearman's correlation test but the analysis did not show any significant deviation among the pollen taxa (Figure 1). Finally, the score plot of the first two components did not show any significant deviation among the pollen taxa (Figure 2).

Table II. The matrix of principal components (PCs), the eigenvalues for each PC, the variance (%) and the cumulative proportion of common variance (%) extracted by each PC.

	Components			
	1	2	3	4
Exine sculpture	-0.188	-0.244	-0.574	-0.434
Pollen grain polar size	-0.255	0.833	0.203	-0.369
Pollen grain volume	-0.203	0.860	0.213	-0.336
Total sugars	0.935	0.001	0.145	0.120
Fructose	0.869	0.078	-0.091	0.186
Glucose	0.868	0.216	-0.009	0.246
Sucrose	0.078	-0.425	0.760	-0.177
Spring	-0.635	-0.045	0.290	0.623
Summer	0.499	-0.344	0.136	-0.760
Autumn	0.240	0.543	-0.615	0.139
Nectarless taxa	-0.284	-0.462	-0.504	-0.083
Eigenvalue	3.319	2.354	1.758	1.571
Variance (%)	30.2	21.4	16.0	14.3
Cumulative variance (%)	30.2	51.6	67.6	81.9

Discussion

As carbohydrates constitute the main component of bee-collected pollen, the knowledge of the sugar profile is of great importance for both the beekeepers

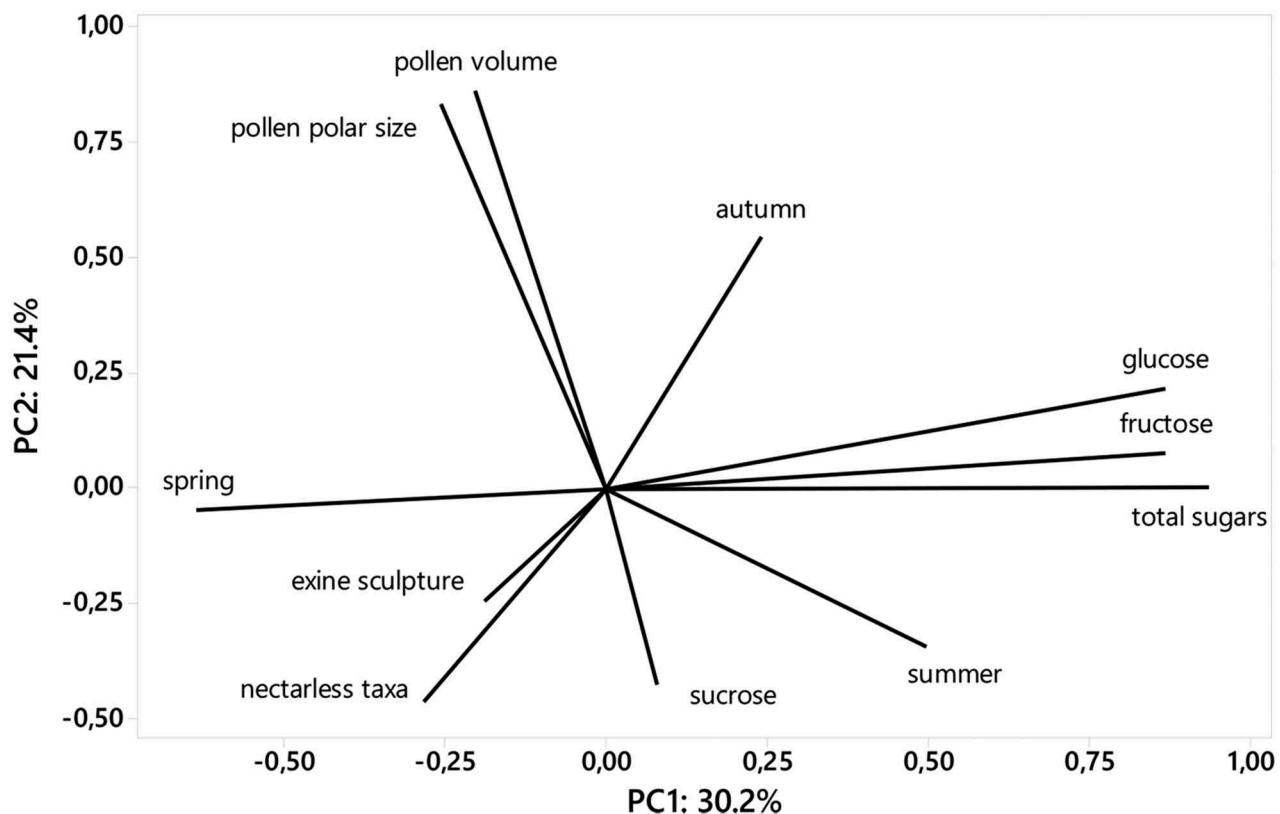


Figure 1. Principal component analysis loading plot of 11 variables.

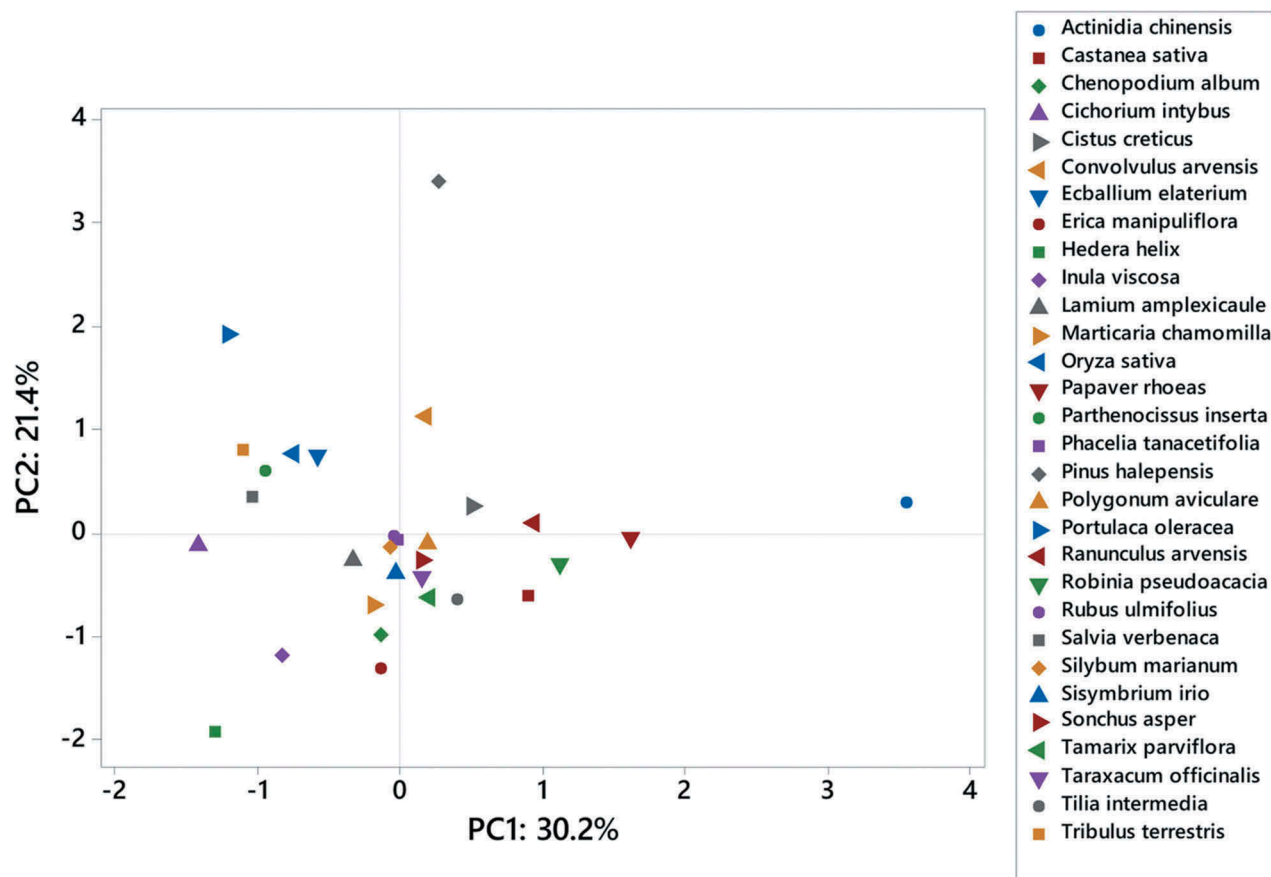


Figure 2. Principal component analysis score plot of 30 bee collected pollen taxa.

and the human consumers. Comparative studies between hand-collected and bee-collected pollen have shown the importance of the added nectar during pollen load formation to the sugar content. Particularly, whereas the sugar profile of hand-collected pollen is characterised by high values of sucrose and low content in reduced sugars, the sugar profile of the bee-collected pollen is characterised mainly from glucose and fructose (Todd & Bretherick 1942; Stanley & Linskens 1974; Nicolson 2011), as we found in our work.

Previous studies have so far demonstrated that the sugar content of bee-collected pollen depends on its botanical origin as well as on the seasonal changes in the surrounding vegetation (Liolios et al. 2018). As the carbohydrate composition of nectar from different plant sources varies, the nectar that is available to the bees from the surrounding vegetation during the formation of the pellet also affects the sugar concentration in bee-collected pollen (Szczęsna et al. 2002; Dominguez-Valhondo et al. 2011; Liolios et al. 2018). This study showed that the volume of pollen grains also affected statistically significantly the sugar content of bee-collected pollen, with the

larger pollen grains having comparatively lower values of sugars. This is probably attributed to the fact that pollen grains of smaller size require higher quantity of nectar or honey for the formation of the pellet.

However, we found no clear evidence that the pollen grain exine morphology may affect the sugar content in bee-collected pollen. Previous studies have pointed out the significance of the pollen grain morphology during the pollen grooming and packing by the bees. For example, pollen collection of taxa such as those from the Malvaceae is more laborious compared to other pollen types, as the long spines of these pollen grains seem to physically interfere with the pollen aggregating process (Vaissière & Vinson 1994; Azo'o et al. 2011; Lunau et al. 2015). The results in this study did not show significant differences between the sugar content between pollen grains with spine exine sculpturing such as those from Asteraceae and other families. This, however, could be due to the size of spines. Indeed, García-García et al. (2004) did not notice significant differences during pollen collection by honeybees regarding the weight of pollen loads between *Carduus meonanthus* Hoffmanns. et Link

and other pollen types without spine exine sculpturing. Vaissière and Vinson (1994) also reported that the grooming process of pollen grains from *Helianthus annuus* L. did not differ significantly compared to other pollen types that lacked spines in their exine sculpture, such as *Rubus* L. or *Zea mays* L.

Finally, the statistical multivariate analysis (PCA) confirmed that sugar content is not the only factor that characterises the bee-collected pollen taxa but interacts with other important parameters as well, such as the volume of pollen grains, the botanical origin, the flowering time and the surrounding vegetation.

Conclusions

Carbohydrates constitute the main component of bee-collected pollen and its content may vary significantly among different pollen types. This variability depends on several parameters such as the botanical origin, the surrounding vegetation and the pollen grain size. These parameters can interfere with each other providing therefore a unique result regarding the sugars content of bee-collected pollen. Hence, further studies including different unifloral pollen taxa may shed more light on the significance of the parameters that affect the sugars content of bee-collected pollen.

Disclosure Statement

No potential conflict of interest was reported by the authors.

ORCID

Maria Dimou  <http://orcid.org/0000-0003-1482-8290>
 Vasilios Liolios  <http://orcid.org/0000-0001-7353-2364>
 Maria-Anna Rodopoulou  <http://orcid.org/0000-0003-1643-4615>
 Chrysoula Tananaki  <http://orcid.org/0000-0002-0056-483X>

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