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Original article

Influence of pollen grain volume on the estimation of the relative importance of its source to bees

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Summary — The influence of pollen grain volume on the quantitative analysis of food stored by bees is examined. It is concluded that percentages calculated from counts of pollen types in mixed samples of bee food are not reliable estimates of the relative importance of each pollen source to bees. Methodologies to weight frequencies by volumetric correction are discussed.

pollen grain / volume / estimation / pollen plant / Apoidea / melissopalynological method

INTRODUCTION

Melissopalynological methods were originally developed for the determination of the botanical and geographic origin of commercial honey samples (Zander, 1950; Louveaux *et al,* 1978). They have been further employed in bionomic and ecological research to characterize trophic niches and trophic niche overlap among bees (*eg* Anasiewicz and Warakomska, 1971; Tasei, 1973; Raw, 1974; Absy and Kerr, 1977; Kapyla, 1978; Engel and Dingemans-Bakels, 1980; Murrell and Szabo, 1981; Severson and Parry, 1981; Ranta and Lundberg, 1981; Tepedino, 1982; Tepedino and Frohlich, 1982; Sommeijer *et al,* 1983; Ramalho *et al,* 1985).

Dry weight is the most accurate way to quantitatively evaluate the importance of a food source for an organism. It is impossible, however, to know the weight of each constituent fraction of a pollen sample when working with bee-stored food. In the methodology commonly used in melissopalynology, the relative importance of each pollen source has been expressed by the frequency of its pollen grains relative to a given number of grains counted in a microscope slide of food sample.

Due to the great variation in size of grains among plant species, the frequencies obtained by this procedure may not represent the real contribution, in weight or in volume, of each plant to the whole sample (Tasei, 1973; Kapyla, 1978). A pollen load carried by a bee, or a bee-

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stored pollen sample, may contain many more grains than another of equal mass or volume but composed of a larger diameter pollen type. To correct for these volume differences, Tasei (1973) utilized quotients of mean diameters to the third power. Some authors (*eg* Kapyla, 1978; Tepedino, 1982; Ramalho *et al*, 1985) have chosen arbitrary frequencies below which pollen types were considered as contamination or as unimportant in the samples.

The objective of this work was to quantify the distortion that pollen volume differences can bring in such procedures.

MATERIALS AND METHODS

An artificial pollen sample was made to quantify the error resulting from neglecting pollen grain volume in the usual counting procedure. Pollen was collected with the aid of conventional traps fitted to the entrance of honey bee hives. Corbicular pollen pellets thus obtained were sorted by colour and texture into homogeneous samples. Before usage these samples had been dried and stored in glass vials at ≈ 4 °C.

Several slides were prepared with pellets randomly taken from each of these samples, to assure that they were composed of a single pollen type. Seven of these unispecific samples were selected. Each of them contained 1 pollen type differing one from another in shape and size. These were pollen from *Mabea* sp (Euphorbiaceae), *Cecropia* sp (Moraceae), and a representative of the Gramineae, Compositae, Myrtaceae, Sterculiaceae and a *Croton*-type (Euphorbiaceae).

One pellet of each selected pollen type was placed in glacial acetic acid for 24 h, acetolyzed, and then prepared on 5 microscope slides. On each slide, measurements were taken of 5 grains, totalling 25 measured grains of each pollen type (plant species).

Another pollen pellet was taken from each of the selected unispecific pollen samples, weighed, and used for making the composite experimental sample. Thus, the proportional contribution by weight of each pollen type to this sample was known.

The experimental sample was homogenized in glacial acetic acid and after 24 h acetolysed and mounted on 5 slides. Nearly 1 000 grains were counted on each of these slides. The mean frequency for each pollen type in these counts was then calculated.

The volumetric correction coefficient *(Q)* used by Tasei (1973) was obtained for each pollen type, as indicated below:

$$Q_i = \frac{(\text{Mean diameter of pollen species } i)^3}{(\text{Mean diameter of pollen species } s)^3}$$

where pollen type *s* is that with the smallest mean diameter, and type *i* stands for any of the 7 types present.

Tasei (1973) does not explain how he calculated mean diameters. Here all 3 pollen dimensions were considered in the final calculated value: thus, in ellipsoidal grains, equatorial diameters were counted twice and polar diameters once.

An estimated volume for each pollen type was calculated by application of the pollen grain measurements to the volumetric formulae for the geometric figure it most resembles (*eg* sphere, ellipsoid, triangular base prism). Figures and corresponding volumetric formulae used for each pollen type are shown in table II.

The percentage composition of the experimental sample was calculated using the number of grains counted, the number of grains counted adjusted by the *Q* coefficient and by the number counted adjusted by the estimated volume. To examine the accuracy of each procedure in estimating the weight composition of the experimental sample, Spearman's rank correlation coefficients were calculated (Siegel, 1956).

RESULTS AND DISCUSSION

In the experimental sample, the *Croton*-type pollen had its actual frequency reduced by a factor of 17 using the slide counting procedure; the Sterculiaceae-type frequency was reduced by a factor of 15,

Table I. Means and standard deviations of the pollen types present in the experimental sample ($n = 25$).

Pollen type	*Equatorial view*				*Polar view*		*Mean diameter*	
	Polar diameter		*Equatorial diameter*		*Triangle height*			
	(μm)	*SD*	*(μm)*	*SD*	*(μm)*	*SD*	*(μm)*	*SD*
Mabea	59.28 ±	20.6	53.32 ±	15.0	–	–	55.30	–
Graminea*	–	–	–	–	–	–	33.23 ±	15.5
Compositae	23.41 ±	7.4	24.75 ±	6.2	–	–	24.30	–
Myrtaceae	14.39 ±	5.7	25.85 ±	7.2	23.67 ±	8.1	21.30	–
Cecropia	13.78 ±	5.4	8.47 ±	4.1	–	–	10.24	–
Croton *	–	–	–	–	–	–	51.17 ±	18.8
Sterculiaceae*	–	–	–	–	–	–	62.96 ±	21.7

* Spheroidal grains had their diameter measured in no specified polar position.

while the *Cecropia*-type had its relative contribution increased 7-fold! The mean deviation, in this procedure, was an absolute factor of ≈ 7.7. This is reflected in the Spearman's rank correlation coefficient ($r_s = 0$). Frequencies calculated from pollen grain counts in slides were not significantly correlated with the weight contribution of each pollen type to the sample.

The percentage contribution of each pollen type to the sample, calculated by its weight contribution, mean number of counted grains, estimated volumetric contribution and *Q* corrected volumetric contribution are compared in table III.

In table IV, Spearman's rank correlation coefficients are presented as a measure of the accuracy with which each procedure

Table II. Mean estimated volumes and Q volumetric correction coefficients for each of the pollen types in the experimental sample (e = equatorial radius; p = polar radius; h = triangle height).

Pollen type	*Approximate geometric figure*	*Volume formula*	*Mean estimated volume (μm³)*	*Mean diameter* (d)	d^3	Q
Mabea	Ellipsoid	$4(\pi e^2 p)/3$	88 244	55.30	169 112	157.46
Graminea	Sphere	$4(\pi e^3)/3$	19 213	33.23	36 694	34.17
Compositae	Ellipsoid	$4(\pi e^2 p)/3$	7 508	24.30	14 349	13.36
Myrtaceae	Triangular Base prism	$2e2ph/2$	4 402	21.30	9 664	9.00
Cecropia	Ellipsoid	$4(\pi e^2 p)/3$	518	10.24	1 074	1.00
Croton	Sphere	$4(\pi e^3)/3$	70 153	51.17	133.982	24.75
Sterculiaceae	Sphere	$4(\pi e^3)/3$	130 675	62.96	249 571	232.38

Table III. Real and estimated contributions of each pollen type to the experimental sample (*w* = weight contribution; *n* = contribution in number of grains counted; *vn* = estimated volumetric contribution; *Qn* = *Q*-corrected volumetric contribution).

Pollen type	w (*mg*)	n	SD	vn (μm)^3	*Q*	*Percentage contribution* %w	%n	%vn	%Qn
Mabea	6.9	16	± 10.89	1 411 904	2 519	18.1	1.6	21.6	21.1
Graminea	4.8	68	± 12.00	1 306 484	2 324	12.6	6.7	20.0	19.5
Compositae	5.5	133	± 24.85	998 564	1 777	14.4	13.2	15.3	14.9
Myrataceae	7.1	235	± 14.52	1 034 470	2 115	18.5	23.3	15.9	17.7
Cecropia	3.1	540	± 45.86	279 720	540	8.1	53.5	4.3	4.5
Croton	7.9	12	± 3.51	841 836	1 497	20.6	1.2	12.9	12.5
Sterculiaceae	2.8	5	± 1.92	653 375	1 162	7.3	0.5	10.0	9.7

Table IV. Spearman's rank correlation coefficients as a measure of the accuracy of grain counts *(n)*, estimated volumes *(nv)* and *Q*-corrected volumes *(Q)* as weight estimators.

	n	*nv*	*Q*
w	0.0*	0.43	0.43*

* $P < 0.05$.

estimates the weight composition of the sample.

These results confirm the conclusions of Tasei (1973) and Kapyla (1978) that percentages obtained from counts are not the same as the proportions of the different species in the total weight or volume of the pollen sample. For this reason, the conclusions from many of the works published on relative importance of pollen sources for bees may be wrong. Thus, plants considered unimportant or plants whose grains were considered as contamination in pollen slides may be actually important food sources for bees, and *vice versa*.

Although Tasei's volumetric correction coefficient *(Q)* and the estimated volumes proposed here slightly improved the accuracy of estimation, the correlation coefficients for these 2 procedures were still not significant (r_s = 0.43; $P > 0.05$). Considering its ease of calculation, as compared to estimated volumes, it would be better to use Tasei's method, if any volumetric corrections are used.

Density differences, counting errors, differential loss in supernatant phases in the various centrifugations during acetolysis, inaccuracy in volume estimation and the addition of nectar by the bees to the pollen loads are among the potential factors causing the observed deviations of *Q* corrected and estimated volumes in weight composition estimations. Another potential source of error is change in pollen grain size or shape caused by storage, acetolysis and the slide-mounting procedures. According to Reitsma (1969), boiling pollen grains for less than 4 min in the acetolysis mixture can cause some size increase (in this study, pollen was boiled for only

2 min); placing grains in glycerine jelly without cover glass supports, as was done here, can also cause slight size increase after some time. As measurements were done in less than 5 days after slide mounting, only the length of boiling should cause volume alteration. Nothing is known about effects of long storage periods at low temperatures.

Since considerable improvement in volume estimation accuracy does not seem easy to achieve for most practical applications, it is suggested that:

1), the collection of pollen loads before storage by bees should be preferred. In this case, pellets can be sorted by colour and texture, and fractions thus obtained can be weighed. Such a procedure was previously used by Severson and Parry (1981); 2), when stored pollen is the only available information source, 2 procedures could be adopted: i), the elaboration of a simple list of pollen-offering species; or ii), quantification by frequency classes based on Tasei's (1973) volumetric correction coefficient. Frequencies or frequency classes based solely on grain counts should not be used.

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Résumé — Influence du volume des grains de pollen sur l'évaluation de l'importance relative de leur source pour les abeilles. Les méthodes mélissopalynologiques, qui ont été développées à l'origine pour l'analyse des miels, ont été utilisées également dans de nombreuses études écologiques et bionomiques. La fréquence de chacun des nombreux types polliniques dans un nombre donné de grains observés sur des lames microscopiques, a été souvent utilisée pour estimer l'importance relative des sources de pollen dans l'alimentation de l'abeille. Étant donnée la grande variation en taille des grains des différentes plantes, les fréquences obtenues par ces procédures ne sont pas représentatives de la contribution réelle, en poids ou en volume, de chaque plante dans l'échantillon total.

Dans ce travail, ont été quantifiées les erreurs potentielles qui sont commises, en l'absence de prise en considération du volume du pollen lors de certaines procédures. La possibilité de corriger les fréquences par des coefficients de correction volumétrique a été égalemment discutée.

Les pelotes de pollen obtenues grâce à l'utilisation de trappes conventionnelles fixées devant les ruches, ont été classées, par couleur et par texture, en échantillons homogènes. Plusieurs lames ont été préparées avec des pelotes prises au hasard dans chacun de ces échantillons, afin de s'assurer qu'ils étaient composés d'un seul type de pollen. Sept types de pollen ont ainsi été sélectionnés par leurs différentes formes et leurs différentes tailles.

Deux pelotes de chacun de ces types ont été utilisées. Après acétolyse, 5 lames ont été préparées à partir de l'une d'entre elles. Pour chaque lame, les mesures ont été faites sur 5 grains, ce qui fait un total de 25 grains mesurés pour chaque type pollinique (tableau I). Les autres pelotes ont été pesées et utilisées pour produire un échantillon expérimental mélangé avec les 6 autres types. Ainsi, la contribution proportionnelle, en poids, de chaque type pollinique et cet échantillon a été connue.

L'échantillon expérimental a été homogénéisé dans l'acide acétique glacial, et, après 24 h, acétolysé et monté sur 5 lames. Environ un millier de grains ont été comptés sur chacune de ces 5 lames. La fréquence moyenne de chaque type pollinique a été calculée.

Le coefficient de correction volumétrique *(Q)*, utilisé par Tasei (1973), a été obtenu pour chaque type pollinique. Le volume estimé de chaque type pollinique a été calculé par l'application, à la mesure des grains, de la formule du volume de la figure géométrique à laquelle ils ressemblent le plus (tableau II).

La composiiton, en pourcentage, de l'échantillon expérimental a été calculée au moyen : du nombre de grains comptés, du nombre de grains comptés, ajusté par le coefficient *Q*, et du nombre compté, ajusté par le volume estimé (tableau III).

Les coefficients de corrélation de rang de Spearman ont été calculés afin de vérifier la précision de chaque procédure (tableau IV).

Les résultats montrent que les fréquences obtenues, au moyen des pourcentages de grains comptés seuls, n'ont aucune corrélation avec la contribution relative en poids, et donnent naissance à de graves erreurs dans l'interprétation des lames de pollen. La correction, par le coefficient *Q* de Tasei et par l'estimation du volume, augmente la précision de l'estimation, mais ne résout pas le problème complètement. Cela était probablement dû à l'absence de précision dans l'estimation du volume.

Puisque l'amélioration considérable requise pour estimer les volumes ne semble pas facile à obtenir dans la pratique, 2 suggestions sont faites pour les études futures.

1), Il est préférable de récolter les pelotes de pollen avant qu'elles ne soient stockées par les abeilles. Dans ce cas, les pelotes devraient être classées par couleur et texture, et ces sous-échantillons devraient être pesés, comme cela a été fait par Severson et Parry (1981).

2), Quand la source disponible d'information est uniquement constituée des pollens stockés par les abeilles, 2 procédures pourraient être adoptées: i), l'élaboration d'une liste des plantes offrant du pollen; ou ii), la quantification par des classes de fréquence basées sur le coefficient de correction volumétrique de Tasei. Les fréquences, ou les classes de fréquences, basées simplement sur le comptage des grains ne devraient pas être utilisées.

grain de pollen / volume / estimation / plante pollinifère / Apoidea / méthode melissopalynologique

Zusammenfassung — Die Bedeutung des Pollenkornvolumens auf die Schätzung der relativen Bedeutung dieses Pollens für die Bienen. Melissopalynologische Methoden, ursprünglich zur Honiganalyse entwickelt, wurden in zahlreichen ökologischen und bionomischen Studien eingesetzt. Die Häufigkeit jeder der zahlreichen Pollentypen wurde unter dem Mikroskop bei einer bestimmten Zahl von Pollenkörnern gezählt und als Maß für die Schätzung ihrer relativen Bedeutung für die Diät der Bienen benutzt. Wegen der außerordentlichen Größenschwankungen der Pollen verschiedener Pflanzen geben die mit diesem Verfahren gewonnenen Häufigkeiten jedoch keinen richtigen Hinweis auf den Beitrag jeder Pflanze, nach Gewicht oder Volumen der Pollenkörner, in der Gesamtprobe.

In dieser Untersuchung werden die möglichen Fehler, entstanden durch Vernachlässigung des Pollenvolumens bei manchen Verfahren, quantifiziert. Gleich-

zeitig wird die Möglichkeit einer Anpassung der Häufigkeiten durch Korrektur der Volumenunterschiede mittels Einführung von Koeffizienten diskutiert.

Pollenhöschen, auf die übliche Weise mittels Pollenfallen vor dem Flugloch gesammelt, wurden nach Farbe und Struktur zu einheitlichen Proben sortiert. Es wurden mikroskopische Präparate aus Höschen, die nach Zufall aus jeder dieser Proben entnommen waren, angefertigt, so daß jedes Präparat nur aus einem einzigen Pollentyp bestand. Sieben verschiedene Pollentypen wurden nach Unterschieden in Form und Größe ausgewählt.

Die Einführung eines "Q"-Koeffizienten und Volumen-Schätzung verbesserten zwar die Genauigkit der Schätzung, lösten aber das Problem nicht völlig. Dies war vermutlich auf mangelhafte Genauigkeit der Volumensbestimmung zurückzuführen.

Da die erforderlichen beträchtlichen Verbesserungen zur Erhöhung der Schätzgenauigkeit nicht leicht zu erreichen sind, werden für spätere Studien folgende Vorschläge gemacht: 1), Die Pollenhöschen sollen vor dem Einlagern durch die Bienen gesammelt werden. Dann kann man die Höschen nach Farbe und Struktur sortieren und anschließend diese Teilproben wiegen, wie dies Severson und Parry (1981) getan haben. 2), Wenn nur eingelagerter Pollen als einzige Informationsquelle zur Verfügung steht, können zwei Prozeduren eingesetzt werden: 1) Eine einfache Liste der pollenspendenden Pflanzen, oder 2) Quantifizierung durch Häufigkeitsklassen mittels volumetrischen Korrektionskoeffizienten nach Tasei. Häufigkeiten oder Häufigkeitsklassen, die nur auf der Zählung der Körner beruhen, sollten nicht benutzt werden.

Melissopalynology / Pollenvolumen / Schätzverfahren / Pollenpflanze / Apoidea

ADDENDUM

Since this paper was submitted, at least 2 new articles have used pollen counts to assess the importance of pollen sources to bees. These are:

Camillo R, Garofalo CA (1989) Analysis of the niche of two sympatric species of *Bombus* (Hymenoptera, Apidae) in southeastern Brazil. *J Trop Ecol* 5 (1), 81-92

Imperatriz-Fonseca VL, Klnert-Giovannini A, Ramalho M (1989) Pollen harvest by eusocial bees in a non-natural community in Brazil. *J Trop Ecol* 5 (2), 239-242

Another recent paper suggests estimation of pollen volume to minimize bias in the analysis of mixed pollen samples. The use of volumetric formulae for solid figures, as here tested, is recommended. This paper is:

O'Rourke MK, Buchmann SL (1991) Standardized analytical techniques for bee-collected pollen. *Environ Entomol* 20 (2), 507-513

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Tepedino VJ (1982) Flower visitation and pollen collection records for bees of high altitude shortgrass prairie in southeastern Wyoming. *Southwest Entomol* 7 (1), 16-25

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