



Analysis of pollen load based on color, physicochemical composition and botanical source

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ABSTRACT

Pollen load samples from 10 hives of *Apis mellifera* (L.) were analyzed based on their physicochemical composition and botanical source, considering color as a parameter for quality control. In seven samples it was possible to establish the occurrence of more than 80% of a single pollen type, characterizing them as unifloral but with protein content variation. One of the samples was exclusively composed of saprophytic fungi (*Cladosporium* sp.). Comparing the mean results of the fungi loads with those of the nutritional components of pollen load, the former presented higher protein, mineral matter and dry matter and lower organic matter, ethereal extract and total carbohydrate values. The monochromatic samples met the physicochemical specifications regulating pollen load quality. The results showed that homogeneous coloration of the pollen load was not found to be a good indication of unifloral pollen, confirming the importance of physicochemical analysis and melissopalynological analysis for characterization of the quality of commercial pollen load.

Key words: *Apis mellifera*, bees, food source, nutritional analysis, palynology.

INTRODUCTION

Pollen collected from plant anthers is essential for the nutrition of *Apis mellifera* (L.), since it is a source of protein mainly for larvae and adults (Zerbo et al. 2001). For humans, many benefits have been attributed to pollen load as an extraordinary fortifier of the organism, stimulant and generator of physical vigor, correcting deficient diets and resulting in functional balance (Linskens and Jorde 1997, Kroyer and Hegedus 2001).

Pollen contains proteins, lipids, including sterols, starch, sugar, several minerals and vitamins (Goodman 2003). The chemical composition of pollen load and its

botanical source can greatly influence load color. When pollen is derived from a single botanical source, the loads are called unifloral, with the organoleptic and biochemical properties being similar to the original plant. When the pollen originates from several botanical species, the loads are heterofloral possessing varying properties (Stanley and Linskens 1974).

According to Almeida-Muradian et al. (2005), knowledge of the nutritional composition of pollen load can be applied for its quality control, principally as a guide for the commercial production of unifloral pollen. Thus, the objectives of this study were to characterize nine pollen load samples of *Apis mellifera* (L.) as to their chemical composition, weight and botanical source as

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well as to discuss the importance of load color as a parameter for pollen load quality control.

MATERIALS AND METHODS

Pollen loads samples were obtained from 12 August to 13 December, 2005 in pollen collectors installed in 10 *Apis mellifera* (L.) hives located in a seasonal semideciduous forest (Mata Atlântica) with 60 years of secondary succession in Viçosa, Minas Gerais, Brazil. To maintain pollen quality, pollen was removed every 2–3 days, hand cleaned and stored in a freezer at temperatures between -22°C and -14°C , until processing.

The pollen load were sorted out by color still humid, with color being determined according to the Red Green Blue (RGB) color classification system. Nine samples with homogenous color were selected and out of these, ten pollen loads of each one of the samples were weighed on an analytical balance to determine wet weight (g), calculating its averages (Mean weight in mg). The samples were dried in an air forced circulation oven for 72 hours at approximately 55°C . The dried samples were sent to the Animal Nutritional Laboratory (UFV, Dept. of Animal Science) for routine physicochemical analysis to determine % of dry weight, organic matter, ashes or mineral matter, crude protein, crude fat or ethereal extract and total carbohydrates (Silva 2002).

Melissopalynological preparation was based on the European standard of Maurizio and Louveaux (1965), without acetolysis application, adapted for pollen load by Almeida-Muradian et al. (2005). Identification of the pollen types was based mainly on the reference collection of microscopic slides with pollen from flowering plants of the region studied as well as from catalogues specialized in pollen morphology of species from several floras (Salgado-Labouriau 1973, Melhem et al. 1984, Roubik and Moreno 1991). Approximately 500 pollen grains/sample were identified and counted. The microscopic slides utilized were kept in the Laboratory of melissopalynology of the Federal University of Viçosa, Department of Animal Biology.

RESULTS AND DISCUSSION

Average wet weight of the pollen loads was 5.80 ± 0.82 mg. Color varied between clear beige (RBG:

E8E4CD), several yellow shades (RBG: EEF359; EDF0A2; E7E258; E0DB84), orange-like (RBG: CFA020; C79F23) and light brown (RBG: 9A7A57). In seven samples the occurrence of over 80% of a single pollen type (samples 2 to 8) characterized them as unifloral (Table I).

Although pollen load storage time must be taken into account, since it causes oxidization changing color, color homogeneity of the wet loads seems to be a good indicator of unifloral pollen, as also reported by Almeida-Muradian et al. (2005).

In this study, sample 9 differed in color from the standard found for the other samples (RBG: 1E1A1A). On analysis, the presence of saprophytic fungi (*Cladosporium* sp.) was confirmed rather than of pollen, as expected (Table I). The reason for that is unknown, but high ambient humidity (average of the relative humidity of air in November = 85%) associated with limited supply of protein sources near the apiary may have influenced the bees to supply the colony needs by collecting fungi from rotten fruit (Shaw 1990). In the region studied there are extensive coffee plantations and *Cladosporium* is a genus recognized as contaminant of these cultures (Chalfoun et al. 2007). Occasional collection of fungi spores by *Apis* bees in several regions of the world (Wingfield et al. 1989, Shaw 1990) or even the occurrence of fungi infecting pollen load (González et al. 2005) were reported. These observations show that melissopalynological analyses of pollen loads displaying unusual color in the collection region must be carried out to avoid consumption of undesirable products, which, although within the Brazilian and Argentine physicochemical regulations (Krell 1996, Brasil 2001), do not specify their botanical sources.

Based on the chemical composition of the samples analyzed, *Myrcia* and *Cirsium* loads presented the main values of ash percentage, compared with the total samples ash average, while *Vernonia* presented the main values of the ethereal extract and total carbohydrate in sample 4. Notably, *Myrcia* loads presented the main crude protein values (Table II), reinforcing the results reported by Modro et al. (2007), and indicating that the Spearman correlation test, proposed by these authors, between chemical composition and the pollen types present in several samples was true for this particular situation.

TABLE I
Color, weight and botanical source of pollen loads collected by *Apis mellifera* (L.) in Viçosa, MG, Brazil, 2005.

Samples	Color	Mean weight (mg)	Number of pollen types present in the sample	Frequency of pollen types (>10% pollen representation in the sample)
1	Yellow RBG: E0DB84	5.61	11	<i>Anadenanthera</i> (49.3%), <i>Eucalyptus</i> (29.7%), <i>Arecaceae</i> (10.0%)
2	Yellow RBG: E7E258	7.68	8	<i>Baccharis</i> (98.3%)
3	Dark orange RBG: CFA020	5.53	6	<i>Cirsium</i> (94.0%)
4	Light brown RBG:9A7A57	5.99	7	<i>Vernonia</i> (88.2%)
5	Light beige RBG: E8E4CD	5.04	10	<i>Vernonia</i> (87.6%)
6	Yellow RBG: EDF0A2	6.37	5	<i>Coffea</i> (96.6%)
7	Orange RBG: C79F23	5.54	6	<i>Myrcia</i> (97.7%)
8	Yellow RBG: EEF359	5.02	7	<i>Alchornea</i> (81.3%), <i>Cecropia</i> (17.8%)
9	Black RBG: 1E1A1A	5.41	3	Fungi (<i>Cladosporium</i> sp.) (99.4%)

TABLE II
Physicochemical composition of pollen loads collected by *Apis mellifera* (L.) in Viçosa, MG, Brazil, 2005.

Samples	Dry matter (%)	Organic matter (%)	Mineral matter (%)	Crude protein (%)	Etherium extract (%)	Total carbohydrate (%)
1	85.88	97.13	2.87	44.44	1.78	50.91
2	87.09	98.44	1.56	19.62	4.07	74.75
3	82.53	96.7	3.3	16.23	4.62	75.85
4	86.66	98.73	1.27	15.39	6.55	76.79
5	86.92	98.73	1.27	20.88	3.01	74.84
6	82.05	99.02	0.98	27.72	1.83	69.47
7	87.07	96.69	3.31	41.23	2.36	53.10
8	78.90	97.86	2.14	21.16	5.40	71.30
9	85.00	97.50	2.50	28.81	2.50	66.19
Mean±SD	84.7±2.9	97.9±0.9	2.1±0.9	26.2±10.5	3.6±1.7	68.1±9.8

Flowering plants found near the apiaries that possibly contributed as pollen source were: *Anadenanthera colubrina* (Vell.) Brenan (sample 1), *Baccharis dracunculifolia* DC. and *B. melastomaefolia* Hook. & Arn. (sample 2), *Eupatorium squalidum* DC., *Mikania cordi-*

folia (L.f.) Willd. and *Wedelia paludosa* DC. (sample 3), *Vernonia condensata* Baker, *V. diffusa* Less., *V. lanuginosa* Gardner and *V. mariana* Mart. Ex Baker (samples 4 and 5), *Coffea* spp. (sample 6), *Myrcia fallax* (Rich.) DC. (sample 7) and *Euphorbia pulcherrima* Willd. Ex

Klotzsch (sample 8) (A.F.H. Modro, unpublished data). Thus, to produce high protein pollen load, the apiarist should cultivate bee plants like *A. colubrine* and *M. fallax*.

Although samples 4 and 5 have the same dominant pollen type (*Vernonia*), their chemical compositions presented variations, possibly due to the presence of other pollen types that constituted the remaining sample, which, even at low concentrations (approximately 12%) were important to characterize the chemical composition of the samples (Tables I and II).

CONCLUSIONS

Color homogeneity of the loads when still humid was not found to be a good indicative of unifloral pollen for the region under study.

Samples of *Anadenanthera* and *Myrcia* loads presented the highest crude protein content.

One of the samples presented black color and was composed exclusively by saprophytic fungi (*Cladosporium* sp.) but it was within the standards established by current Brazilian legislation.

Fungi loads presented higher contents of protein, mineral matter and dry matter contents and lower contents of organic matter, ethereal extract and total carbohydrate as compared to the mean results of the nutritional components of the pollen load.

The physicochemical composition of the pollen load samples is influenced by the set of pollen types present in the sample.

Melissopalynological analysis of pollen load samples is necessary to characterize product quality even in cases when the chemical composition meets the official Brazilian specifications.

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RESUMO

Amostras de cargas de pólen de 10 colméias de *Apis mellifera* (L.) foram analisadas quanto a sua composição físico-química e origem botânica, tomando-se a coloração como parâmetro para o controle de qualidade. Em sete amostras foi possível estabelecer a ocorrência de mais de 80% de um único tipo polínico, caracterizando-as como monoflorais, porém com variações nos valores protéicos. Uma das amostras era composta exclusivamente por fungos saprofiticos (*Cladosporium* sp.). Comparando-se as bolotas de fungos com os resultados médios dos componentes nutricionais das bolotas de pólen, as primeiras apresentaram maior valor protéico, matéria mineral e matéria seca e menores valores de matéria orgânica, extrato etéreo e carboidratos totais. As amostras monocromáticas estiveram de acordo com as especificações físico-químicas reguladoras da qualidade de pólen apícola. Os resultados demonstram que a coloração homogênea das cargas de pólen não se apresentou como um bom indicativo de pólen monofloral e confirma a importância das análises físico-químicas e melissopalínológicas para a caracterização da qualidade do pólen apícola a ser comercializado.

Palavras-chave: *Apis mellifera*, abelhas, recurso alimentar, análise nutricional, palinologia.

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