PALYNOLOGICAL EVALUATION OF BEE POLLEN LOAD BATCHES FROM THE VENEZUELAN ANDES OF MISINTÁ

Ortrud M. Barth, Alex da Silva de Freitas, Érika de Oliveira Sales and *Patricia Vit*

SUMMARY

Besides honey, bee pollen is the beehive product best known by the public for nutritional and medicinal purposes. Ten samples of fresh bee pollen loads were collected using pollen traps during ten months from December 2004 to October 2005 in the Páramo of Misintá, Mérida, Venezuela. They were evaluated in the present study using two techniques, one considering single pollen loads, and the other a pool of two grams of mixed pollen loads. Pollen loads changed from light green and yellow to brownish and ochre; Rosaceae, Eucalyptus *and* Hyptis *pollen load colors changed monthly. A unique pollen type was commonly predominant in each pollen load, therefore confirming the preference of the bees to collect pollen from one species until reaching the final weight to be carried in their corbicula. Pollen load color analysis showed four bifloral and six heterofloral batches. Pollen load pool analysis emphazised three monofloral batches of* Brassica napus *in March, April and September, and two bifloral pollen batches in June and November, beside the five heterofloral ones. This evaluation revealed better the potential of the regional pollen production.*

Introduction

Besides honey, bee pollen is the beehive product best known by the public for nutritional and medicinal purposes. Its botanical origin, related to flower richness, local and regional vegetation, provides information about the capacity of the area where the apiaries are located, regarding qualitative and quantitative aspects of honey, bee pollen, royal jelly and bee wax production (Manrique, 1996; Rodríguez, 2006).

Identification of bee plants may be obtained using the pollen analysis technique, characterizing the local and regional vegetation and its potential for beekeeping (Barth, 2004). Few previous data about palynological bee pollen analysis in the Venezuelan Andes could be obtained (Vit and Santiago, 2008). Results of bee pollen load sample analyses of neighboring countries of Venezuela have been published mainly in Brazil (Bastos *et al*., 2004; Luz *et al*., 2007; Barth *et al*., 2009), as well as in Surinam (Engel and Dingemans-Bakels, 1980) and Colombia (Sierra and Pardo, 2009).

The Páramo of Misintá, Municipality of Rangel, state of Mérida, in the Venezuelan Andes, comprises altitudes around 3300-3400masl. Beekeeping activities in this region are limited. In the last 20 years, honeybees shifted their foraging habits to collection of more pollen than nectar (Juan C. Schwartzenberg, personal communication). Consequently in the apiaries, honey crops dropped while harvests of bee pollen increased.

Using two palynological techniques, the present paper intends to investigate the validity of bee preferences when collecting pollen grains from plants in the Misintá region of the Venezuelan Andes. The chemical composition (moisture, ash, fat, pH, proteins) of the same pollen samples from this region has been published elsewhere (Vit and Santiago, 2008).

Material and Methods

The apiary is located (Figure 1) in the Páramo of Misintá, Mérida state, Venezuela. Samples of fresh bee pollen loads were collected during three days in ten hives during ten months, from December 2004 to October 2005, using pollen traps. The samples were kept frozen until botanical analysis was carried out applying two different techniques.

For the first procedure, that of pollen load batch botanical origin evaluation (Barth *et al*., 2009), pollen loads of two grams of each sample were distributed, according to color, in batches or sub-samples. A unique load of each colored batch was used for pollen grain identification. Pollen grains were macerated with the aid of one drop of 70% ethanol directly on a microscope slide.

For the second technique, pollen loads of two grams of each sample were homogenized in 70% ethanol using ultrasound. After a second wash in ethanol and ultrasonic treatment, the sediment obtained was left for a half hour in a solution of distilled water/glycerin 1:1. One drop of the well mixed

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EVALUACIÓN PALINOLÓGICA DE DE LOTES DE CARGA DE POLEN APÍCOLA DE LOS ANDES VENEZOLANOS DE MISINTÁ

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RESUMEN

Además de la miel, el polen apícola es el producto mejor conocido por los consulmidores en relación a sus propiedades nutricionales y medicinales. Se recolectaron diez muestras de polen apícola fresco por medio de una trampa caza polen durante diez meses, de diciembre 2004 a octubre 2005, en el Páramo de Misintá, Mérida, Venezuela. Las muestras fueron evaluadas en el presente trabajo utilizando dos técnicas, una considerando las cargas aisladas de polen y otra usando dos gramos de cargas bien mezcladas ("pool") por lote. El color de las cargas de polen varió de verde claro y amarillo a marrón y ocre; los colores de las cargas de polen de Rosaceae, Eucalyptus *e* Hyptis *variaron mensualmente. En general, dominó un tipo único de polen en cada carga, confirmando de esta manera la preferencia de las abejas por la colecta de polen de una especie hasta llegar a el peso final que puede ser llevado en sus corbículas. El análisis del color de las cargas de polen resultó en cuatro lotes biflorales y seis heteroflorales. El análisis de "pool" de cargas de polen resultó en tres lotes monoflorales de* Brassica napus *en marzo, abril y setiembre, y dos biflorales en junio y noviembre, además de cinco heteroflorales. Esta evaluación del polen apícola reveló mejor el potencial regional para la producción de polen.*

AVALIAÇÃO PALINOLÓGICA DE PARTIDAS DE CARGAS DE PÓLEN APÍCOLA DOS ANDES VENEZUELANOS DE MISINTÁ

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RESUMO

Além de mel, o pólen apícola é o produto mais bem conhecido pelos consumidores em relação a suas propriedades nutricionais e medicinais. Foram coletadas dez amostras de pólen apícola fresco, por meio de um caça-pólen, durante dez meses, de dezembro 2004 a outubro 2005, no Páramo de Misintá, Mérida, Venezuela. As amostras foram avaliadas no presente trabalho usando duas técnicas, uma considerando cargas isoladas de pólen e a outra usando dois gramas de cargas bem misturadas ("pool") por partida. A cor das cargas de pólen variou de verde claro e amarelo a marrom e ocre; as cores das cargas de pólen de Rosaceae, Eucalyptus *e* Hyptis *variaram mensalmente. Em geral dominava um único tipo polínico em cada carga de pólen, desta maneira confirmando a preferência das abelhas pela coleta de pólen de uma só espécie até chegar ao peso final que possa ser levado em suas corbículas. A análise por cor das cargas de pólen resultou em quatro partidas biflorais e seis heteroflorais. A análise do "pool" de cargas de pólen resultou em três partidas monoflorais de* Brassica napus *em março, abril e setembro e duas biflorais em junho e novembro, além de cinco heteroflorais. Esta avaliação do pólen apícola revelou melhor o potencial regional para a produção de pólen.*

pollen grain suspension was placed on a microscope slide, covered with a 22x22mm cover slide, and sealed with enamel. The slide must be maintained in a horizontal position. Pollen has to be examined within one month or less, when drying starts and the pollen grains are selectively pressed against the air bubbles.

The evaluation of single pollen loads does not require counting. More than 500 pollen grains of each sample were counted (pollen sum) for pool evaluation. For identification of the botanical *taxa* the plant species list of Vareschi (1970) and pollen descriptions and illustrations in Vit (2005) were used.

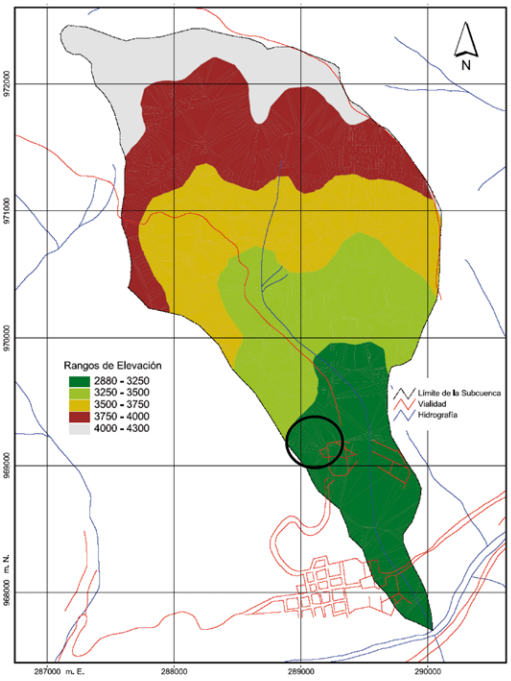

Figure 1. Misintá is located to the north of Mucuchíes, Rangel County, Mérida State. From Vit and Santiago (2008).

Results and Discussion

The color of pollen loads changed during the ten months of this study from yellow and ochre, light gray and greenish gray, to orange and brown. The data obtained are presented in Table I, considering the pollen types representing ≥3% of the pollen sum. Twelve pollen types were detected by single pollen load analysis and eight pollen types (one was not identified) by pollen load pool analysis. A total of fourteen pollen types were detected using both techniques. This aspect illustrates a very low richness of bee plant species in the studied region when compared with data of areas covered with tropical vegetation, where 37 plant species were related by Engel and Dingemans-Bakels (1980).

Pollen types could be identified at the level of family, genus or species of plants occurring in the vegetation of Misintá (Vareschi, 1970; Vit *et al*., 2008). Nearly all single pollen loads analyzed were composed of a unique plant species pollen (Table I, columns 2 and 3). Only in September bees have mixed pollen grains of

TABLE I
COMPARISON OF TEN SAMPLES OF POLLEN LOADS (2g OF EACH BATCH) FROM *Apis mellifera* EVALUATED BY ITS COLOR AND BY A POOL OF 2g OF EACH BATCH*

Samples	Single pollen load analysis according to color		Analysis of pollen load pools	
	Sub-samples	Pollen types (++++)	Pollen types identified	Evaluation of the sample
December 2004	yellow ochre light gray brown	*Brassica napus* *Hyptis* Rosaceae *Myrcia acuminata* and yeast	*Brassica napus* (++) *Eucalyptus* (++) *Myrcia acuminata* (++) Rosaceae (++)	Heterofloral
January 2005	yellow greenish gray	*B. napus* Rosaceae	*B. napus* (+++) *Eucalyptus* (++) Rosaceae (++)	Heterofloral with dominance of *Brassica napus*
February 2005	yellow greenish gray orange	*B. napus* *Fragaria vesca* aff. *Guazuma ulmifolia*	*B. napus* (+++) *Eucalyptus* (++) Rosaceae (++)	Heterofloral of *B. napus*, *Eucalyptus* and Rosaceae
March 2005	ochre/yellow light brown dark brown	*B. napus* *B. napus* *Eucalyptus*	*B. napus* (++++) *Carica papaya* (+) *Eucalyptus* (+) Rosaceae (+)	Monofloral of *B. napus*
April 2005	yellow greenish gray	*B. napus* *Eucalyptus*	*B. napus* (++++) *Eucalyptus* (+) Rosaceae (+)	Monofloral of *B. napus*
May 2005	ochre brown	*B. napus* *Medicago denticulata*	*B. napus* (+++) *Carica papaya* (+) *Eucalyptus* (+) *Medicago denticulata* (+) *Rumex acetosella* (+)	Heterofloral with dominance of *B. napus*
June 2005	brown dark brown	*F. vesca* *Parkinsonia aculeata*	Rosaceae (+++) *B. napus* (++) *Myrcia acuminata* (+) *R. acetosella* (+)	Bifloral of Rosaceae and *B. napus*
September 2005	yellow ochre greenish gray orange	*B. napus* several pollen types Rosaceae *Hyptis*	*B. napus* (++++) *Eucalyptus* (+) Rosaceae (+) *M. acuminata* (+)	Monofloral of *B. napus*
October 2005	ochre greenish gray brown	*Cercidium praecox* Rosaceae *Coriandrum sativum*	Rosaceae (++) *B. napus* (+) *Eucalyptus* (+) *M. denticulata* (+)	Heterofloral
November 2005	ochre light brown dark brown	*C. praecox* *Rumex acetosella* unknown	*B. napus* (+++) Rosaceae (++) *R. acetosella* (+) unknown (+)	Bifloral of *B. Napus* and Rosaceae

Only the pollen types with frequency >3% were considered, as very frequent (++++, >85%), frequent (+++, 45-85%), less frequent (++, 15-45%), rare (+, 3-15%).

several plant *taxa* in a single ochre colored pollen load.

Brassicaceae pollen grains were the most frequent all over the year. The species *Brassica napus* is abundant in the studied region. It blooms in yellow and gives to the mountains a characteristic pattern. The pollen load pool analysis detected this species mainly in the months from November until May (Table I, column 4), and in lower concentration from June to October. Analyzing the pollen loads according to its color, this period was shortened from December to May, and no pollen grains of this species were detected in June, October and November. Sensorial parameters of *B. napus* pollen loads in comparison with those of Rosaceae (*Fraxinus americana*), *Zea mays* and *Datura arborea*, obtained at Mérida state, showed significant differences (Vit *et al*., 2008).

The second most frequent pollen type was from Rosaceae, comprising at least three pollen types. This taxon was found during the pool analysis as dominant in June and October and in lower concentration during all months, except May. Using color analysis (Table I, column 3) it was only observed in some months.

The third important taxon of pollen contribution was *Eucalyptus* (Barth and Barbosa, 1972; Barth, 1989), which is an introduced plant species. The pool analysis did not detect these pollen grains in June and November, and by the color analysis only in the months of March and April. Therefore, *Eucalyptus* trees may be of lower value for pollen production in the Misintá region.

Considering the pool analysis, the remaining four pollen types with >3% frequency (*Carica papaya, Medicago denticulatea, Myrcia acuminata* and *Rumex acetosella*), never reached a significant representation. *B. napus* was the dominant plant species in three of the ten batches analyzed, and were the only monofloral ones, occurring in the months of March, April and September. Bifloral pollen loads of *B. napus* and Rosaceae were found in June and November, and heterofloral samples in December, January, February, May and October.

Color analysis of pollen loads showed no monofloral batches. The use of weight of different colored pollen batches may be helpful, as it was exemplified by Barth *et al*. (2009). The error in this analysis consists in the presentation of different colors by pollen grains of a given species. In the present study this observation can be exemplified (Table I, columns 2 and 3) with yellow, ochre and light brown colored pollen loads of *B. napus*, dark brown and greenish gray pollen loads of *Eucalyptus*, greenish gray and brown ones of *Fragaria vesca*, and ochre and orange of *Hyptis*. The identification of pollen grains of different colored pollen loads results in a larger number of pollen types.

Therefore, the pollen load pool analysis is much more

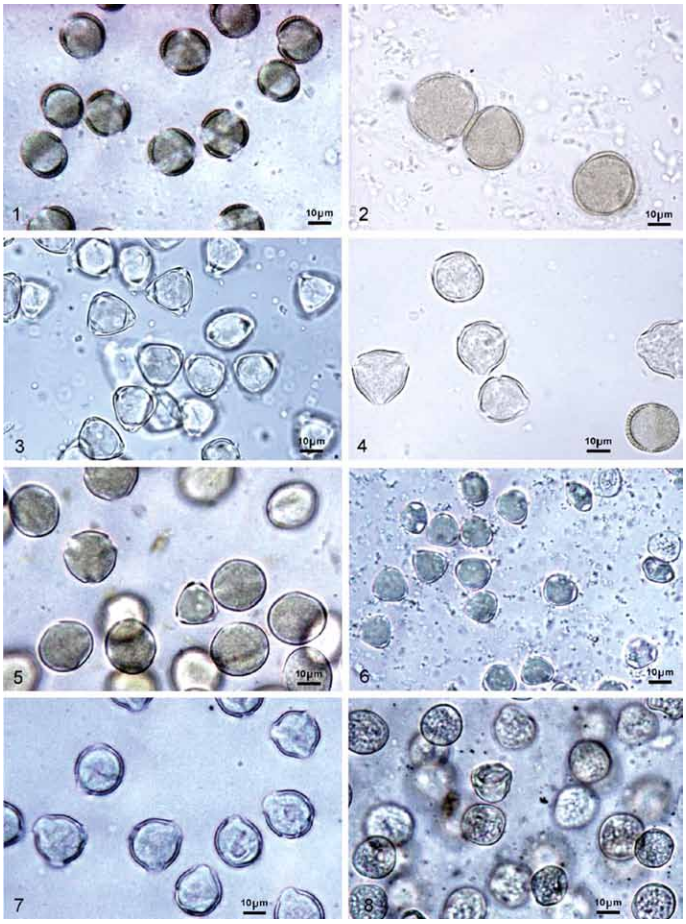

Figure 2. Main pollen types identified in bee pollen loads collected in the region of Misintá, Venezuelan Andes, from December 2004 until November 2005 (the selected pollen types are in agreement with column 4 of Table I). 1: *Brassica napus*, 2: *Carica papaya*, 3: *Eucalyptus* sp., 4: Rosaceae (pollen type *Malus silvestris*), 5: *Medicago denticulate*, 6: *Myrcia acuminate*, 7: Rosaceae (pollen type *Prunus persica*), 8: *Rumex acetosella*. All figures are of the same magnification.

accurate quantitatively for commercial interest than the color analysis. On the other hand, the identification of pollen grains of different colored pollen loads provides a larger number of pollen types, showing a better resolution of the bee floral composition.

In summary, the analysis of different colored pollen loads provides better information about the regional vegetation, while the analysis of a pollen load pool reveals the regional potential for pollen production. The practical result for beekeepers is, first, to know through pool analysis which plant species really are the best for obtaining pollen, and second, to find out, using pollen load color analysis, which plant species were visited by the bees. However, some plant species may be of less value in a study area, and of higher importance in another region.

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