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A melissopalynological analysis of *Apis mellifera* L. loads of dried bee pollen in the southern Brazilian macro-region

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Abstract
Sixty-one samples of dried bee pollen collected from various apiaries in the southern Brazilian macro-region were examined to detect the botanical origin of the pollen and to enable more accurate product certification. The palynological analysis of the samples followed the standard methodology and was conducted without the use of acetolysis. The samples were washed once or twice with ethanol and then with water. The sediment obtained was homogenised in a water/glycerine solution for microscopic observation. The target number of pollen grains to be counted was 500 pollen grains or more per sample. The results demonstrated that 35 samples consisted of a unique pollen type representing more than 90% of the pollen sum (or more than 60% if no accessory pollen was present). These samples were considered monofloral. Several pollen types grouped in a sample were related to heterofloral pollen batches. The most frequent pollen types were of *Mimosa scabrella, Eucalyptus, Andira, Machaerium, Myrcia* and *Piptocarpha*. The results of the current study were related to the surrounding vegetation of the apiaries and reflected the resources available to the bees. Furthermore, these results are relevant to apicultural activities and are commercially significant.

Keywords: Apis*, palynology, pollen loads, southern Brazil, vegetation*

Bees provide pollination services to plants and receive food in exchange. This relationship is reflected by the honey produced in the hive, as the honey contains evidence of the plants visited, namely, pollen found in the honey. For this reason, the analysis of pollen provides a powerful tool for determining the plants that the bees are visiting (Pirani & Cortopassi-Laurino 1993).

The Brazilian flora is geographically diverse and is divided into five distinct macro-regions (north, northeast, midwest, southeast and south). The southern region has a tropical to subtropical climate with vegetation formations classified as ombrophilous forest, field vegetation ('Cerrado'), and 'Restinga' (Veloso 1962). A small occurrence of 'Cerrado' is limited to western Paraná (Pinha & Siminski 2011).

Paraná is a transitional region in southern Brazil. It is located between the tropical and subtropical regions. The vegetation formations found in this state range from areas of mangroves through areas of salty marshes and ombrophilous forests. The most representative plant families are Myrtaceae, Myrsinaceae, Lauraceae, Euphorbiaceae, Asteraceae, Melastomataceae, Araceae and Arecaceae (Troppmair 1990). Other species present in the 'Cerrado' areas include: *Myrsine umbellata* L., *Myrceugenia alpigena* O.Berg, *Ouratea spectabilis* (Mart.) Engl., *Plenckia populnea* Reissek, *Erythroxylum suberosum* A.St.-Hil., *Copaifera langsdorffii* Desf., *Calyptranthes concinna* DC. and *Myrciaria cuspidate* O.Berg

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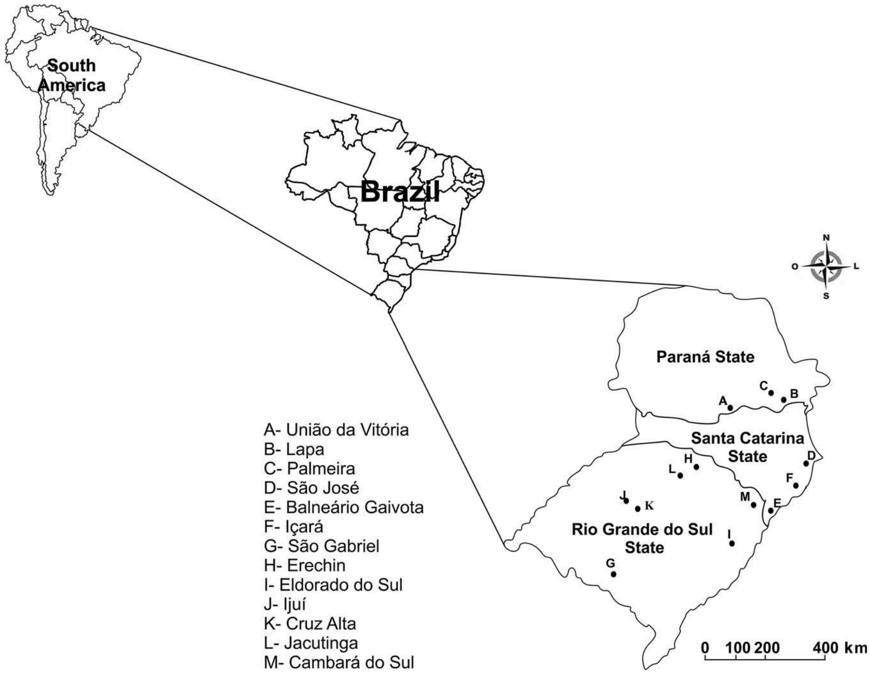

Figure 1. Map of the Southern Brazilian Region with the locations of sample collection sites.

and are considered representative in this vegetation formation (Carmo et al. 2012).

In Santa Catarina, ombrophilous forests and a strong presence of *Araucaria angustifolia* Juss. forest are characteristic and are found throughout the state (Negrelle & Silva 1992). Other plant families that stand out by virtue of their species richness are (especially) Asteraceae and, in addition, Myrtaceae, Fabaceae, Solanaceae, Melastomataceae, Lauraceae, Orchidaceae, Rubiaceae, Poaceae, Cyperaceae and Piperaceae (Gasper et al. 2013). The vegetation of Rio Grande do Sul consists of ombrophilous forests and a large area of Savannah (Pinha & Siminski 2011). The most representative arboreal families are Myrtaceae, Fabaceae, Lauraceae and Euphorbiaceae (Grings & Brack 2009). Other families with high species richness in the 'Restinga' formation are Asteraceae, Poaceae, Fabaceae, Cyperaceae and Myrtaceae (Jacobi et al. 2013). Each of these vegetation communities has a typical flowering period. Large areas in this region are used for crop production and *Apis mellifera* L. is increasingly used to provide pollination services (Rosa et al. 2011).

Pollen grains are collected in the corbicula of the hind legs of the bees during field activity and are stored in hive cells, where they exist as 'bee bread' (Luz & Barth 2012). *Apis mellifera*, as well as other bee species and other insects, use pollen grains as their main resource for proteins and lipids. These products are required by the bee larvae and are essential to the normal growth and development of colonies (Marchini et al. 2006).

The Brazilian apiculture market is not only interested in the pollination of crop plants. Brazil's large-scale agricultural production is expanding on a broad front in response to the increased demands for exports in recent years. Currently, the favourable consumer market for natural products that can serve as diet supplements or furnish therapeutic benefits has been encouraging and promoting beekeeping activity (Barreto et al. 2005). To obtain quality control of bee pollen, the botanical origin of the pollen grains must be investigated. Palynological analysis is used to identify the morphological characteristics of pollen grains (Barth 1989; Luz et al. 2007). Almeida-Muradian et al. (2005) have emphasised the importance of physicochemical analyses of bee pollen loads in improving quality control.

Melissopalynological analysis serves to enhance current knowledge of the botanical origin of pollen loads. For example, recent investigations by Santos (2011) in the Brazilian northeast region, in the southeast by Luz et al. (2010), and in the south by Freitas et al. (2013) have examined *Apis mellifera* pollen loads. However, there is little information about bee pollen loads from southern Brazil.

According to Falkenberg and Simões (2011), who conducted a preliminary botanical survey of southern Brazilian vegetation, *Piptocarpha angustifolia* Dusén ex Malme, *P. tomentosa* Baker, *Baccharis uncinella* DC., *B. dracunculifolia* DC., *Mimosa scabrella* Benth. and *Vernonanthura* spp. are prominent pioneering small tree and brush species occurring in early or middle successional stages. Eventually, they are also found in advanced

Table I. Pollen types identified in the pollen batches samples collected by *Apis mellifera* in the Paraná State.

Municipality/GPS coordinates	Collection period	Palynologycal evaluation	Predominant botanical origin
União da Vitória (1) 26° 10′ 38.80″ S 51° 05′ 46.30″ W	Spring 2011	PP: *Machaerium* (49.9%); PA: *Eucalyptus* (26.9%); PI: *Phoradendron* (5.8%); *Senecio* (13.4%)	Heterofloral sample with with primary contributions from *Machaerium* and *Eucalyptus*
União da Vitória (2) 26° 10′ 38.80″ S 51° 05′ 46.30″ W	Spring 2011	PP: *Piptocarpha* (72.0%); PI: *Eucalyptus* (9.0%); *Crotalaria* (13.6%)	Monofloral sample from *Piptocarpha*
União da Vitória (3) 26° 10′ 38.80″ S 51° 05′ 46.30″ W	Spring 2011	PP: *Machaerium* (62.1%); PI: *Eucalyptus* (14.6%); Rosaceae (5.6%); *Ilex* (7.4%);	Monofloral sample from *Machaerium*
União da Vitória (4) 26° 10′ 38.80″ S 51° 05′ 46.30″ W	Spring 2011	PP: *Machaerium* (65.8%); PA: *Eucalyptus* (21.8%); Ilex (7.1%)	Heterofloral sample with primary contributions from *Machaerium* and *Eucalyptus*
Lapa 25° 48′ 50.27″ S 49° 46′ 48.27″ W	Spring 2011	PP: *Eucalyptus* (83.9%); PI: *Machaerium* (5.1%)	Monofloral sample from *Eucalyptus*
Palmeira 25° 41′ 12.58″ S 50° 09′ 32.56″ W	Spring 2011	PA: *Euterpe* (38%); *Ilex* (23.9%); *Celtis* (22.6%)	Heterofloral sample with primary contributions from *Euterpe, Ilex* and *Celtis*

stages of succession at forest edges and in clearings within the remaining forest fragments. A substantial concentration of individuals and massive flowering characterise these populations. In addition, arboreal species such as *Syagrus romanzoffiana* (Cham.) Glassman, *Casearia sylvestris* Sw., *Campomanesia xanthocarpa* O.Berg, *Mimosa bimucronata* (DC.) Kuntze, *Schinus terebinthifolius* Raddi, *Luhea divaricata* Mart. and *Cupania vernalis* Cambess, the sub-shrubs *Senecio brasiliensis* Less., *Solidago chilensis* Meyen and *Cyrtocymura scorpioides* (Lam.) Pers. and the herbaceous *Paspalum notatum* Flüggé are also common. *Senecio brasiliensis* and *Solidago chilensis* are typical of the 'Cerrado' vegetation and occur in countryside weed vegetation, along roadsides and in open areas (Falkenberg & Simões 2011).

The present study aims to investigate the botanical and geographical origin of bee pollen collected in the southern Brazilian macro-region to provide results that will help to improve the certification of apicultural products that are associated with the use of this pollen by the bees.

Material and methods

A total of 61 dried pollen load samples of *Apis mellifera* were collected with pollen traps in 14 municipalities in southern Brazil (Figure 1) in the years 2011 and 2012. Samples from Eldorado do Sul were collected in 2014. All samples were analysed using the standard pollen analysis methodology proposed by Barth et al. (2010). Two grammes of each well-mixed sample of dried bee pollen was homogenised by stirring in 70% ethanol. Centrifuge tubes were filled to 13 ml, and the material was allowed to stand for 30 minutes or overnight. Each sample was then sonicated for five minutes to dissociate pollen grain agglomerates. After centrifugation, the samples containing a large amount of oil were subjected twice to ethanol extraction. The resulting sediment was diluted in a 1:1 mixture of water/glycerine for 30 minutes. One drop of this well-homogenised pollen grain suspension was applied to a microscope slide, covered with a 22 mm × 22 mm cover glass and sealed with nail varnish. The stock pollen suspension was kept for a long time in glycerine at room temperature in Eppendorf vials. Two microscope slides were prepared, and more than 500 pollen grains of each sample were counted. Samples were observed using light and polarised light microscopy.

The pollen classes used in this study followed the classification given in Barth et al. (2010): meaning PP (predominant pollen present at a frequency of more than 90%, or a frequency of more than 60% if no accessory pollen was present), AP (accessory pollen, 15–45%) and IP (important pollen, 3–15%). These classes were used for qualitative and quantitative analyses. Samples were classified as monofloral or heterofloral batches according to their pollen grain percentages. Pollen grain illustrations presented in Barth (1989), Roubik and Moreno (1991) and Moreti et al. (2002) were used for pollen identification. Micrographs of non-acetolysed pollen grains were obtained with a digital camera at 400× magnification.

Table II. Pollen types identified in the pollen batches samples collected by *Apis mellifera* in the Santa Catarina State.

Municipalities/GPS coordinates	Collection period	Palynologycal evaluation	Predominant botanical origin
São José 27° 34′ 00.35″ S 48° 43′ 18.27″ W	Spring 2011	PA: *Euterpe* (32.6%); *Eupatorium* (16.1%); PI: *Machaerium* (10.2%); *Piper* (14.7%); *Eucalyptus* (15.0%); *Senecio* (3.9%) *Ilex* (4.5%)	Heterofloral sample with primary contributions from *Euterpe* and *Eupatorium*
São José 27° 34′ 00.35″ S 48° 43′ 18.27″ W	Spring 2011	PA: *Euterpe* (38.0%); *Ilex* (23.9%); *Celtis* (22.6%); PI: *Eucalyptus* (13.0%)	Heterofloral sample with primary contributions from *Euterpe* and *Ilex*
São José 27° 34′ 00.35″ S 48° 43′ 18.27″ W	Spring 2011	PA: Poaceae (31.4%); *Eucalyptus* (20.9%); Melastomataceae (25.6%); PI: *Euterpe* (10.1%); *Piper* (6.3%)	Heterofloral sample with primary contributions from Poaceae, *Eucalyptus* and Melastomataceae
Balneário Gaivota 29° 09′ 25.2″ S 49° 34′ 51.2″ W	Spring 2011	PA: *Montanoa* (21.2%); *Lithraea* (31.8%); *Eucalyptus* (22.2%); Undetermined (17.0%)	Heterofloral sample with primary contributions from *Montanoa, Lithraea* and *Eucalyptus*
Içara 28° 43′ 37.7″ S 49° 18′ 52.4″ W	Winter 2011	PP: *Brassica* (77.3%); PA: Asteraceae (15.8%); *Eucalyptus* (6.3%)	Heterofloral sample with primary contributions from *Brassica* and Asteraceae

Results

The samples of dried pollen loads were first grouped according to the southern Brazilian states in which the samples were collected (Tables I–III).

Paraná (Table I)

Two samples from the União da Vitória municipality showed predominant pollen types of *Piptocarpha* (Asteraceae) and *Machaerium* (Fabaceae, Faboideae). *Eucalyptus* (Myrtaceae) pollen occurred in several samples (n = 5) and was predominant only in the sample from Lapa municipality. No predominant pollen type was detected in the sample from Palmeira municipality, where a large contribution of *Euterpe* (Arecaceae), *Ilex* (Aquifoliaceae) and *Celtis* (Ulmaceae) pollen types was observed.

Santa Catarina (Table II)

All samples were considered heterofloral and contained *Eucalyptus* pollen grains. The four samples from São José municipality all contained the *Euterpe* pollen type. The *Ilex* type occurred in two samples along with a large representation of *Celtis, Eupatorium* (Asteraceae), Melastomataceae, *Piper* (Piperaceae) and Poaceae. A different pollen spectrum was found in the other two municipalities. A strong contribution of *Lithraea* (Anacardiaceae) and *Montanoa* (Asteraceae) was observed in the Balneário Gaivota sample. However, in the Içara municipality, *Brassica napus* L. (Brassicaceae) was predominant along with a presence of Asteraceae pollen grains.

Rio Grande do Sul (Table III)

This state had the highest number of samples analysed (n = 49). Thirty samples were considered monofloral. *Eucalyptus* pollen was found in the majority of samples (n = 41), followed by Poaceae (n = 11), *Myrcia* (n = 6), *Brassica napus* (n = 6) and *Mimosa scabrella* (Mimosaceae) (n = 5). One monofloral sample of *Brassica napus* (São Gabriel 2) only occurred in the São Gabriel municipality. This pollen type was found in two other samples (São Gabriel 1 and 2), where it was associated with several pollen types. *Eucalyptus* pollen was present in all four analysed samples. Erechim municipality had a high contribution of *Eucalyptus* and the *Dalbergia* pollen type. The *Andira* (Fabaceae) pollen type represented 98.5% of the pollen content and yielded a monofloral sample in the Ijuí municipality.

The samples from Cruz Alta municipality contained one monofloral sample of *Eucalyptus* pollen. The other two samples consisted of either the *Eucalyptus* or *Brassica napus* pollen type, with variable percentages (Cruz Alta 2 and 3). No monofloral samples were present in the Jacutinga municipality. Heterofloral samples contained large contributions of Melastomataceae, *Myrcia* and *Piper* pollen types. Cambará do Sul municipality had monofloral samples of the *Mimosa scabrella* pollen type in four pollen batches (Cambará do Sul 1–4). *Eucalyptus* pollen was present in one batch (Cambará do Sul 6). The Cambará do Sul 5 sample had a large contribution of *Ilex* and Asteraceae pollen grains. The Cambará do Sul 7 sample contained large amounts of *Eucalyptus* and of the *Eupatorium* pollen type. The other sample (Cambará do Sul 8) had a high representation of *Ilex* and *Mimosa scabrella* pollen grains.

Table III. Pollen types identified in pollen batches collected by *Apis mellifera* in the Rio Grande do Sul State and botanical evaluation.

Municipalities/GPS coordinates	Collection period	Palynological evaluation	Predominant botanical origin
São Gabriel (1) 30° 15′ 27.72″ S 54° 28′ 59.41″ W	Winter 2011	**PP**: *Brassica* (80.5%) **PA**: *Eucalyptus* (19.4%)	Heterofloral sample with primary contribution of *Brassica* and *Eucalyptus*
São Gabriel (2) 30° 15′ 27.72″ S 54° 28′ 59.41″ W	Winter 2011	**PP**: *Brassica* (84.6%) **PI**: *Eucalyptus* (9.2%); *Machaerium* (5.2%)	Monofloral sample of *Brassica*
São Gabriel (3) 30° 15′ 27.72″ S 54° 28′ 59.41″ W	Winter 2011	**PP**: *Brassica* (48.5%) **PA**: *Machaerium* (16.6%); *Eucalyptus* (16.4%) **PI**: *Caryca* (13.3%); *Psychotria* (4.7%)	Heterofloral sample with primary contribution of *Brassica*, *Machaerium* and *Eucalyptus*
São Gabriel (4) 30° 15′ 27.72″ S 54° 28′ 59.41″ W	Spring 2011	**PP**: Caesalpiniaceae (56.1%) **PA**: *Eucalyptus* (32.3%)	Heterofloral sample with primary contribution of a Caesalpiniaceae and *Eucalyptus*
Erechim 27° 37′ 17.05″ S 52° 15′ 28.91″ W	Spring 2011	**PP**: *Eucalyptus* (70.6%) **PA**: *Dalbergia* (16.4%) **PI**: *Lithraea* (10.5%)	Heterofloral sample with primary contribution of *Eucalyptus* and *Dalbergia*
Ijuí 28° 21′ 41.14″ S 53° 50′ 34.84″ W	Spring 2011	**PP**: *Andira* (98.5%)	Monofloral sample of *Andira*
Cruz Alta (1) 28° 37′ 35. 94″ S 53° 37′ 57.66″ W	Spring 2011	**PP**: *Eucalyptus* (83.9%) **PI**: *Machaerium* (5.1%); *Brassica napus* (6.0%)	Monofloral sample of *Eucalyptus*
Cruz Alta (2) 28° 37′ 35. 94″ S 53° 37′ 57.66″ W	Spring 2011	**PA**: *Eucalyptus* (21.8%); *Euterpe* (16.0%) **PI**: *Machaerium* (13.1%); *Brassica napus* (12.3%); *Borreria* (5.3%); Asteraceae (4.8%); *Protium* (4.4%); *Tapirira* (3.8%); Phrygilanthus (3.5%)	Heterofloral sample with primary contribution of *Eucalyptus* and *Euterpe*
Cruz Alta (3) 28° 37′ 35. 94″ S 53° 37′ 57.66″ W	Summer 2012	**PP**: *Brassica* (56.6%) **PA**: *Eucalyptus* (41.8%)	Heterofloral sample with primary contribution of *Brassica* and *Eucalyptus*
Jacutinga 27° 43′ 38.3″ S 52° 32′ 15.3″ W	Summer 2012	**PA**: Melastomataceae (40.2%); *Myrcia* (32.7%); *Piper* (22.9%)	Heterofloral sample with primary contribution of Melastomataceae, *Myrcia* and *Piper*
Cambará do Sul (1) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PP**: *Mimosa scabrella* (81.6%); **PI**: Poaceae (5.8%); undetermined (3.7%)	Monofloral sample of *Mimosa scabrella*
Cambará do Sul (2) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PP**: *Mimosa scabrella* (94.8%)	Monofloral sample of *Mimosa scabrella*
Cambará do Sul (3) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PP**: *Mimosa scabrella* (86%) **PI**: *Ilex* (7.1%); *Baccharis* (4.7%)	Monofloral sample of *Mimosa scabrella*
Cambará do Sul (4) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PP**: *Mimosa scabrella* (99.2%)	Monofloral sample of *Mimosa scabrella*
Cambará do Sul (5) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PP**: *Ilex* (71.8%); PA: Asteraceae (20.5%); **PI**: *Podocarpus* (7.3%); *Protium* (3.8%)	Heterofloral sample with primary contribution of *Ilex* and Asteraceae
Cambará do Sul (6) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PP**: *Eucalyptus* (75.7%); **PI**: *Lamanonia* (15%); Ilex (4.5%); Asteraceae (3%)	Monofloral sample of *Eucalyptus*
Cambará do Sul (7) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PP**: *Eucalyptus* (69.8%); **PA**: *Eupatorium* (29.2%)	Heterofloral sample with primary contribution of *Eucalyptus* and *Eupatorium*
Cambará do Sul (8) 28° 54′ 45.06″ S 50° 04′ 58.45″ W	Spring 2012	**PA**: *Mimosa scabrella* (43.7%); *Ilex* (42.0%) **PI**: *Eupatorium* (6.4%);	Heterofloral sample with primary contribution of *Mimosa scabrella* and *Ilex*

(*Continued*)

Table III. (*Continued*).

Municipalities/GPS coordinates	Collection period	Palynological evaluation	Predominant botanical origin
Eldorado do Sul samples (1–25) 30° 4′ 54.05″ S 51° 39′ 55.81″ W	Winter 2014	**PP**: *Eucalyptus* (86–100%), without any accessory pollen type	25 monofloral samples of *Eucalyptus*
Eldorado do Sul samples (26–27) 30° 4′ 54.05″ S 51° 39′ 55.81″ W	Winter 2014	**PP**: *Myrcia* (78.8–88%), without any accessory pollen type	Two monofloral samples of *Myrcia*
Eldorado do Sul samples (28–31) 30° 4′ 54.05″ S 51° 39′ 55.81″ W	Winter 2014	*Eucalyptus* (18.6–63.7%), *Myrcia* (31.1–88%), *Zea mays*, (3.1–34.4%)	Four heterofloral samples with primary contribution of *Eucalyptus*, *Myrcia* and *Zea mays*

Thirty-one samples were obtained in the Eldorado do Sul municipality. The monofloral samples consisted of *Eucalyptus* in 25 samples and *Myrcia* in two samples. Heterofloral pollen batches were represented by these pollen types and, in addition, by Poaceae and *Zea mays* L. pollen grains.

Discussion

Although the southern Brazilian region shows a strong variety of vegetation (Veloso 1962; Rizzini 1997), some pollen types indicate that certain local plant taxa are particularly visited by the bees to collect and harvest pollen grains. Barth (2004) highlighted that analyses of bee pollen loads in the southern region have not been frequent. Recently, Freitas et al. (2013) examined the composition of some monofloral pollen batches obtained in the States of Santa Catarina and Rio Grande do Sul.

Paraná

Three monofloral samples of *Piptocarpha*, *Machaerium* and *Eucalyptus* pollen types occurred. The genus *Piptocarpha* is characteristic of Paraná; these trees occur in both the ombrophilous forest and in disturbed areas of secondary forests (Grokoviski et al. 2009). The *Machaerium* pollen type was also observed in honey samples from Paraná also by Borsato et al. (2014) and in samples from several environments by Bortoluzzi et al. (2004); these plants are widely used for the restoration of degraded areas (Lorenzi 1992, 1998).

Several species of *Eucalyptus* are cultivated in Brazil, mainly in the south and southeast regions. They have been introduced to Brazil since the beginning of the nineteenth century. This pollen type was observed in several honey samples from southern Brazil (Barth 1989) and is frequently visited by *Apis mellifera*, for which it serves as a pollen and protein resource (Pacheco et al. 1986).

Santa Catarina

No monofloral samples were obtained. Heterogeneity of pollen batches was found to be shown by the frequent pollen types of *Brassica, Celtis, Eucalyptus, Eupatorium, Euterpe, Ilex, Montanoa*, Anacardiaceae, Asteraceae, Melastomataceae and Poaceae. Freitas et al. (2013) recognised a monofloral pollen batch of a Caesalpiniaceae and another of the *Mimosa caesalpiniaefolia* Benth. pollen type in this state. The heterofloral samples also presented Asteraceae and Brassica pollen types, as in one sample from Içara (Santa Catarina State).

The *Euterpe* pollen type and some genera of Asteraceae (*Eupatorium, Senecio, Montanoa*) were representative of the Santa Catarina samples. *Euterpe* palm trees are of significant distribution in the ombrophilous forest (Raupp et al. 2009), but tree specimens were less representative in these samples. Asteraceae are particularly well represented in savannas and grasslands and, comparatively, not very significant in humid tropical forest lowlands (Jeffrey 2006). The pollen types *Eupatorium* (herbaceous to arboreal), *Senecio* (herbaceous) and *Montanoa* (shrub) found in the Santa Catarina samples may be indicating proximity to a forest region (Liebsch & Acra 2004), as they were generally characterised as pioneers genera.

Several pollen types were of low frequency in the samples analysed. These pollen types are useful for characterising the phytogeographical region surrounding the apiaries in each municipality. A mixture of ombrophilous forest (*Machaerium, Phoradendron, Piper, Ilex* and *Psychotria*) with field vegetation (Poaceae, Asteraceae) and crop elements (*Eucalyptus* and *Brassica napus*) could be observed. The bees, as well as the foraging plants (Veloso 1962; Rizzini 1997), are adapted to a cold climate in this region.

Rio Grande do Sul

The 35 monofloral samples consisted primarily of pollen grains of *Eucalyptus, Brassica, Myrcia, Machaerium, Andira, Piptocarpha* and the *Mimosa scabrella* pollen type. In addition, pollen types of *Eupatorium* and *Dalbergia* as well as of *Zea mays, Ilex, Euterpe*, Asteraceae, Melastomataceae and Poaceae also occurred at lower concentrations.

Three of four dried pollen batches of the São Gabriel municipality presented *Brassica* and *Eucalyptus* pollen grains at high concentrations; one sample (of *Brassica*) was considered monofloral. Large areas of Rio Grande do Sul are covered by crops such as *Brassica napus* ('canola') (Rosa et al. 2010, 2011) that are widely visited by *Apis mellifera*. All the samples from the Cruz Alta municipally contained high concentrations of *Eucalyptus* and *Brassica* pollen grains; one sample was considered monofloral of *Eucalyptus*. Cambará do Sul municipality contributed eight samples, four of them monofloral of the *Mimosa scabrella* pollen type, one of *Eucalyptus*.

Mimosa scabrella pollen was frequently found in honey and pollen samples from southern Brazil (Barth 1989; Freitas et al. 2013; Borsato et al. 2014). This species is a pioneer, mainly related to secondary vegetation; it is very tolerant of various soil conditions (Machado et al. 2008). Monofloral batches of *M. scabrella* have been analysed by Freitas et al. (2013) in one sample from São Paulo. Ramalho et al. (1990) highlighted that in the Neotropical region, the *M. scabrella* pollen type is commonly collected by *Apis mellifera*.

One sample from Ijuí presented a monofloral batch of *Andira* pollen type. Species of this genus are not frequent in pollen samples from southern Brazil. They were commonly found in open land areas and in secondary forests; this information indicates a preference for wet soils (Lorenzi 2002).

Thirty-one samples obtained from several apiaries of the Eldorado do Sul municipality were predominantly monofloral: 25 of *Eucalyptus*, two *Myrcia*. The Myrtaceae family is well represented in the ombrophilous forest (Grings & Brack 2009). The high frequency of the *Eucalyptus* pollen type may be associated with the large-scale replacement of native vegetation by other forms of land occupation. Large areas of forests have been converted into food production areas, especially into grain agriculture, and/or have been planted with *Pinus* and *Eucalyptus* species (Pinha & Siminski 2011).

Conclusion

The pollen analysis of the 49 pollen loads obtained in the state of Rio Grande do Sul showed how significant the information is to be gained from the study of a major number of colonies in different municipalities. If sufficient source material is available, the bees specialise on a given plant species: e.g. in our study, *Eucalyptus*, followed by *Brassica, Mimosa scabrella* and *Myrcia*. Further studies can potentially yield better resolution through field observation and plant species identification based on the pollen morphology of monofloral bee products. The heterofloral pollen batches represent the heterogeneity of the local bee-resource flora; they also indicate the context within which a uniform product can be obtained for consumption. However, a major incentive is needed to develop and implement a comprehensive and detailed botanical certification programme to increase the commercial value of bee pollen products.

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