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Do different landscapes influence the response of native and non-native bee species in the *Eucalyptus* pollen foraging, in southern Brazil?



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ABSTRACT

Eucalyptus is an exotic genus in Brazil with massive flowerings that are visited by bees in search of food. To verify the use of *Eucalyptus* spp. in the pollinic diet of *Apis mellifera* L. and *Melipona obscurior* Moure, we evaluated the proportion of pollen that both bee species collected from *Eucalyptus* spp. in relation to the relative abundance of *Eucalyptus* spp. in the study areas. The study occurred in the localities of Rioz-inho and Rolante, RS, Brazil, whose land cover was characterized by remote sensing. Every two weeks from April/2009 to March/2010, pollen was collected from foragers of three hives of each bee species for posterior palynological analysis. The median percentage of *Eucalyptus* spp. pollen collected in Riozinho was 16.3% (0–55.3%; n = 18) for *A. mellifera* and 2.6% (0–72.3%; n = 15) for *M. obscurior*. In Rolante, the median was 21.9% (0–66.7%; n = 19) for *A. mellifera* and 17.6% (0–82.9%; n = 17) for *M. obscurior*. The difference between these values was significant only when considered the collection period. The index of use was similar for both species and both areas. The attractiveness and availability of flowering *Eucalyptus* spp. throughout the year allowed both species of bees to use pollen from this genus in both areas, regardless of the landscape characteristics. In southern Brazil, sustainable practices for the management of eucalypts can contribute to the survival of social bees during the periods of lower pollen production by other sources.

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1. Introduction

The growth of exotic forest species is one of the primary causes of the decrease in native vegetal cover and subsequent habitat fragmentation (Foroughbakhch et al., 2001). This is followed by the loss of biodiversity due to the dissolution of mutualistic relationships between plants and pollinators (Morales and Aizen, 2002; Aizen et al., 2012). The insects are the most vulnerable component in this interaction (Taki and Kevan, 2007). A similar impact results from the effects of agroforestry and the homogenization of the landscape, reducing the diversity and abundance of native bees (Steffan-Dewenter and Tscharntke, 1999; Kremen et al., 2002).

Eucalypts dominate sectors of planted forest, a practice that finds itself in expansion (Marchini and Moreti, 2003) in most Brazilian states. Native to Australia, the genus *Eucalyptus* is cultivated on approximately 4.873.952 ha of Brazilian territory; Rio Grande do Sul is the country's fourth biggest producer with 280.198 ha of *Eucalyptus* plantings (ABRAF, 2012). The cultivation of *Eucalyptus* spp. is driven by the demand for wood for civil con-

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struction, woodcraft and cellulose extraction for paper production (ABRAF, 2012). The high melliferous potential of *Eucalyptus* species (Falkenberg and Simões, 2011) is utilized by beekeepers that associate *Apis mellifera* L. hives with forest nurseries during the blooming season (Dongock et al., 2007; Anjos et al., 2009). There are partnership proposals between companies and beekeepers with the goal of developing sustainable projects with social inclusion to raise the income of rural communities by producing honey (ABRAF, 2012). In degraded areas, *Eucalyptus* can be used on the restoration of ecosystem services.

Despite this use, the implementation of *Eucalyptus* spp. planting has raised several environmental questions such as concerns about the allopathic effect of their metabolites on the development of herbaceous plants (Souto et al., 2001; Zhang and Fu, 2009) and whether the eucalypts are capable of affecting the biodiversity associated with the cultivation areas (Falkenberg and Simões, 2011). Exotic vegetal species that are abundant sources of nectar and pollen for both native and introduced floral visitors can lead to alterations in the relationships between anthophilous insects and plants (Morales and Aizen, 2002, 2006; Aizen et al., 2012). Similar result can occur due to the distance between native forest fragments, diminished mobility of pollinators and decreased

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landscape heterogeneity due to the presence of these large scale cultivations (Steffan-Dewenter and Tscharntke, 1999; Kremen et al., 2002; Brosi et al., 2008). In this context, remote sensing technology has been an important tool to monitor ecosystems on a spatial scale (Malenovsky et al., 2009; Ustin and Gamon, 2010) and to study the human impact on the environment and its richness, diversity and bee abundance (Winfree et al., 2008, 2011; Steffan-Dewenter and Westphal, 2008).

Eucalyptus spp. have flowering patterns that vary in the length of the productive season (from three weeks to six months) and the number of flowers produced (from 100 to 1.5 million units in a single individual during the flowering period) (Potts and Gore, 1995). These characteristics have important implications on the foraging behavior of bees (Jha and Vandermeer, 2009), their main pollinators. The use of *Eucalyptus* by bees has been documented in the work of Cortopassi-Laurino and Ramalho (1988), which showed a predominance of pollen grains from species of *Eucalyptus* in the pollen samples collected by A. mellifera and Trigona spinipes Fabricius in São Paulo. To identify the nectariferous sources most visited by Hypotrigona gribodoi Magetti and Melipona ferruginea Lepeletier, Kajobe (2006) analyzed the pollen from the bodies of individuals from these species and found that the genus *Eucalyptus* was the most representative of the Myrtaceae family. Additionally, the study by Kleinert-Giovannini and Imperatriz-Fonseca (1987) on pollen samples from colonies of Melipona marginata Lepeletier over the period of one year in São Paulo, Brazil, found that Eucalyptus was the most common genus of pollen found on these samples.

In Rio Grande do Sul, Eucalyptus species are distributed throughout nearly the entire state, including the Atlantic rain forest areas inhabited by Meliponini species such as Melipona obscurior Moure (Witter and Blochtein, 2009). The presence of this species in the Atlantic Tropical Domain in southern Brazil is endangered by the ecosystem fragmentation that isolates their populations (Blochtein and Harter-Marques, 2003). M. obscurior shares its habitat with the Africanized A. mellifera, an exotic hybrid between subspecies from Europe and the African Apis mellifera scutellata (Schneider et al., 2004; Francoy et al., 2012), that is widely distributed among many Brazilian ecosystems. A. mellifera is a social species with perennial colonies that need nourishment for the whole year to support maintenance and reproduction (Imperatriz-Fonseca et al., 1994); this species exhibits generalist behavior regarding the use of floral sources (Kleinert et al., 2009). A. mellifera possesses up to 100,000 individuals per colony and is mainly associated with open areas. On the other hand, M. obscurior colonies have hundreds of individuals (Nogueira-Neto, 1997), and, like other Meliponini, they inhabit forest areas (Roubik, 2006; Brosi, 2009). The foraging radius for A. mellifera is around 1700 m (Roubik, 1989) and can be increased through induction to 10 km (Winston, 2003). Although there are no indications of the flight radius of M. obscurior, other Melipona species have flight radii of a few hundred meters to nearly 2 km (Roubik 1989; Imperatriz-Fonseca et al., 1994).

To evaluate the use of *Eucalyptus* on the pollinic diet of *A. mellifera* and *M. obscurior*, we analyzed the relationship between the proportion of pollen collected by both species and the relative vegetal covering.

2. Material and methods

2.1. Study areas

This study was conducted in two Atlantic rain forest areas in northeast Rio Grande do Sul, Brazil, in the hydrographic basin of the Rio dos Sinos located approximately 15 km from each other. The study areas are located in the townships of Riozinho (29°40'36.63″S and 50°27'32.58″O) and Rolante (29°38'3.20″S and

50°34′24.91″O), at altitudes of 570 m and 70 m above sea level, respectively. The region has subtropical humid weather and is classified as type Cfa according to the Köppen classification. The average temperature of the coldest month is above 3 °C and the hottest day is greater than 22 °C (Moreno, 1961). The region's phytophysionomy is considered to be semidecidual seasonal forest (Oliveira-Filho, 2009) with elevated vegetal diversity distributed among 143 woodland species belonging to 48 families (SEMA, 2012). The landscape in both places is also marked by areas with *Eucalyptus reforesting in which the most frequent species are Eucalyptus grandis* Hill ex maiden, *Eucalyptus saligna* Sm, *Eucaltptus citriodora* (Hook), *Eucalyptus viminalis* Labill and *Eucalyptus robusta* Sm (Sergio Koch, personal communication on 04/11/2012).

2.2. Bee colonies

In both study areas, three colonies of *A. mellifera* were used in Langstroth hives, and three *M. obscurior* colonies were kept in standard hives according to the model of Venturieri et al. (2003). The distance between the *A. mellifera* and *M. obscurior* hives was approximately 200 m in Riozinho and 150 m in Rolante.

2.3. Acquisition of pollen samples

The entrance to each A. mellifera hive was attached to a pollen collector suitable for Langstroth hives. This equipment consists of a wooden box attached to the inferior part of the hive with dimensions of approximately $34 \times 9 \times 7$ cm and an acrylic plate with 221 circular holes of 4.6 mm diameter attached directly in front of the hive entrance. When entering the hive by flying through the board, the pollen carried by the foraging bees would fall into the collector box. On each collecting day, the acrylic plates were put on at dawn and removed at twilight. To obtain pollen samples collected by M. obscurior, the entrance to the hive was blocked with a small wooden stick for 15 min every hour during the period of flight activity. Foragers returning to the hive were then captured with entomological nets, their pollen loads were manually removed and the bees were immediately set free. This procedure was performed sequentially at the three hives with a 5 min interval between hives. The pollen sampling of both species occurred biweekly from April 2009 to March 2010. Because the external activity of hives is related to weather conditions, some months had single or no sampling.

2.4. Laboratory procedures

The pollinic material from each hive was dried at a temperature of 38 °C for 48 h. After weighing, the samples were disaggregated and homogenized with a magnetic agitator at 10,000 rpm. Once solubilized, 0.1 g of pollen was collected and subjected to acetolysis of the grains (Erdtman, 1966). The palynological slides were prepared using glycerin gelatin assemblage stained with fuchsin over which a small fraction of the pollinic material was deposited; three slides were prepared for each sample.

2.5. Palynologic analysis

Transects were established on each slide, and 1200 pollen grains were counted and identified for each sample. Pollinic catalogs and a pollen reference collection were used to identify the pollinic types.

2.6. Classification of ground covering

With the objective of relating the data obtained from hives of *A. mellifera* and *M. obscurior* with the classes of ground usage, a radius

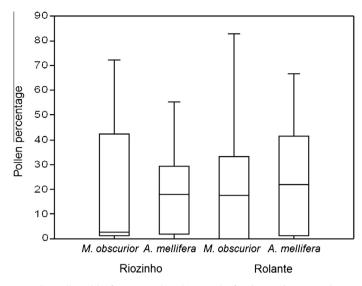


Fig. 1. Average percentage of *Eucalyptus* spp. pollen collected by foragers in three hives each of *Melipona obscurior* and *Apis mellifera* from April 2009 to March 2010 in Riozinho and Rolante, RS, Brazil. Horizontal internal line = median; external limit of the columns = minimum and maximum values.

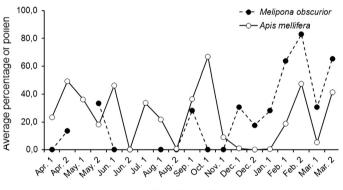
Table 1

Relationship between the *Eucalyptus* spp. pollen percentage with the variables date, bee species, local and their interactions as determined by 3-way ANOVA (n = 168). The data from months during which no *Eucalyptus* pollen was collected are not shown. Significant values are with asterisk.

	df	f	р
Date	13	5.550	<0.001*
Specie	1	1.173	0.281
Local	1	3.588	0.061
Date [*] specie	13	2.602	0.003*
Date [°] local	13	3.472	< 0.001
Specie [®] local	1	0.014	0.906
Date [*] specie [*] local	13	2.003	0.027*

* Values with significative difference.

of 3 km around the hives was mapped based on the flight measures reported for *A. mellifera* (higher than *Melipona* bees) in Roubik (1989), Imperatriz-Fonseca et al. (1994) and Winston (2003). This was accomplished using a Landsat TM5 image of each area with a point-orbit of 220/081 that was obtained on 04/28/2001 and is available on the INPE (National Institute of Space Research) website. The images were processed by using the computer program ENVI® (*Environment for Visualizing Images*) version 4.8. A GeoCover base image was used for georeferencing. The ground truths, band



Sampling periods

Fig. 3. Average of the percentages of *Eucalyptus* spp. pollen obtained from three *Melipona obscurior* (filled symbols) and three *Apis mellifera* (open symbols) hives from April 2009 to March 2010 in Rolante, RS, Brazil. The number beside the month indicates the fifteen days of each collection.

compositions and NDVI were used for the selection of the samples during the maximum Gaussian probability supervised classification. The analysis of the Landsat TM 5 images from interpretation

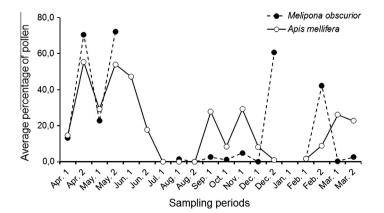


Fig. 2. Average of the percentages of *Eucalyptus* spp. pollen obtained from three *Melipona obscurior* (filled symbols) and three *Apis mellifera* (open symbols) hives from April 2009 to March 2010 in Riozinho, RS, Brazil. The number beside the month indicates the fifteen days of each collection.

patterns (color, tone, texture and form) coupled with field observations allowed for the identification and grouping of the following classes of use and ground cover: agropastoral, water, urban area, *Eucalyptus* spp., native forest and *Pinus* spp. Due to the spatial resolution of 30 m in Landsat TM 5 images, some types of ground usage observed in field visits, such as swamps, were included by the digital classifier in the adjacent ground usage classes or a similar spectral answer.

2.7. Statistical analysis

Data from pollen counts were used to create a data matrix considering the three hives of each bee species, the study area and period of each sampling. For all analysis was used the program Statistical Package for the Social Sciences (SPSS) 17.0. To verify the effect of the collection date, bee species, place and respective interactions on the use of *Eucalyptus* spp. pollen, a 3-way ANOVA was performed on the percentage Eucalyptus spp. pollen. A square root transformation was used on this variable to satisfy the test assumptions. Only collecting dates for which there were data from both places and for both bee species were used in this analysis. To control for the effect of the date on the analysis, the species and places were also compared using the Wilcoxon test based on the average of the values from the three hives observed for each collecting date. To analyze the relationship between the use of Eucalyptus spp. pollen by both bee species and the influence of percentage of area occupied by *Eucalyptus* considering the distinct landscapes, the variable "index of use" (Iu) was created using the following formula:

Iu = (% of Eucalyptus pollen/100)

\times (% of area occupied by *Eucalyptus*).

This index was used to indicate how much the ground cover may influence the bee's pollen diet. *Eucalyptus* occupied 4.33% and 3.62% of the studied areas in Riozinho and Rolante, respectively. A 3-way ANOVA was performed on **Iu** to compare dates, bee species and places as well as interactions between these factors. A square root transformation was used on **Iu**.

3. Results

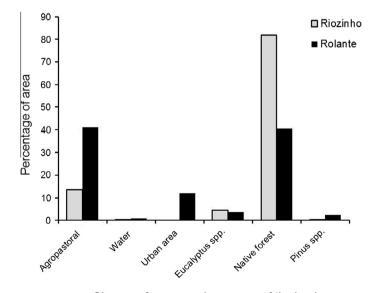
Considering the total pollen collected in a year of sampling in Riozinho, the median percent of pollen from *Eucalyptus* spp. used by *M. obscurior* and *A. mellifera* were 2.6% (minimum = zero, maximum = 72.3; n = 15) and 16.3% (minimum = zero, maximum = 55.3; n = 18), respectively. In Rolante, the median percentage of *Eucalyptus* spp. pollen collected was 17.6% by *M. obscurior* (minimum = zero, maximum = 82.9; n = 17) and was 21.9% for *A. mellifera* (minimum = zero, maximum = 66.7; n = 19) (Fig. 1).

Although the relative frequency of *Eucalyptus* spp. pollen collected by both bee species was greater in Rolante than in Riozinho, the difference between the areas was not significant by ANOVA (p = 0.061). Similarly, the difference between the values found for the percentage of *Eucalyptus* spp. pollen collected by *A. mellifera* compared to *M. obscurior* was not significant (p = 0.281) even when separated according to location (p = 0.906) (Table 1).

Wilcoxon's test was applied to the *Eucalyptus* spp. pollen percentage collected by both species, and, after controlling for the collection date, no significant difference was found between *A. mellifera* and *M. obscurior* in Riozinho (z = 0.314; n = 14; p = 0.787) or in Rolante (z = 0.471; n = 14; p = 0.671). There was also no difference between the percentages of collection of this pollinic type among the different locations for *A. mellifera* (z = 0.79; n = 14; p = 0.463) or *M. obscurior* (z = 0.454; n = 14; p = 0.685).

The percentages of pollen from *Eucalyptus* spp. collected by both bee species oscillated throughout the year for both areas. The percentage of *Eucalyptus* spp. pollen in the Riozinho samples was largest in April and May for both *A. mellifera* and *M. obscurior* (Fig. 2). At Rolante, the collection peaks were different for the two species; the largest percentages of *Eucalyptus* spp. pollen collected by *A. mellifera* occurred in April and October, and *M. obscurior* collection peaked in February and March (Fig. 3).

Plantations of *Eucalyptus* species are found in the landscapes around the hives in both areas in similar proportions along with other classes of ground occupation (water, urban area, native forest and *Pinus* spp.). In Riozinho (Fig. 4), the area with *Eucalyptus* spp. corresponds to 4.3%, and native species occupy the majority (81.8%) of the ground cover. In Rolante, the area occupied by



Classes of usage and covarage of the land

Fig. 4. Usage and coverage of the land surrounding the Apis mellifera and Melipona obscurior hives in Riozinho and Rolante, RS, Brazil. The classification was performed according to a Landsat TM 5 image from 04/28/2011.

Eucalyptus is 3.6%, native forest occupies 40.4%, and classes associated with anthropic action (52.9%) occupy the majority of the area, indicating a greater degree of fragmentation in this area than in Riozinho (Figs. 5 and 6).

The analysis of the relationship between percentage of *Eucalyptus* spp. pollen and the area occupied by this arboreal, by ANOVA showed no significant difference in the index of use of *Eucalyptus* spp. for the variables species (p = 0.707), place (p = 0.314) and the interaction of these variables (p = 0.850). However, the difference was significant when comparing the index of use between date of collection (p < 0.001) and a significance was also found in the interaction between date of collection and species. Thus, the level of significance of the index of use of *Eucalyptus* spp. was dependent on the date (Table 2).

4. Discussion

The occurrence of *Eucalyptus* pollen in most samples collected from *A. mellifera* and *M. obscurior* over the year and the high

representativeness of this pollen in relation to the average percentage of total pollen collected may be associated with the appeal of the *Eucalyptus* pollen and nectar. Its intense flowering, with flowers that stay productive for more than three days, are greatly attractive to floral visitors (Marchini and Moreti, 2003), and bees respond positively to the presence of massive flowerings with high rates of visits to the flowers (Westphal et al., 2003; Holzschuh et al., 2007; Kohler et al., 2008; Jha and Vandermeer, 2009). This can also explain the similarity between the percentages of *Eucalyptus* pollen collected by *A. mellifera* and *M. obscurior*, not only due to its attractiveness, but also because it supports the concomitant exploration of this resource (Ramalho et al., 2007) by both bee species.

Although *Eucalyptus* is an exotic genus, it occurs in a variety of Brazilian ecosystems and is commonly found in pollinic samples (Wilms and Wiechers, 1997; Carvalho et al., 1999; Ferreira et al., 2010; Luz et al., 2011) and in observations of bees making floral visits (Imperatriz-Fonseca et al., 1994; Marchini and Moreti, 2003; Lopes et al., 2007; Mendes et al., 2008). The use of nourish-

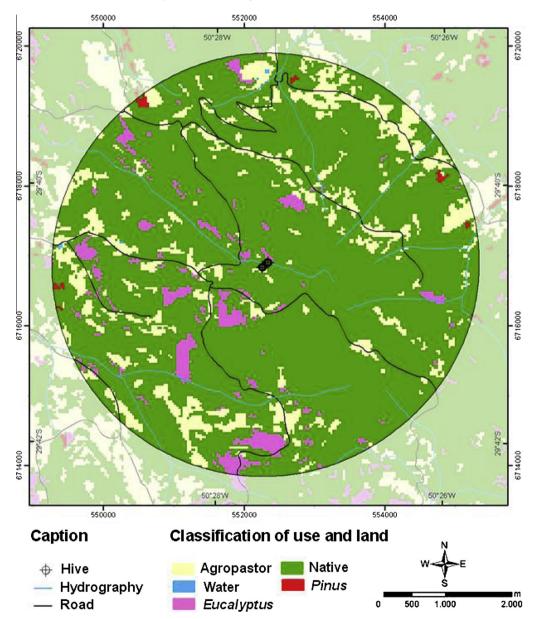


Fig. 5. Classification of use and land coverage of the Apis mellifera and Melipona obscurior hives' surroundings in Riozinho, RS, Brazil based on a Landsat TM 5 image, orbit-point 220/081, from 04/28/2011.

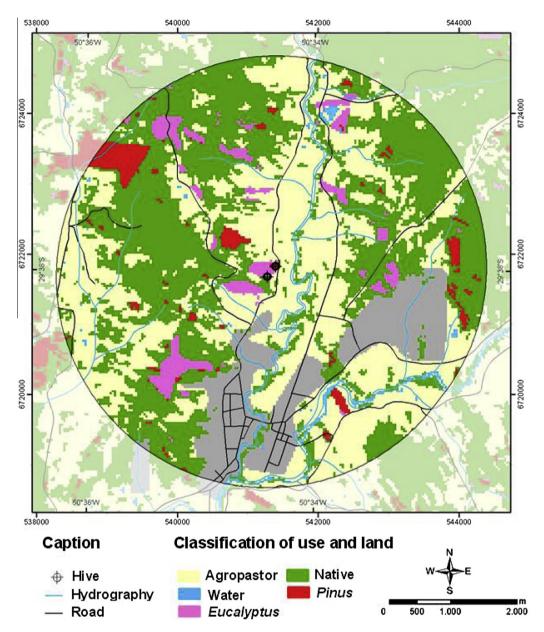


Fig. 6. Classification of use and land coverage of the Apis mellifera and Melipona obscurior hives' surroundings in Rolante, RS, Brazil based on a Landsat TM 5 image, orbit-point 220/081, from 04/28/2011.

Table 2

Relationship between the index of use (**Iu**) of *Eucalyptus* spp. pollen and the variables date, bee species and local and their interactions as determined by 3-way ANOVA (n = 168). The data from months in which no *Eucalyptus* pollen was collected are not shown. Significant values are with asterisk.

	df	f	р
Date	13	5.073	< 0.001*
Specie	1	1.142	0.707
Local	1	1.023	0.314
Date [*] specie	13	2.415	0.007*
Date [*] local	13	3.459	< 0.001*
Specie [*] local	1	0.036	0.85
Date [*] specie [*] local	13	1.784	0.054

* Values with significative difference.

ment resources provided by *Eucalyptus* spp. has been reported for both *A. mellifera* and native bees in Brazil. This interaction was found by Souza et al. (2004) in an urban area in São Paulo involving

Eucalyptus tereticornis Smith and its floral visitors, composed of 11 Meliponini species and *A. mellifera*. As in the more preserved land-scape of Riozinho, Wilms and Wiechers (1997) found in a tropical forest preserved in São Paulo, 60% of the pollen collected by *A. mellifera* and *Melipona quadrifasciata* Lepeletier came from *Eucalyptus*. This was also one of the most frequent pollen sources for *A. mellifera*, *Plebeia droryana* (Friese), *Partamona helleri* (Friese), *Tetragonisca angustula* Latreille and *Nanotrigona testaceicornis* Lepeletier in the city of Piracicaba, São Paulo (Carvalho et al., 1999). These results are aligned with the idea that the **Iu** of *Eucalyptus* by both bee species is independent of the ground cover.

The analysis of pollen in 10 hives of *Melipona capixaba* Moure and Camargo located near forest fragments containing *Eucalyptus* species in Espírito Santo, Brazil, revealed that this genus contributed from 53.4% to 96.6% of the total pollen stored in each hive (Luz et al., 2011). In the analysis of pollen collected by *Scaptotrigona depilis* (Moure) in Midwestern Brazil, Ferreira et al. (2010) found, among 42 plant species represented, *Eucalyptus* had higher percentage of pollen (46.5% of the pollen total).

The many species of the genus Eucalyptus cultivated in the region of this study have different flowering periods that resulted in the variable availability of their floral resources throughout the year in both areas. This explains the observation that the average percentages of Eucalyptus spp. pollen in the samples varies throughout the year in both areas and is evidenced in the significant influence of the date on the collected pollen percentages and on the indices of use by both bee species in both areas. Such influence could be related to the availability of new attractive blossoms of other plant species that comprised the different pollinic diet of each bee species in different periods. Furthermore, southern Brazil shows seasonality in the distribution of temperatures, and *M. obscurior* is sensitive to more extreme weather conditions and may exhibit facultative reproductive diapause during periods of low temperatures (Borges and Blochtein, 2005). Considering temperatures. A. mellifera can withstand extreme cold and heat (Goulson, 2003) and has advantage over *M. obscurior*.

Even with the observation that the structure of the landscape in Riozinho is more preserved than that of Rolante and has native forest occupying the majority of the ground cover, the index of use of Eucalyptus spp. was similar to both bee species in both areas. This, once again, demonstrates the degree of attractiveness of *Eucalyptus* spp. in relation to other vegetal species and the floral preference of A. mellifera and M. obscurior. In a study of the interactions between native and exotic species of Patagonia, especially in areas with more anthropic activity, Morales and Aizen (2006) pose the existence of a "mutualistic complex invader" that can favor the exotic pairs with the greatest intensity in altered habitats. The authors found that A. mellifera had strongest association with exotic plants compared to other floral visitors. Similar results were described by Medan et al. (2011) for the Argentinean Pampa. They found more interactions between exotic pollinators (including A. mellifera) and exotic plants than with native vegetal species. However, they observed that many native pollinators started to relate opportunistically with exotic plants. This observation corroborates our results through the similarity of the representativeness of Eucalyptus pollen in the total pollen collected and its index of use by M. obscurior and A. mellifera.

Despite eucalypts competes with the native vegetation for pollinators (Souza et al., 1994; Lopes et al., 2007), it can receives pollinator species whose native vegetation has been excluded from their local habitats (Holzschuh et al., 2007; Winfree et al., 2007). This relationship is described by Kremen et al. (2007) for anthropized landscapes and, according to Tscheulin et al. (2011) and Öckinger et al. (2012), shows how the characteristics of the landscape around habitats can influence the persistence of populations in fragmented areas. Such can be due to the capability of Eucalyptus in supporting the presence of more than one collector species resulting in low or inexistent competition by resource (Colwell and Futuyma, 1971; Townsend et al., 2006; Ramalho et al., 2007). From this conjecture, the present study evaluating the utilization proportion and the real use of floral sources from its representativeness on the landscape conclude that, the response of M. obscurior and A. mellifera species in the Eucalyptus pollen foraging is the same, even in different landscapes and ground cover.

Considering that *Eucalyptus* can help on the maintenance of native bees and that it has broad utilization in current Brazilian rural models, it is suggested that the implementation of this kind of cultivation takes into account the different flowering periods of plants and the necessity of planned cutting in order to guarantee continuous nourishment for the bees.

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