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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 Riozinho 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.

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 construction, 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 × 9 × 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