Comparative analysis of two sampling techniques for pollen gathered by *Nannotrigona testaceicornis* Lepeletier (Apidae, Meliponini)

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**ABSTRACT.** Pollen counts from samples taken from storage pots throughout one year (from October to September) were adjusted by Tasei’s volumetric correction coefficient for the determination of pollen sources exploited by two colonies of *Nannotrigona testaceicornis* in São Paulo, Brazil. The results obtained by this sampling technique for seven months (December to June) were compared with those from corbicula load samples taken within the same period. This species visited a large variety of plant species, but few of them were frequently used. As a rule, pollen sources that appeared at frequencies greater than 1% were found with both sampling methods and significant positive correlations (Spearman correlation coefficient) were found between their values. The pollen load sample
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data showed that \textit{N. testaceicornis} gathered pollen throughout the external activity period.

Key words: Stingless bees; Pollen analysis; Pollen exploitation; Sampling methods

INTRODUCTION

Pollen analysis of food sources visited by the social Apidae has been widely used in studies about bee feeding habits (Kleinert-Giovannini and Imperatriz-Fonseca, 1987; Kleinert et al., 1987; Cortopassi-Laurino and Ramalho, 1988; Guibu et al., 1988; Imperatriz-Fonseca et al., 1989; Ramalho, 1990; Roubik and Moreno, 1990; Martínez-Hernández et al., 1994; Wilms and Wiechers, 1997; Ramalho et al., 1989, 1990, 2007). This kind of analysis revealed that these social insects have generalist feeding habits, which is the result of their eusocial habits and prolonged activity period, although they concentrate on certain flowers over given periods (Imperatriz-Fonseca et al., 1989).

However, these studies employed different sampling techniques, sometimes using pollen taken from corbiculae (Absy and Kerr, 1977; Sommeijer et al., 1983; Roubik et al., 1986; Ramalho et al., 2007), and other times using pollen sampled from storage pots (Engels and Dingemans-Backels, 1980; Absy et al., 1984; Kleinert-Giovannini and Imperatriz-Fonseca, 1987; Cortopassi-Laurino and Ramalho, 1988; Guibu et al., 1988; Ramalho, 1990; Martínez-Hernández et al., 1994).

Malagodi-Braga and Kleinert (1993), weekly following the usage of food pots from \textit{Schwarziana quadripunctata} verified that the same pot was used for pollen storage or honey storage during variable periods. Martínez-Hernández et al. (1994) found that \textit{Nannotrigona testaceicornis} used pollen from open pots, that were not yet full, for brood cell provisioning.

The use of two sampling techniques, of pollen from corbicula loads and of pollen from storage pots should help us understand the dynamics of pollen usage; as Wilms and Wiechers (1997) pointed out, using these two methods together should allow a better measure of the real proportion of pollen sources exploited by bees.

In general, studies about patterns of usage of pollen sources by bees utilize the frequency of pollen grains in food samples to establish the relative importance of each source, by counting a certain number of grains. According to Silveira (1991), due to the great size variability among pollen grains from different plant species, frequencies obtained by this procedure may not represent the real contribution in weight or volume of each plant in the samples.

Biesmeijer et al. (1992) found that several plant species, which were important pollen sources for \textit{Apis mellifera} in volume estimates, were not frequent in grain counts. They also noted that volume estimates resulted in greater plant diversity used as pollen sources and demonstrated a more homogeneous use of these sources by bees.

Buchmann and O’Rourke (1991) and Biesmeijer et al. (1992) considered volume estimates as the best method to determine the patterns of utilization of pollen sources by bees, despite the difficulties in estimating grain volume precisely. Silveira (1991) suggested the use of Tasei’s (1973) volume correction coefficient (Q), which utilizes the
quotients of mean diameters of grains to the third power.

We evaluated the contribution of two sampling techniques, sampling from corbicula loads and from storage pots, for the identification of pollen sources exploited by two colonies of *N. testaceicornis*, using grain counting as well as adjustment with the Q coefficient (Tasei, 1973).

**MATERIAL AND METHODS**

The samples for this study were taken from colonies kept at the Bee Laboratory of the Biosciences Institute (IBUSP) at the University of São Paulo (Brazil). The two *N. testaceicornis* colonies, named C1 and C2, were located close to each other.

There is great plant diversity at the IBUSP gardens, where native and exotic plant species can be found. Close to the gardens, there is a 10 hectare semi-deciduous forest with many native species.

Pollen was sampled from the colonies by means of two techniques: sampling from storage pots (along one year, from October to September) and from corbicula loads (for seven months, from December to June). Pollen pot samples (about 3 mg) were taken every 15 days from the newer pots, which were mapped weekly through direct observation. Once a week, pollen loads were collected every hour (from 8:00 am to 16:00 pm): for 15 min, the foragers that were returning to the hive were captured near the entrance with a net and their pollen loads were immediately removed and the total number of bees carrying pollen was recorded.

All samples were kept in glacial acetic acid in glass vials, until undergoing acetolysis, following Erdtman (1960). Pollen grains were identified by comparison to the reference slide collection of the Bee Laboratory and with the aid of specialized literature. Those pollen grains that could not be identified received an identification number. One thousand pollen grains were counted following Vergeron (1964) and the results were added up monthly to determine the relative frequency of pollen types in the monthly pollen spectrum; the counting of each of the two pot samples for each month was added and the same was done with each of the four pollen load samples.

The pollen grains with relative frequency above 1% in the two colonies had their number counts adjusted by Tasei’s (1973) Q coefficient in both sampling methods. These grains had their mean diameters estimated following Silveira (1991), and the Q coefficient was calculated for each month sample.

The Spearman correlation coefficient was used to check for similarity between the results of the two sampling methods in both colonies.

**RESULTS**

One hundred and seven pollen types were found in the storage pots along the year. Only 24 types occurred with a relative frequency above 1%. From December to June, 72 pollen types were found in pot samples (seven with over 1%) and 54 in pollen-load samples (11 above 1%). Most of the pollen sources exploited (with percentage of occurrence above 1%) by *N. testaceicornis* were native trees (Table 1).

In the results adjusted by Tasei’s coefficient, the major pollen sources (relative frequency above 10%) in samples taken from storage pots throughout the year were: *Bauhinia*
Table 1. Pollen type frequency from pollen pots of two Nannotrigona testaceicornis colonies (C1 and C2).

<table>
<thead>
<tr>
<th>Plant family</th>
<th>Pollen type</th>
<th>Habitat</th>
<th>Growth habit</th>
<th>Origin</th>
<th>Pots Oct to Dec</th>
<th>Pots Dec to Jun</th>
<th>Loads Dec to Jun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anacardiaceae</td>
<td><em>Schinus terebinthifolius</em></td>
<td>mid forest tree native</td>
<td></td>
<td></td>
<td>*</td>
<td>X*</td>
<td>X*</td>
</tr>
<tr>
<td>Apiaceae</td>
<td><em>Foeniculum vulgare</em></td>
<td>gardens shrub introduced</td>
<td></td>
<td></td>
<td>N*</td>
<td>N*</td>
<td></td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Vernonia polyantha</em></td>
<td>gardens shrub native *</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td><em>Alchornea sidaefolia</em></td>
<td>mid forest shrub native</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Fabaceae</td>
<td><em>Bauhinia</em> sp</td>
<td>gardens shrub native X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Caesalpinia peltophoroides</em></td>
<td>gardens shrub native X</td>
<td></td>
<td></td>
<td>X*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td><em>Leucaena leucocephala</em></td>
<td>gardens shrub native X</td>
<td></td>
<td></td>
<td>X*</td>
<td>X*</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Piptadenia gonoancthan</em></td>
<td>gardens shrub native X</td>
<td></td>
<td></td>
<td>X*</td>
<td>X*</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Schizolobium parahyba</em></td>
<td>gardens shrub native X*</td>
<td></td>
<td></td>
<td>X*</td>
<td>X*</td>
<td>X</td>
</tr>
<tr>
<td>Liliaceae</td>
<td><em>Aloe</em> sp</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Loranthaceae</td>
<td><em>Struthanthus andrastylus</em></td>
<td>gardens shrub Tipuana</td>
<td></td>
<td></td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
</tr>
<tr>
<td>Meliaceae</td>
<td><em>Cedrela</em> sp</td>
<td>mid forest shrub native</td>
<td></td>
<td></td>
<td>X</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Moraceae</td>
<td><em>Cecropia</em> sp</td>
<td>gardens shrub native X</td>
<td></td>
<td></td>
<td>N*</td>
<td>N*</td>
<td>N*</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Eucalyptus</em> sp</td>
<td>gardens shrub native X</td>
<td></td>
<td></td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
</tr>
<tr>
<td>Rhamnaceae</td>
<td><em>Hovenia dulcis</em></td>
<td>gardens shrub introduced</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Rosaceae</td>
<td><em>Eryobotrya japonica</em></td>
<td>gardens shrub introduced</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Sapindaceae</td>
<td><em>Serjania</em> sp</td>
<td>gardens shrub introduced</td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Not identified</td>
<td>No. 1</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No. 2</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No. 29</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>No. 39</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>No. 67</td>
<td>-</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Counts adjusted by the Q coefficient. X: monthly frequency always 10% or higher; *: monthly frequency always between 1 and 10%; X*: monthly frequency both higher than 10 and between 1 and 10% during the sampling period; N*: monthly frequency between 1 and 10% when the counts were not adjusted.

When the two sampling methods were compared for the same seven months (December to June), the number of major pollen sources in corbicula loads was twice that found in samples from storage pots. *Eucalyptus* sp, *P. gonoancthan*, and *S. andradystylus* appeared as the major sources in the pot samples during this period. The latter species occurred as an alternative source in load samples and the others were major contributors in the load samples (Table 1).
The genus *Eucalyptus* stood out among the major pollen sources because it was always present in percentages above 10% for both colonies, except in October. From February to June its relative frequency was above 90% for both methods and for results with or without volumetric correction (Figures 1 and 2).

**Figure 1.** Pollen types with monthly frequencies 10% or higher in storage pot samples from two colonies (C1 and C2) of *Nannotrigona testaceicornis* (October 1991 to September 1992): A. Based solely on grain counts; B. Adjusted by Tasei’s (1973) volumetric correction coefficient (Q).
Comparative analysis of pollen sampling methods

Bees carrying pollen were captured from 8:00 am until 4:00 pm on collection days (Figure 3). Through seven months the mean number of bees captured each hour was 10 for colony C1 and 19 for C2.

Figure 2. Pollen types with monthly frequency equal or greater than 10% based on Tasei’s (1973) volumetric correction coefficient from two colonies (C1 and C2) of *Nannotrigona testaceicornis* (December 1991 to June 1992): A. In storage pot samples; B. In corbicula load samples.
When we compared the results of the two sampling methods, we found that the same plant species were exploited monthly for pollen collection in both colonies, although at different frequencies. Only two unidentified grain types were exceptions: a) #67 occurred in June only in the load samples and it appeared in pot samples after this month and b) #2 was only present in the load samples of colony C2.

There was a significant positive correlation between the two sampling methods: nearly 66% of the values of colony C1 (rs = 0.658, P = 0.0056) and about 72% of those of colony C2 (rs = 0.723, P = 0.001).

DISCUSSION

Nannotrigona testaceicornis explored a huge range of different plants to gather pollen, although concentrating its exploitation on a few pollen sources. Most of the major pollen sources were trees that usually produce a great number of flowers. There are several trees near the Bee Laboratory, including Caesalpinia peltophoroides, Piptadenia gonoacantha, Schizolobium parahyba, and Eucalyptus spp. Roubik et al. (1986) and Martínez-Hernández et al. (1994) also observed that the arboreal stratum was the major pollen source for N. testaceicornis. Other stingless bee species have also been found to be the dominant flower visitors of trees (Roubik et al., 1986; Wilms et al., 1996; Ramalho, 2004).
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Despite the relatively great number of introduced plant species, the number of native plants exploited as pollen sources by this bee was more than 60%. However, from February to June, an introduced plant genus, *Eucalyptus*, was the only major pollen source for *N. testaceicornis* (except in June in C2 load samples). This is a relatively accessible resource (open flowers, numerous anthers), nearby the hives and abundant (Guibu et al., 1988). Moreover, it flowers during a period when there are not many other species in bloom (Rossi, 1987).

In other studies at this site, the genus *Eucalyptus* was one of the most important pollen sources during fall (March through August), both for several Meliponini species and *Apis mellifera* (Kleinert-Giovannini and Imperatriz-Fonseca, 1987; Cortopassi-Laurino and Ramalho, 1988; Guibu et al., 1988; Ramalho, 1990). One can suppose that pollen production by *Eucalyptus* is high enough to allow its exploitation by the bee community of the area without interspecific competition (Kleinert-Giovannini and Imperatriz-Fonseca, 1987). Pollen exploitation is usually more diversified in the other months, and the available plant resources show a more homogeneous (more equitable) distribution in the bees’ diet, due to the higher number of plant species in bloom.

Biesmeijer et al. (1992) pointed out that studies that use only the counting of pollen grains could overestimate the relative importance of small grains, such as *Eucalyptus* spp and *Cecropia* sp. In this study *Cecropia* sp with the smallest pollen grain in the samples had their relative importance reduced to a frequency below 1% when the values were adjusted with the Q coefficient, but *Eucalyptus* spp generally remained as a major pollen source throughout the year, due to the great number of their grains in the samples and the shortage of other pollen types.

According to Rossi (1987) and Knoll et al. (1993), from August to January IBUSP’s gardens and forest show a higher abundance of species in blossom. Within this period both colonies of *N. testaceicornis* visited a higher number of plant species, resulting in higher variation in the relative frequencies of their pollen sources.

Along the year, 57% of pollen sources with relative frequencies above 1% were exclusively alternative sources (always with relative frequencies between 1 and 10%). This suggests that they have an important role in the development and support of colony populations of this species, maybe supplementing its pollen diet in periods when the diversity of species in bloom is reduced. From February to May a smaller number of alternative sources in the pot samples were observed, owing to a decrease in the number of species in bloom and to the plentiful flower offer of *Eucalyptus* trees. Arriaga (1989) found that alternative sources, exploited by *Plebeia* sp for several months, had an important role in supporting colonies of this species. These sources might contribute to diversify bees’ diet, providing pollen grains with different digestibility degrees (Velthus, 1992).

The results here confirmed the phenology of most pollen sources exploited by *N. testaceicornis* in samples taken from storage pots at the same period. Sommeijer et al. (1983) collected pollen loads from 2 to 5 bees, twice a day, and considered the method appropriate in determining the relative importance of different plant species for the bees’ diet.

Statistical analysis showed that the values of two sampling methods were significantly positively correlated; there was statistical similarity in the relative frequency of pollen grains among corbicula load samples versus storage pot samples from the two colonies.

Moreover, pollen loads can provide much more information about foraging behavior of bees and daily variations, such as the period of the day during which pollen is collected, the peak hours of collection, etc. Sommeijer et al. (1983) observed that *Melipona favosa* collected pollen from 8:30 am to 12:30 pm and nectar from 11:00 am to 6:00 pm. They suggested that
this clear separation of nectar and pollen foraging throughout the day in stingless bees may demonstrate a consistent difference from foraging behavior of *Apis* colonies. Bruijn and Sommeijer (1997) saw this temporal division among nectar, pollen and resin foraging in *Melipona beecheii* and *M. fasciata*. Hilário et al. (2000) observed the same for *Melipona bicolor*, with a strong tendency to collect pollen in the early morning. Fidalgo and Kleinert (2007) found that workers of *Melipona rufiventris* had both peaks of pollen and nectar collection at the same time during the coldest and driest period of the year, with shorter days, henceforth moving apart gradually along with increases in temperature, rainfall and length of day. This was not observed in *N. testaceicornis*, as a similar number of workers gathered pollen throughout the day, or in colonies of *Plebeia pugnax* (Hilário et al., 2001).

Should there be a difference in foraging pattern between bees of the genus *Melipona* and other Meliponini species? *Melipona* workers show a specific way of pollen collection by buzzing, which allows them to collect pollen from poricide anthers. Certain weather conditions at specific hours of the day may facilitate this means of pollen collection (Sommeijer et al., 1983). Ramalho et al. (2007) comparing pollen collection by *Melipona scutellaris* and *Apis mellifera* at different places and in different periods found that colonies of the former species showed high intraspecific similarity, thus indicating selectivity or floral preference.

Small bees such as *N. testaceicornis* and *P. pugnax* need a higher temperature to fly than *Melipona* bees, starting their external activities later (Hilário et al., 2000, 2001; Fidalgo and Kleinert, 2007). With bigger colonies and a less efficient communication system than *Melipona* bees, they should spend more time collecting pollen throughout the day.

The variation in relative frequencies observed among pollen sources in the two sampling methods could be explained by several factors: a) higher sampling deviation when collecting pollen loads from foragers, especially because weather conditions affect flight activity and pollen release, b) using corbicula load pollen grains of very short lasting resources could have resulted in oversampling or undersampling if they were sampled at specific times, and not just as they were available, whereas pollen of long lasting resources could have been undersampled if it was not very frequently sampled, and c) different pollen types can be stored for different periods or can be consumed without storage (Malagodi-Braga and Kleinert, 1993).

Some stingless bees may consume pollen directly after it is brought into the colony or may feed brood with pollen resources that have been stored only a short time. Martínez-Hernández et al. (1994) observed that *N. testaceicornis* used fresh pollen and pollen resources stored for very short periods to feed its brood. Sommeijer and Bruijn (1994) found that *Melipona* house bees have a very strong tendency to close storage pots after a continuing strong pollen flow and to eat pollen of other types that will be hardly stored. These data can be lost if only pollen of storage pots are sampled, but to confirm this hypothesis we would need to check the pollen types in the larval food too. Other comparative studies would be useful to understand the exact contribution of each sampling method in the determination of pollen sources exploited by bees.

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