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RESEARCH ARTICLE - BEES

The effect of toxic nectar and pollen from *Spathodea campanulata* on the worker survival of *Melipona fasciculata* Smith and *Melipona seminigra* Friese, two Amazonian stingless bees (Hymenoptera: Apidae: Meliponini)

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Abstract

Spathodea campanulata is an African plant introduced into South America and other tropical and subtropical areas for ornamental purposes. This plant has been linked to insect mortality, bees included. However, its effects on the Neotropical *Melipona* are as yet unknown. Thus, the aim of this study was to evaluate the effect of *S. campanulata* nectar and pollen on the survival of *Melipona fasciculata* Smith and *Melipona seminigra* Friese workers. A total of 120 newly emerged workers of each species were divided into groups of 10 individuals and confined in boxes. They were submitted to the following diet treatments: *S. campanulata* nectar or 11% sucrose solution (nectar control); 11% sucrose solution and *S. campanulata* pollen or 11% sucrose solution and the species' original pollen (pollen control). A higher mortality of workers was detected in the groups fed with toxics nectar and pollen (*M. fasciculata*, $p<0.01$; *M. seminigra*, $p<0.01$) than on the respective controls. Our results demonstrate that nectar and pollen from *S. campanulata* affected the survival of *M. fasciculata* and *M. seminigra* worker bees. We thus recommend that *S. campanulata* should not be provided as food source for stingless bees.

Introduction

Nectar and pollen collected from plants are the main food resources of social bees. Pollen is used mainly as a protein source and nectar a carbohydrate source (Roubik, 1989). Some plants, however, are reported as toxic for bees, and the potentially toxic compounds may be present in the pollen or nectar (Roubik, 1989). Many plant species may poison bees due to the toxicity of pollen or nectar, extrafloral nectaries, tree sap or honeydew (Barker, 1990). Pollen or nectar toxicity for bees is a widespread phenomenon, although it is poorly understood. Thus, many hypotheses have been proposed to explain this phenomenon.

According to Adler (2000), toxic nectar would promote pollinator specialization, prevent nectar theft and degradation, and corrupt pollination behaviors. Johnson et al. (2006) demonstrated that secondary compounds in nectar are effective visitor filters, which lead to a specialization in the pollination system. Toxicity for several animals is normally due to the secondary compounds found in all plant parts, especially on those most important for survival and reproduction (Levin, 1976). These secondary compounds associated with resistance to herbivory have been frequently observed in floral nectar. Adler (2000) detected nectar that was toxic or had secondary compounds in at least 21 different plant families. According to Ott (1988), at least three psychoactive phytotoxin categories occurred in toxic honeys, and, consequently, in the nectar from which it was produced.

Toxins found amongst some plants are nicotine, rotenones, pyrethrins and tannins (Bueno et al., 1990). *Dimorphandra mollis* Benth. (Fabaceae; popularly called in Brazil "fake barbatimão") and *Stryphnodendron adstringens* (Martius) Coville (Fabaceae; the "real barbatimão"), are rich in tannins and may cause serious losses to beekeepers due to larvae mortality and a reduction in adult *Apis mellifera* Linnaeus longevity. The toxicity of barbatimão is attributed to the pollen and nectar, and the pollen is

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considered more harmful (Carvalho & Message, 2004; Santoro et al., 2004; Cintra et al., 2005).

Spathodea campanulata Beauv. (an exotic species of African origin introduced for ornamental purposes [Nogueira-Neto, 1997]) has been reported as toxic to stingless bees (Tribe Meliponini, *sensu* Michener, 2007). Portugal-Araújo (1963) was one of the pioneers in reporting these effects. He recorded dead stingless bees on *S. campanulata* flowers in Gabon, along with Nogueira-Neto (1997) and Oliveira et al. (1991) in Brazil. Trigo and Santos (2000) monitored dead insects in *S. campanulata* flowers for to up to five days after anthesis and stated that meliponine bees represented 97% of the dead insects. Calligaris (2001) confirmed its nectar toxicity on *Scaptotrigona postica* (Latreille) and *A. mellifera* worker bees in laboratory bioassays, although pollen toxicity was not verified.

Thus, in this study we evaluated the effects of *S. campanulata* nectar and pollen consumption on the survival of two species of *Melipona* Illiger worker bees from the Brazilian Amazon used in meliponiculture, *Melipona fasciculata* Smith and *Melipona seminigra* Friese.

Material and Methods

Studied species and study site

We used *M. seminigra* and *M. fasciculata* workers to study the effect of *S. campanulata* nectar and pollen on the worker survival. *M. seminigra* occurs in the Brazilian Amazon States of Acre, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, Roraima, Tocantins, and *M. fasciculata* in the Brazilian States of Maranhão, Mato Grosso, Pará, Piauí and Tocantins (Camargo & Pedro, 2012). All experiments were carried out from January to May 2012 in the meliponary of Embrapa Amazônia Oriental (1º26'11.52''S, 48º26'35.50''W). The area is composed of secondary forests patches of native plants (several hundred species, including trees and shrubs) and several agricultural crops such as assai trees (*Euterpe oleraceae*, Arecaceae) and other species.

Spathodea campanulata is a large tree (up to 20m) with numerous large flowers, externally red and internally yellow (Francis, 1990). In its region of origin (Africa), *S. campanulata* is pollinated by birds and bats, but is also visited by bees attracted by the abundant nectar and colorful flowers (Ayensu, 1974; Rangaiah et al., 2004; Corlett, 2005). In the study site, there was only one tree of *S. campanulata*, situated 20 meters from the nests.

Experimental design
Effect of nectar and pollen on the survival of workers

To analyze the effect of *S. campanulata* nectar and pollen on the survival of *M. fasciculata* and *M. seminigra* workers, we used newly emerged workers, obtained from eight different nests, four for each bee species. A total of 120 bees of each species were used (30 from each colony), from which 60 were destined for two control groups (30 for each species) and 60 for each experimental group: *S. campanulata* nectar and pollen. The workers were divided into groups of 10 individuals and confined in polyethylene boxes (8 x 8 x 4 cm) without the queen, and. The boxes were kept in a BOD incubator (model DL-SEDT 02) at 28 ± 1°C. Every day the number of live bees was checked, any dead individuals were removed, the plastic box's rubbish dump area was cleared and water was added to maintain humidity (method adapted from Costa & Venturieri, 2009).

Inflorescences with flower buds and newly opened flowers were gathered from trees located at the research campus – Embrapa Amazônia Oriental – to prepare the *S. campanulata* nectar that was offered to *M. fasciculata* and *M. seminigra* workers. The nectar was removed with an automatic micropipette and the percentage of total sugars was measured with a field refractometer adapted to small volumes (Bellinghan-Stanley™). For pollen sampling, anthers of the flower buds were removed and kept in 2 ml microtubes. A total of 1ml of water with 11% sucrose was added to the tubes with the anthers (the same concentration of sugar found in *S. campanulata* nectar) to wash and assist pollen extraction. The material was centrifuged for five minutes at 2000 rpm, the liquid part of the microtube was drained and the accumulated pollen on the bottom was collected and offered to the bees as protein source. A botanical sample of the plant's reproductive structures was identified and stored at the IAN Herbarium (Embrapa) under the number 187659.

Workers were submitted to the following diet, according to the treatment: NSc - bees fed on *S. campanulata* nectar; NeC - bees fed on 11% sucrose solution; PSc - bees fed on an 11% sucrose solution and *S. campanulata* pollen; PoC - bees fed on 11% sucrose solution (nectar control) and its own pollen (*M. fasciculata* or *M. seminigra* pollen; pollen control). Workers were daily fed with: (1) NSc: 240 µL of nectar and (2) NeC: 240 µL of 11% sucrose solution. The workers from the PSc treatments were offered 240 µL of 11% sucrose solution and 0.1 g of *S. campanulata* pollen daily. For the PoC treatment, 240 µL of 11% sucrose solution and 0.1g of the species' pollen was offered. The food was weighed daily in an analytical balance with a 10^{-3} g precision to check the consumption of each item. The carbohydrate (11% sucrose solution or *S. campanulata* nectar) and protein (pollen of *S. campanulata* and *M. fasciculata M. seminigra* colonies) foods were renewed whenever completely eaten (pollen), or daily (sucrose).

Analyses

Kaplan-Meier survival curves were made for each species' different treatments. The survival of control workers (pollen and nectar) was monitored until half or more of the treatment individuals died. A Cox-Mantel test was carried out, using the software STATISTICA® 8.0, to compare the survival curves of the treatments used for each species (5% significance level). The data regarding workers that were alive at the end of the experiment were treated as censured and from the workers monitored until death as complete data for the survival curve setting (Crawley, 2007).

Results

Effect of ***S. campanulata*** *nectar and pollen on the survival of workers*

There was food consumption in all the studied groups (Table 1). The death rate was high for the 30 *M. fasciculata* bees that received *S. campanulata* nectar as a carbohydrate source (NSc). Only nine bees from the group NSc remained alive at the end of the experiment. Furthermore, from the 30 bees from group NeC, only three died on the second day, so that 27 bees remained alive at the end of the experiment (Fig 1a). A similar pattern of mortality due to *S. campanulata* nectar was detected for *M. seminigra* (Fig 1b). Mortality of *M. fasciculata* and *M. seminigra* workers was significantly higher in the experimental group (*S. campanulata* nectar) than in the control group (Cox-Mantel: *M. fasciculata*, $p<0.01$; *M. seminigra*, $p<0.01$).

Only nine of the 30 *M. fasciculata* workers submitted on the treatment with *S. campanulata* pollen (PSc) remained alive at the end of the experiment. There was high mortality on the four experiment days. Mortality was lower on the pollen control group (PoC), with 24 live workers at the end of the experiment (Fig 2a). Again, the pattern was similar for *M. seminigra* (Fig 2b). For both species the survival of the control group was significantly higher than for the experimental group (*S. campanulata* pollen) (Cox-Mantel: *M. fasciculata*, $p<0.01$; *M. seminigra*, $p<0.01$).

There was no difference on *M. fasciculata* survival between *S. campanulata* nectar (NSc) and pollen (PSc) treatments (Cox-Mantel, $p=0.55$). However, the intake of *S. campanulata* nectar by *M. seminigra* had a stronger impact on the survival of workers than pollen (Cox-Mantel, $p<0.01$). There were no significant differences between the longevity of bees that consumed 11% sucrose solution and pollen from their own boxes for the control groups (NSc and PSc) (Cox-Mantel: *M. fasciculata*: $p=0.29$; *M. seminigra*: $p=0.45$).

Table 1. Daily consumption rate per worker (mean±S.D.) of *Melipona fasciculata* and *Melipona seminigra*, confined in groups of 10 individuals and submitted to dietary treatments. NSc- bees fed on *Spathodea campanulata* nectar; NeC- bees fed on 11% sucrose solution; PSc - bees fed on an 11% sucrose solution and S. campanulata pollen; PoC - bees fed on 11% sucrose solution (nectar control) and its own pollen (pollen control).

Species/ Treatment	NSc (µl)	NeC (µl)	PSc (mg)	PoC mg)
M. fasciculata	19.62 ± 1.91	29.73 ± 8.45	9.11 ± 11.40	5.20 ± 3.70
M. seminigra	27.01 ± 3.41	30.24 ± 6.81	5.00 ± 3.70	1.40 ± 0.70

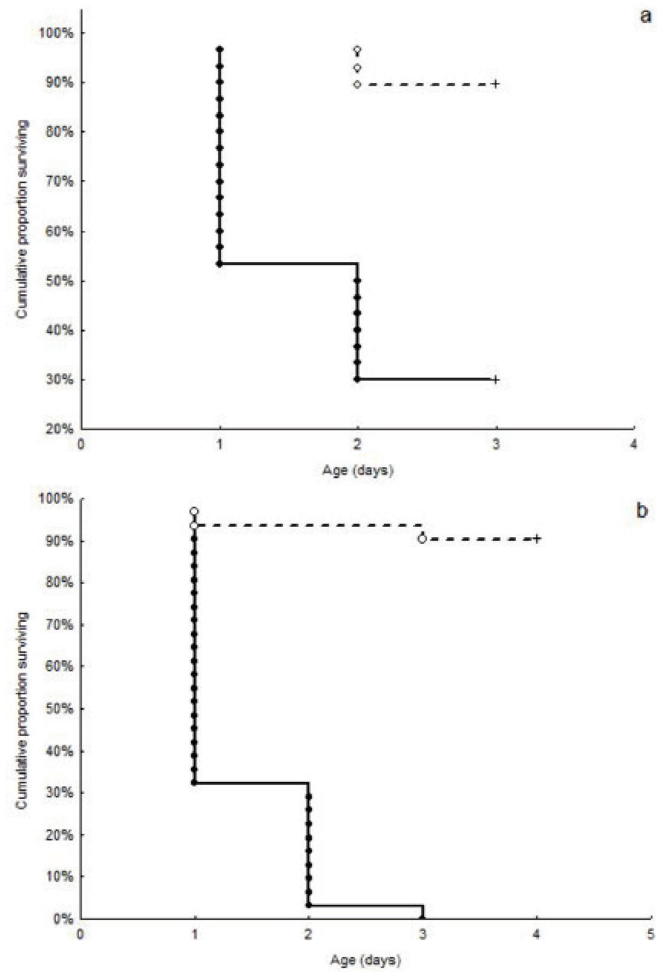

Fig 1. Survival curves for workers confined in groups of 10 individuals and submitted to dietary treatments. NSc- bees fed on *S. campanulata* nectar (filled circles); NeC- bees fed on 11% sucrose solution (empty circles). A - *Melipona fasciculata*. B - *Melipona seminigra.*

Discussion

In this study we were able to prove the effect of *S. campanulata* pollen and nectar in reducing the survival of two meliponine species (*M. fasciculata* and *M. seminigra*) when fed on it. Both pollen and nectar of *S. campanulata* reduced the survival of worker bees undergoing these treatments. Since the food was consumed in the experimental cages, this indicates that the tested bee groups actually died due to *S. campanulata* nectar and pollen ingestion, and due to starvation.

In general, *S. campanulata* nectar and flower bud secretion are referred to as toxic, although little has been studied about its pollen. Calligaris (2001) found *S. postica* and *A. mellifera* survival reduction when *S. campanulata* nectar was added to the bees' diet in bioassays. Portugal-Araújo (1963) attributed the death of insects in *S. campanulata* flowers, including stingless bees, to floral bud secretion toxicity. In a periodic survey, Nogueira-Neto (1997) also reported many Meliponini bees in fallen flowers, highlighting *Plebeia droryana* (Friese)*, Tetragonisca angustula* (Latreille)*, S. postica, Trigona spinipes* (Fabricius) and *Friesella schrottkyi*

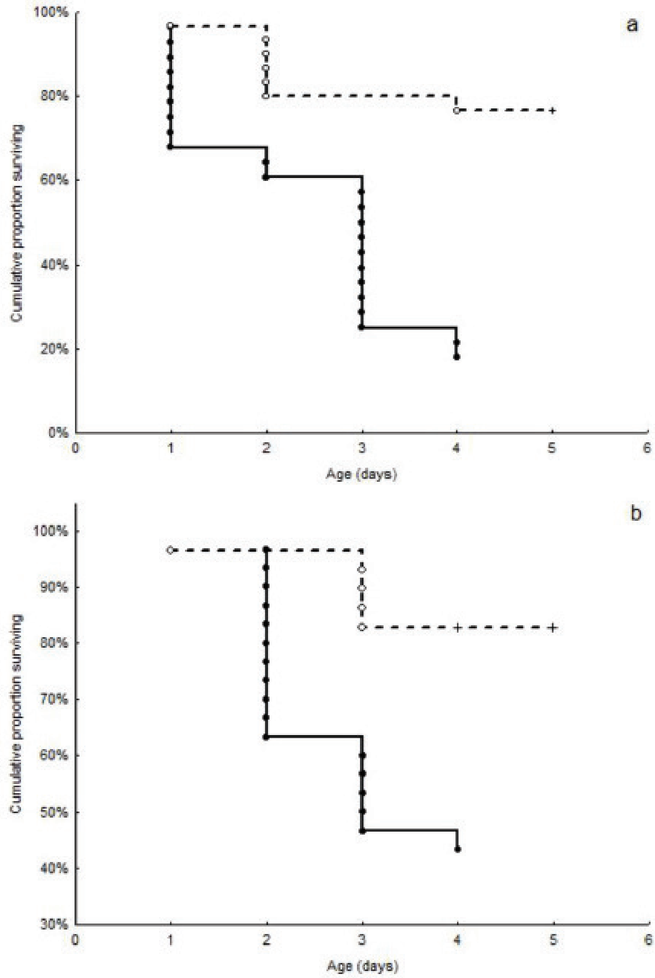

Fig 2. Survival curves for workers confined in groups of 10 individuals and submitted to dietary treatments. PSc - bees fed on 11% sucrose solution and *S. campanulata* pollen (filled circles); PoC- bees fed on 11% sucrose solution and its own pollen (empty circles). A - *Melipona fasciculata.* B - *Melipona seminigra.*

(Friese). Stingless bees represented up to 97% of the dead insects in the flowers, especially *S. postica* (Trigo & Santos, 2000).

Trigo and Santos (2000) tested different flower mucilage concentrations on newly emerged *S. postica* workers, in laboratory bioassays. Mucilage at a concentration of 25% reduced bee longevity by 52.9%, while pure mucilage reduced it by 95.2%. However, this last result is considered ambiguous, since there is no evidence that bee feed on pure mucilage.

Both pollen and nectar of *S. campanulata* are considered toxic. Calligaris (2001) did not detect a reduction in the survival of *S. postica* and *A. mellifera* when fed on 5% pollen. However, Oliveira et al. (1991) reported the death of *T. spinipes* bees due to the *S. campanulata* pollen found in its gizzard. In this study we found a marked reduction of *M. fasciculata* and *M. seminigra* survival rates when fed on pure pollen.

Trigo and Santos (2000) suggested the existence of a defense mechanism in *S. campanulata* that protects flower buds from nectar and pollen thieves. Otherwise these resources could be stolen by some meliponine bees, such as *S. postica*, or other efficient pillagers, before flower opening. In this case, vertebrate pollination would be reduced or even prevented. Indeed, Endress (1994) noted that some plants, including Bignoniaceae, produce a mucilaginous or watery liquid to protect juvenile flower organs before anthesis. Flower bud secretion would thus be a plant defense system, of chemical or physical nature, suffocating the bees (Trigo & Santos, 2000).

Considering these effects and the actual expansion of meliponiculture in Brazil (Contrera et al., 2011; Venturieri et al., 2012), the use of *S. campanulata* trees is not recommended in areas foraged by stingless bees. Such a recommendation has already been made regarding *A. mellifera* (Modro et al., 2011).

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