**Article**

**Title:** Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen-rewarding plant family: a case study using Miconia theizans

**Creators:** de Brito, V. L. G., Rech, A. R., Ollerton, J. and Sazima, M.

**DOI:** 10.1007/s00606-017-1405-z

**Example citation:** de Brito, V. L. G., Rech, A. R., Ollerton, J. and Sazima, M. (2017) Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen-rewarding plant family: a case study using Miconia theizans. *Plant Systematics and Evolution.* **303**(6), pp. 709-718. 0378-2697.

**Version:** Accepted version

**Official URL:** http://dx.doi.org/10.1007/s00606-017-1405-z

**Note:** This is a post-peer-review, pre-copyedit version of an article published in Plant Systematics and Evolution. The final authenticated version is available online at: https://doi.org/10.1007/s00606-017-1405-z.

# Plant Systematics and Evolution

## Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen rewarding plant family: a case study using Miconia theizans

--Manuscript Draft--

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|---|---|
| **Manuscript Number:** | PLSY-D-16-00233R4 |
| **Full Title:** | Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen rewarding plant family: a case study using Miconia theizans |
| **Short Title:** | Nectar production in Miconia theizans |
| **Article Type:** | Original Article |
| **Keywords:** | generalisation - Melastomataceae - Miconia theizans Cogn. - nectar dynamics - pollination syndromes - reproductive success |
| **Corresponding Author:** | Vinícius Lourenço Garcia Brito, Dr.<br>Universidade Federal de Uberlandia<br>Campinas, São Paulo BRAZIL |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | Universidade Federal de Uberlandia |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Vinícius Lourenço Garcia Brito, Dr. |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Vinícius Lourenço Garcia Brito, Dr. |
| | André Rodrigo Rech, Dr. |
| | Jeff Ollerton, Dr. |
| | Marlies Sazima, Dr. |
| **Order of Authors Secondary Information:** | |
| **Funding Information:** | Fundação de Amparo à Pesquisa do Estado de São Paulo (BR) (2010/51494-5) — Mr. Vinícius Lourenço Garcia Brito |
| | Fundação de Amparo à Pesquisa do Estado de São Paulo (BR) (2009/54491-0) — Mr. André Rodrigo Rech |
| | Fundação de Amparo à Pesquisa do Estado de São Paulo (BR) (2013/14442-5) — Dr. Jeff Ollerton |
| | Fundação de Amparo à Pesquisa do Estado de São Paulo (BR) (2012/50425-5) — Ms. Marlies Sazima |
| | Conselho Nacional de Desenvolvimento Científico e Tecnológico (BR) (303084/2011-1) — Ms. Marlies Sazima |
| **Abstract:** | Generalist plant-pollinator interactions are prevalent in nature. Here, we untangle the role of nectar production in the visitation and pollen release/deposition in Miconia theizans, a nectar rewarding plant within the specialised pollen rewarding plant family Melastomataceae. We described the visitation rate, nectar dynamics and pollen release from the poricidal anthers and deposition onto stigmas during flower anthesis. Afterwards, we used a linear mixed model selection approach to understand the relationship between pollen and nectar availability and insect visitation rate, and the relationship between visitation rate and reproductive success. Miconia theizans was visited by 86 insect species, including buzzing and non-buzzing bees, wasps, flies, hoverflies, ants, beetles, hemipterans, cockroaches, and butterflies. The nectar produced explained the visitation rate, and the pollen release from the anthers was |

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| | best explained by the visitation rate of pollinivorous species. However, the visitation rates could not predict pollen deposition onto stigmas. Nectar production may explain the high insect diversity and led to an increase in reproductive success, even with unpredictable pollen deposition, indicating the adaptive value of a generalised pollination system. |

Uberlândia, 15/03/2017

| To: M. Koch; M.A. Lysak; K. Marhold | Pablo Vargas |
|---|---|
| Editor-in-Chief | Associate Editor |

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Managing Editor
Plant Systematics and Evolution

Dear Editorial Board of Plant Systematics and Evolution,

We thank the Editor in Chief for the considerations on our manuscript: "Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen rewarding plant family". In the new version, we double-checked the citations and references and addressed the other editor suggestions. The answers to the Editor is presented below in blue.

We would be happy to consider any further changes, and we look forward to hearing from you.

Sincerely yours,

Vinicius L. G. Brito on behalf of co-authors

COMMENTS FOR THE EDITOR:

1. Please check carefully the formats, especially concerning citations and references. Please see Instructions to Authors or some recent issues or papers of the journal for style.

References:
Use the standard abbreviation of a journal's name according B-P-H (Bridson GDR, 2004), available also at http://www.ipni.org/.

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3. Electronic supplementary material:
- EMS5 - please provide the Table and figures as 3 separate Online Resources. Alternatively name them Online Resource 5a, 5b, 5c (also in the main text refer to Online Resource 5a, 5b, 5c).

We are sorry for this mistake. Actually, Online Resource 5 only refers to the table with the AIC values for pollen release and deposition. The other figures is part of Online Resource 2 and 3 and they were wrong placed in this file.

Original Research Manuscript

# Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen rewarding plant family: a case study using *Miconia theizans*

**Vinícius Lourenço Garcia de Brito[1,2*], André Rodrigo Rech[3], Jeff Ollerton[4], Marlies Sazima[5]**

[1]Programa de Pós-Graduação em Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas - SP, 13.083-970, Brazil

[2]Programa de Pós-Graduação em Biologia Vegetal, Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia-MG, 38 400-902, Brazil

[3]Faculdade Interdisciplinar de Humanidades, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina-MG, 39100-000, Brazil

[4]Landscape and Biodiversity Research Group, School of Science and Technology, The University of Northampton, Avenue Campus, Northampton, NN2 7AL, United Kingdom

[5]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas - SP, 13.083-970, Brazil

**Running title:** Nectar production in *Miconia theizans*

*Corresponding author. Email viniciusduartina@gmail.com

Telephone: (34) 3225-8640

## Abstract

Generalist plant-pollinator interactions are prevalent in nature. Here, we untangle the role of nectar production in the visitation and pollen release/deposition in *Miconia theizans*, a nectar rewarding plant within the specialised pollen rewarding plant family Melastomataceae. We described the visitation rate, nectar dynamics and pollen release from the poricidal anthers and deposition onto stigmas during flower anthesis. Afterwards, we used a linear mixed model selection approach to understand the relationship between pollen and nectar availability and insect visitation rate, and the relationship between visitation rate and reproductive success. *Miconia theizans* was visited by 86 insect species, including buzzing and non-buzzing bees, wasps, flies, hoverflies, ants, beetles, hemipterans, cockroaches, and butterflies. The nectar produced explained the visitation rate, and the pollen release from the anthers was best explained by the visitation rate of pollinivorous species. However, the visitation rates could not predict pollen deposition onto stigmas. Nectar production may explain the high insect diversity and led to an increase in reproductive success, even with unpredictable pollen deposition, indicating the adaptive value of a generalised pollination system.

**Keywords:** generalisation - Melastomataceae - *Miconia theizans* Cogn. - nectar dynamics - pollination syndromes - reproductive success

## Introduction

Plant-pollinator interactions vary along a broad range of possibilities from obligate specialists, when a single species of pollinator depends on only one species of plant and vice-versa, to facultative generalists, when a flower or pollinator interacts with many species belonging to different functional or phylogenetic groups (Waser et al. 1996; Ollerton et al. 2007). Specialised interactions, in addition to the concept of pollination syndromes, have been the focus of pollination researchers since the concept was formalized (Faegri and van der Pijl 1979; Fenster et al. 2004). However, despite the wider prevalence of generalist systems in plant-pollinator interactions (Waser et al. 1996), their complexity and variability over time and space prevented their appreciation as case studies from an evolutionary perspective (Alarcón et al. 2008; but see Amorim et al. 2012; King et al. 2013; Zych et al. 2014).

The degree of generalisation in pollination systems has been the subject of intense discussion among ecologists (Waser et al. 1996; Rosas-Guerrero et al. 2014; Ollerton et al. 2015; Aguilar et al. 2015). Under the assumption of constant flower specialisation to the most effective pollinator (Stebbins 1970), there was a general idea that highly specialised pollination systems could be evolutionary dead-ends and that transitions from generalist systems to specialist ones are more frequent than the reverse (Futuyma and Moreno 1988; but see Tripp and Manos 2008). This idea was partially corroborated since most reported transitions happened among functionally specialised pollination systems (Chase and Hills 1992; Armbruster 1988; Kay et al. 2005; Wilson et al. 2007; Whitall and Hodges 2007; Tripp and Manos 2008; Martén-Rodriguez et al. 2010; Smith 2010). To the best of our knowledge, there are only three phylogeny-supported situations where transitions from specialised to generalised systems were described (Armbruster and Baldwin 1998; Martén-Rodriguez et al. 2010; Brito et al. 2016). Two of these cases were recorded on islands and explained as alternative strategies to the lack of specialised pollinators.

Plants of the family Melastomataceae present poricidal anthers, often offer only pollen as a reward, and are mainly pollinated by bees able to vibrate their wing muscles to release the pollen ("buzz-pollination", Renner 1989; Larsson and Barrett 1999; Melo et al. 1999; Fracasso and Sazima 2004; Luo et al. 2008; Franco et al. 2011; Pereira et al. 2011; Brito and Sazima 2012). Despite being a phenotypically specialised (sensu Ollerton et al. 2007) pollen-based pollination system, nectar production has been reported for some genera and is associated with the colonization of high altitude habitats where bees are supposed to be less predictable (Varassin et al. 2008; Kriebel and Zumbado 2014). However, such new pollinator groups are often as specialised as vibrating bees (e.g. bats and hummingbirds, Varassin et al. 2008). These discoveries increased the number of known transitions among specialised systems but still corroborate the ‘dead-end’ hypothesis. On the other hand, there are reports of visitation by different insect orders for nectar in flowers of *Miconia* Ruiz and Pav. (Goldenberg and Sheperd 1998; Varassin et al. 2008; Santos et al. 2010; Kriebel and Zumbado 2014). In this case, the changes in the pollination strategy from a pollen- to a nectar-based reward was associated with more open anthers in small, white flowers, allowing animals that are unable to vibrate to access the pollen through the anther aperture (Goldenberg et al. 2008; Brito et al. 2016).

Despite the reported mechanism, structure and location of nectar production in *Miconia* flowers (Varassin et al. 2008; Kriebel and Zumbado 2014), little is known about the dynamics of nectar production during anthesis and its relation to insect visitation and plant reproductive success. Nectar production could be another evolutionary strategy, other than heteranthery, to solve the "pollen dilemma" in Melastomataceae flowers, where the pollen must feed the bees' larvae and, at the same time, be the plant male gametophyte (Luo et al. 2008; Vallejo-Marin et al. 2009). In addition, increased nectar availability may result in longer visit durations and in higher pollen removal and deposition (Ollerton et al. 2007). In this sense, we expect that the nectar production associated to the dynamics of pollen as a resource in a generalist flower of Melastomataceae would be related to the variation of the visitor spectrum and consequently to the pollen removal from anthers and its deposition on stigmas. This would reinforce the role of nectar production in the evolution of generalised pollinations system from specialised ones in this family (Brito et al. 2016; Ollerton et al. 2007).

The genus *Miconia* Ruiz and Pav. is the largest in the Melastomataceae with more than 1050 species (Goldenberg et al. 2008). The clade Miconia III is mostly restricted to the Andes and Central America, with a few species widespread in South America. *Miconia theizans* Cogn. is a nectar producing Melastomataceae species with small, pale flowers that are visited by a varied suite of insects, which suggests a generalised pollination system. The phylogeny of the genus shows that nectar production is not monophyletic and *M. theizans* is neither basal nor much derived inside the genus (Goldenberg et al. 2008). In this work, we describe the dynamics of nectar production and relate it to the pollination system and reproductive success of this species. We seek to test the hypothesis that 1) nectar production is an important factor to improve flower visitation and 2) reproductive success is improved by the increase of visitor spectra in *Miconia theizans*, which would highlight the adaptive value of generalised pollination systems.

## Material and methods

### Study system

Field work was carried out at the Núcleo Santa Virgínia (NSV), Serra do Mar State Park, in the municipality of São Luís do Paraitinga at the top of the Serra do Mar mountain range (23º20’S, 45º50’W). The local vegetation is classified as ombrophilous montane forest (Padgurschi et al. 2011). At the study site, the altitude ranges from 870 m to 1100 m above sea level, the mean monthly temperature is 16.1 ºC and the mean monthly precipitation is 172.5 mm (CPTEC 2010). The regional climate is subtropical wet without a dry season (Alvares et al. 2013) and January to February are the wettest months, while June to August are the driest (Tabarelli and Mantovani 1999).

*Miconia theizans* Cogn. is a very common small tree at the study site ranging from 1.5 m to 3.0 m tall. Its flowers last less than one day and produce a weak perfume; the corolla is pale and less than 3 mm in diameter. Inflorescences are the visual unit (from a human perspective) and can present more than 60 open flowers per day at the peak of flowering time, when they are visited by a large number of insects from different orders (Online Resource 1, Table 1, Fig. 1). They also produce large amounts of fleshy fruits, which are eaten by a diverse array of birds (Borges and Melo 2012).

### Sampling methods

We observed and monitored 25 *Miconia theizans* individuals in flower during two flowering seasons: December 2012 – January 2013 and December 2013 – January 2014. Each individual was monitored from 0700 h to 1300 h, after which most of the flowers wilted. During this interval, from hour to hour, we measured the amount of nectar in three different bagged and unbagged flowers to estimate the dynamics of nectar production and the nectar standing crop per flower. Nectar was measured using strips of filter paper (Whatman no. 1). We touched the nectar on the petal surface and marked the wet part of the strip with a pencil. The marked pieces of paper were dried and brought to the lab. In the lab, the marked portion of each dried piece of paper strip was weighed using a high precision weighing machine. As *M. theizans* produces minute amounts of nectar, we measured nectar sugar concentration from a subset of 20 flowers collected from at least 10 different individuals. Knowing its nectar concentration, we prepared a solution with the same sugar concentration in the laboratory and used drops from 1 to 10 µl to wet the same type of strips of filter paper used in the field. We then dried the these filter papers strips and marked, measured and weighted it to set a calibration curve between nectar volume and filter paper weight. Then, we estimate the nectar volume produced by flowers using the weight of filter paper marked in the field and the calculated relationship between nectar volume and filter paper weight (Online Resource 2, Adjusted R2 = 0.87, p <0.01).

At the same hour-time interval, we also collected two anthers (representing each anther cycle) and one stigma from the same bagged and the same unbagged flower used for nectar measurements in each individual. Each anther was stored in a microcentrifuge tube filled with 1 ml of 70% ethanol. Afterwards, these anthers were macerated and the total number of pollen grains was estimated in laboratory using a haemocytometer and a 10uL aliquot from the homogenized solution (Brito and Sazima 2012). The stigmas collected in the field were placed on glass microscope slides previously prepared with fuchsin jelly allowing a semi-permanent preparation to count the pollen grains in the laboratory (Dafni et al. 2005). As *Miconia theizans* presents large pored anthers and may possibly self-pollinate, the stigmas of bagged flowers gives the amount of self-deposited pollen grains, while the stigmas of unbagged flowers gives the amount of self-deposited pollen plus the amount of pollen deposited by flower visitors. In the same way, the anthers of bagged flowers give the remaining pollen grains after self-dispersal while the anthers of unbagged flowers give the remaining pollen grains after self-dispersal and pollinator collection.

We recorded flower visitors and the visitation rate to flowers during 10 minutes every half hour between 0700 h and 1300 h, in one of the 25 different individuals. Every individual was observed and sampled during one day. Insects were morphotyped and assigned to functional groups based on their flower visitation behaviour: a) those collecting exclusively nectar (N); b) those collecting exclusively pollen (P); and c) those collecting both pollen and nectar (B). We calculated the relative richness and frequency of each species and functional category during the study period. Afterwards, we used this data to estimate the total visitor richness following Chao (1987). Samples of the most frequent flower visitor species were collected either from individual plants not used for pollinator observations or from observed plants on non-observation days. Sampling was performed using an entomological net; visitors were killed in vials containing ethyl acetate, then dried and pinned for later identification by specialists (Maicon Diego Grella - Laboratório de Entomologia, Unicamp, Silvia Pedro - FFCL-USP Ribeirão Preto and Mateus Clemente - UNESP - Rio Claro). Vouchers of these visitors were deposited in the collection of the Museu de Zoologia - Unicamp, L2B-DBA - Laboratório de Entomologia, Departamento de Biologia Animal – UNICAMP, Museu de Entomologia -USP-Ribeirão Preto, Laboratório de Entomologia UNESP-RC). Vouchers of these visitors were deposited in the collection of the Museu de Zoologia -

Unicamp, L2B-DBA - Laboratório de Entomologia, Departamento de Biologia Animal – UNICAMP, Museu de Entomologia -USP-Ribeirão Preto, Laboratório de Entomologia UNESP-RC). A voucher specimen from the plant population is deposited at the Herbarium of the University of Campinas (UEC 182795). Parallel to data collection we also recorded the local temperature and humidity using a digital thermo-hygrometer (Online Resource 3).

### Nectar, pollen and visitation dynamics

We performed analysis of variance (ANOVA type 3 for unbalanced data) using linear mixed models to assess the pattern of variation in nectar dynamic as well the number of pollen grains released from the anthers and deposited on stigmas among time intervals. To examine the dynamics of nectar production and standing crop, we considered time and treatment (bagged and unbagged) as fixed effects, the picked flower as a random effect and the nectar volume (estimated from the filter paper weight) as the response variable. In the pollen release analysis, we considered the time interval and the treatment (bagged and unbagged) as fixed factors, the quadrant of the haemocytometer was considered a random factor, and the number of pollen grains was the response variable. In the pollen deposition analysis, we built a linear model considering the time interval and the treatment (bagged and unbagged) as factors and the number of deposited pollen grains as the response variable. For the visitation dynamics, we also built a linear model considering time interval and the functional categories as factors and the number of visits per flower as the response variable.

### Visitation rates, and pollen release and deposition by visitors

To investigate the determinants of the visitation dynamics of *M. theizans* we built linear mixed models with decreasing complexity considering the time interval and day as random factors in all models. The fixed factors for each model were as follows: a) *full*: temperature, humidity, number of available pollen grains inside anthers of unbagged flowers and nectar standing crop; b) *pollen*: only the number of available pollen grains inside anthers of unbagged flowers; c) *nectar*: only the nectar standing crop; d) *both*: pollen and nectar available to visitors; e) *weather*: only temperature and humidity and; f) *null*: no fixed effects.

We also built linear mixed models to understand the influence of visitation rate of each functional group on the number of pollen grains released from the anthers by flower visitor activity (*i.e*. the number of pollen grains inside anthers of unbagged flowers minus the number of pollen grains inside anthers of bagged flowers). Using the same rationale, we calculated the number of pollen grains deposited onto the stigmas due to flower visitor activity and related it to the visitation rate of each functional group. Time intervals and days were considered random factors in all models while the fixed factors were as follows: a) *full*: sum of the visits by N, P and B; b) *N*: only the visitation dynamics of N; c) *P*: only the visitation dynamics of P; d) *B*: only the visitation dynamics of B; e) *NB*: the visitation dynamics of N and B; f) *PB*: the visitation dynamics of P and B; g) *NP*: the visitation dynamics of N and P and; h) *null*: no fixed effects.

Afterwards, we used the Akaike information criterion (AIC) to evaluate the prediction ability of each model and the ΔAIC (the difference between the AIC for the $i^{th}$ model and the minimum AIC among all the models) to compare them and choose the best fit. Values of ΔAIC within 0-2 have substantial support; within 4-7 considerably less support; and greater than 10 essentially no support (Burnham and Anderson 2002). We validated each model by visually checking the dispersion of residuals against the fitted values. All the statistical analyses were run in the R environment using *lme4*, *lmeTest* and *bbmle* packages (http://www.r-project.org/).

## Results

Nectar, pollen and visitation dynamics

We recorded 86 species of anther buzzing and non-buzzing bees, wasps, flies, hoverflies, ants, beetles, hemipterans, cockroaches, and butterflies visiting the flowers of *Miconia theizans* (Table 1, Fig. 1; for the complete list and visitation frequency see Online Resource 1). The total richness estimation was 125.6 ± 18.4 species. Visitation started in the morning with the sunrise and continued until around 1400 h when the flowers started to wilt. We recorded 10,875 visits; 17% of them were performed by the introduced bee *Apis mellifera*, followed by the wasp *Angelaia vicina* (13% of total visits) and the ant *Camponotus rufipes* (10% of total visits). The most frequent group was the wasps with 36.5% of the visits, followed by bees and ants with 35.8% and 13.0% of total visits respectively (Table 1). However, the richest visiting order was Diptera with 35 species, followed by bees and wasps in Hymenoptera with 22 and 11 species, respectively (Table 1).

By categorizing the flower visitors according to the resource they collected, it was possible to see that nectarivorous insects were the main visitor group in terms of both species richness and frequency (Fig. 2A). The visitation dynamics show that nectarivorous insects continued to visit the flowers long after the peak of visitation of the other two groups (P and B) (Fig. 2B). There was a significant effect of time (F=1.95,

$p<0.03$) and of the functional group considered ($F=71.97$, $p<0.01$) on flower visitation, but no interaction between both factors ($F=0.77$; $p>0.05$).

The flowers of *Miconia theizans* produce minute amounts of highly concentrated nectar (about 60%) that is continuously depleted during anthesis. In unbagged flowers, the nectar availability was almost zero after 0900 h (crystals could remain on the petal surface due to the synergic action of reduction by visitors and evaporation), while the nectar was available until the last time interval inside bagged flowers (Fig. 3A). Time and treatment were both significant factors influencing nectar dynamics (time: $F=54.68$, $p<0.01$; treatment: $F=36.35$, $p<0.01$), but there was no interaction between these factors ($F=2.07$; $p>0.05$). Pollen was also depleted during anthesis. In general, a large amount of pollen was released before 0900 h but bagged flowers released less pollen than unbagged ones (Fig. 3B). Time and treatment explained the pollen dynamics from the anthers (time: $F=14.45$, $p<0.01$; treatment: $F=19.00$, $p<0.01$), but there was no interaction between these factors ($F=0.84$; $p>0.05$). Pollen deposition on stigmas showed the inverse pattern, increasing during anthesis, with the unbagged flowers receiving more pollen than bagged flowers (Fig. 3C). Again, there was an effect of time and treatment (time: $F=8.40$, $p<0.01$; treatment: $F=43.76$, $p<0.01$) and no interaction between factors ($F=0.94$; $p>0.05$).

### Visitation and pollen release and deposition by visitor activity

The full model, which considers weather variables (temperature and humidity), the available resources (nectar and pollen) and the random factors (time and day) was the best fitted to the total flower visitation ($\Delta AIC= 0.0$, df= 8, weight = 1; Fig. 4; Online Resource 4). The number of pollen grains released from the anthers by visitor activity was best explained by the visitation dynamics of exclusively pollinivorous insects (ΔAIC= 0.0, df= 5, weight = 0.92) with no other competing model below the substantial support threshold (Fig. 5A; Online Resource 5). On the other hand, the number of pollen grains deposited onto the stigmas by visitor activity was explained by all the models (model *null*: ΔAIC= 0.0, df= 4, weight = 0.24; model NP: ΔAIC= 1.3, df= 5, weight = 0.13; model *full*: ΔAIC= 1.4, df= 5, weight = 0.12; model N: ΔAIC= 1.5, df= 5, weight = 0.11; model NB: ΔAIC= 1.6, df= 5, weight = 0.11; model P: ΔAIC= 1.7, df= 5, weight = 0.10; model PB: ΔAIC= 1.9, df= 5, weight = 0.09; weight = 0.10; model B: ΔAIC= 2.0, df= 5, weight = 0.09; Fig. 5B; Online Resource 5).

## Discussion

*Miconia theizans* flowers offer both minute amounts of nectar as well as pollen as rewards. Nectar and pollen availability varies throughout the day, as does insect visitation. Although we have sampled a number of individuals of *M. theizans*, a simple richness estimator index suggests that this plant could be visited by more than 120 species. This indicates that many more visitor species should be expected if more plants would be sampled, and that this pollination system is even more ecologically generalised than we recorded (Ollerton et al. 2007; Herrera 2005). Our results suggest that high richness and frequency of flower visitors is associated with nectar production, since the majority of the visitation concerns insects feeding exclusively on nectar (Thomson et al. 1989; Kriebel and Zumbado 2014). The visitation rate suggests that nectarivorous visitors may remain visiting and possibly depositing pollen on stigmas long after pollinivorous ones have already stopped visiting the flowers. It is also notable that visitors classified as collecting both nectar and pollen (mainly bees) switched from pollen collectors at early morning to nectar collectors after pollen availability decreased (a time-structured behaviour). Hence, nectar production can also be seen as an effective strategy stimulating some of the pollen collectors to keep visiting the flowers.

The evolution of new pollination systems from a typical buzz-pollinated flower *bauplan* (Endress 1994; Varassin et al. 2008; Waser et al. 2011), mediated by nectar production, is an example of an unusual transition from a functionally specialised to a functionally generalised pollination system, confirming the previously suggested role of nectar in these transitions in the Melastomataceae (Varassin et al. 2008; Kriebel and Zumbado 2014; Brito et al. 2016). Nectar production, in contrast to pollen, is not ontogenetically limited, thus plants can have more control on visitation, allowing visits of a larger suite of animals and enabling longer periods of visitation (Ollerton et al. 2007). In our study, we assumed a conservative estimate of the role of nectar since the paper strips do not have perfect capillarity and were likely underestimating the available volume of nectar in the flowers (Galetto and Bernadello 2005). The small amounts of exposed nectar reduced by visitor activity associated with high temperatures promoted evaporation and increased the nectar concentration to levels that it could be seen on the flowers but no longer infiltrated the filter paper used to quantify its availability. Moreover, visitors such as flies and wasps kept visiting the flowers and collecting very dense nectar on the petals and wasps can even collect remaining sugar crystals. Such behaviour was also seen in other fly and wasp pollinated species such as *Hedera helix* (Araliaceae – J. Ollerton personal observation).

The change from pollen- to nectar-rewarding flowers could also be seen as a way to deal with the “pollen dilemma” (Lunau et al. 2015). Since pollen flowers have their own gametophytes eaten by flower visitors, strategies that diminish the importance of pollen as a reward should be evolutionarily favoured (Thorp 1979; Harder and Thomson et al. 1989; Westerkamp 1996). In this study, the pollen removal from the anthers was explained by the visitation frequency of the exclusively pollen-feeding visitors. This was already expected since these visitors are the ones able to vibrate the anthers and take most of the pollen out of them (Buchmann 1983). However, as these bees have an optimized behaviour in order to produce their offspring, they could leave almost nothing for pollination, and consequently play a minor part in fruit set (Hargreaves et al. 2009; Westerkamp and Claßen-Bockhoff 2007; Schlindwein et al. 2005). We also cannot rule out the possibility that nectar feeding visitors were major pollen depositors over the entire anthesis period because we were not able to separate the contributions of each group to the total pollen deposition on stigmas, a matter for further studies.

On the other hand, according to the pollen deposition curve, pollen was deposited throughout all the observation period. However, all the models explained the number of pollen grains deposited onto the stigmas, which indicates that our variables could not predict this component of reproductive success during flower anthesis. In fact, large pored Melastomataceae flowers should favour diffuse pollen deposition by many unspecialised non-vibratory insects and decrease pollen transfer predictability during anthesis (Thomson et al. 2000), which in turn should have favoured the evolution of fruits with fewer seeds in such species in the tribe Miconieae (Brito et al. 2016). It can also be added that nectar probably helps to change the attention of visitors when landing on the flower. When such visitors are collecting nectar, they randomly touch the anthers and get pollen all over their bodies and the same should happen with stigmas, promoting a diffuse but safer pollen transfer. In this sense, our results suggest that nectar production could be another solution to the “pollen dilemma” in Melastomataceae flowers, because it should decrease the pressures of pollen consumption by specialised bees via flower use among pollinators of different functional groups (Thomson et al. 1989, Lunau et al. 2015).

A previous study at the same site had already indicated less predictability in pollination by buzzing-bees compared to the closest lowlands populations in another Melastomataceae species (*Tibouchina pulchra*, Brito and Sazima 2012). Our study corroborates this trend since buzzing-bees were a small proportion of flower visitors (14%). At elevated areas, nectar production in *M. theizans* can contribute to enlarge the number of pollinators and therefore improve the whole system’s resistance to the loss of specialised buzz-pollinators. Hence, we provide support to Baker's law (Pannell et al. 2015), by including generalisation (mediated by nectar production) as another strategy to ensure plant establishment in places where specialised pollinator faunas are less predictable and it may be adaptive in a stochastic pollination environment (Busch and Delph 2012; Cheptou 2012). Related to this, *M. theizans* has been pointed out as one potential Melastomataceae species for ecological restoration of degraded riparian forests (Albuquerque et al. 2013).

Although sexual organs of *M. theizans* are very exposed and every visitor is likely to be a pollinator, a further study evaluating the visitors’ interspecific variation in effectiveness is required to ensure that the strategy is truly generalised (Niemirski and Zych 2011). Once it is confirmed, we will be able to strengthen the evidence that specialisation by buzz-pollination is not a dead-end for pollination systems (Tripp and Manos 2008). Such specialisation in flower-pollinator interactions is one of the predominant ideas to explain the high divergence rates in plant clades and the diversity of their floral patterns, and this seems to be the case of Melastomataceae (Berger et al. 2016). However, our results suggest that the generalization of pollination systems may also be evolutionarily favoured even in places other than islands, as disturbed, open and elevated mainland areas where the predictability of specialist pollinators is also low (Armbruster and Baldwin 1998; Martén-Rodriguez et al. 2010).

This study has shown that nectar production can increase the richness and frequency of potential pollinators and keep such pollinators visiting the flowers even after pollen depletion. Despite the diffuse pollen deposition onto stigmas by non-specialised visitors, such increase in the pollinator fauna biodiversity should affect reproductive success of the plants, especially in habitats where specialised pollinators are scarce or even absent (Bartomeus et al. 2013). Moreover, in habitats where specialised bees are present, the presence of other pollinators oriented by different reward might also decrease pollen consumption (Westerkamp 1996). Therefore, this study reinforces the adaptive meaning of generalised pollination systems as effective reproductive strategies suitable and likely to evolve under certain conditions (Waser and Ollerton 2006; Tripp and Manos 2008).

## Acknowledgements

The authors kindly thank our friends for help in the field and lab: Pedro J. Bergamo, Maraísa Braga, Priscila Vieira, Camila Oliveira, Rafael Pereira and specially Carine Carriere and Cristiano Silva. We thank Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP - for funding support to V.L.G.B. (Proc. 2010/51494-5), A.R.R. (Proc. 2009/54491-0), J.O. (Visiting Researcher - Proc. 2013/14442-5) and M.S. (2012/50425-5). MS thanks the funding support from Conselho Nacional de

Desenvolvimento Científico e Tecnológico - CNPq (303084/2011-1). The authors declare no conflict of interest.

## Legends to Electronic Supplementary Material

**Online Resource 1.** Taxa visiting *Miconia theizans* flowers, the reward(s) consumed and their relative frequency at Serra do Mar State Park (Santa Virgína station) São Paulo. Brazil.

**Online Resource 2.** The relationship between nectar volume and the filter paper weight used in the field to estimate the nectar volume produced by flowers of *Miconia theizans* at Serra do Mar State Park (Santa Virgína station) São Paulo, Brazil.

**Online Resource 3.** Weather conditions during field data collection of *Miconia theizans* at Serra do Mar State Park (Santa Virgína station) São Paulo, Brazil.

**Online Resource 4.** Results of selection of the best fitting linear mixed model to visitation rate on *Miconia theizans* at Serra do Mar State Park (Santa Virgína station), São Paulo, Brazil.

**Online Resource 5.** Results of selection of the best fitting linear mixed model to pollen release and deposition of *Miconia theizans* at Serra do Mar State Park (Santa Virgína station), São Paulo, Brazil.

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## Figure captions

**Fig. 1** Flower visitors of *Miconia theizans* at Serra do Mar State Park (Santa Virgína station) São Paulo, Brazil. **a-b** beetles (Coleoptera), **c-d** Flies (Diptera: Tabanidae and Syrphidae), **e-f** wasps (Hymenoptera: Vespidae) and **g-i** bees (Hymenoptera: Apidae). Scale bars = 3mm

**Fig. 2** Dynamics of visitation to flowers of *Miconia theizans* at Serra do Mar State Park (Santa Virgína station), São Paulo, Brazil. **a** Proportional species richness and relative frequency of the flower visitors grouped by the resource they were collecting from the flowers. **b** Visitation rate over time of each visitor functional group. Bars indicate standard errors

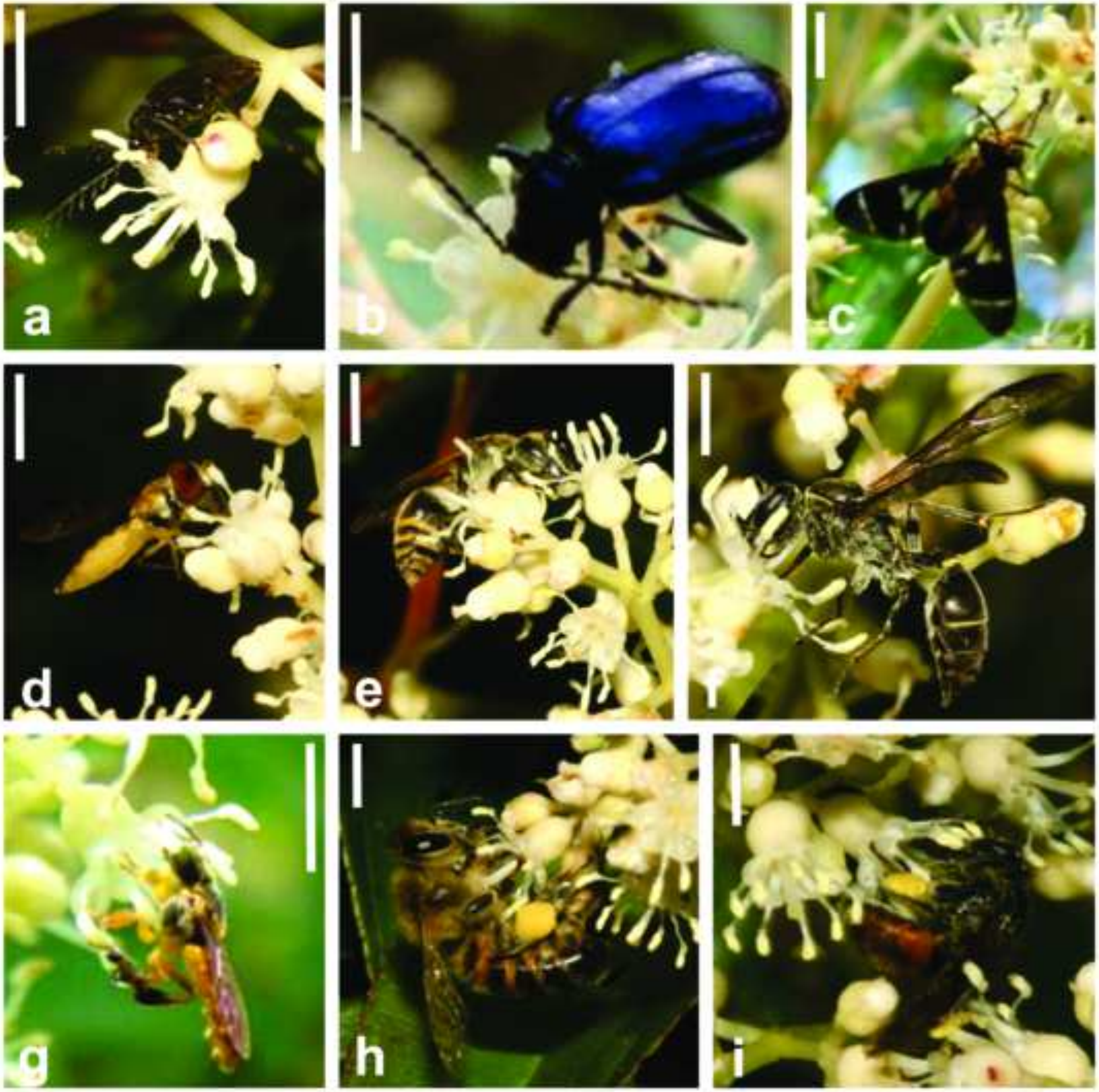
**Fig. 3** Nectar and pollen dynamics in *Miconia theizans* flowers at Serra do Mar State Park (Santa Virgína station), São Paulo, Brazil. **a** Nectar produced in bagged and in unbagged flowers (standing crop). **b** Estimated number of pollen grains from anthers in bagged and unbagged flowers. **c** Number of pollen grains deposited on stigmas of bagged and unbagged flowers. Bars indicate standard errors

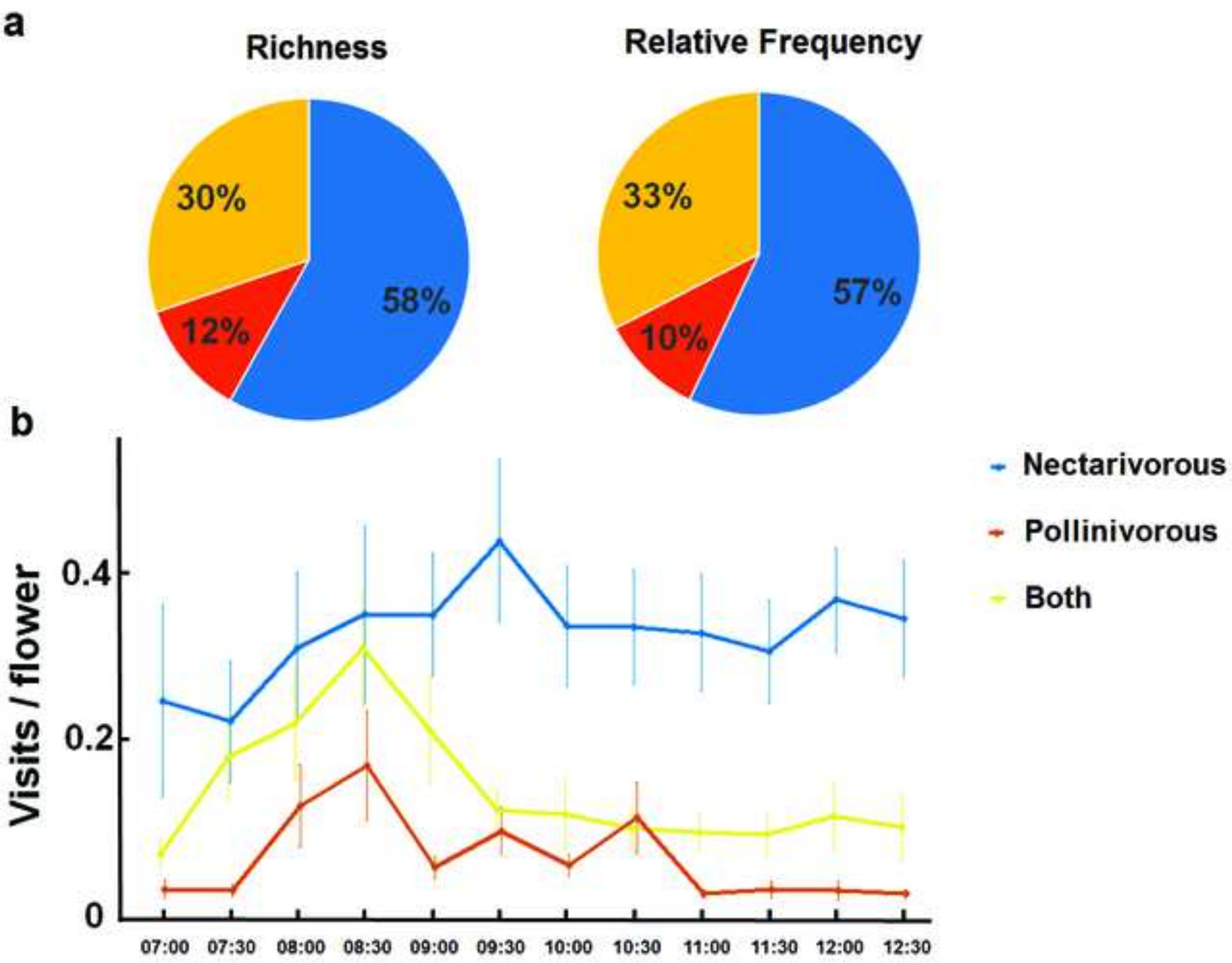
**Fig. 4** Selection of the best fitting linear mixed model for the visitation rate on *Miconia theizans* at Serra do Mar State Park (Santa Virgína station), São Paulo, Brazil. ΔAIC value below the dashed line (ΔAIC = 2) indicates the best model with substantial support based on Akaike information criteria. All models considered day and time as random effects. Model fixed factors are as follows: *full*: temperature, humidity, number of available pollen grains inside anthers of unbagged flowers and nectar standing crop;

*pollen*: only the number of available pollen grains inside anthers of unbagged flowers; *nectar*: only the nectar standing crop; *both*: pollen and nectar available to visitors; *weather*: only temperature and humidity and; *null*: no fixed effects

**Fig. 5** Selection of the best fitting linear mixed model to pollen release and deposition by visitor action in *Miconia theizans* at Serra do Mar State Park (Santa Virgína station), São Paulo, Brazil. ΔAIC values below the dashed line (ΔAIC = 2) indicate competing models with substantial support based on Akaike information criteria. **a** Number of pollen grains released from the anthers by visitors action. **b** Number of pollen grains deposited on stigmas by visitors action. All models considered day and time as fixed effects. Model random factors are as follows: *full*: sum of the visits by N, P and B; *N*: only the visitation dynamics of N; *P*: only the visitation dynamics of P; *B*: only the visitation dynamics of B; *NB*: the visitation dynamics of N and B; *PB*: the visitation dynamics of P and B; *NP*: the visitation dynamics of N and P and; *null*: no random effects. N – nectarivorous insects; P – pollinivorous insects; B – insects collecting nectar and pollen

Figure 1

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Nectarivorous
Pollinivorous
Both

Figure 3 Click here to download Figure Fig3.tif

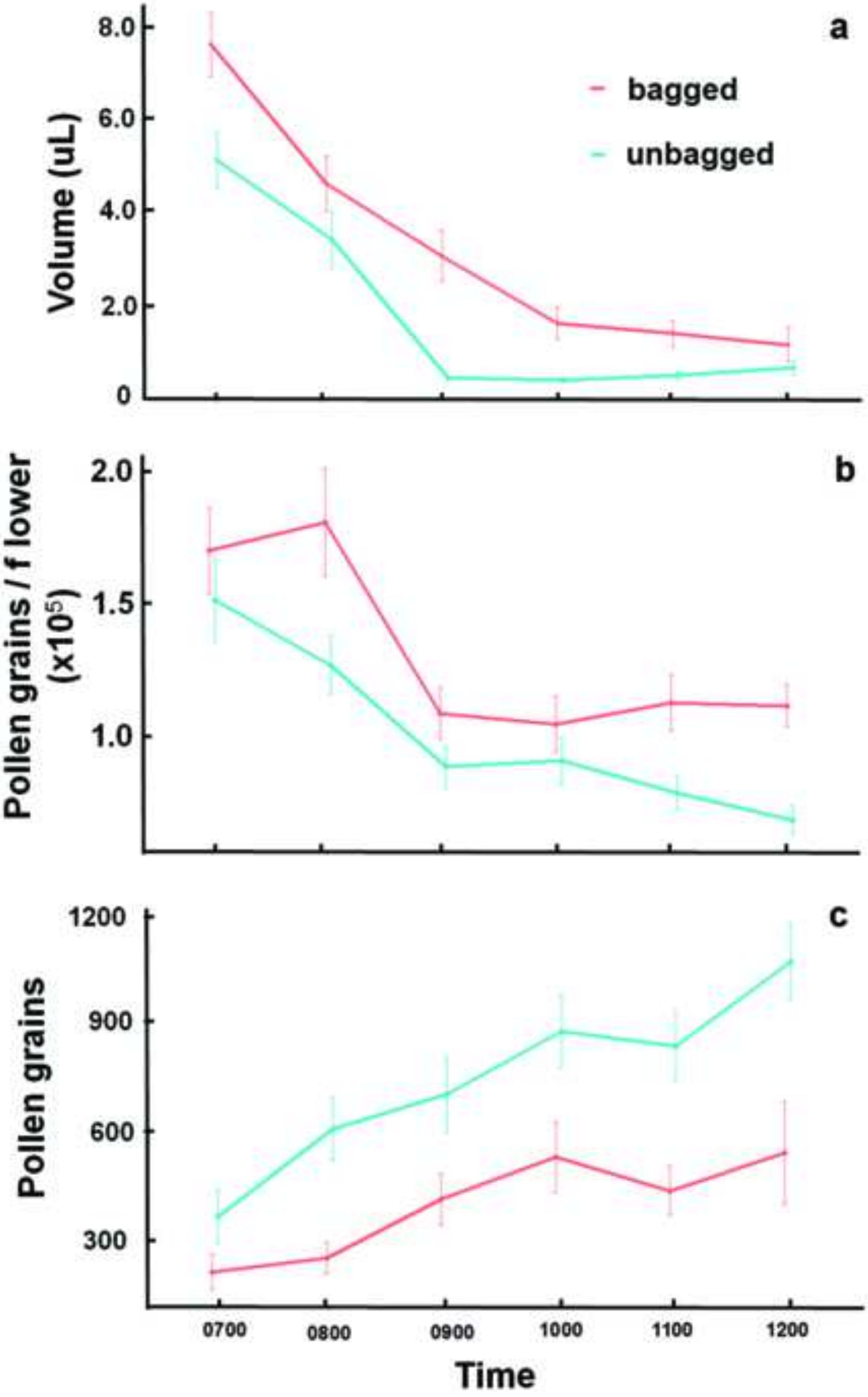
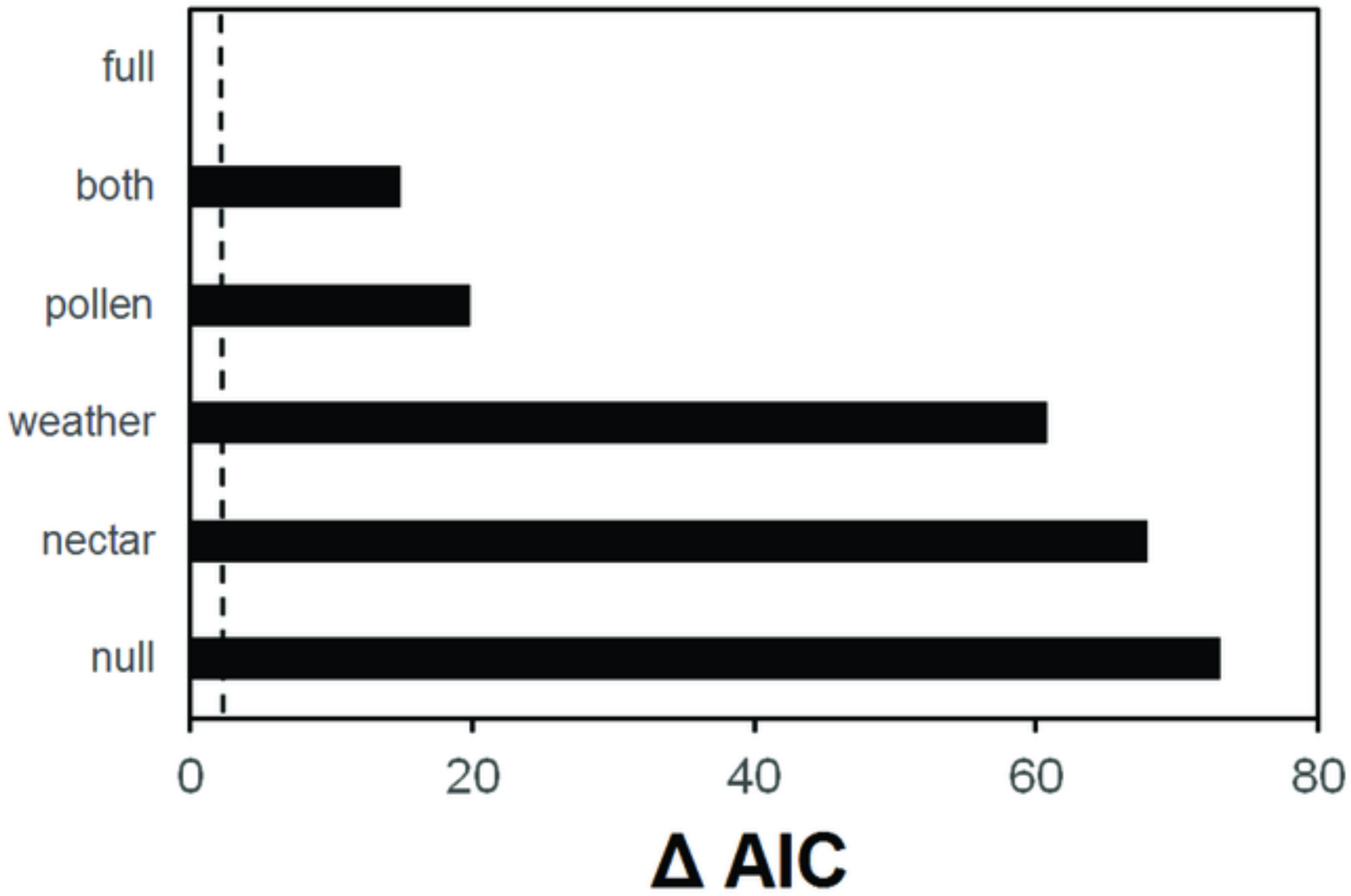


Figure 4



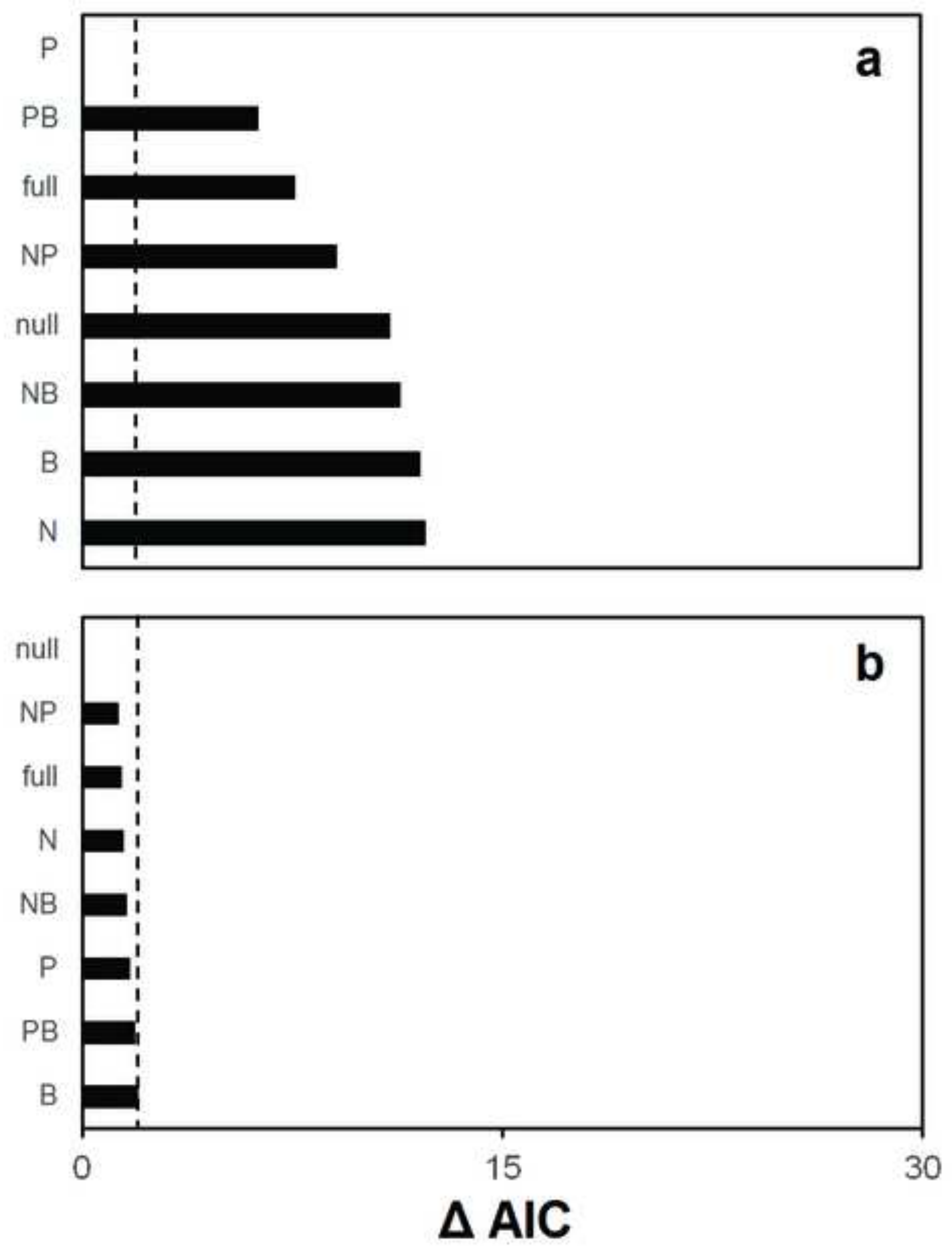

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**Table 1** Number of species visiting *Miconia theizans* flowers at Serra do Mar State Park (Santa Virgína station), São Paulo, Brazil. P: pollinivorous insects; N: nectarivorous insects; B: insects as collecting both pollen and nectar.

| **Order** | **Number of species** | | | **Richness** | **Relative Frequency ($x10^3$)** |
|---|---|---|---|---|---|
| | **P** | **N** | **B** | | |
| Blattodea | - | 3 | - | 3 | 4.14 |
| Coleoptera | - | 7 | 1 | 8 | 35.03 |
| Diptera | 1 | 22 | 12 | 35 | 105.47 |
| Hemiptera | - | 2 | - | 2 | 1.47 |
| Hymenoptera - bees | 13 | - | 9 | 22 | 358.16 |
| Hymenoptera – ants | - | 4 | - | 4 | 130.02 |
| Hymenoptera –wasps | - | 11 | - | 11 | 365.33 |
| Lepidoptera | - | 1 | - | 1 | 0.37 |
| **Total** | **14** | **54** | **29** | **86** | **1000** |

Electronic Supplementary Material 5