

## The importance of plant diversity in maintaining the pollinator bee, *Eulaema nigrata* (Hymenoptera: Apidae) in sweet passion fruit fields

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**Abstract:** The euglossine bee *Eulaema nigrata* plays an important role for the pollination of native and economically important plants, such as the sweet passion-fruit *Passiflora alata*. *E. nigrata* uniquely collects the nectar from the flowers of *P. alata*, nevertheless, it needs to visit other plants to collect pollen, nectar and other resources for its survival. There are two methods to identify the species of plants used by bees in their diet: by direct observation of the bees in the flowers, and through identification of pollen grains present in brood cells, feces, or in the bees' body. In order to identify the other plants that *E. nigrata* visits, we analyzed samples of pollen grains removed from the bee's body in the course of the flowering period of *P. alata*. Among our results, the flora visited by *E. nigrata* comprised 40 species from 32 genera and 19 families, some of them used as a pollen source or just nectar. In spite of being a polylectic species, *E. nigrata* exhibited preference for some plant species with poricidal anthers. *P. alata* which has high sugar concentration nectar was the main source of nectar for this bee in the studied area. Nonetheless, the pollinic analysis indicated that others nectariferous plant species are necessary to keep the populations of *E. nigrata*. Studies such as this one are important since they indicate supplementary pollen-nectar sources which must be used for the conservation of the populations of *E. nigrata* in crops neighbouring areas. In the absence of pollinators, growers are forced to pay for hand pollination, which increases production costs; keeping pollinators in cultivated areas is still more feasible to ensure sweet passion fruit production. Rev. Biol. Trop. 60 (4): 1553-1565. Epub 2012 December 01.

**Key words:** bee, conservation, maintenance of pollinators, pollinic analysis.

Animal pollination is one of nature's crucial ecosystem services. The majority of wild plant species and even many crops depend on animals to be pollinated (Crane & Walker 1984, Daily 1997, Nabham & Buchmann 1997, Kearns *et al.* 1998, Kremen *et al.* 2007, Ricketts *et al.* 2008, Ollerton *et al.* 2011). According to Roubik (1995), approximately half of all pollinators of tropical plants are bees, which improve the pollination of at least 72% of 1 330 crop species. Among bees, those belonging to the tribes Centridini and Euglossini can be

considered key pollinators in tropical ecosystems (Schlindwein 2000).

*Eulaema nigrata* Lepeletier, 1841 is an important euglossine species of the Brazilian bee fauna. The geographic range of this species extends from Costa Rica to Northern Argentina (Roubik & Hanson 2004), within which the bees are most commonly encountered in open areas, such as the Cerrado vegetation of Brazil (Rebêlo & Garófalo 1997, Nemésio & Faria-Jr 2004, Alvarenga *et al.* 2007). *E. nigrata* plays an important role for the pollination of native

and economically important plants such as, *Campomanesia pubescens* (Myrtaceae) (Torezan-Silingardi & Del-Claro 1998, Gressler *et al.* 2006), *Chamaecrista debilis* (Vogel) Irwin & Barneby (Fabaceae-Caesalpinaceae) (Nascimento & Del-Claro 2007), *Adenocalymma bracteatum* (Bignoniaceae) (Almeida-Soares *et al.* 2010), achiote *Bixa orellana* L. (Bixaceae) (Almeida & Pinheiro 1992), Brazil nut *Bertholletia excelsa* H.B.K. (Lecythidaceae) (Maués 2002), guava *Psidium guajava* L. (Myrtaceae) (Boti 2001), and sweet passion-fruit *Passiflora alata* Curtis (Passifloraceae) (Varassin & Silva 1999). Among these plant species, sweet passion-fruit obligatorily depends on cross-pollination for successful fruit production.

In recent studies carried out in Uberlândia, state of Minas Gerais, and São Francisco do Itabapoana, state of Rio de Janeiro, Brazil, Gaglianone *et al.* (2010) reported that *Epicharis flava* Friese, 1900 (Centridini) was the most frequent pollinator of *P. alata* in both areas and *Eulaema* species were also considered as potential pollinators. According to those authors, *E. nigrita* visited the flowers of *P. alata* exclusively for nectar collection. This indicates that pollen, another resource necessary for adult survival and brood production, must be obtained from other plants as it was already observed to *Xylocopa* species, important pollinators of other *Passiflora* species, like *Passiflora edulis* f. *flavicarpa* Deg. (Silva *et al.* 2010a).

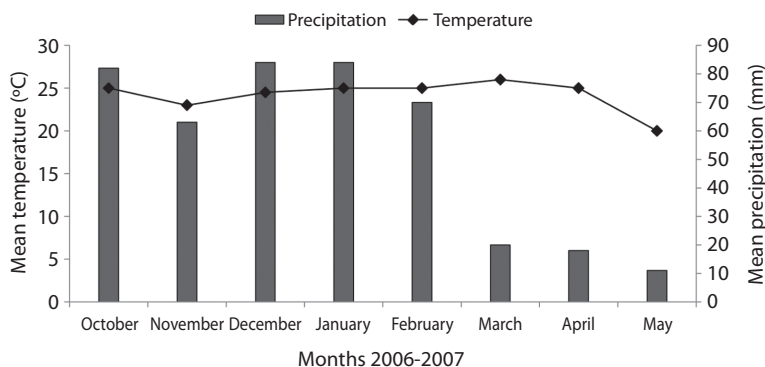
Conservation of natural areas at the surroundings of crops and the pattern of floral resources used by bees vary according to food plants' availability at a spatiotemporal scale (Kremen *et al.* 2007, Ricketts *et al.* 2008, Silva 2009). The knowledge of such patterns is of fundamental importance since it provides support to determine the plants that may be used in the conservation and maintenance of bee populations at the surrounding areas of passion-fruit crops. There are two methods to identify the species of plants used by bees in their diet: by direct observation of the bees in the flowers (Antonini & Martins 2003, Andena *et al.* 2005) and through identification of

pollen grains present in brood cells, feces, or in the bees' body (males and females) (Silva *et al.* 2010a). During the collection of floral resources, both male and female touch the anthers leaving the pollen grains adhered to their body. So, the pollen grains deposited on the males' body indicate the nectar sources, while those deposited on the females indicated both, nectar and pollen sources. Therefore, the pollen grains are natural markers of floral resources used by bees as food for adults and immatures, as observed by Silva *et al.* (2010a, b). For that reason the pollinic analysis is an important tool in the investigation of the plants which maintain the bees. This study aimed to identify the species of native plants that are visited and used by *E. nigrita* in their diet during the flowering of sweet passion fruit. This information will be very important since they will indicate supplementary pollen-nectar sources which must be used for the conservation of the populations of *E. nigrita* in neighbouring areas to the crops.

## MATERIAL AND METHODS

**Study area:** This study was carried out at Campo Alegre farm, Uberlândia-Minas Gerais State, Brazil (18°59'17" S - 48°14'35" W) during the flowering period of *P. alata* in the study region (October 2006-May 2007). The farm comprises a total area of 114ha, of which 20% is protected as reserve, one hectare is occupied by commercial cultivation of *P. alata* and seven hectares are occupied by *Passiflora edulis* f. *flavicarpa*.

The local climate has two well-defined seasons: a cool/dry season extending from March-September, and a hot/wet season extending from October-February. During the study period, the mean monthly temperature ranged from 20.0-26°C, and the precipitation ranged from 11-84mm (Fig. 1). Records on temperature and precipitation were obtained from the Laboratório de Climatologia e Recursos Hídricos, da Estação Climatológica da Universidade Federal de Uberlândia, MG (Fig. 1).



**Fig. 1.** Climate data (monthly mean temperature and rainfall) for the flowering period of *Passiflora alata*, in Uberlândia, Southeastern Brazil.

**Availability of floral resources in the surroundings of the *Passiflora alata* culture:**

The surroundings of *P. alata* plantations at Campo Alegre farm were predominantly pastures and scattered patches of Cerrado *stricto sensu*. In order to evaluate the availability of floral resources potentially used by *E. nigrita*, we studied the floristic composition and the flowering phenology in a Cerrado reserve at “Clube de Caça e Pesca Itororó de Uberlândia” (CCPIU), located at 5 000m away from the studied plantation of *P. alata*. This distance between CCPIU and the studied area is within the flight range of many euglossine bees, which may forage at up to 23km from their nest (Janzen 1971). Native flowering plants were sampled along a transect of 1 000m long and 10m wide on each side, thus covering a total of 2ha of Cerrado flora, the dominant vegetation in the region studied. The transect was sampled once a month during the flowering period of *P. alata* (October 2006-May 2007), recording all flowering species as well as the number of individuals per species. The voucher specimens were deposited in the Herbarium Uberlandense at the Federal University of Uberlândia, Brazil.

**Collection of bees for sampling pollen grains:** For the collection of pollen grain samples from bees, we captured specimens of *E. nigrita* once a month during the flowering period of *P. alata* from 8:00am-4:00pm (126

sampling hours in total). During that period, the bees were collected in the flowers of *P. alata*, for 15min per hour. This method was adopted in order to not interfere with pollination and fruit production in the study area, because it is a commercial area of *P. alata*. The bees were collected with an insect net and carefully verified in which part of the body (head, thorax, metasoma, corbiculae) the pollen grains were deposited. The pollen grains were sampled following the method suggested by Silva *et al.* (2010a), which consists of placing the bees into transparent vials containing 1mL of water in order to remove the pollen grains attached to the body of the individuals. The individuals were then released and 4mL of 70% alcohol were added in each vial. The material of each collected individual corresponded to one pollen sample, being analysed a total of 47 samples.

**Pollen analysis:** From each plant species found in the surrounding area, we removed the anthers of at least four individuals in order to set up a reference collection of pollen grains, using acetolysis as proposed by Erdtman (1960). The same method was used for pollen grains samples of the bees. All prepared microscope slides were deposited at the Pollen Slide Collection of ‘Laboratório de Morfologia Vegetal, Microscopia e Imagem’ (LAMOVI) at the Federal University of Uberlândia (UFU).

From each bee pollen sample, we mounted three microscope slides that were qualitatively and quantitatively analyzed. For the qualitative analysis we compared the pollen grains removed from the individuals, with the pollen from plants sampled in the surrounding area (Pollen Slide Collection), and also with pollen grains from the region's Pollen List described by Silva *et al.* (2010b). For the quantitative analysis, we counted the first 400 pollen grains found on each microscope slide as suggested by Montero & Tormo (1990). From these, we determined the percentages following classification of Barth (1970) and Louveaux *et al.* (1970, 1978): predominant pollen (>45% of total pollen grains on the slide), secondary pollen (from 15-45%), important minor pollen (3-15%) and minor pollen (<3%). In this study, those plant species whose pollen grains were found at percentages of more than 15% in the samples each month, were considered the preferably used plants for obtaining floral resources. In order to evaluate the importance of the native plant species in the surrounding area for the maintenance of *E. nigrita* populations, we did not consider the pollen grains of the two *Passiflora* species cultivated in the area in the quantitative analysis.

To determine the use of nectar by *E. nigrita* from flowers of species of native plants, floral traits were evaluated to check if the floral morphology favoured just the collection of nectar, for example, in species with tubular corollas which have nectariferous chamber or narrow floral tube. The flowers were also analyzed as determine the position of the anthers, to understand the process of visit, and if was possible, or not that *E. nigrita* also collect pollen beyond the nectar. Direct observations were done in the flowers on surrounding cultures whenever possible, to check the behaviour of *E. nigrita* in the flowers. Additional data were obtained in the literature.

Statistical tests were done using the statistical software BioEstat 5.0 (Aires *et al.* 2005). Pearson correlation test was applied to assess the degree of association among climate (monthly mean temperature and rainfall) and

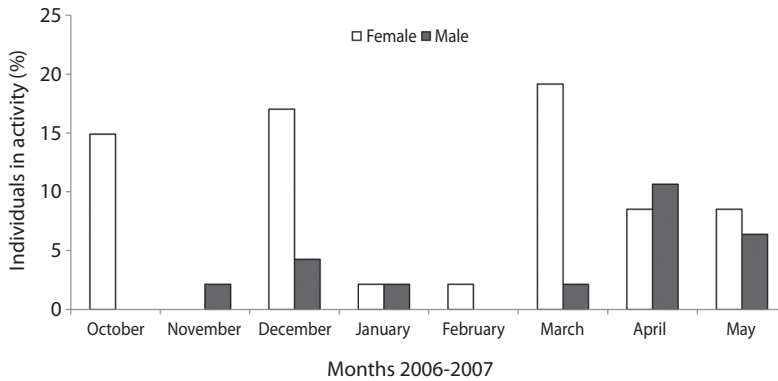
availability of floral resources in the areas surrounding the *P. alata* plantation, and the number of bees sampled per month during flowering period of *P. alata*.

## RESULTS

In the study area, flowering of *P. alata* occurred continuously from October 2006 to May 2007, although some peaks of open flowers were observed during the rainy season (October-February). At the beginning of the dry season (March-May) a decrease on flowering was observed. Thirty four females and 13 males of *E. nigrita* were collected. Of the sampled females, 24 individuals carried pollen on their corbiculae. Fifteen of these pollen-loaded females were collected from 8:00am to 11:00am and nine of them from 12:00 noon to 4:00 pm. Female bees were most frequently observed on *P. alata* flowers in October, December, and March whereas males were most abundant in April and May (Fig. 2). No correlation was observed between the number of active bees on flowers and climatic conditions ( $r=0.43$ ,  $p=0.28$ , for precipitation and  $r=0.13$ ,  $p=0.75$ , for temperature).

During the collection of bees, we observed that pollen grains were distributed throughout the body of male individuals, but especially on the thorax ( $n=13$ ); while in the body of females, the pollen grains were observed on the thorax, metasoma and predominantly in the corbiculae ( $n=34$ ). Among the types of pollen grains accumulated on the thorax of males and females, that of *P. alata* was the predominant. In addition to the pollen of *P. alata*, we found pollen grains from 35 native plant species on females and from 14 species on males. There was an overlap between males and females in the visit of some plant species, being identified in total 40 plant species distributed in 32 genera and 19 botanical families, excluding the pollen grains of the passion fruit species (Table 1).

The use of floral resources by *E. nigrita* varied in the course of the studied period, with the highest number of pollen types in March, although in this month we observed

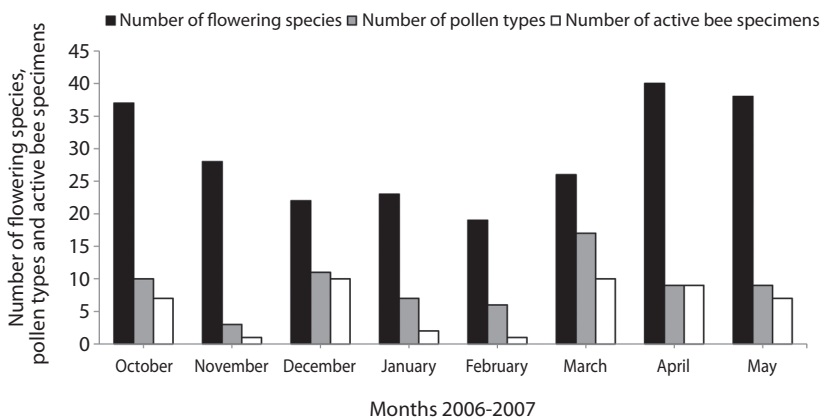


**Fig. 2.** Percentage of females and males of *Eulaema nigrita* visiting flowers of *P. alata* from October 2006 to May 2007, in Campo Alegre Farm, Uberlândia, Southeastern Brazil.

one of the lowest numbers of flowering species in the plantation surroundings (Fig. 3). There was no correlation between the number of active individuals and the number of individuals flowering in the area surrounding the sweet passion-fruit plantation ( $r=0.40$ ,  $p=0.33$ ) (Fig. 3). However, the number of active bees was correlated with the number of pollen types identified in the bee samples ( $r=0.83$ ,  $p=0.01$ ) (Fig. 3). The amount of different pollen types carried by bees was strongly associated with the number of plant species providing pollen

as pollinator-attractant ( $r=0.98$ ,  $p<0.0001$ ), but not with the number of species providing nectar ( $r=0.55$ ,  $p=0.18$ ).

The flowers visited by *E. nigrita* share several characteristics such as color, shape, type of anther, and size (Table 1). Those plant species represented 25% of the total of plants visited in each month. The most visited plant species to collect nectar have long corolla, which facilitates access to this flower resource by bees with long tongue, as *E. nigrita*. The species of plants that provide the pollen as attractive floral



**Fig. 3.** Number of flowering plants in the surroundings of the plantation of *P. alata* compared with number of pollen types identified in the samples and number of specimens of *Eulaema nigrita* in activity in the Campo Alegre Farm, Uberlândia, Southeastern Brazil.

TABLE 1  
Native plant species used as floral sources by *E. nigrita* during the flowering period of *P. alata*

Family	Frg	Floral characteristics Sh/Sy/Co/Ad	Species	Percentual of pollen type/month											
				Oct	Nov	Dec	Jan	Feb	Mar	Apr	May				
Acanthaceae	ne	tu, zig, li, long	<i>Ruellia brevifolia</i> (Pohl) C.Ezeurra	6.08	-	-	-	-	-	-	-	-	-		
Apocynaceae	ne	tu, zig, re, long	<i>Aphelandra longiflora</i> (L.indl.) Profice	-	-	0.05	-	-	-	-	-	-	-		
Asteraceae	ne, po	tu, zig, ye, long	<i>Mandevilla hirsuta</i> (Rich.) K.Schum.	-	-	-	-	-	-	-	-	-	0.34		
Bignoniaceae	ne	tu, act, ni, long	<i>Chromolaena</i> sp.	-	-	-	-	4.76	-	-	-	-	-		
	ne	tu, zig, wh, long	<i>Amphilophium elongatum</i> (Vahl) L.Lohmann	-	-	0.19	6.06	-	-	-	-	-	-		
	ne	tu, zig, pu, long	<i>Cuspidaria pulchra</i> (Cham.) L.Lohmann	-	-	-	-	-	-	-	-	0.68	-		
	ne	tu, zig, li, long	<i>Jacaranda rufa</i> Silva Manso	-	-	0.05	-	-	-	-	-	-	-		
Bromeliaceae	ne	tu, act, li, long	<i>Ananas ananassoides</i> (Baker) L.B.Sm.	0.31	-	-	-	-	-	-	-	-	-		
	ne	tu, act, or, long	<i>Dyckia leptostachya</i> Baker	-	-	0.05	-	-	-	-	-	-	-		
Clusiaceae	po	di, act, wh, long	<i>Kielmeyera coriacea</i> Mart.	-	-	7.40	-	4.76	-	-	-	-	-		
Convolvulaceae	ne	camp, act, ni, long	<i>Ipomoea</i> sp.	-	-	-	-	38.10	-	-	-	-	-		
Dilleniaceae	po	di, act, ye, long	<i>Davilla elliptica</i> A.St-Hil.	-	-	-	-	-	1.06	-	-	-	-		
Erythroxylaceae	ne, po	di, act, cr, long	<i>Erythroxylum suberosum</i> St-Hil.	-	25.00	-	-	-	-	-	-	-	-		
Leguminosae (Fabaceae)	ne, po	di, act, wh, long	<i>Copaifera langsdorffii</i> Desf.	-	-	-	-	-	0.05	-	-	-	-		
	ne, po	stan, zigo, ye, long	<i>Crotalaria brachystachya</i> Benth.	-	-	-	-	-	0.42	-	-	-	-		
	ne, po	stan, zig, ye, long	<i>Crotalaria micans</i> Link	-	-	-	-	-	-	-	-	32.92	-		
Malpighiaceae	po	di, zig, ye, por	<i>Senna obtusifolia</i> (L.) H.Irwin & Barneby	-	-	-	6.06	9.52	0.05	0.23	21.47	-	-		
	po	di, zig, ye, por	<i>Senna rugosa</i> (G.Don) Irwin & Barneby	0.05	-	12.48	-	-	11.42	-	-	-	-		
	po	di, zig, ye, por	<i>Senna silvestris</i> (Vell.) H.S.Irwin & Barneby	0.41	-	14.09	-	-	-	-	-	-	-		
	po	di, zig, ye, por	<i>Senna velutina</i> (Vogel) H.S.Irwin & Barneby	-	-	-	-	-	5.63	1.80	-	-	-		
	po	di, zig, ye, long	<i>Byrsonima verbascifolia</i> (L.) Rich.	0.31	-	0.05	-	-	-	-	-	-	-		
	po	di, zig, ye, long	<i>Byrsonima basiloba</i> A. Juss.	-	-	0.09	-	-	0.11	-	-	-	-		
	po	di, zig, ye, long	<i>Heteropteris pteropetala</i> A. Juss.	-	-	-	-	-	0.11	-	-	-	-		
Malvaceae	po	di, act, wh, long	<i>Eriotheca gracilipes</i> (K.Schum.) A.Robyns	-	-	-	-	-	0.05	-	-	-	-		
Melastomataceae	po	di, zig, or, por	<i>Cambessedexia hilariana</i> (Kunth.) DC.	-	-	-	28.79	-	25.23	-	-	-	-		
	po	di, zig, pu, por	<i>Microlicia isophylla</i> DC.	-	-	-	-	-	2.02	-	-	-	-		
	po	di, zig, li, por	<i>Rhynchanthera grandiflora</i> (Aubl.) DC.	0.05	-	-	-	-	41.42	58.06	63.84	-	-		
	po	di, zig, li, por	<i>Tibouchina gracilis</i> Cogn.	-	-	-	-	-	-	5.19	1.58	-	-		
	po	di, zig, wh, por	<i>Trembleya parviflora</i> (D.Don) Cogn.	-	-	-	43.94	-	-	-	-	-	-		

TABLE 1 (Continued)  
Native plant species used as floral sources by *E. nigrita* during the flowering period of *P. alata*

Family	Frg	Floral characteristics Sh/Sy/Co/Ad	Species	Percentual of pollen type/month											
				Oct	Nov	Dec	Jan	Feb	Mar	Apr	May				
Myrtaceae	po	di, act, wh, long	<i>Eucalyptus</i> sp.	-	-	0.05	-	-	-	-	-	-	-		
	po	di, act, wh, long	<i>Eugenia calycina</i> Cambess.	-	-	-	-	-	-	-	-	-	0.34		
	po	di, act, wh, long	<i>Eugenia puniceifolia</i> (Kunth) DC.	-	-	-	-	9.52	0.16	0.11	-	-	-		
	po	di, act, wh, long	<i>Myrcia guianensis</i> (Aubl.) DC.	-	-	-	1.52	33.33	-	-	-	-	-		
Ochnaceae	po	di, act, ye, por	<i>Ouratea hexasperma</i> (St-Hil.) Baill.	1.08	-	-	12.12	-	-	-	-	-	-		
	po	di, act, ye, por	<i>Ouratea spectabilis</i> (Mart.) Engl.	14.06	-	-	-	-	-	-	0.79	0.11	-		
Rubiaceae	ne	tu, act, ye, long	<i>Palicourea rigida</i> Kunth.	-	-	-	-	-	-	0.11	-	-	-		
Solanaceae	po	di, zig, li, por	<i>Solanum lycocarpum</i> A.St-Hil.	67.20	50.00	65.51	1.52	-	11.74	0.23	12.09	-	-		
	po	di, zig, li, por	<i>Solanum paniculatum</i> L.	10.45	-	-	-	-	0.32	-	0.11	-	-		
Styracaceae	ne	di, act, wh, long	<i>Syrax ferrugineus</i> Nees & Mart.	-	-	-	-	-	-	-	-	-	0.11		
Vochysiaceae	ne	tu, zig, ye, long	<i>Vochysia tucanorum</i> Mart.	-	25.00	-	-	-	0.11	-	-	-	-		
Total family: 19				10	3	11	7	6	17	9	9	9	9		
Total genus: 32															
Total species: 40															
Total species/month															

Frg: Floral resource gathered by *E. nigrita*. Sh: floral shape (tu: tube, di: dish, stan: standart, camp: campanulate); Sy: floral symmetry (act: actinomorphic, zig: zigomorphe); Co: color (li: lilac, re: red, ye: yellow, ni: no information, wh: white, pu: purple, or: orange, cr: cream); Ad: anther dehiscence (long: longitudinal, por: poricidal).

resources in this study have poricidal anthers, that are vibrated by females during visits for pollen grains removal.

In general, *E. nigrita* visited more native plant species to collect pollen (28 species) than to collect nectar (17 species) (Table 1). The pollen grains most frequently encountered in the bee pollen-samples were from *Solanum lycocarpum* St. Hil. (Solanaceae) and *Rhynchanthera grandiflora* (Aubl.) DC. (Melastomataceae). The flowers of these two plant species have poricidal anthers and provide only pollen as floral resource. From the set of species used by *E. nigrita*, *S. lycocarpum* and *R. grandiflora* were preferably visited during their flowering periods as indicated by the frequency of pollen grains present in the bee pollen samples (Table 1). Between the flowering season end of *S. lycocarpum* and the beginning of *R. grandiflora* (January and February), other species, such as *Trembleya parviflora* (D. Don) Cogn. (Melastomataceae), *Myrcia guianensis* (Aubl.) DC. (Myrtaceae) and *Cambessedesia hilariana* (Kunth) DC. (Melastomataceae), were the most visited. These three species were visited and/or used as source of floral resources only when *S. lycocarpum* and *R. grandiflora* were not bloom (Fig. 4). Concerning nectar collection, with the exception of *P. alata* that was predominant in all samples, we observed that *Crotalaria micans* Link (Fabaceae) in April (32.9%), and *Vochysia tucanorum* Mart. (Vochysiaceae) in November (25.0%), were the plants more visited by *E. nigrita* (Table 1).

## DISCUSSION

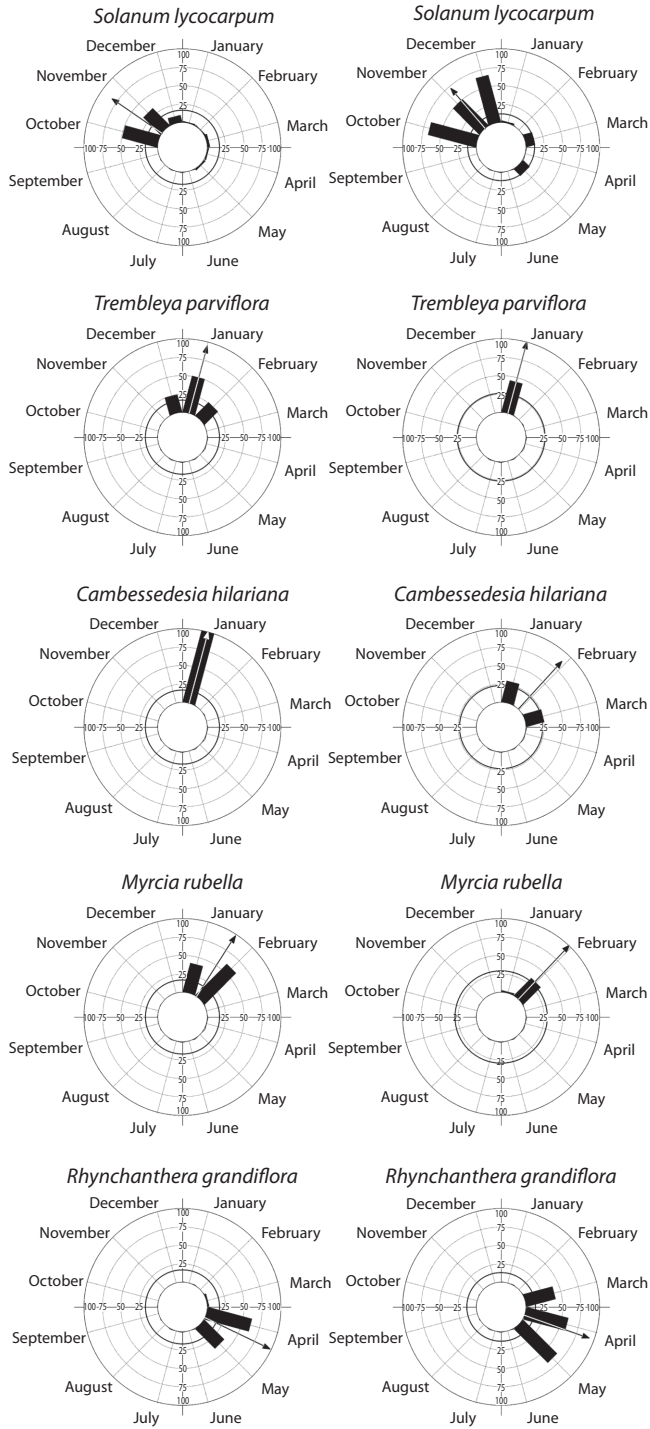
The polylectic foraging pattern of *E. nigrita* observed in this study, was also reported by Ramírez *et al.* (2002) and Roubik & Hanson (2004). This pattern was also exhibited by other species of euglossine bees such as *Euglossa atrovoneta* Dressler, 1978 (Arriaga & Hernández 1998) and *Euglossa annectans* Dressler, 1982 (Cortopassi-Laurino *et al.* 2009).

*Eulaema nigrita* females collect floral resources on a variety of plants that are not used by males. These males collect only nectar

and floral fragrances, as males of other Euglossini species (Dodson *et al.* 1969, Dressler 1982, Ackerman 1985, Roubik & Hanson 2004).

The collection of high amounts of pollen and nectar by females of *E. nigrita* in the study site reflects, mainly, the demand for these resources to the provision of brood cells. Nectar plays an important role not only to feed the brood, but also as an energetic source to the maintenance of adults. The energy expenditures during female foraging for nest-building material and for the provision of cells are very significant due to female's performance of long trips to collect excrement, mud, resin and larval food (Pereira-Martins & Kerr 1991, Santos & Garófalo 1994). Euglossine bees collect nectar from tubular flowers and these flowers have nectar with a relatively low concentration of sugar (Roubik *et al.* 1995), leading females to extend the search for flowers in the field. On the other side, in an opportunistic way, *E. nigrita* may exhibit floral constancy in sources of nectar that are more concentrated and produced in high quantities, as it occurs in flowers of *P. alata* that exhibit on average a sucrose concentration of  $39.65\% \pm 2.63$  and a volume of  $408\mu\text{l} \pm 76.71$  per flower (Rocha 2006). According to Roubik *et al.* (1995), *E. nigrita* collects nectar at a concentration ranging from 20-49%. Another factor that may influence the collection of nectar in *P. alata* is the proximity of the nests to the cultivated area. As reported by Arriaga & Hernández (1998), the most important plants used by *Euglossa atrovoneta* were near to the nests, and this may be related to energy saving in the flight. However, the pollen analysis indicated that the nectar produced by *P. alata* flowers is not enough to supply the necessary energy to maintain the population of *E. nigrita*. Thus, although *P. alata* has a relatively long flowering period, the samples revealed a wide variety of pollen types from nectar-producing native species, what corroborates the need to preserve the nectariferous plant species in the natural areas near to cultures, especially the species of Acanthaceae, Apocynaceae, Bignoniaceae, Bromeliaceae, Convolvulaceae and Vochysiaceae. All plant species representatives





**Fig. 4.** Percentage of individuals of these most frequently plant species and constantly used by *Eulaema nigrita* in the surroundings of the plantation of *Passiflora alata* in Campo Alegre Farm, Uberlândia, Southeastern Brazil. The column left indicate the percentage of flowering individuals of the plant species more visited. The column right indicate the percentage of the pollen grains in this plant species samples.

of these families identified in this study, analysing the pollen grains removed from the body of *E. nigrita*, exhibit floral morphology that allows only the collecting of nectar by this bee.

It is important to emphasize again that *P. alata* flowers are used by *E. nigrita* exclusively to collect nectar and that these bees, as well as the others, need pollen for a balanced diet to survive (Weiner *et al.* 2010). From June to September *E. nigrita* depends on other sources than *P. alata* for the maintenance of nests, and even during its flowering period these bees still need other sources. Quantitative analyses of pollen grains in the samples were important because they revealed the floral constancy in species with poricidal anthers, such as the species of the families Fabaceae (subfamily Caesalpinioideae), Melastomataceae, Ochnaceae and Solanaceae. Pollen grains of these plants were included in the predominant category, corresponding to 12.5% of all species used by *E. nigrita*. This shows that these bees sporadically visit a wide variety of plants per month, but prefer a small fraction of them, for example *S. lycocarpum* and *R. grandiflora*. Roulston *et al.* (2000) observed that pollen grains from species with poricidal anthers exhibit high protein content (47.8%), and bees use pollen grains with a percentage between 12-61%. The protein directly influences the larval development of bees (Michener 2000, Minckley & Roulston 2006). This may justify the floral constancy of *E. nigrita* on *S. lycocarpum* and *R. grandiflora*, which blooming during several months throughout the year. The foraging movement between these two species may have occurred due to the variation in the percentage of flowering individuals. This reinforces the preference of *E. nigrita* females for plants with poricidal anthers. Similar data were observed for other bee species with vibration behavior, in which the predominant pollen grains were the ones from plant species with poricidal anthers, as for example *Xylocopa* spp. (Silva *et al.* 2010a) and *Centris tarsata* Smith, 1874 (Dórea *et al.* 2009). The pollen analysis enables to obtain quantitative data that are important because they confirm the fact that the plant species that

are the most used by bees with vibration behaviour are the ones with more expensive resources, both in terms of protein content of pollen and in terms of energetic content of nectar. The quality of the floral resources must be taken into consideration when making management and conservation plans for pollinators, which has been declining with habitat alteration and reduction of ecological resources used for both feeding and nesting (Cane 2001, Ricketts *et al.* 2008, Potts *et al.* 2010, Bommarco *et al.* 2010).

Despite not being the most often pollinator of *P. alata*, *E. nigrita* is a potential pollinator for management in the plantations, since the main pollinator, *Epicharis flava* (Gaglianone *et al.* 2010), build their nest in soil, while *E. nigrita* nests in preexisting cavities. Regarding nesting sites, cement blocks with inside cavities used in constructions can be disposed in the field as trap-nests to attract nesting females of *E. nigrita*, as suggested by Garófalo *et al.* (2011).

Concerning feeding, the plants preferably visited and used by *E. nigrita*, which were identified in the present study, must be preserved or reintroduced in the proximities of *P. alata* to favour the maintenance of that species. The management of pollinators in the surroundings of *P. alata* is important, since this plant is a self-incompatible species (Varassin & Silva 1999), and in the absence of pollinators, growers are forced to pay for hand pollination, which increases production costs (Pereira-Vieira *et al.* 2010). Keeping pollinators in cultivated areas is still more feasible to ensure sweet passion fruit production.

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## RESUMEN

La abeja euglosina *Eulaema nigrita* juega un importante papel para la polinización de las plantas nativas y de importancia económica, como es el caso de la fruta de la pasión o maracuyá *Passiflora alata*. *E. nigrita* únicamente recoge el néctar las flores de *P. alata*, sin embargo, tiene que visitar otras plantas para recoger polen, néctar y otros recursos para su supervivencia. Hay dos métodos para identificar las especies de plantas utilizadas por las abejas en su dieta: por observación directa de las abejas en las flores, y a través de la identificación de los granos de polen presentes en las celdas de cría, heces o en el cuerpo de las abejas. Con el fin de identificar las otras plantas que *E. nigrita* visita, se analizaron muestras de granos de polen extraído del cuerpo de la abeja durante el período de floración de *P. alata*. Entre nuestros resultados, la flora visitada por *E. nigrita* está compuesta por 40 especies de 32 géneros y 19 familias, algunas de ellas utilizadas como fuente de polen o solamente de néctar. A pesar de ser una especie polifilética, *E. nigrita* exhibe preferencia por algunas especies de plantas con anteras poricidas. El néctar de *P. alata* tiene la más alta concentración de azúcar y fue la principal fuente de este recurso para las abejas en el área de estudio. Sin embargo, el análisis polínico indicó que otras especies de plantas nectaríferas son necesarias para mantener las poblaciones de *E. nigrita*. Estudios como éste son importantes pues indican cuales son las fuentes complementarias de néctar y polen que deben ser utilizadas para la conservación de las poblaciones de *E. nigrita* en los cultivos de las zonas vecinas. En ausencia de polinizadores, los productores se ven obligados a pagar por la polinización manual, lo que aumenta los costos de producción, por lo tanto el mantenimiento de polinizadores en las zonas cultivadas es más factible para asegurar la producción de la fruta de la pasión.

**Palabras clave:** abeja, análisis polínico, conservación, mantenimiento de los polinizadores.

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