Orchid bee baits attracting bees of the genus *Megalopta* (Hymenoptera, Halictidae) in Bauru region, São Paulo, Brazil: abundance, seasonality, and the importance of odors for dim-light bees

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ABSTRACT. Orchid bee baits attracting bees of the genus *Megalopta* (Hymenoptera, Halictidae) in Bauru region, São Paulo, Brazil: abundance, seasonality, and the importance of odors for dim-light bees. Nocturnal bees in the genus *Megalopta* Smith, 1853 are generally collected using artificial light sources. However, between 1993 and 2000, a total of 946 females (no males were captured) were captured using aromatic baits commonly used for orchid bees (Euglossini) in five localities in Bauru region, São Paulo, Brazil. Aromatic compounds used in bait traps were: benzyl acetate, eucalyptol, eugenol, skatole, methyl salicylate, and vanillin. The *Megalopta* species collected were: *M. guimaraesi* (71.2% of total number of specimens), *M. amoena* (28.1%), and *M. aegis* (0.6%). Using the data from these traps, we showed that there was a positive and significant correlation between the abundance of individuals and meteorological factors, rainfall and temperature. Bees were more commonly collected in the spring (September to December) and summer (December to March) than in the autumn and winter, the latter characterized for being a drier and colder period. Variations in the abundance were also detected among localities and years. The most attractive compounds were eugenol (54%), methyl salicylate (22%), and eucalyptol (16%). The ability to detect smells may have an important role in searching for flowers during dim-light conditions. We suggest the use of aromatic compounds in future studies on the biology of *Megalopta* in the Neotropical region.

KEYWORDS. Abundance; chemical baits; scent; seasonality.

RESUMO. Abelhas do gênero *Megalopta* (Hymenoptera, Halictidae) atraídas por iscas químicas usadas para euglossíneos na região de Bauru, SP: abundância, sazonalidade e importância de odores para abelhas crepusculares. Abelhas noturnas do gênero *Megalopta* (Smith, 1853) são geralmente coletadas usando fontes artificiais de luz. Porém entre os anos de 1993 e 2000, um total de 946 fêmeas de *Megalopta* foram capturadas (machos não foram capturados) usando iscas aromáticas frequentemente usadas para atração de machos de Euglossini, em cinco localidades na região de Bauru, São Paulo, Brasil. Os compostos aromáticos utilizados foram: acetato de benzila, eucaliptol, eugenol, escatol, salicilato de metila e vanilina. As espécies encontradas foram *M. guimaraesi* (71.2% do total de indivíduos), *M. amoena* (28.1%) and *M. aegis* (0.6%). De modo geral, os resultados mostraram correlação positiva e significativa entre a abundância de indivíduos e os fatores meteorológicos considerados — precipitação pluviométrica e temperatura. As abelhas foram geralmente capturadas em maior número na primavera e verão e raramente no outono e inverno, período mais seco e frio. Variações na abundância também foram detectadas entre as localidades e entre os anos estudados. As substâncias mais atrativas foram: eugenol (54%), salicilato de metila (22%) e eucaliptol (16%). A capacidade de detectar cheiros pode ter um importante papel na busca por flores em condições crepusculares. Sugerimos o uso destes compostos aromáticos em pesquisas futuras sobre a biologia de *Megalopta* na região Neotropical.

PALAVRAS-CHAVE: Abundância; iscas químicas; olfato; sazonalidade.

Individuals of the nocturnal bee genus *Megalopta* Smith, 1853, forage in dim-light conditions when most other bees do not forage (Wcislo *et al*. 2004; Wcislo & Tierney 2009). Females are either solitary or facultatively social (Arneson & Wcislo 2003; Wcislo & Gonzalez 2006) and construct nests in dead wood (Janzen 1968; Wcislo *et al.* 2004; Santos *et al.* 2010). *Megalopta* occurs from tropical parts of Mexico to the state of Santa Catarina, Brazil, and contains 30 described species (Michener 2007; Engel 2011). Despite this diversity, the number of undescribed species is presumably high, and still today little is known about their natural history in South America (Santos *et al*. 2010). Most of what is known about their biology comes from studies on two species (*M. genalis* Meado-Waldo, 1916 and *M. centralis* Friese, 1926) from a single locality in Central America, Barro Colorado Island, Panama (Arneson & Wcislo 2003; Wcislo *et al.* 2004; Wcislo & Gonzalez 2006).

Since most species are believed to be nocturnal or crepuscular because of their anatomical features shared with other dim-light foraging aculeate Hymenoptera (e.g., enlarged ocelli and compound eyes, pale body color, etc.), they are normally captured using artificial light sources (Wcislo &

Tierney 2009). However, in the region of Bauru, São Paulo state (SP), Brazil, a large number of *Megalopta* specimens have been collected using chemical baits that are commonly used to attract male orchid bees (Apidae, Euglossini). Such a technique has been used since the identification of various pure forms of orchid odor components (Dodson *et al.* 1969), and it is widely used in the Neotropical region (Nemésio 2007). The main goal of the application of these chemical baits is to collect euglossine bees, but other bees are also rarely attracted: *Megalopta* and *Megaloptilla* Moure & Hurd, 1987 (Halictidae, Augochlorini), *Ptiloglossa* Smith, 1853 (Colletidae, Caupolicanini), *Lestrimellita* Friese, 1903 (Apidae, Apini), and *Acanthopus* Klug, 1807 (Apidae, Ericrocidini) (Campos *et al.* 1989; Melo 1995; Engel & Brooks 1999; Gonzalez *et al.* 2010b; Nemésio & Siqueira 2011); however, the significance of these unintentionally collected bees is often neglected by melittologists.

Herein we report the use of chemical baits to collect *Megalopta* bees. We also provide information on the meteorological (rainfall and temperature) influence on the abundance and seasonality of *Megalopta* based on samplings conducted between 1993 and 2000 with orchid bee baits, in five localities in Bauru region, São Paulo, Brazil. We also discuss the importance of the olfactory system in *Megalopta* in foraging flowers with odors in dim-light conditions.

MATERIAL AND METHODS

The study was conducted in five localities of west central São Paulo state (Bauru region). The dominant vegetation in all localities is semidecidual forest (Veloso *et al.* 1991), and the climate is humid subtropical (Cwa) following the classification of Köppen-Geiger, which is defined by hot summers and dry winters (Peel *et al.* 2007). In general, the dry season covers the period from April to August, while the wet season is from September to March.

The bees were captured in the following four fragmented areas and in the university campus in the municipalities of Bauru, Agudos, Gália, and Alvinlândia in São Paulo, Brazil.

1. Permanent Preservation Area Vargem Limpa-Campo Novo (PPAVL) in Bauru, SP. It has a total preservation area of about 1274 ha [22°21'S; 49°01'W; 580 m]. The native vegetation is a forested savanna (cerradão) with a very dense tree stratum, usually with *Copaifera langsdorfii*, *Coussarea hydrangeafolia*, *Siparuna guianensis*, *Xylopia aromatica*, *Vochysia tucanorum,* and *Myrcia guianensis* (Cavassan *et al*. 2006). The landscape is composed of farms, pastures, and urban area.

2. Permanent Preservation Area of Duratex (PPAD). This is situated in Agudos [22°29'–22°47'S; 48°51'–48°99'W] and has an altitude of 580 m. The fragment studied covers 94.22 ha, and it is located inside an area of 920 ha of native vegetation and introduced *Pinus* and *Eucalyptus*. It is a fragment of seasonal semideciduous forest, with the most important botanical families being Leguminosae, Rubiaceae, and Myrtaceae (Cavassan *et al*. 1984).

3. The Ecological Station of Bauru (ESB) is located in Bauru [22°07'–22°15'S; 49°04'–49°05'W] and has a mean altitude of 530 m. It is a remnant of a seasonal semideciduous forest with an area of 287 ha. The most important botanical families are Leguminosae, Rutaceae, Myrtaceae, Boraginaceae, Meliceae, and Lauraceae. It is surrounded by pastures.

4. The Ecological Station of Caetetus (ESC) is located between the municipalities of Galia and Alvinlândia [22°22'–22°27'S, 49°40'–49°43'W] and has a mean altitude of 690 m. It has an area of 2308 ha. It can be described as a seasonal semideciduous forest because it has high trees with complex stratification and high floristic diversity. The most important tree species of the upper stratum are *Metrodorea nigra*, *Savia dictyocarpa*, *Ocotea indecora*, *Aspidosperma polyneuron*, and *Trichilia catigua* (Durigan *et al*. 2000). It is surrounded by agricultural areas, mostly growing coffee.

5. Paulista State University (UNESP) Campus in Bauru (UCB) [22°21'S; 49°01'W] has an altitude of 580 m. Its vegetation is originally forested savanna with forest representatives, but it also has ornamental and fruit-bearing plants. The size of the area is 52.47 ha and is separated from the preceding (PPAVL) by a highway (width of 50 to 60 m) and surrounded by an urban area.

The bees were collected in bait traps, similar as those proposed by Campos *et al.* (1989). The chemical baits used were eucalyptol (cineol), eugenol, methyl salicylate, and vanillin (four different chemical compounds per station). Skatole was also used in ESB and UCB and benzyl acetate in UCB.

Traps were run from 1993 and 2000 and were located about 1.70 m above the ground, remaining in the field during the entire sampling period. In all areas the bait traps were separated from each other by a distance of 50 to 100 m. In the preserved areas the sampling stations were established along a trail, as close to the center of the reserve as possible. A total of six stations were located in PPAVL (four in the interior of the bushes and two on the edge), four in both ESB and PPAD, eight in ESC, and three in UCB.

The traps were always placed on days without rainfall, approximately at 08:00 am, remaining at the site for a period from three to five days. After this period, during the daytime, the bait traps were checked and the bees collected. This procedure was repeated monthly, always at the same stations previously chosen. The monthly sample was a total number of bees collected in all stations and traps.

Monthly values of accumulated rainfall (mm) and the average of minimum and maximum temperature (°C) were correlated with monthly samples of abundance for *M. amoena* and *M. guimaraesi.* We used a nonparametric Spearman's rank correlation coefficient for localities studies and for cluster of data for all areas studied. Meteorological data were obtained from the Instituto de Pesquisas Meteorológicas da UNESP (IPEMET), except for ESC which was obtained from the Instituto Agronômico de Campinas (IAC).

The collected bees are deposited in Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia da Universidade Federal do Paraná (DZUP), Brazil.

RESULTS

Bee Activity. A total of 946 individuals, all females (12% of all collected bees), of the following three *Megalopta* species were collected: *M. aegis* (Vachal, 1904), *M. amoena* (Spinola, 1853), and *M. guimaraesi* Santos & Silveira, 2009. Most specimens belonged to *M. guimaraesi* (n = 674) and the majority were captured in PPAD (Table I). *Megalopta guimaraesi* was the most abundant species in all four localities while *M. amoena* was more abundant only in PPAD; *M. aegis* was rarely collected and was only present in PPAVL and ESB (Table I).

Table I. Number of *Megalopta* bees collected in five localities in Bauru region (SP) Brazil. Permanent Preservation Area of Vargem Limpa – Campo Novo (PPAVL); Permanent Preservation Area of Duratex (PPAD); Ecological Station of Bauru (ESB); Ecological Station of Caetetus (ESC) and University Campus of UNESP in Bauru, SP (UCB).

	M. guimaraesi	*M. amoena*	*M. aegis*	Total
PPAVL	248	45	5	298
PPAD	161	199	0	360
ESB	192	9	1	202
ESC	19	7	0	26
UCB	54	6	0	60
Total	674	266	6	946

M. guimaraesi was captured between 32 and 503 mm of rainfall and in temperatures between 13.6°C and 31.9°C, the average monthly minimum and maximum temperature, respectively. The abundance of *M. guimaraesi* was correlated with rainfall in all areas of study. The correlation was higher and meaningful for the minimum average temperature in PPAVL, ESB, and UCB (Table II).

M. amoena was captured between 22 and 475 mm of rainfall and in temperatures between 12.0° C and 31.1°C. The correlation of abundance and rainfall was positive and meaningful only in PPAD. The correlation of abundance and temperature was higher and meaningful in PPAVL for the average temperature minimum and maximum (Table III).

Megalopta bees were more commonly captured in the wet months of spring (September to November) and summer (December to February) than in autumn (March to May) and winter (June to August) (Figs. 1–5). The correlation between monthly abundance of *M. guimaraesi* and *M. amoena* (r_s = 0.718, P = 0.001) sustains the similar seasonality standard between these two species. However, data show differences in the abundance among years, as observed in the spring-summer of 1998/99, which was especially favorable for both species in the forest areas (PPAD, ESB and ESC), while in the cerrado of PPAVL, the highest abundance peak occurred in 1997/98 spring-summer. It also calls our attention the long periods of little abundance, or even the complete absence of

Table II. Meteorological data (rainfall and temperatures) observed in monthly samples, abundance of individuals (number and mean ± SD) and values of Spearman's rank correlation coefficient (rs) of *M. guimaraesi* for all localities in Bauru region (SP), Brazil (legends are the same of Table I).

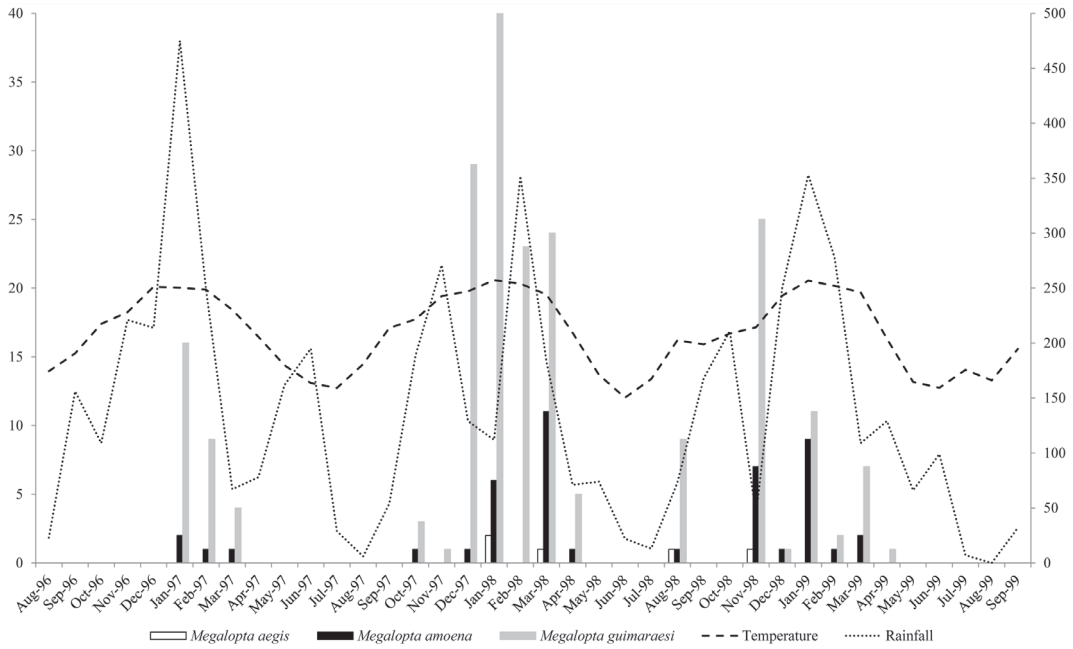
	PPAVL	PPAD	ESB	ESC	UCB
Number of individuals	248	161	192	19	54
Individuals mean (± Sd)	6.5 ± 14.4	5.8 ± 10.8	8 ± 17.1	0.5 ± 1.83	1.4 ± 2.03
Number of samples	38	28	24	39	77
Min. and max. rainfall (mm)	44 –475	44 – 336	32 – 353	49 – 503	54 – 475
rs	0.406*	0.478*	0.520**	0.386*	0.372**
Average of min.temp. (°C)	16.2	13.7	15.6	13.6	19.8
rs	0.734**	n.s.	0.542**	n.s.	0.534**
Average of max. temp. (°C)	31.1	30.4	30.6	30.8	31.9
rs	0.666**	n.s.	n.s.	n.s.	0.459**

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level (2-tailed; n.s. Correlation is not significant).

Table III. Meteorological data (rainfall and temperatures) observed in monthly samples, abundance of individuals (number and mean±SD) and values of Spearman's rank correlation coefficient (rs) of *M. amoena* for all localities in Bauru region (SP), Brazil (legends are the same of Table 1).

	PPAVL	PPAD	ESB	ESC	UCB
Number of individual	45	199	9	7	6
Individuals mean (± Sd)	1.2 ± 2.6	7.1 ± 12.0	0.38 ± 1.24	0.18 ± 0.6	0.0 ± 0.27
Number of samples	38	28	24	39	77
Min. and max. rainfall (mm)	44 – 475	22 – 353	44 – 353	45 – 315	112 – 253
rs	n.s	0.408*	n.s.	n.s.	n.s.
Average of min. temp. (°C)	16.2	12.0	16.7	13.9	19.8
rs	0.646**	n.s.	n.s.	n.s.	0.283*
Average of max. temp. (°C)	31.1	30.4	29.9	30.6	31.1
rs	0.590**	n.s.	n.s.	n.s.	0.358**

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level (2-tailed; n.s. Correlation is not significant).



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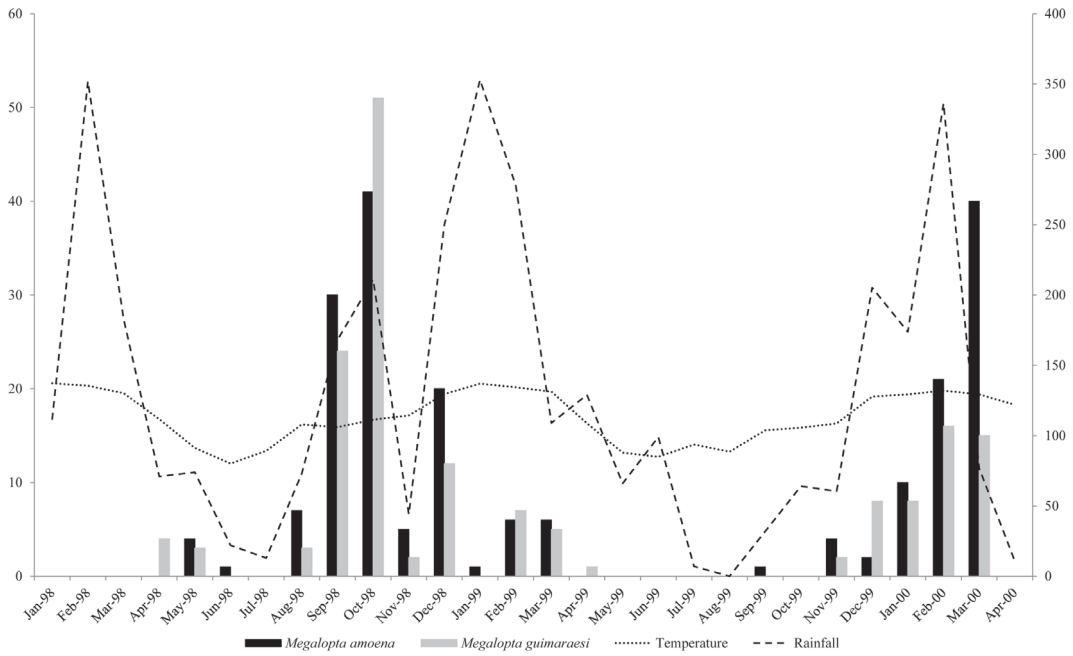

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Figs. 1–2. Abundance of *Megalopta*, accumulated rainfall (mm) and average of minimum temperature (°C) at the: 1, Permanent Preservation Area Vargem Limpa-Campo Novo (PPAVL), Bauru (SP), Brazil; 2, Permanent Preservation Area of Duratex (PPAD) in Agudos (SP), Brazil.

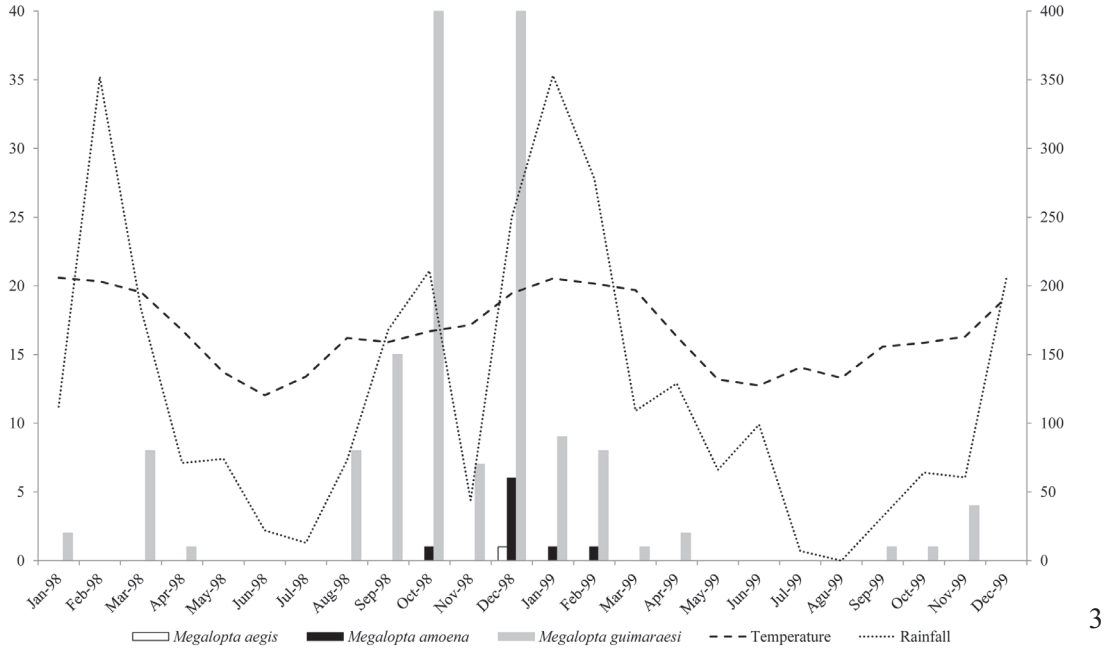
bees, observed at UCB (during the years of 1995, 1996, and 1999) and at ESC where bees were completely absent in collections amidst 1997 and 2000 (Figs. 4–5).

Coumpond Atractiveness. In general, the more attractive compounds for *Megalopta* were eugenol (54%), methyl salicylate (22%), and eucalyptol (16%); vanillin, benzyl acetate, and skatole attracted few individuals all together (8%) (Table IV).

At PPAD, the preference for eugenol was more evident for *M. guimaraesi* (54%) and *M. amoena* (88%). *M. aegis* was captured with benzyl acetate (n = 3), eucalyptol (n = 2), and eugenol (n = 1). No significant differences were detected between them (Pearson chi-square = 217.592; G.L. = 20; P = 0.001).

DISCUSSION

This study shows the importance of the use of bait traps to sample other bees, including *Megalopta* and *Megaloptilla* (Halictidae, Augochlorini), *Ptiloglossa* (Colletidae, Caupolicanini), *Lestrimellita* (Apidae, Apini), and *Acanthopus* (Apidae, Ericrocidini) (Campos *et al.* 1989; Melo 1995; Engel & Brooks 1999; Gonzalez *et al.* 2010b; Nemésio & Siqueira



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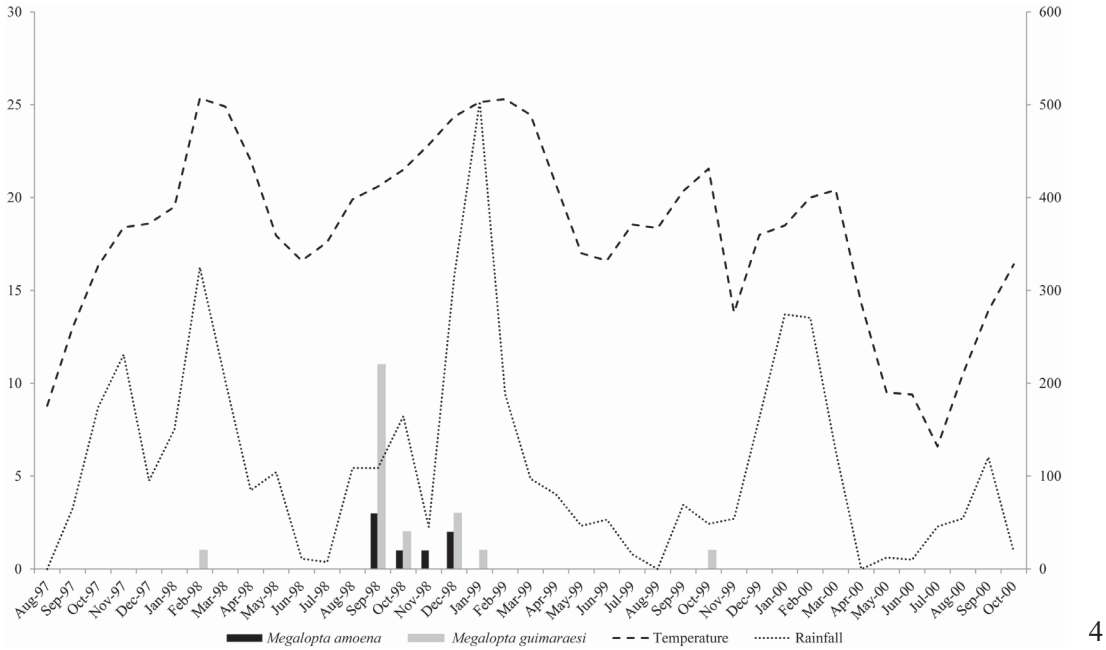

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Figs. 3–4. Abundance of *Megalopta*, accumulated rainfall (mm) and average of minimum temperature (°C) at the: 3, Ecological Station of Bauru (ESB), Bauru, (SP), Brazil; 4, Ecological Station of Caetetus (ESC), Alvinlândia, Gália (SP), Brazil.

2011). Bait traps for orchid bees could be left overnight to attract dim-light bees and such data use as an additional set of information in biodiversity surveys. Sampling *Megalopta* with orchid bee baits could potentially be included in the established collecting protocols for rapid assessment of the bee fauna, as suggested by Santos & Silveira (2009).

Bee Abundance and Seasonality. In Bauru region, the activity of *Megalopta* began in the later months of the dry season and the highest abundance peak occurred during the wet season. The same seasonal patterns related with rainfall have been reported from Panama, where these bees have been intensively studied (Wolda & Roubik 1986; Wcislo *et al.* 2004).

Wcislo *et al.* (2004) observed that nest provisioning began at the start of the dry season, when the rains end. The oviposition was more frequent during the dry season and the first months of the wet season, and no eggs were found in nests at the end of the wet season. The estimated time of emergence to adult is 35 days (Wcislo *et al.* 2004). Such information helps us to explain the seasonal pattern observed in this study. Thus, our data suggest that *Megalopta* in Bauru region have an annual life cycle with more than one repro-

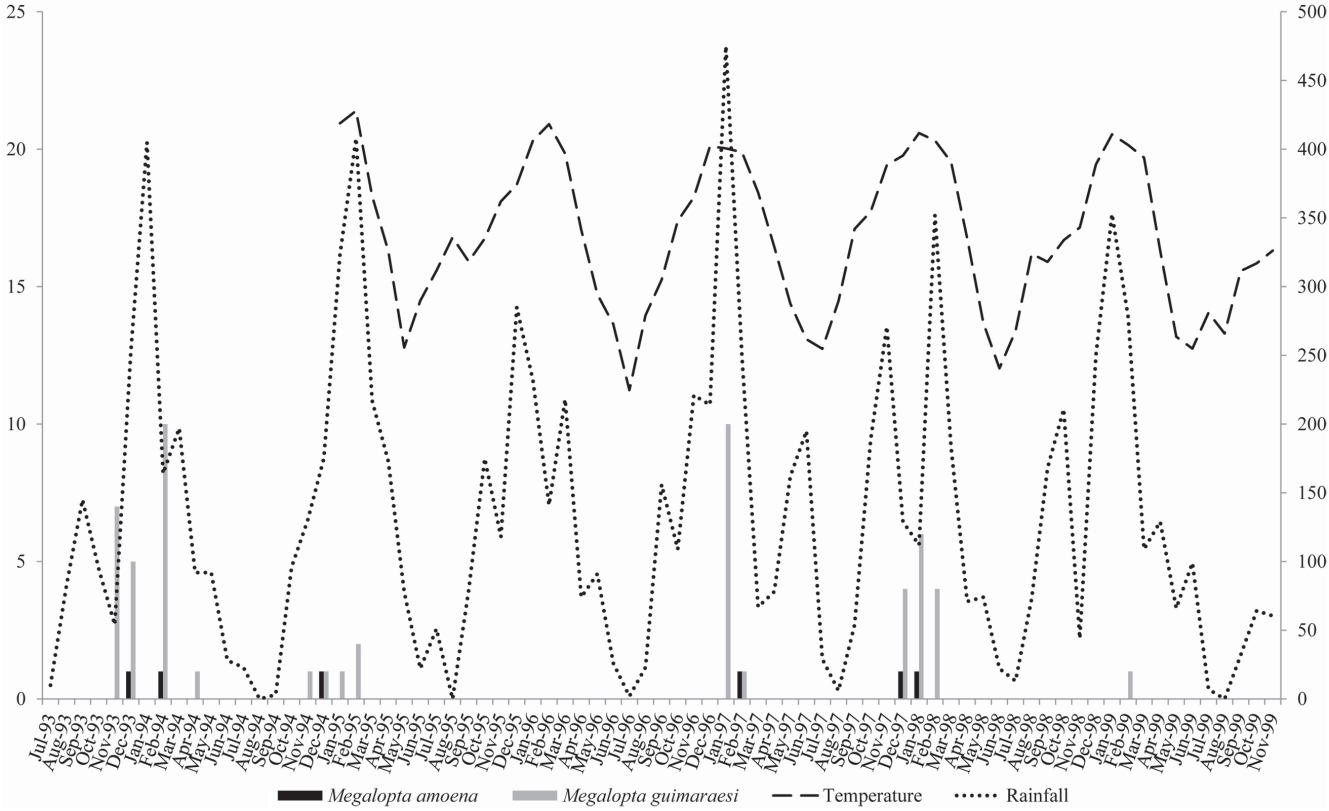

Fig. 5. Abundance of *Megalopta*, accumulated rainfall (mm) and average of minimum temperature (°C) at the University Campus of Bauru (UCB), Bauru (SP), Brazil.

Table IV. Number of individuals of *M. guimaraese* and *M. amoena* attracted by aromatic compounds used in bait traps for all localities in Bauru region (SP), Brazil (legends are the same of Table I).

Aromatic compounds	PPAVL	PPAD	ESB	ESC	UCB	Total
Megalopta guimaraesi						
Eugenol	91 (36%)	87 (54%)	93 (42%)	8 (42%)	8 (15%)	287 (43%)
Methyl salicylate	89 (36%)	43 (27%)	42 (16%)	3 (16%)	12 (22%)	189 (28%)
Eucalyptol	64 (26%)	16 (10%)	46 (37%)	7 (37%)	6 (11%)	139 (21%)
Vanillin	4 (2%)	15 (9%)	10 (5%)	1 (6%)	18 (33%)	48 (7%)
Benzyl acetate	–	–	0	–	8 (15%)	8 (1%)
Skatole	–	–	1 (0%)	–	2 (4%)	3 (0%)
Total	248	161	192	19	54	674
Megalopta amoena						
Eugenol	37 (82%)	176 (88%)	6 (67%)	2 (29%)	2 (33%)	223 (84%)
Methyl salicylate	5 (11%)	10 (5%)	2 (22%)	1 (14%)	0	18 (7%)
Eucalyptol	2 (4%)	7 (4%)	0	2 (29%)	0	11 (4%)
Vanillin	1 (2%)	6 (3%)	1 (11%)	2 (29%)	3 (50%)	13 (5%)
Benzyl acetate	–	–	0	–	1 (17%)	1 (0%)
Skatole	–	–	0	–	0	0
Total	45	199	9	7	6	266

ductive period during the wet season. The seasonality and the interannual variation in the abundance of *Megalopta* have been also observed in Panama (Roubik & Wolda 2001).

In the Cerrado habitat, the abiotic factors, namely temperature, humidity, and rainfall, influence the induction and control of flowering plants and consequently the interaction with pollinators —bees (Gottsberger & Silberbauer-Gottsberger 2006). In the Cerrado, it was observed that the blooming presented a continuous temporal standard of distribution, with the predominance of species that show annual events of blooming and fruit production; however, the peak of flowers bud and anthesis happened in months of hy-

dric stress, July and August (Pirani *et al.* 2009), on the contrary to the seasonal pattern of *Megalopta*, which increases in abundance from this period and with higher peaks in December – January. Though the authors have verified flowers every year, they testified evidences of supra-annual variation in the amount of produced flowers (Pirani *et al.* 2009). This temporal dynamic of resources must be related to the interannual variation in the *Megalopta* individual abundance, both in diapause periods and abundance periods. In this way, abiotics factors induced the flowering and, consequently, the abundance of *Megalopta* bees.

The floral odors and compound attractiveness. Females forage on flowers to collect nectar and pollen, which is necessary for larval development. In Mexico and Panama, *Megalopta* uses many plant species for pollen (Gonzalez *et al*. 2010a; Tierney *et al.* 2012), which have both diurnal and nocturnal anthesis (Falcão *et al.* 1992; Ervik & Feil 1997; Hopkins *et al.* 2000; Gressler *et al.* 2006; Oliveira *et al.* 2009), during either the dry or wet season (Wcislo *et al*. 2004). Given the similar seasonal pattern of *Megalopta* observed in this study in comparison with that recorded in Central America (Wolda & Roubik 1986), we assume that the attractiveness of bait traps might be related to the fragrance produced by the flowers that produce nectar, pollen or both.

In flowers, pollen produces fragrances for multiple functions in pollination and defense (Dobson & Bergström 1999). Furthermore, insects can perceive pollen odor and use it to discriminate between different pollen types and host plants, so pollen odor influences bee foraging, including the location of pollen sources (Dobson & Bergström 1999). The fact that only females have been captured in the orchid bee baits suggests that they use olfactory clues to search for pollen during dim-light conditions. Some species of plants used by dim light bees that also produce scents are *Prestoea schultzeana* (Arecaceae), *Parkia velutina* (Fabaceae), and *Ternstroemia dentata* (Theaceae) (Ervik & Feil 1997; Melo 1995; Hopkins *et al*. 2000). If this is correct, the olfactory system may have also played as important a role in the evolution of *Megalopta* as the visual system (Greiner *et al.* 2004, 2005; Kelber *et al.* 2005; Warrant 2004).

The compound that attracted more *Megalopta* was eugenol (a benzenoid substance) (Dobson & Bergström 1999), possibly because it lasts longer in the field when compared to eucalyptol, which is a highly volatile. The others compounds used have low volatility. The attractiveness for chemical compounds observed in this study may reflect the preference, and/or presence, for chemical compounds found in flowers generally visited by *Megalopta*. Futures studies can test such hypotheses.

ACKNOWLEDGEMENTS

We are thankful to the "Instituto Agronômico de Campinas" and "Instituto de Pesquisas Meteorológicas da UNESP" (IPEMET) for meteorological data. To VH Gonzalez and anonymous reviewers who made significant comments that helped to improve this note. Partial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ph.D. Scholarship 140949/2010–0 (to L.M.S).

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Received 20/12/2011; accepted 13/11/2012
Editor: Maria Cristina Gaglianone