Floral Volatiles: A Promising Method to Access the Rare Nocturnal and Crepuscular Bees

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Crepuscular and/or nocturnal bees fly during the dusk, the dawn or part of the night. Due to their short foraging time and sampling bias toward diurnal bees, nocturnal bees are rarely collected and poorly studied. So far, they have been mostly sampled with light and Malaise traps. However, synthetic chemical compounds resembling floral volatiles were recently found to be a promising alternative to attract these bees. By reviewing available literature and collecting original data, we present information on the attraction and sampling of nocturnal bees with scent-baited traps. Bees were actively captured with entomological nets while approaching to filter papers moistened with distinct chemical compound, or passively caught in bottles with scent baits left during the night. So far, all data available are from the Neotropics. Nocturnal bees belonging to three genera, i.e., Ptiloglossa, Megalopta, and Megommation were attracted to at least ten different synthetic compounds and mixtures thereof, identified from bouquets of flowers with nocturnal anthesis. Aromatic compounds, such as 2-phenylethanol, eugenol and methyl salicylate, and the monoterpenoid eucalyptol were the most successful in attracting nocturnal bees. We highlight the effectiveness of olfactory methods to survey crepuscular and nocturnal bees using chemical compounds typically reported as floral scent constituents, and the possibility to record olfactory preferences of each bee species to specific compounds. We suggest to include this method in apifauna surveys in order to improve our current knowledge on the diversity of nocturnal bees in different ecosystems.

Keywords: nocturnal bees inventory, crepuscular bees, apifauna survey, sampling method, floral scents, volatile organic compounds, 2-phenylethanol
INTRODUCTION

The nocturnal and/or crepuscular behavior in bees arose independently in four of the seven bee families: Andrenidae, Apidae, Colletidae, and Halictidae (Wcislo et al., 2004; Warrant, 2007; Danforth et al., 2019). There are about 250 described nocturnal bee species and they fly during the dusk, the dawn, or part of the night. These bees can be obligatory nocturnal, nocturnal bee species and they fly during the dusk, the dawn, or part of the night. These bees can be obligatory nocturnal, nocturnal bee species and they fly during the dusk, the dawn, or part of the night. These bees can be obligatory nocturnal, nocturnal bee species and they fly during the dusk, the dawn, or part of the night. These bees can be obligatory nocturnal, nocturnal bee species and they fly during the dusk, the dawn, or part of the night. These bees can be obligatory nocturnal, nocturnal bee species and they fly during the dusk, the dawn, or part of the night. 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corresponding to the activity time of nocturnal bee species. The bees sampled were transferred to glass vials containing ethyl acetate. We offered compounds resembling floral volatiles previously identified as attractants to nocturnal bees (Cordeiro et al., 2017; Krug et al., 2018), i.e., 1-hexanol (Sigma-Aldrich, ≥99%), 2-phenylethanol (Acros Organics, 99%), 1-octanol (Sigma-Aldrich, ≥99%), and linalool (Sigma-Aldrich, 97%). Our sampling effort summed up to 87 h within 23 days. We are calling this sampling as active method, since the collector remains present during the exposure of the scent and actively collects the approaching bees.

The collected bees were mounted and identified with the taxonomic keys proposed by Mouré (1945, 1964), Santos and Silveira (2009), Gonçalves and Santos (2010), Gonzalez et al. (2010) and Santos and Melo (2015), and deposited in the Paulo Nogueira-Neto Entomological Collection at the University of São Paulo (CEPANN), in São Paulo, Brazil.

Literature Review
A systematic review of the literature on nocturnal bees lured with chemical compounds was conducted on Google Scholar, JSTOR, NCBI, Scopus, and Web of science, with the following combinations of keywords: “Compounds” OR “Chemical lures” OR “Floral scent” OR “Floral volatile” OR “Nocturnal bees” OR “Nocturnal and Crepuscular bees” OR “Nocturnal anthesis” OR “Volatile organic compounds.” From the selected articles we recovered information about the localities, species, number of individuals attracted, and attractive compounds (Table 1 and Supplementary Table 1). In these studies, the nocturnal bees were mostly sampled with bottles scent traps (similar to those used in Euglossini bees sampling) left during the night. This trap is built with PET bottles, in which a cotton ball impregnated with a synthetic compound is inserted (Figure 1B). Chemical compounds used as lures in these previous studies were mostly: eucalyptol, eugenol and methyl salicylate. This sampling protocol is referred hereafter as passive method, since the traps are left in the field without direct supervision and removed in the day after.

RESULTS
In the present and previous studies (literature review), 1115 individuals of 12 species of nocturnal bees were attracted to chemical compounds. The genus with the highest number of individuals and species registered was Megalopta (1050 individuals, 8 species) (Table 1).

The active sampling method attracted 103 individuals belonging to ten species of nocturnal bees, comprising our sampling (41 individuals, five species) and previous studies (62 individuals, six species). The bees most commonly recorded during the active methods were Megommation insigne (33 individuals) and Megalopta aeneicollis (25 individuals). Most individuals (39 individuals) were attracted by the aromatic compound 2-phenylethanol (Table 1).

The passive method, applied in all previous studies, attracted 1012 individuals, among them Megalopta amoena and M. guimaraesi, which accounted together for 994 individuals (Table 1).

The aromatic compounds eugenol and methyl salicylate trapped 530 individuals from 4 species and 233 individuals from 5 species, respectively (Table 1). The only monoterpene tested as single compound, eucalyptol, attracted 152 individuals of three Megalopta species. The aliphatic compound 1-octanol, attracted bees of the genera Megommation and Ptiloglossa. The different mixtures of compounds attracted 57 individuals from seven species, including some unique species, such as Megalopta cuprea and M. pira in which were only collected with specific mixtures, but not single compounds. Compounds from other chemical classes, such as irregular terpenes and nitrogen-bearing compounds, attracted only few bees (4 individuals) (Table 1).

DISCUSSION
The results demonstrate that chemical compounds are appropriate to sample nocturnal bees, and sampling of bees attracted by volatile organic compounds should be incorporated as an additional method to apifauna surveys. It is worthy to offer compounds identified in plants that serve as host for nocturnal bees and use them individually or as blends.

The capture of nocturnal bees has usually been done with black and fluorescent light traps. Chandler (1961) collected 392 individuals of Sphecodogastra texana in LaPorte Indiana between 1959 and 1960. However, according to the author there is a possible interference by the killing agent cyanide in the attraction of the bees. Likewise, Wolda and Roubik (1986) collected an astonishing number of individuals of two Megalopeta species on the island of Barro Colorado, in Panama: 7,713 and 2,487 individuals of M. ecuadoria and M. genalis, respectively, were sampled. One possible reason that explains the high attraction of these two species of Megalopeta to light traps on the island was the synchronization with the flowering of the Tachigalia versicolor where the light trap was installed (Wolda and Roubik, 1986). The floral volatiles emitted by this plant may have helped attracting the bees to the light trap. Another explanation is that a high abundance of nests of these two species might have been close to the light traps (Roulston, 1997; Wcislo et al., 2004).

Floral synthetic compounds almost exclusively attracted female nocturnal bees (Table 1). This differs with the sex of individuals attracted in the Euglossini tribe. Nemésio (2012) mentioned that attracting only males in this tribe has led to taxonomic problems that involve describing species based on male specimens and making it difficult to match males with females. This situation is similar in the nocturnal bees, especially of the genus Ptiloglossa, where the taxonomic identification keys were constructed only for male specimens (Moure, 1945). However, the increase in female nocturnal bees in collections, e.g., by using chemical attractants, allows the construction of taxonomic keys that include male and female specimens, as done by Velez-Ruiz (2015).

Our study suggests that chemical compounds sample a higher diversity of species than light traps. Overall 12 species of 3 genera (Megalopeta, Megommation, and Ptiloglossa) were
TABLE 1 | Number of individuals of nocturnal bee species lured with synthetic chemical compounds and collected with active and passive methods.

| Family/Species | Compound/mixtures (chemical classes) | Active method | Passive method | References |
|---------------|--------------------------------------|---------------|----------------|------------|
| **Colletidae** |                                      |               |                |            |
| Ptiloglossa torquata Moure | methyl salicylate (Aro) | 1 | | Almeida et al. (2020) |
| | vanillin (Aro) | | | Almeida et al. (2020) |
| Ptiloglossa latecalcarata Moure | Mix 1 | 10 | | Cordeiro et al. (2017) |
| | 1-octanol (Ali) | | | Cordeiro et al. (2017) |
| | 2-phenylethanol (Aro) | | | This study |
| Ptiloglossa pretiosa Friese | 2-phenylethanol (Aro) | | 2♂ | This study |
| | 2-phenylethanol (Aro) | | | This study |
| **Halictidae** |                                      |               |                |            |
| Megalopta aegis (Vachal) | eugenol (Aro) | 1 | | Knoll and Santos (2012) |
| | methyl salicylate (Aro) | | | Carvalho et al. (2012) |
| | eucalyptol (Mon) | | | Knoll and Santos (2012) |
| | benzyl acetate (Aro) | | | Knoll and Santos (2012) |
| | benzyl benzoate (Aro) | | | Carvalho et al. (2012) |
| Megalopta aeneicollis Friese | Mix 2 | | 9 | Krug et al. (2018) |
| | Mix 3 | | 16 | Krug et al. (2018) |
| Megalopta amoena (Spinola) | eugenol (Aro) | 238 | | Almeida et al. (2020); Knoll and Santos (2012) |
| | methyl salicylate (Aro) | | 34 | Almeida et al. (2020); Carvalho et al. (2012); Knoll and Santos (2012) |
| | eucalyptol (Mon) | | 11 | Knoll and Santos (2012) |
| | vanillin (Aro) | | 13 | Knoll and Santos (2012) |
| | benzyl acetate (Aro) | | 7 | Carvalho et al. (2012); Knoll and Santos (2012) |
| | benzyl benzoate (Aro) | | 4 | Carvalho et al. (2012) |
| **Megalopta cuprea Friese** | Mix 2 | | 1 | Krug et al. (2018) |
| **Megalopta guimaraesi Santos and Silveira** | benzyl benzoate (Aro) | | 1 | Carvalho et al. (2012) |
| | methyl salicylate (Aro) | | 196 | Knoll and Santos (2012); Carvalho et al. (2012) |
| | benzyl acetate (Aro) | | 12 | Carvalho et al. (2012); Knoll and Santos (2012) |
| | β-ionone (Ite) | | 1 | Carvalho et al. (2012) |
| | eugenol (Aro) | | 287 | Knoll and Santos (2012) |
| | eucalyptol (Mon) | | 139 | Knoll and Santos (2012) |
| | vanillin (Aro) | | 48 | Knoll and Santos (2012) |
| | skatole (Nbc) | | 3 | Knoll and Santos (2012) |
| **Megalopta piraha Santos and Melo** | Mix 3 | | 1 | Krug et al. (2018) |
| **Megalopta socialis (Vachal)** | Mix 3 | | 1 | Krug et al. (2018) |
| | eugenol (Aro) | | 1 | Almeida et al. (2020) |
| | methyl salicylate (Aro) | | 1 | Almeida et al. (2020) |
| | 2-phenylethanol (Aro) | | 5 | This study |
| | 1-octanol (Ali) | | 2 | This study |
| **Megalopta sp. 1** | Mix 4 | | 3 | Krug et al. (2018) |
| | Mix 2 | | 2 | Krug et al. (2018) |
| **Megommation insigne (Smith)** | Mix 1 | | 14 | Cordeiro et al. (2017) |
| | 1-octanol (Ali) | | 3 | Cordeiro et al. (2017) |
| | 2-phenylethanol (Aro) | | 16 | This study |

All the specimens collected are female, except the two individuals of Ptiloglossa pretiosa.

**Chemical classes**: Ali = aliphatic; Aro = aromatic; Mon = monoterpenes; Ite = irregular terpenes; Nbc = nitrogen bearing compounds.

**Mixtures**: Mix 1 (Aro-Ali) = benzyl alcohol (29%), 2-phenylethanol (35%), hexanal (2%), 1-hexanol (13%), (Z)-3-hexen-1-ol (8%), and 1-octanol (13%); Mix 2 (Mon-Aro-Ite-Nbc) = (Z)-linalool oxide furanoid (6%), methyl benzoate (19%), linalool (71%), phenylacetonitrile (2%), 4-oxoisophorone (2%), and (Z)-linalool oxide pyranoid (0.4%); Mix 3 (Mon-Se) = (E)-β-ocimene (57%), (Z/E)-linalool oxide furanoid (6%), linalool (30%), and (E)-β-caryophyllene (7%); Mix 4 (Mon-Aro-Ite-Nbc) = (E)-β-ocimene (4%), (Z/E)-linalool oxide furanoid (5%), methyl benzoate (17%), linalool (64%), epoxy-oxoisophorone (5%), phenylacetonitrile (2%), 4-oxoisophorone (2%), and (Z/E)-linalool oxide pyranoid (0.4%).
collected on chemical lures so far, while the surveys conducted with light traps recorded five species, most of them belonging to the genus *Megalopta* (Chandler, 1961; Kerfoot, 1967; Wolda and Roubik, 1986; Roulston, 1997). Although the sample design is not comparable in terms of time, area, climatic conditions, etc., the superiority in the number of species attracted by the chemical method suggests that this method is more effective in attracting high numbers of species.

The ability of chemical compounds according to the diversity and abundance of attracted nocturnal bees varied among the chemical classes used. However, this may be due to the differences in the design and duration of the sampling carried out by each author in the literary review. Nevertheless, most of the nocturnal bees were attracted to aromatic compounds, such as eugenol, methyl salicylate, 2-phenylethanol, and monoterpenes such as eucalyptol.

The aromatic compounds eugenol and methyl salicylate and the monoterpenes eucalyptol, widely used for the attraction of male Euglossine bees (Nemésio, 2012), lured 527, 236, and 152 individuals of nocturnal bees, respectively (Carvalho et al., 2012; Knoll and Santos, 2012; Almeida et al., 2020), all from the genus *Megalopta*, with a single exception of one individual of *Ptiloglossa* sampled with methyl salicylate (Almeida et al., 2020). The species most abundantly attracted to the abovementioned three compounds were *M. amoena* and *M. guimaraeasi*. Although little is known about the abundance of these compounds in plants with nocturnal anthesis, they are present in a wide variety of plants with diurnal and nocturnal anthesis (Knudsen et al., 2006; El-Sayed, 2021). We believe that the sampling of floral scents in a broader spectrum of plants visited by nocturnal bees might reveal these attractive compounds at least in some representatives.

Another aromatic worth mentioning is 2-phenylethanol. Unlike eugenol, methyl salicylate, and eucalyptol, 2-phenylethanol tends to be less specific and attracts more than one genus and family of nocturnal bees, including rare species such as *P. pretiosa*. Preliminary results also show that this compound is capable of eliciting physiological responses in electroantennography assays (EAG) with *M. insigne* (Supplementary Figure 1). This general efficiency of 2-phenylethanol in attracting nocturnal bees may be due to their widespread occurrence among floral scents (Knudsen et al., 2006), sometimes also as major component of bee-pollinated plants (Dobson, 2006), also including some with nocturnal anthesis (Shaver et al., 1997; Cordeiro et al., 2017, 2019). This aromatic compound is also a known attractant for diurnal bees (Dötterl and Vereecken, 2010; Rocha-Filho and Garófalo, 2014).

Previous studies demonstrate that not only single compounds but also synthetic mixtures of compounds are capable of attracting nocturnal bee pollinators. The mixtures attracted 41 specimens of nocturnal bees of at least three genera and two families, including some *Megalopta* collected exclusively with these mixtures. Rare species such as *M. cuprea* and *M. piraha* were exclusively attracted to mixtures. Furthermore, unlike the individual compounds, these mixtures have the advantage of resembling the natural aroma emitted by the flowers and attracting potential pollinators (Cordeiro et al., 2017; Krug et al., 2018).

All methods used for apiﬂora surveys have advantages and disadvantages. The passive method was able to sample a high number of individuals. However, as Euglossini surveys, it can sample hundreds of individuals in one day (Viana et al., 2002; Nemésio and Vasconcelos, 2013), therefore it must be applied with care in fragmented forests with potentially small populations. The active sampling method attracted smaller number of individuals but more species. In addition, in the active sampling, it is possible (for some species) to determine the specimens directly in the field and avoid killing all attracted individuals. Finally, it enables isolating the caught bees in single vials, allowing pollen analyses of each individual. The passive sampling method allows the bottle traps with chemical compound to be left overnight. Thus it is less time consuming than active methods, where collectors spend hours in front of the baits.

To conclude, our study shows that synthetic chemical compounds lure a wide diversity of nocturnal bees. Nocturnal bee species are successfully attracted by aromatic compounds and monoterpenes such as eugenol, methyl salicylate, 2-phenylethanol and eucalyptol. Offering volatile compounds in an active and passive way should be included in nocturnal apiﬂora surveys, as this approach attracts species otherwise difﬁcult to obtain, and helps clarifying taxonomic issues and the dynamics of their populations of these important pollinators.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of the article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CM-M, HM, IA-d-S, and RK: conceptualization. CM-M, CS, CK, EF, GC, HM, IA-d-S, RK, SD, and SM: investigation, methodology, and carried out collection of data. MS: electroantennographic assay. CM-M, GC, HM, IA-d-S, MS, PM-P, RK, and SD: writing – original draft preparation and review. IA-d-S: Supervision. All authors have read, revised, and agreed the final version of the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.676743/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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