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Chemical Characterisation of the Floral Oil of the Nance (*Byrsonima sericea*): Discovering the Constituents Used in Reproduction by Oil-Collecting Bees

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Abstract

The recent decline in population of generalist bees such as those of the genera *Apis* and *Bombus* has shown the need to breed and manage a larger number of bee species. Among the species with potential use for agricultural pollination in the Neotropics, a peculiar small group has specialised in collecting floral oil. Therefore, the aim of this study was to analyse the chemical profile and to identify the main constituents of the floral oil of the nance (*Byrsonima sericea*), an abundant species in the Northeast of Brazil and widely used by oil-collecting bees. A sample of 400 flowers of the nance were collected between October 2017 and January 2018. The samples were derivatised (MSTFA) and analysed by gas chromatography-mass spectrometry in a Model 7890B GC Gas Chromatograph System coupled to a Model 5977A MSD mass spectrometer. The compounds were separated using an HP-5ms capillary column and identified by comparing the mass spectra with the National Institute of Standards and Technology (NIST) database, and by comparison of the retention indices (RI). From the chromatographic analysis, it was possible to identify 23 constituents, especially fatty acids and carboxylic acids. The results indicate the presence of tricosanoic acid, palmitic acid and heneicosanoic acid as the main constituents of the oil under study. There is still a need for studies that would better explain the relationship of these constituents with the bees that use the oil.

Introduction

The great majority of plants on the planet depend on pollination by animals to produce fruit and seeds, and bees are the main agents involved in this process for both wild and cultivated plants (Klein et al., 2006; Ricketts et al., 2008). The economic value of pollination carried out by animals is estimated at USD 235-577 billion annually, with bees accounting for 90% of that value, but despite the existence of about 20,000 bee species in the world, only a few species such as those belonging to the genera *Apis* and *Bombus*, are bred for agricultural pollination (Klein et al., 2006; Magalhães & Freitas, 2013; Potts et al., 2016). However, a recent decline in the population of these bees in certain regions of the planet has drawn attention to the risk underlying the over dependence of the world agricultural production to only a few bee species, and highlighted the need to breed and manage a larger number of bee species for crop pollination (IPBES, 2016).

Among the bee species with potential use for agricultural pollination in the Neotropics, a peculiar small group has specialised in collecting floral oil for both nesting and feeding purposes (Wnson et al., 1997; Dotterl &Vereecken, 2010). These bees, abundant in the region and known as oil-collecting bees, are non-social species and have evolved independently in five different groups belonging to two taxonomic families: Melittinae (Melittidae), Ctenoplectrini, Centridini, Tapinotaspidini e Tetrapediini (Apidae) (Alves Dos Santos et al., 2002; Vinson et al., 2006; Reis et al., 2007). Due to their peculiarity of collecting floral oils, these bees present a tight relationship to plant species that provide this resource.

Eleven botanical families are known to have evolved and specialised in offering this reward to their pollinators. The supply of floral oils has been interpreted as a highly successful evolutionary strategy to enhance pollination since these lipids are produced especifically to increase the attractiveness of the flowers to the pollinators (Buchmann, 1987; Dotterl & Schaffler, 2007).

One such family is Malpighiaceae. Around 85% of its species occur in the neotropical region and bear floral characteristics in accordance to the mellitophilous pollination syndrome (Lombello & Forni-Martins, 2003). In fact, cultivated species of this family, such as acerola (*Malpighia emarginata*), wild species exploited by local populations, such as nance (*Byrsonima* spp.), or by the fauna, such as *Byrsonima cydoniifolia*, are totally dependent on oil-collecting bees to produce fruit (Freitas et al., 1999; Pereira & Freitas, 2002; Sazan et al., 2014). In addition, species belonging to other botanical families, such as cashew (*Anacardium occidentale*), brazil nut (*Bertholettia excelsa*) and sweet-passion-fruit (*Passiflora alata*), although not exclusively dependent on these bees, also benefit from the pollination they carry out when visiting the flowers in search of pollen and/or nectar (Freitas & Paxton, 1998; Ganglione et al., 2010; Cavalcante et al., 2012). As such, species of oil-collecting bees are important pollinators of various plant species and crops in the Neotropics.

However, oil-collecting bees are neither bred rationally nor used for agricultural pollination. Although studies of some species of the genus *Centris* have shown that they are abundant and may even be managed in trap nests in areas of nance and acerola (Pereira & Freitas, 2002; Magalhães & Freitas, 2013), the dependence of these bees on floral oils for bulding their nests and feeding their brood has been seen as a limiting factor for their use on a larger scale and with other agricultural crops (Freitas & Pereira, 2004; Lourenço, et al., 2019). Therefore, knowledge of the chemical composition of floral oils may be important in order to get around this obstacle.

Studies of the chemical characteristics of the floral oils poduced by some plant species have pointed to a diverse combination of long-chain saturated and unsaturated fatty acids with or without β-acetoxy and mono- or diglyceride substituents, in addition to the presence of a smaller number of other groups (Dumri et al., 2008; Capellari et al., 2011). The different chemical compounds present in these oils are probably related to differences in their attractiveness to pollinators, suggesting the existence of components in these rewards that are responsible for acting as mediators between the different species of plants and their pollinators. However, it is not yet known whether this mediation is carried out by the action of isolated compounds or if the characteristics of the mixture of compounds that play the main role in the attraction (Cappellari et al., 2011; Leonard & Masek, 2014). While the components responsible for attracting pollinators play animportant role for the plants, those related to larval nutrition and nest building are crucial for bee reproduction (Michener, 2000).

Studies aimed at knowledge of the chemical constituents present in floral oils are fundamental for a better understanding of how the plant-pollinator interactions work in this highly specialised system, as well as for generating information that may serve as a basis for the development of technologies which could aid in the conservation of both animal and plant species, in addition to facilitating the management and permanence of these bees in agricultural crops that benefit from their pollination services. Therefore, the aim of the present study was to analyse the chemical profile and to identify the main constituents of the floral oil of *Byrsonima sericea*, a species abundant in the Northeast of Brazil and widely used by oil-collecting bees (Rosa et al., 2007; Lourenço et al., 2019).

Material and methods

The plant material was collected between October 2017 and January 2018, from three specimens of the nance (*Byrsonima sericea*) that were in full bloom during this period in the Pici Campus belonging to the Federal University of Ceará (UFC). The nance species was identified at the Prisco Bezerra Herbarium, also in UFC, from dried and mounted plant samples collected from the specimens studied. These plants are part of the local vegetation which is formed by a predominantly pre-coastal-plain forest with a semi-deciduous forest physiognomy (Moro et al., 2015). According to the Köppen classification (1948), the climate in the region is type Aw', hot sub-humid tropical, with a rainy period from January to May.

Samples of floral oil were obtained by collecting the nance inflorescences which are the type terminal racemic and measure between 7 and 11 cm in length. These inflorescences were protected in net bags before floral anthesis (opening of the floral buds), to prevent bees or other agents from removing the oil from the flowers. After anthesis of most of the inflorescence buds, they were cut with pruning shears in the region of the peduncle and placed in zipper bags, avoiding direct contact with any material that could be contaminated. Then, the inflorescences were taken to the Bee Laboratory of the Federal University of Ceará located less than 1km away to the farthest sampled nance specimen for oil collection.

The oil from each flower was collected directly from the epithelial glands (n = ± 4,000 glands, from of a total of 400 flowers) which were pierced using capillary tubes (10 µL capacity). The capillary tubes were filled with the oil by rubbing them against the glands (Fig 1A) simulating the collecting behaviour of the bees. Although this collecting procedure is laborious, it allows the obtention of pure oil. After the oil was collected in the capillary tubes, it was transferred to a 4 ml vial (Fig 1B) and kept in a freezer at -14°C for chromatographic analysis.

Chromatographic analysis of the oil samples were performed at the Multi-user Natural Product Chemistry Laboratory of Embrapa Agroindústria Tropical. To characterise the compounds in the oil we collected, samples were derivatised

using the following procedure: the samples were first weighed and separated into aliquots of 10 mg which were then solubilised in 50 µL of ethyl acetate ($C_4H_8O_2$), homogenised and placed in a rotary evaporator. Once the solvent had evaporated, 200 µL of pyridine followed by 200 µL of the silylating agent, MSTFA [N-methyl-N (trimethylsilyl) trifluoroacetamide] were added. The solution was then homogenised and kept for 30 min at 37°C. At the end of this period, the mixture was analysed by gas chromatography-mass spectrometry (GCMS).

The derivatised samples were analysed by GCMS using a Model 7890B GC System gas chromatograph (Agilent Technologies, Santa Clara, Calif., USA) coupled to a Model 5977A MSD mass spectrometer (Agilent Technologies, Santa Clara, Calif., USA). The compounds were separated using a 30m x 250µm x 0.25µm HP-5ms capillary column (Agilent J & W GC Columns, Santa Clara, CA), with the samples injected in splitless mode, using helium as the entrainment gas at a flow rate of 1 mL min^{-1}.

The oven was programmed for floral-exudate analysis with a heating ramp of 50°C to 260°C at 4°C/min and maintained at 260°C for 15 min. The mass spectrometer was operated in scan mode (40-650 m/z) with electron impact (EI) at 70 eV. The total time of the analysis was 65.947 min.

The retention indices (RI) of the compounds were obtained by co-injecting the samples with a mixture of C_7-C_{30} standard n-alkanes, and calculated as per Van Den Dool & Kratz, 1963 (Adams, 2007). The derivatised compounds (MSTFA) were identified by comparing the mass spectra with the National Institute of Standards and Technology (NIST) database, and by comparing the calculated retention indices with those found in the literature.

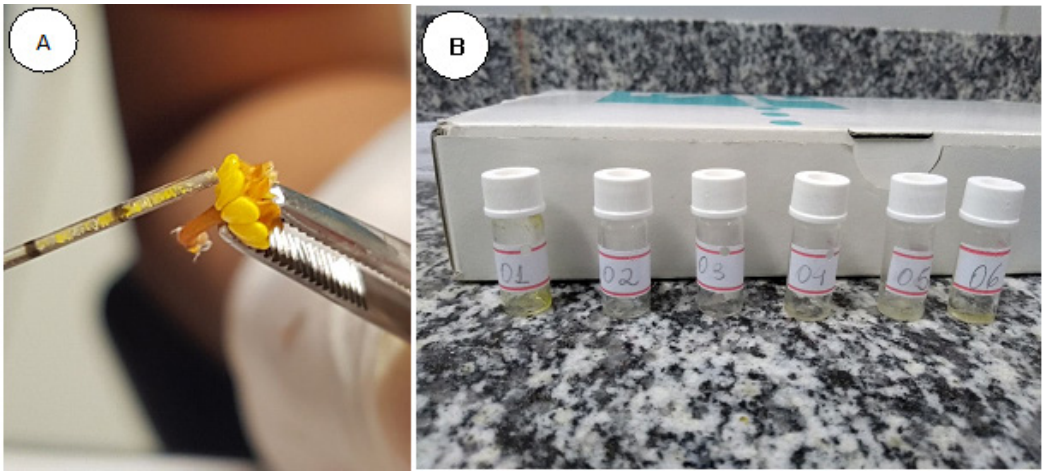

Fig 1. Collecting oil from nance (*Byrsonima sericea*) flowers. A) using a capillary tube (10 µL) for removing the sample directly from the epithelial gland. B) floral oil kept in a 4 ml vial.

Results

The amount of pure oil obtained from the total number of nance (*Byrsonima sericea*) flowers used in the study (N = 400) was 2.8829 g, with a mean of 0.0072 g per flower. The oil was translucent and yellowish in colour.

By means of the chromatographic analysis carried out on the derivatised floral oil of *Byrsonima sericea*, more than 50 chemical compounds were detected, but it was only possible to confirm the identification of 23 constituents (Table 1). Important among these were fatty acids (11) and carboxylic acids (6). The other constituents are fatty alcohols (2), ketones (2), diterpene (1) and aldehyde (1). The samples required 65.947 min on average for elucidation of all the structures.

When the total ion chromatogram (TIC) was evaluated (Fig 2), the first 15 min of the chromatographic run were ignored. This range was disregarded because it only presented compounds formed from the silylating agents.The compounds that were identified, together with the analytical characteristics used for their identification, are listed in Table 1, and numbered following the order of retention time (RT). Tricosanoic acid, which is a long-chain fatty acid, presented the largest relative area. This compound eluted at a high retention time (57.83 min) and the electron impact fragmentation included a base m/z fragment of 411 and a less intense m/z of 75. The mass spectrometric analysis of this compound is presented in Fig 3, and shows the similarities to the library that was used in the identification.

As expected, the most representative chemical components of the oil under study were the fatty acids. The results indicate the presence of tricosanoic acid (38.18%), palmitic acid (4.62%) and heneicosanoic acid (4.21%) as major components (Table 1). A less intense 43 m/z fragmentation signal was frequent in most of the structures in the analysed matrix. This signal is usually generated in the presence of an acetyl group in the chain. Most of the constituents, especially the fatty acids, were identified in TMS-derivative form due to the use of the silylating agent, and it is possible to find in their mass spectra the fragmentation pattern that includes the 75 m/z signal, which is a characteristic ion in this type of reaction.

Table 1. Chemical compounds identified in oils collected from nance (Byrsonima sericea) flowers, with their respective retention times (RT in min), retention indices (experimental and from the literature) (RI), relative areas and representative ions (m/z). RT = Retention time; RI_exp = experimental linear retention index; RI_lit = retention index found in the literature; *compounds derived from TMS.

Peak	Compound	RT (min)	RI_{exp}	RI_{lit}	Area	Match	R. Match	Representative ions (*m/z*)
1	Hexanoic acid*	18.439	1299	1290	8.4 x 10^5	819	874	43, 75, 131, 173(BP), 230($M^{+\cdot}$),
2	2-Butenoic acid*	29.272	1658	1646	2.8 x 10^6	900	918	73, 133, 147, 199, 273(BP), 330($M^{+\cdot}$)
3	2-Pentadecanone	30.547	1704	1698	2.1 x 10^6	678	855	43, 58(BP), 71, 85, 226($M^{+\cdot}$), 259
4	Hexadecanal	33.588	1820	1818	1.0 x 10^7	890	926	43, 57, 68, 82(BP), 96, 109, 240($M^{+\cdot}$)
5	2-Heptadecanone	35.74	1906	1899	3.8 x 10^6	853	907	43, 58(BP), 71, 85, 254($M^{+\cdot}$)
6	Hexadecanoic acid, methylester	36.333	1931	1920	1.9 x 10^6	820	883	43, 55, 74(BP), 87, 143, 227.2, 270.3($M^{+\cdot}$)
7	Hexanedioic acid*	37.139	1964	1964	4.8 x 10^6	912	923	73, 75, 111, 141, 317.2(BP), 374($M^{+\cdot}$)
8	Geranyl linallol	38.767	2034	2034	6.6 x 10^6	812	882	41, 55, 69(BP), 81, 93, 107, 121, 290($M^{+\cdot}$), 309
9	Myristic acid*	39.084	2048	2085	7.3 x 10^5	780	866	43, 73, 75, 129, 285(BP), 342($M^{+\cdot}$)
10	Linoleic acid, methylester	40.249	2098	2098	9.9 x 10^6	936	942	41, 55, 67(BP), 81, 95, 263.2, 294.3($M^{+\cdot}$)
11	Citramalic acid*	40.732	2120	2127	1.1 x 10^6	694	746	73(BP), 115, 147, 299.2, 301, 331.2, 433.2, 490($M^{+\cdot}$)
12	Pentadecanoic acid*	41.545	2157	2184	1.1 x 10^6	840	880	73, 75, 129, 299.3(BP), 356($M^{+\cdot}$)
13	9-Hexadecenoic acid, (Z)*	43.752	2260	2268	2.8 x 10^6	770	846	43, 73, 75, 129, 185, 311(BP), 335, 368($M^{+\cdot}$)
14	Nonanedioic acid*	43.966	2270	2272	5.5 x 10^6	806	896	73, 75, 129, 311.2, 359.2(BP), 416($M^{+\cdot}$)
15	Palmitic acid*	44.345	2288	2288	5.5 x 10^7	949	959	75, 129, 313.3(BP), 370($M^{+\cdot}$)
16	Oleic acid, (E)*	47.848	2462	2464	2.0 x 10^6	875	897	73, 75, 129, 339.3(BP), 396($M^{+\cdot}$)
17	Stearic acid*	48.379	2490	2486	2.6 x 10^7	934	935	75, 129, 341.3(BP), 398($M^{+\cdot}$)
18	Oleic acid, (Z)*	49.351	2541	2466	2.2 x 10^7	812	815	75, 129, 155, 339.3(BP), 396($M^{+\cdot}$)
19	Heneicosanoic acid*	52.302	2696	2708	5.0 x 10^7	696	783	43, 75, 267.3, 365.3, 383.3(BP), 440($M^{+\cdot}$)
20	Docosanol*	54.605	2796	2784	9.4 x 10^6	874	902	43, 73, 75, 95, 267.3, 365.3, 383.3(BP), 440($M^{+\cdot}$)
21	Tricosanoic acid*	57.832	2906	2920	4.5 x 10^8	725	810	43, 57, 75, 125, 257.2, 295.3, 393.4, 411.4(BP), 468($M^{+\cdot}$)
22	1-Tetracosanol*	59.067	2940	2988	1.1 x 10^8	684	831	43, 57, 73, 75, 97, 125, 257.2, 295.3, 411.4(BP), 468($M^{+\cdot}$)
23	Tetracosanoic acid*	65.838	>3000	3088	1.6 x 10^6	860	909	43, 75, 129, 425.4(BP), 482($M^{+\cdot}$)

It was not possible to identify some of the compounds present in the oil under study simply from a comparison with the NIST library database and the retention indices. Among these unidentified chemicals, three (Fig 4) presented a relevant relative area compared to the others (17.35%, 19.59% and 33.38%).

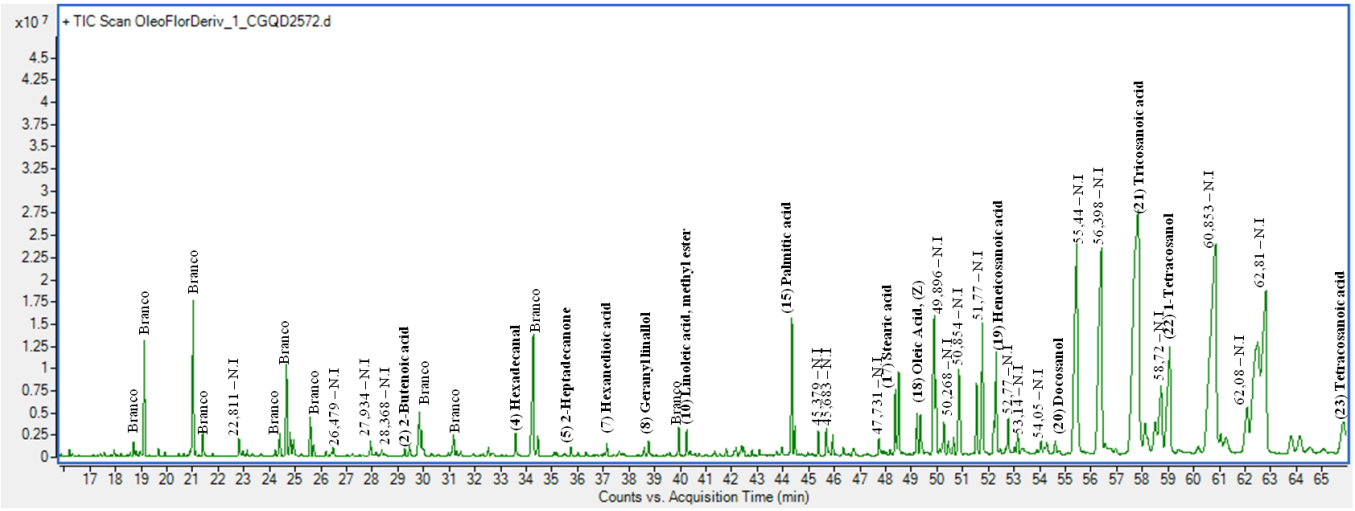

Fig 2. Total ion chromatogram of the floral oil of nance (*Byrsonima sericea*) derivatised with TMS silylating agent. White = compound present in the white sample, N.I = unidentified compound.

The unknown compounds 1 and 2 showed a fragmentation pattern that included the base peak (BP) m/z of 117. This fragmentation pattern is commonly found in compounds identified as diacylglycerol. Because this group of analytes has also been previously identified in samples of floral oils, it is believed that these unknown compounds may be isomers. However, following the identification criteria adopted in this work, the similarity when compared to the NIST library database was low and their identification was therefore not reliable. To identify these components, a deeper investigation with more detailed spectroscopic techniques is necessary.

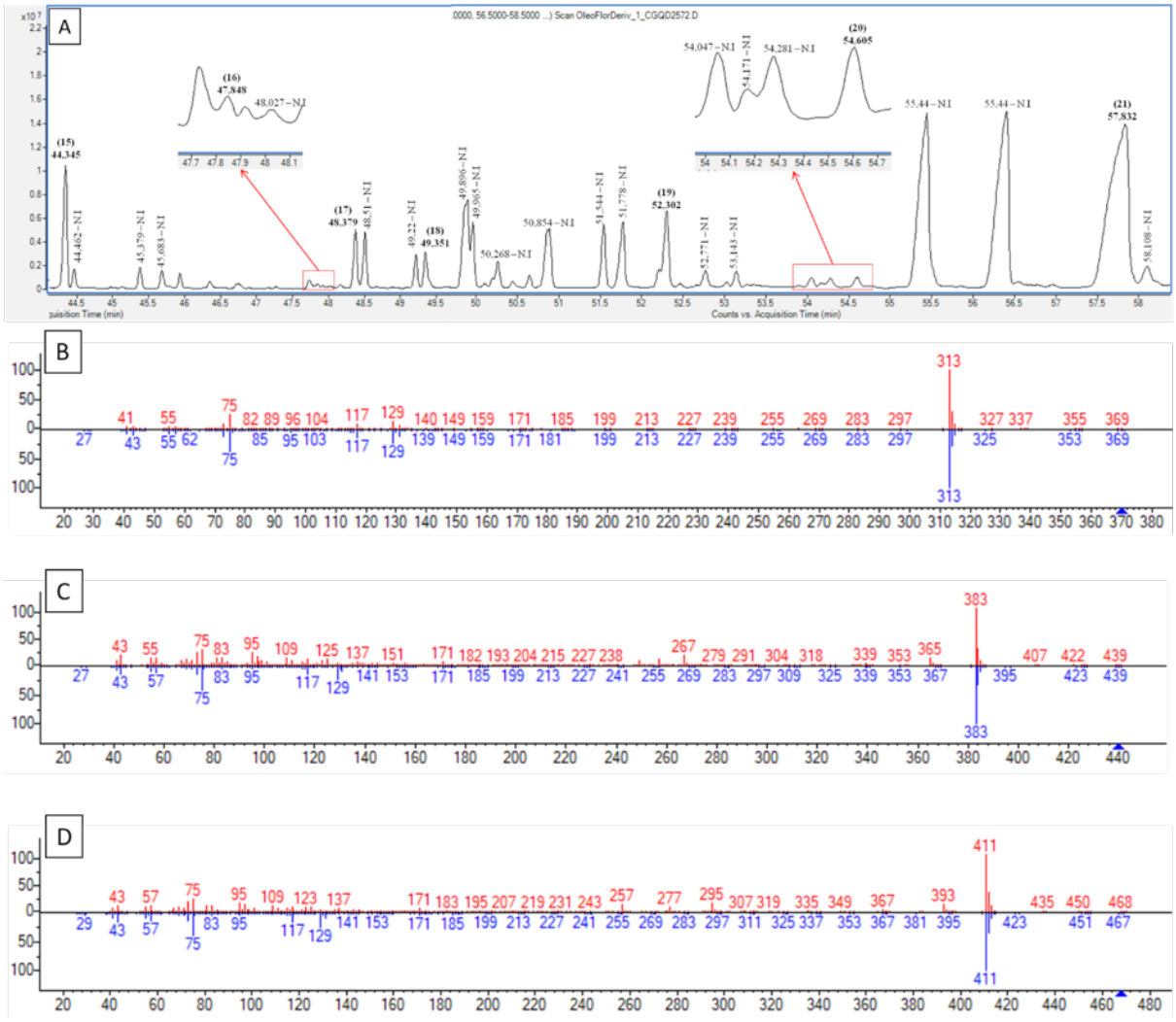

Fig 3. Expansion of the total ion chromatogram (A) and similarity of the mass spectra of the compounds with the largest relative areas identified in the floral oil of nance (*Byrsonima sericea*), showing the mass spectra from the NIST library in blue. B) Palmitic acid; C) Heneicosanoic acid; D) Ticosanoic acid.

Discussion

The species of nance investigated in this study, *Byrsonima sericea*, had a higher yield of floral oil than other previously studied species, perhaps due to a greater quantity of flowers produced per individual (Rosa & Ramalho, 2011; Barônio et al., 2017). The availability of floral resources is one of the factors that can influence the nesting of bees that use this resource (Scheper et al., 2015, Dainese et al., 2018). In the particular case of oil-collecting bees, studies of nesting activity in bee species of the genus *Centris* in trap nests have shown that they do not nest in the absence of this resource, with its abundance seeming to regulate the nesting rate (Magalhães & Freitas, 2013; Lourenço et al., 2019). *Byrsonima sericea*, in turn, appears to be a good source of this floral resource for these bees, since Lourenço et al., 2019, working in the same area as the present research, saw *C. analis* nesting in trap nests throughout all six months of the study, with the peak of nest construction and brood production coinciding with the start of flowering in this plant species.

It is not yet known what role many of the floral-oil chemical components found in this study play for the bees. However, Manning (2001) suggests that fatty acids may be involved in energy metabolism, as well as the synthesis of fat reserves, glycogen and cell-membrane structure, which are important in the development, reproduction and nutrition of bees.

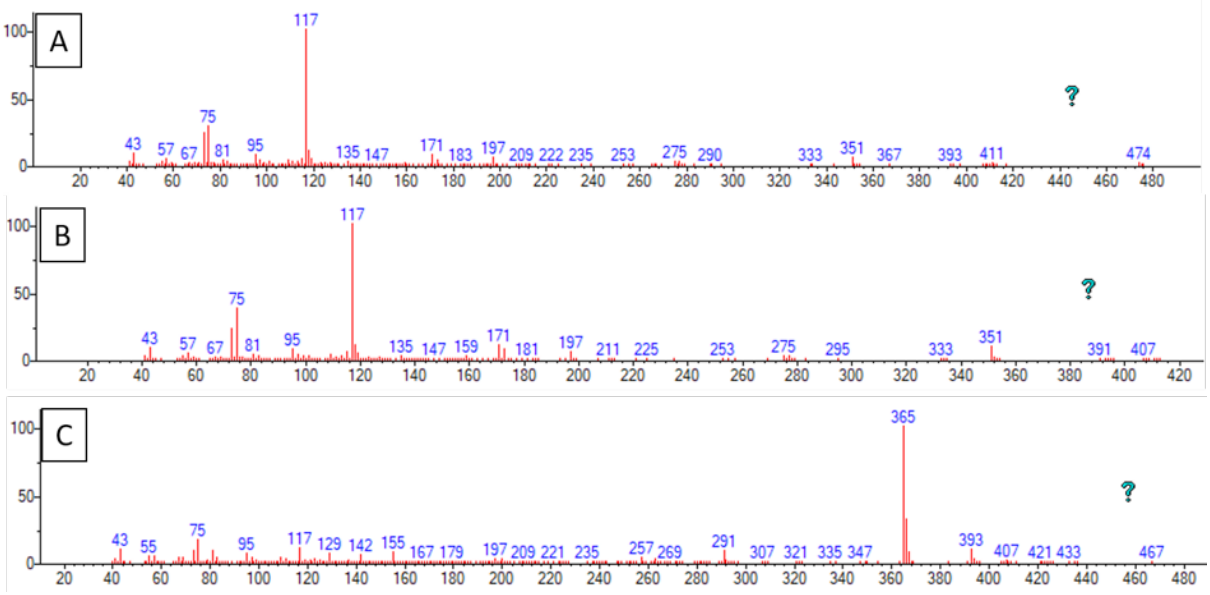

Fig 4. Mass spectra of the unidentified compounds. A) unknown 1, RT = 55.44 min; B) unknown 2, RT = 56.405 min; C) unknown 3, RT = 60.85 min.

In the case of oil-collecting bees, this refers to larval nutrition, since adults do not use the oil as food (Vogel, 1990; Alves-Dos-Santos et al., 2007). However, nutrition at the larval stage is important in many solitary bee species, and can have serious consequences on the emergence rate, longevity, and reproductive capacity of the adult individual (T'ai & Cane, 2002; Radmacher & Strohm, 2010).

The literature indicates a combination of long-chain saturated and unsaturated fatty acids, with or without β-acetoxy substituents and mono- or diglycerides, as the main constituents of floral oils, as well as the smaller presence of other groups (Dumri et al., 2008; Capellari et al., 2011). However, these fatty acids can undergo biotransformation due to reactions caused by enzymes bees add to the oil when using their mandibles to mix and place it in the cells inside their nests, giving rise to products that are different from those originally secreted by the plant and harvested by the bees (Reis et al., 2007).

In the present study, ion chromatograms showed that the compounds classified as fatty acids were those with the highest relative area, which would suggest the importance of these compounds in the floral oil of *B. sericea* and probably other plant species that produce floral oils. Among other characteristics, these fatty acids varied in saturation and chain length, between 14 and 24 carbons. Regarding the size of the carbon chain, tetradecanoic acid, or myristic acid as it is also known, was one of the shortest fatty acids found in the sample. This acid has a medium saturated carbon chain with 14 carbons and, together with linoleic acid, which was also identified, is mainly related to antimicrobial properties (Desbois & Smith, 2010). Considering that oil-collecting bees usually nest in the soil or in natural wood cavities, the properties of these acids must be very important to the bees as they help keep the developmental environment of the brood protected from fungi and bacteria.

Tricosanoic acid was the most representative in the matrix, and together with tetracosanoic acid was the longest in relation to chain size. These constituents, due mainly to the size of their chain, required a high retention time to elucidate their structure; they are long-chain linear molecular compounds with the presence of 23 and 24 carbons respectively. There is no literature indicating the role of long-chain fatty acids in bee reproduction, but nutritionally they are metabolised mainly during larval development, and constitute an important source of energy (Cantrill et al., 1981). Perhaps, from the quantities present in the nance oil, these acids may act as source of energy for the brood.

Various fatty acids found in the floral oil of *B. sericea*, such as tetradecanoic, palmitic, stearic, tricosanoic, oleic and linoleic acid, among others, appear to be common constituents of floral oils produced by other plant species. Therefore, although in different concentrations, they are also present in the floral oil of *Phymatidium delicatulum*, *Phymatidium tillandsioides, Pterandra pyroidea, Byrsonima crassifolia* and *Diascia* spp. (Vison et al, 1997; Reis et al., 2006; Dumri et al., 2008; Capellari et al., 2011). However, these constituents can vary between the different genera and botanical families. The family Malpighiaceae, for example, includes genera that present distinct fatty acids and that can cause chemical variations among the floral oils (Reis et al., 2007; Renner & Schaefer, 2010; Capellari et al., 2011). These differences may be used to better attract and win the preference of their pollinators. In addition, some species seem to produce far more specific and unique fatty acids. Reis et al. (2007) isolated the fatty acid (3R, 7R)-3,7-diacetoxy-docosanoic acid, named as birsonic acid, as the main constituent of the floral oil of *B. intermedia*. This acid was not isolated in any other study carried out with floral oils, and although the species studied here belongs to the same genus as that investigated by Reis

et al. (2007), it was not possible to identify the presence of birsonic acid in our oil samples. This result can be due either to differences in the spectroscopic analysis used in the two studies, or, more likely, because this fatty acid is specific to *B. intermedia*.

It was also possible to identify two ketones (2-pentadecanone and 2-heptadecanone) in the oil of *B. sericea* under analysis. It is known that ketones are not important for bee feeding, but are present in small amounts in some floral oils. This group of compounds may be involved in other functions related to bee nesting, since the oils are also used in preparing the nest cells (Alves Dos Santos et al., 2002; Melo & Gaglianone, 2005).

Using mass spectrometry, it was also possible to confirm the presence of geranyl linallol in the oil under study. Geranyl linallol is a volatile molecule that is derived from linallol, which in turn is an important terpenoid, commonly found in the composition of floral aromas (Raguso, 2016; Krug et al., 2018). Floral aromas comprise a mixture of volatile compounds which are present in pollen, nectar, petals, sepals and other floral structures (Farré-Armengol et al., 2015), and which play an important role in the plant-animal relationship, being mainly involved in defensive ecological interactions against pathogens and predators and in attracting pollinators (War et al., 2012, Song & Ryu, 2013, Fernandes et al., 2019).

Although volatiles have a great influence on the collection of floral resources, it is believed that in most cases, attraction of the visitors to the flowers is not due to one compound or class of compounds, but to a set of compounds belonging to different classes (Reis et al., 2004; Fernandes et al., 2019). However, considering that no other compound with a similar function was identified in the samples, it is possible that geranyl linallol is the only floral attractant in the oil of *B. sericea*. If other volatile compounds are also involved in attracting visitors to the flowers of this plant species, they must be present in other parts and products of the flowers.

Despite some of the unidentified compounds have characteristic fragmentation peaks that resemble diacylglycerol isomers, it is possible that they are artefacts or products of the derivatisation reaction. Although derivatisation, especially silylation, is typically used to reduce polarity and alter the properties of the analyte to get better separation and improve the sensitivity of the method, in some cases this reaction may generate artefacts under the influence of the reagents, and may cause a reading error (Moldoveuanu & David, 2018). In addition, it can be difficult to identify compounds in the analysed matrix simply by means of the spectrometric method used; the identification of these analytes is also hampered, as the mass spectra of many silylated compounds are not present in the usual libraries (Moldoveuanu & David, 2018).

A knowledge of food sources is essential for understanding the associations between bees and plants (Lima et al., 2017). As such, awareness of the chemical composition of floral oils, an essential resource for nesting and brood feeding in oil-collecting bees, can allow measures to be taken for conserving bee species of this group by adopting practices that favour the presence of plant species that produces the specific oils they prefer, as well as developing techniques for agricultural pollination which employ the bees. This may involve cultivating the species that supply the floral oils, and developing artificial oils that include the chemical compounds responsible for attracting the bees, are involved in nest construction and possess the antimicrobial and nutritional properties for development of the brood, the minimum conditions required for these bee species to nest in agricultural areas.

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