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Indicators of plant-insect relationship by analysis of floral scent volatiles from three *Plumeria* species

Indicadores da relação planta-inseto por análise de voláteis de perfume floral de três espécies de *Plumeria*

Elson Santiago Alvarenga[1]*, Nilton Cesar Ribeiro[1], Antonio Jacinto Demuner[1], Cristiane Isaac Cerceau,[1] Marcelo Henrique dos Santos,[1] Slavko Komarnytsky[2]

RESUMO

As espécies do gênero *Plumeria* são conhecidas pela fragrância forte e agradável; no entanto, sua composição volátil e variação de aroma são amplamente desconhecidas. Os perfis diurno e noturno dos aromas florais de três espécies de *Plumeria* foram avaliados com base na composição química (por HS-SPME / GC-MS). Vinte e quatro compostos foram identificados. Linalol foi o composto majoritário em *P. alba* (86,5% dia e 71,0% noite), menor em *P. rubra* (8,3% dia e 13,0% noite) e ausente em *P. obtusa*. O composto majoritário encontrado em *P. rubra* foi o benzoato de metila (55,9% dia e 62,3% noite) e em *P. obtusa* foi salicilato de metila (30,2% dia e 20,6% noite). Todas as espécies revelaram variações diurnas e noturnas na composição de seus metabólitos voláteis, embora a diferença tenha sido mais proeminente em *Plumeria alba*. Diferenças nos voláteis das espécies *Plumeria* podem ser a causa da infestação preferencial de plantas de *P. alba* pela mariposa tetrio sphinx.

Palavras-chave: HS-SPME/GC-MS; *Plumeria*; *Apocynaceae*; *Pseudosphinx tetrio*

ABSTRACT

Genus *Plumeria* Species are known for strong pleasant fragrance; however, its volatile composition and scent variation are largely unknown. The day and night profiles of the flower scents of three species of *Plumeria* were evaluated based on chemical composition (by HS-SPME / GC-MS). Twenty-four compounds were identified. Linalool was the major compound in *P. alba* (86.5% day and 71.0% night), minor in *P. rubra* (8.3% day and 13.0% night), and absent in *P. obtusa*. The major compound found in *P. rubra* was methyl benzoate (55.9% day and 62.3% night) and in *P. obtusa* was methyl salicylate (30.2% day and 20.6% night). All species revealed diurnal and nocturnal variations in composition of their volatile metabolites, although the difference was more prominent in *Plumeria alba*. Differences in the volatiles of the *Plumeria* species could be the cause of the preferential infestation of *P. alba* plants by tetrio sphinx moth.

Keywords: HS-SPME/GC-MS; *Plumeria*; *Apocynaceae*; *Pseudosphinx tetrio*

[1] Universidade Federal de Viçosa – UFV
[2] North Carolina State University
*E-mail: elson@ufv.br

INTRODUÇÃO

Plumeria L. is a genus of the family *Apocynaceae* belonging to the *Magnoliophyta* division and Magnoliopsida class. These shrubs or small trees are native to Mexico and are well known for their strong scented flowers presenting different colors (SHINDE et al., 2014). Several species of this genus, for example, *P. alba, P. odorless, P. obtusa, P. prudish, P. rubra, P. stenopetala, P. stenophylla,* and *P. lancifolia* are generally called the love flowers (CHATTERJEE et al., 2013; SHINDE et al., 2014). *Plumeria* is also the main host of tetrio sphinx moth *Pseudosphinx tetrio* L (SQUYRES, 2014). Volatile organic compounds serve important roles in plant communication, but little is known about the biological functions of most of these substances. Fragrances emitted by flowers serve as a means of orientation and attraction for their pollinators of interest (D'AURIA et al., 2020). Some volatile compounds emitted by plants act as a defensive material, either to ward off predators, promote allelopathic interactions, or attract insects to act on their behalf (MEEKIJJAROENROJ et al., 2007). In addition, the scents of different flowers have altered compositions depending on their growth stage, climate, nutrient availability, or predator attacks (RAGUSO et al., 2003; HASSAN et al., 2008; STEENHUISEN et al., 2012). Therefore, the composition and timing of these volatile fingerprints is variable and intrinsically linked to diverse ecological conditions (GRULOVA et al., 2014; CARRASCO et al., 2015). Multiple metabolic routes produce volatile compounds, and various *Plumeria* species and subspecies emit distinctive scents that lead to different olfactory sensations.

In the literature there are reports of the identification of constituents of essential oils from flowers of *P. rubra* (OMATA et al., 1991; OMATA et al., 1992; BARRETO et al., 2014; GOSWAMI et al., 2016) *P. Alba* (LAWAL et al., 2014; SAHOO et al., 2021) *P. obtusa* L., *P. acuminata* Ait. (yellow flower), *P. rubra* L. (pink flower), and *P. rubra* (orange flower) (TOHAR et al., 2006). However, to the best of our knowledge, only one report on the floral scent composition of *Plumeria tuberculata* was analyzed by HS-SPME (BÁEZ et al., 2012).

In this study the volatile compounds from floral scents of *P. alba*, *P. obtusa*, and *P. rubra*, collected at different periods, were identified by HS-SPME / GC-MS to understand the temporal variation in the floral scent production, associating it with a preferential infestation of *P. alba* by caterpillar of tetrio sphinx moth.

MATERIAL AND METHODS

Plant material

The flowers of *P. alba*, *P. rubra*, and *P. obtusa* were collected in Porto Firme (20° 38' 54" South, 43° 5' 18" West), Minas Gerais, Brazil. The samples were collected in the morning and at night in February when they were in full bloom (WANG et al., 2021; JIAO et al., 2022). Voucher specimens of all species were deposited at the herbarium of the Department of Botany, Federal University of Viçosa, under voucher specimen VIC 47840, VIC 47841, and VIC 47847. The Genetic Patrimony/CTA of the *P. alba*, *P. rubra*, and *P. obtusa* were registered in SisGen No. A31B26F.

The evaluation of the *Plumeria* specie preferentially infested by tetrio sphinx moth was carried out in the same place and period that the flowers were collected. For this, three plants of each species were observed twice a week for one month (the larval period of the moth). Tetrio sphinx caterpillar ate all the leaves of *P. alba*. *P. obtusa* and *P. rubra* were not attacked by the caterpillar.

HS-SPME analysis

The extraction was performed with 85-µm carboxen-polydimethylsiloxane (CAR/PDMS), 65-µm polydimethylsiloxane/divinylbenzene (PDMS/DVB), and 50/30 µm Supelco divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) (Bellefonte, PA, USA) fibers. The flowers (2 g) were inserted into individual vials. The fibers were exposed to floral scent volatiles for 30 min in the headspace (HS) and evaluated at three different temperatures: 26 °C (ambient), 30 °C, and 40 °C. Following the exposure, the fibers were inserted into the GC-MS injector for further analysis (SERRANO et al., 2015). Thus, the fiber and its headspace exposure temperature were selected for their performance (greater number and intensity of individual metabolite peaks) in extracting volatile organic compounds (VOCs) from flowers.

GC-MS analysis

A gas chromatograph with mass detector Shimadzu QP 5050A equipped with a SE-54 column (length 30 m, internal diameter 0.25 mm) was used for the GC-MS analysis. The fiber containing the volatiles was exposed to the injector for 5 min at 250 °C in the splitless mode. The GC-MS transfer line temperature was maintained at 290 °C. The column temperature was initially set to 70 °C, increasing by 5 °C every minute until

reaching 150 °C, then increasing 15 °C per minute until reaching 250 °C, remaining during 5 min at 250 °C. Helium was used as the carrier gas at a constant rate of 1.2 mL min^{-1}. The electron impact ionization (EI) mode was used for measurements, and the scanning was performed at 40-470 *m/z* (CHOU & LEE, 2005).

The volatile compounds were identified by comparison of their mass spectra with standard spectra in the NiST11 and Wiley analytical libraries, and also by comparing their retention index (RI), calculated by using the standard series of C7-C30 hydrocarbons (Bellefonte, PA, USA) (ADAMS, 2012). The relative amounts of each compound were calculated by integrating the peak area (ÁLVAREZ et al., 2015; CERCEAU et al., 2016; CERCEAU et al., 2020).

RESULTS AND DISCUSSION

The initial headspace (HS) and solid-phase micro extraction (SPME) analyses were performed to verify the best SPME fiber to be used in the extraction of volatile components from *Plumeria* flowers. Three fibers (DVB-CAR-PDMS 50/30 µm, DVB-PDMS 65 µm, and PDMS-CAR 85 µm) were shortlisted based on the nature of their coating and affinity to the volatile polarity. Total ion chromatograms (TIC) obtained with 50/30 µm DVB-CAR-PDMS fiber exposed to 40 °C in the head space and GC-MS detection resulted in a larger number and intensity of the individual metabolite peaks (Figure S1, Supporting Information). All subsequent identifications of volatiles from the flowers were therefore performed at 40 °C with the SPME 50/30 µm DVB-CAR-PDMS fiber.

A total of 16 volatiles were identified in the flowers of *P. alba* collected at nighttime (Figure S2, SI), monoterpene and sesquiterpene metabolites were detected (Table 1). These molecules played an important role in volatile signaling during plant-plant and plant-animal interactions, their detection and quantification were therefore critical to understanding plant metabolism (STEENHUISEN et al., 2012; PINTO-ZEVALLOS et al., 2016). Among all compounds identified in the flowers of *P. alba*, linalool and 2-phenylethanol were the major diurnal constituents accounting for 86.5% and 5.8%, respectively.

At nighttime, the proportion of linalool present in the floral scent volatile fraction decreased to 71.0%, while 2-phenylethanol and 2-phenylacetaldehyde increased to 15.4% and 2.4%, respectively (Table 1). Both phenylpropanoids pathway derivatives seemed to

be major contributors to the enhanced floral scent at night. They also act as powerful insect attractants by attracting different sets of pollinating and predatory insects (TIEMAN et al., 2006).

The volatiles of red flowers from *P. rubra* collected during the morning comprised a total of 9 compounds (Figure S3, SI), with methyl benzoate (55.9%), 2-phenylethanol (21.7%), and linalool (8.3%) as the major constituents (Table 1). Nighttime collection has not changed this profile for methyl benzoate and linalool, detected at 62.3% and 13.0%, respectively (Table 1). However, 2-phenylethanol production (13.0%) was measured just above half the diurnal levels (21.7%) (Table 1). Low levels of linalool production comparing to *P. alba*, as well as a strong decrease in 2-phenylethanol during the nighttime, may explain tetrio sphinx moth's preference to damage and defoliate *P. alba*.

A total of 12 compounds were detected in the volatiles of *P. obtusa* during daytime (Figure S4, SI), with methyl salicylate (30.2%), methyl benzoate (14.6%), benzyl benzoate (10.7%), and 2-phenylethanol (10.3%) being the major constituents. An interesting observation was a complete lack of linalool in the floral scents from this species (Table 1). These compounds were also derived from phenylpropanoids pathway via formation of *trans*-cinnamic acid following a branching point to benzoic and salicylic acids that are direct precursors for the synthesis of important floral scents as methyl salicylate and methyl benzoate. We also observed a significant increase in methyl benzoate (27.2%) and a respective decrease in methyl salicylate (20.6%) in *P. obtusa* floral scent collected at night. In addition, pentyl salicylate appeared only in volatiles of daytime collection, while *β*-farnesene was present only in flowers collected at night.

The infestation of three species of *Plumeria* by the tetrio sphinx moth was studied by direct observations carried out twice a week during the month of February. It was observed that while *P. rubra* and *P. obtusa* remained intact, the leaves of *P. alba* were completely devastated by the moth. This observation indicates that tetrio sphinx moth prefers to feed on the leaves of *P. alba*. The white color of the flowers facilitates the approach of the adult insect with nocturnal habit. The combination of linalool and 2-phenylethanol together with the color of the flowers influence the plant-animal relationship.

Table 1- Content (%) of identified volatile compounds of three *Plumeria* specie

Compounds				*Plumeria alba* (%)		*Plumeria obtusa* (%)		*Plumeria rubra* (%)	
				Day	Night	Day	Night	Day	Night
	RT	AI_C	AI_T						
E-Hex-3-enol	5.83	853	850	1.0 ± 0.03	1.3 ± 0.02	-	-	-	-
Benzaldehyde	8.30	951	952	-	-	2.4 ± 0.03	2.7 ± 0.02	-	-
Benzyl alcohol	10.54	1033	1026	-	-	4.4 ± 0.04	4.7 ± 0.03	1.6 ± 0.03	-
β-Ocimene	10.67	1039	1044	0.1 ± 0.00	-	-	-	-	-
2-Phenylacetaldehyde	**10.80**	**1040**	**1036**	**-**	**2.4 ± 0.12**	**0.8 ± 0.10**	**1.3 ± 0.02**	**0.6 ± 0.04**	**0.8 ± 0.04**
E-Linalool oxide	12.28	1091	1084	0.5 ± 0.01	1.2 ± 0.02	-	-	-	-
Methyl benzoate	**12.58**	**1092**	**1088**	**-**	**-**	**14.6 ± 0.04**	**27.2 ± 0.03**	**55.9 ± 0.04**	**62.3 ± 0.04**
Linalool	**12.79**	**1102**	**1095**	**86.5 ± 0.04**	**71.0 ± 0.03**	**-**	**-**	**8.3 ± 0.03**	**13.0 ± 0.03**
2-Phenylethanol	**13.17**	**1110**	**1106**	**5.8 ± 0.03**	**15.4 ± 0.06**	**10.3 ± 0.03**	**9.6 ± 0.04**	**21.7 ± 0.03**	**13.0 ± 0.06**
2-Phenylacetic acid methyl ester	14.99	1179	1175	0.5 ± 0.01	0.4 ± 0.02	-	-	0.7 ± 0.02	-
Methyl salicylate	**15.52**	**1197**	**1190**	**0.1 ± 0.01**	**0.3 ± 0.01**	**30.2 ± 0.06**	**20.6 ± 0.05**	**2.2 ± 0.05**	**2.5 ± 0.04**
Cinnamyl alcohol	17.20	1254	1259	-	0.4 ± 0.02	-	-	-	-
Eugenol	20.25	1359	1356	-	0.04 ± 0.00	-	-	-	-
α-Copaene	20.86	1380	1374	0.08 ± 0.01	0.09 ± 0.02	1.2 ± 0.04	3.4 ± 0.01	-	-
β-Elemene	21.29	1393	1389	0.08 ± 0.01	0.07 ± 0.00	-	-	-	-
β-Farnesene	22.08	1438	1440	-	0.08 ± 0.01	-	2.4 ± 0.12	-	-
β-Caryophyllene	22.09	1421	1417	0.05 ± 0.00	-	-	-	-	-
Pentyl benzoate	**22.45**	**1474**	**1476**	**-**	**-**	**4.7 ± 0.08**	**8.9 ± 0.05**	**1.2 ± 0.01**	**0.9 ± 0.04**
α-Farnesene	23.86	1509	1505	0.2 ± 0.01	0.2 ± 0.01	0.4 ± 0.01	1.6 ± 0.12	-	-
δ-Cadinene	24.20	1523	1522	0.07 ± 0.01	-	-	-	-	-
Pentyl salicylate	24.36	1570	1574	-	-	3.8 ± 0.10	-	-	-
Dendrolasin	25.00	1573	1570	2.1 ± 0.02	2.8 ± 0.13	-	-	-	-
Benzyl benzoate	**27.31**	**1754**	**1759**	**0.04 ± 0.00**	**0.2 ± 0.01**	**10.7 ± 0.12**	**5.9 ± 0.10**	**0.6 ± 0.02**	**-**
Benzyl salicylate	28.32	1869	1864	-	0.08 ± 0.01	0.8 ± 0.10	-	-	-
Total				**97.5**	**96.8**	**86.0**	**88.8**	**92.4**	**92.8**

RT = Retention time in minute; AI_C = Calculated Arithmetic Index; AI_T = Tabled Arithmetic Index (Adams, 2012)

CONCLUSION

A total of 24 compounds were identified in the floral scents of three *Plumeria* species collected during the morning and at night. All species showed diurnal and nocturnal variations in the relative composition of their volatile metabolites. The difference was most prominent in the floral scents from *Plumeria alba* flowers. Linalool in *P. alba*, methyl salicylate in *P. obtusa*, and methyl benzoate in *P. rubra* were present in the greatest amounts in the morning and at night. The difference in color and volatile content in *Plumeria alba* may be the cause of tetrio's attraction. Understanding variation of volatiles emission and plant-animal interaction may help in the development of new products and good practices of agricultural management

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