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Article

Evaluation of the Mandibular Pheromones Produced by Queens of Africanized and European Honeybees under Normal Conditions and During Absconding Processes by HPLC-UV Methodology

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Pheromones are essential for colony organization in honeybees. The pheromones 9-ODA ((*E*)-9-oxodec-2-enoic acid) and 9-HDA ((*E*)-9-hydroxydec-2-enoic acid), produced by the mandibular gland of the queen, have various functions within the colony, including inhibition of ovarian development of workers, attraction of swarms, and stabilization of the cluster group. An example of reaction of the colony to adverse conditions is absconding, which is defined as mass exit of all of the adult individuals, leaving even brood and food. In this study, the pheromones 9-ODA and 9-HDA were determined in virgin and normal mated, egg-laying European Carniolan and Africanized queens, as well as in mated queens that were in absconding colonies, using liquid chromatography with UV detection developed methodology. Absconding was induced by maintaining free-flying five-standard-Langstroth-frame colonies in a chamber artificially heated to 45 ºC. The obtained results showed that the amount of 9-ODA in Africanized queens (6.56 μg bee^{-1}) is very low compared with European queens. However, large amounts of 9-HDA were found in the queens of absconding Africanized swarms (107.4 μg bee^{-1}). According to our results, the quantities of 9-ODA and 9-HDA in Africanized honeybees in Brazil may be contributing to the high rates of absconding, promoting low effect of stabilizing agent, and high effect of attraction of dispersers cluster.

Keywords: pheromones, absconding, European bees, Africanized bees, 9-ODA, 9-HDA, liquid chromatography

Introduction

The organization of the complex social life of honeybee colonies (*Apis mellifera*) is based on a highly sophisticated chemical communication system that involves numerous pheromones.[1] Probably, the most prominent chemical substance in the context of colony coordination is the queen's mandibular pheromone (QMP), a mixture of aliphatic acids, 9-ODA ((*E*)-9-oxodec-2-enoic acid), 9-HDA ((*E*)-9-hydroxydec-2-enoic acid), and the respective optical isomers (*R*-(−) and *S*-(+)).[2] The primary pheromone components of QMP are of low volatility and, accordingly, predominantly act at short range.[2] The components 9-ODA, 9-HDA, methyl-*p*-hydroxybenzoate, and 4-hydroxy-3-methoxyphenylethanol have been shown to initiate a behavior termed "clustering", which is characterized by a group of worker bees antennating, licking, and cleaning the queen. Thereby, QMP is transferred from the queen to her retinue and, subsequently, spreads through the whole colony via contacts between the workers.[2-7] The queen's mandibular pheromone controls not only the behavior of the queen's hive mates but also regulates the bee's physiology.[8] Exposure to QMP, for instance, affects the function of dopamine in the brain of worker bees, which, subsequently, induces behavioral changes.[9]

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The authors dedicate this study to Prof Carol Collins's memory.
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9-ODA, secreted by the mandibular gland in bees, is the major long-distance sex attractant for all honeybee species and participates in a complex communication system that ranges from the attraction of drones to their signaling within the colony, promoting the queen's behavioral relationship with the workers.[10] In the interaction between queens and workers, it has been shown that a blend of five mandibular gland substances is necessary to elicit worker retinue behavior (9-ODA, 9-HDA (85% (*R*)-(–), 15% (*S*-(+)), HOB (methyl-*p*-hydroxybenzoate) and HVA (4-hydroxy-3-methoxyphenylethanol)).[2,11,12] In swarming, the use of pheromones is of utmost importance for the formation of the cluster and maintenance of the swarm both during the flight and in its stabilization. Continuous data were found to be rich sources of information about colony health and activity with weight, CO_2 and temperature and the swarming of honeybees hives, demonstrating the importance of recognizing behaviors in swarming.[13-18]

Colony fission by swarming constitutes a delicate process in the life of a honeybee's colony, since thousands of bees including the active queen, leave the old nest to establish a new home. Swarming, therefore, requires a high level of coordination among colony members in order that the entire group, or at least the better part, reaches the new nesting site. During the process of swarming, pheromones of both the workers Nasonov glands and the queen's mandibular glands are important to coordinate the movement of the swarm and guarantee its cohesion.[14-18]

Pheromones from the Nasonov glands of worker bees are crucial for the orientation of the swarm and its directed movement towards the new home.[14-18] In addition to this worker-produced chemical guidance, several studies[14,18] point to the importance of the queen's mandibular pheromones 9-ODA and 9-HDA for swarming. After leaving the parental nest, the swarm forms a temporary cluster, being attracted to the queen wherever she has alighted. In this process, 9-ODA attracts the flying workers, whereas 9-HDA stimulates alighting and clustering. Once the temporary cluster has formed, 9-HDA apparently stabilizes the cluster, preventing the workers from leaving it before the entire swarm moves to its new home.[14,15] In addition, 9-HDA stimulates workers who have recently settled on a cluster to release Nasonov pheromone, thereby attracting other workers to the cluster. Consequently, the attraction and stabilization of clusters require 9-ODA, 9-HDA as well as pheromones produced by the workers Nasanov glands.[16,17] When the swarm lifts off for the new nesting site, it is kept together only in case the queen is present, which is indicated to the workers through 9-ODA. Therefore, not only clustering but also the coordinated movement of the swarm as a whole depends on both queen- and worker-produced pheromones.[18]

In honeybees (*Apis mellifera*), the mandibular gland secretion of the queen is essential for regulating the reproductive division of labor in the colony inhibiting ovary development in workers.[19] Secretions from the mandibular glands of honeybees, which include 9-ODA and 9-HDA among other pheromones, and their functionalities and chemical signals have been extensively studied.[10,20-26] Grozinger *et al.*[26] review the swarming behavior mechanisms regulating in honeybees and report that the future direction of swarming studies provides a fascinating example of collective behavior involving thousands of individuals in an array of different behavioral and physiological states.

Due to the matrix complexity and the low level of the compounds present in these samples (0.38 μg bee^{-1}), chromatographic methods, such as liquid chromatography coupled to UV detector seems to be a potential tool for this determination.

Studies with pheromones, the timing of absconding and chemical communication between the bees are still not well known by the researchers. A full comprehension of the chemical aspects involved in this phenomenon could contribute to planning actions which keep the colonies' safety. Thus, the objective of this study was to develop a method for the determination of pheromones 9-ODA and 9-HDA in mandibular honeybee queens, employing liquid chromatography and UV-Vis detection, of Africanized (virgin, and reproductive queens in normal conditions and in absconding process) and European Carniolan (virgin and reproductive queens) queen honeybees, and the evaluation of the correlation of the presence of these pheromones in the behavior of Africanized honeybees in absconding.

Experimental

Climatic conditions

The climate according to the Köppen classification, very hot and dry semiarid, type BSwh, is characterized by the scarcity and irregularity of precipitation, with rains occurring in the summer and strong evaporation, due to the high temperatures.

Bee samples

Samples of bees were obtained from colonies of European honeybees (*Apis mellifera carnica*, European carniolan) and Africanized honeybees (*Apis mellifera* L.), established at the campus of the University of São Paulo in Ribeirão Preto, Brazil (*Apis mellifera carnica* and *Apis mellifera* L.), and at in Mossoró, Rio Grande do Norte, Brazil (Africanized honeybees only). Queens derived from

A. m. carnica mother queens were provided by Prof Dr Peter Rosenkranz (University of Hohenheim, Germany). In all text, Carniolan has been replaced by European Carniolan *Apis mellifera*, *carnica* is a European subspecies (Germany) and is interesting for comparison with the literature.

For the analysis of 9-ODA, virgin and reproductive queens were used. The specimens of bees were collected under different conditions and the pheromone analyzes are presented according to Table 1. The bees were stored individually in 1:10 (v/v) Ringer's solution in 10% NaCl solution at –20 °C until the analysis.

HPLC-UV analysis

High performance liquid chromatography coupled to UV detector (HPLC-UV) was performed using a Varian model 230 chromatograph (Varian, Palo Alto, California, USA) coupled to an UV-diode array detector (DAD model 310/330 ProStar). Detection was carried out at a wavelength of λ = 230 nm. The methodology parameters were based on a previously published study.[27] The samples were separated using a pre-column model LiChroCART®4-4 (Merck KGaA, Darmstadt, Germany) and an analytical column model Lichrospher®60 RP-select B (5 µm, 250 × 4 mm) (Merck KGaA, Darmstadt, Germany). Separation was carried out in isocratic mode at a flow rate of 0.7 mL min^{-1}, using purified water (Milli-Q System, Millipore, Bedford, USA) acidified with phosphoric acid (pH = 2.5; Mallinckrodt Baker, Mexico City, Mexico) and methanol (50:50% v/v) (JT Backer, Phillipsburg, USA) as mobile phase, temperature 25 ºC, the chromatograms data was processed by ProStar software (Varian, Palo Alto, California, USA).

For the analysis, the frozen honeybees were manually macerated in 2.0 mL of methanol (HPLC grade: JT Backer, Phillipsburg, USA). The samples were then placed in an ultrasonic bath for 20 min, and the supernatant was filtered through a cellulose membrane (Millex HV 0.45 µm Milipore, Barueri, Brazil). Thereafter, 30 µL of the internal standard (alpha naphthol, 30 µg mL^{-1}, Sigma-Aldrich, USA) were added to the extract, and an aliquot of 50 µL were injected into the chromatographic system. The final sample volume was adjusted to 2 mL considering all added solutions.

Determination of pheromones

The calibration curves used for the pheromone identification were obtained following the methodology described by Koshio and Almeida-Muradian study.[27] First, standard stock solutions of 9-ODA and 9-HDA (analytical standards from Phero Tech Inc., Delta, Canada) in methanol (HPLC grade: JT Backer, Phillipsburg, USA) were prepared at a concentration of 3 mg mL^{-1} and stored at –20 ºC. From these, the following dilutions were used: 25.0, 10.0, 1.0, 0.8, 0.6, 0.3, 0.2, and 0.1 mg mL^{-1}. For each dilution, 30 µL of internal standard (alpha naftol 30 µg mL^{-1}, Sigma-Aldrich, Burlington, MA, USA) and 120 µL of the mobile phase (see above) were mixed resulting in 250 µL of total volume. The samples were subsequently filtered through a membrane (Millex HV 0.45 µm, Millipore, Barueri, Brazil), and 50 µL of the extract was injected in the chromatographic system.

The analytical curves were obtained by honeybee macerated samples adding the pheromones solutions at concentrations of calibration curves, and the internal standard solution at 30 µg mL^{-1}. The final sample volume was adjusted to 2 mL using the mobile phase, and only 50 µL were injected into the HPLC system. For quantitative purposes, this dilution factor was considered. These curves were used for pheromones determination in queen bee samples. Accuracy and interday precision were determined by analytical curves using quintuplicate assays.

The interday precision was expressed in terms of the coefficient of variation (CV) in three concentration levels (0.1, 0.6 and 1.0 µg mL^{-1}) evaluated in the quintuplicate assay (n = 5), and the intra-day precision was determined according to equation 1:

Table 1. Bee samples for the analysis of 9-ODA and 9-HDA were performed with virgin and reproductive queens of European carniola (*Apis mellifera carnica*) and Africanized honeybees (*Apis mellifera* L.)

Queen	Honeybee	No.	Day of age	Condition
Reproductive	*Apis mellifera carnica*	1	180	normal
	Apis mellifera L.	8	180	normal
	Apis mellifera L.	3	≥ 180	absconded due to the of lack of food
	Apis mellifera L.	3	≥ 180	absconded after an increase in ambient temperature (41 ºC)
Virgin	*Apis mellifera carnica*	3	1	normal
	Apis mellifera L.	5	1	normal

No: number of individuals; normal condition: queens under normal and uninduced conditions.

$$CV\ (\%) = (s/\bar{x}) \times 100 \quad (1)$$

where s is the standard deviation between replicates and $\bar{x}$ is the average concentration between the injections. The accuracy was determined by the following equation 2:

$$Accuracy\ (\%) = (EC/TC) \times 100 \quad (2)$$

where EC is the obtained or experimental concentration, and TC is the theorical or expected concentration.

The statistical analyses were performed using the software Microsoft Excel 2000.[28] Linear regression, correlation coefficients (r^2) and coefficient of variation (%) were performed with the 9-ODA and 9-HDA patterns.

Results and Discussion

Figures of merit of the HPLC-UV methodology

The analytical curves with pheromone concentrations ranging from 0.1 to 25 μg mL^{-1} showed adequate linearity (linear correlation coefficients $r^2 > 0.98$; Table 2).

Table 2. Linear regressions and correlation coefficients (r^2) obtained from the analytical curves of the developed method for 9-ODA and 9-HDA in honeybees determination

Pattern	Linear regression	Correlation coefficient (r^2)
9-ODA	$y = -0.07289 + 0.46856x$	0.99982
9-HDA	$y = 0.5576 + 0.1859x$	0.98532

9-ODA: (*E*)-9-oxodec-2-enoic acid; 9-HDA:((*E*)-9-hydroxydec-2-enoic acid.

Interday precision was assessed by replicate analyses (n = 5) of Ringer's solution and methanol solution spiked with standard solutions and internal standard at three different concentrations levels (0.1, 0.6, and 1.0 μg mL^{-1}), and filtrated through a Millex membrane. The variation of the obtained calibration curves was smaller than 5.5% (see coefficient of variation in Table 3). These results indicate that the present method shows high accuracy and adequate interday precision (Table 3).

Under the given chromatographic conditions, elution order 9-ODA, 9-HDA (internal standard, alpha naphthol) (Figures 1-4). Since there were no interfering peaks at the respective retention times of the analytes (Figures 1-4), the analytical method presents adequate sensitivity and can be considered to identify both 9-ODA and 9-HDA. The obtained coefficients of variation were below 5.5% in all evaluated concentration, which demonstrate the precision of the developed method. At the chromatographic condition, it was observed an enhancement in the retention time with the analysis routine due to the mobile phase pH value (2.5). However, it did not compromise the method sensibility once the elution order was not changed and no interference was observed in the chromatogram peak times.

Table 3. Accuracy and interday precision of the proposed method to identify pheromone components of Africanized honeybee queens

Pattern	Added concentration / (μg mL^{-1})	Accuracy / %	Coefficient of variation / %
9-ODA	0.1	90	4.3
	0.6	92	3.9
	1.0	95	1.2
9-HDA	0.1	93	5.5
	0.6	91	2.1
	1.0	96	0.8

9-ODA: (*E*)-9-oxodec-2-enoic acid; 9-HDA:(*E*)-9-hydroxydec-2-enoic acid.

Honeybee queen pheromones

Preliminary studies indicated that the queen has a role of dominance and adjusts the cluster to your departure and pheromones to attract and stabilization appear to be heavily involved in these mechanisms.[26,27,29-33] The pheromones are the primary means of communication within the nest of chemical species of social bees and are responsible for maintenance and operation of a colony, which, despite being composed of thousands of individuals, operates as a cohesive and efficient unit.

The quantitative determination of the pheromones presents in honeybee queens showed reduced concentration of 9-ODA and 9-HDA in virgin queens of Africanized honeybees when compared to queens of *Apis mellifera carnica* (Table 4, Figure 5). The concentration of pheromone 9-ODA in virgin (6.56 μg bee^{-1}) and reproductive Africanized queens (10.63 μg bee^{-1}) are higher than concentrations in swarming by absconding queens, absconding by lack of food (2.60 μg bee^{-1}) or temperature (4.30 μg bee^{-1}). Already in the virgin queens European carniolan (*Apis mellifera carnica*), 9-ODA obtained was greater obtained (mean (X) = 67.63 μg bee^{-1}, standard deviation (SD): 1.09) compared to the virgin Africanized (mean (X) = 6.56 μg bee^{-1}; SD = 1.25). The virgin queen of European carniolan was not used for swarming induction by absconding.

An important fact is that the data of reproductive queens European carniolan in Table 4 and Figure 5 indicate that the amount of 9-HDA in reproductive queens European carniolan probable is low and 9-ODA higher (9-ODA = 15.97 μg bee^{-1} and 9-HDA = 7.22 μg bee^{-1}),

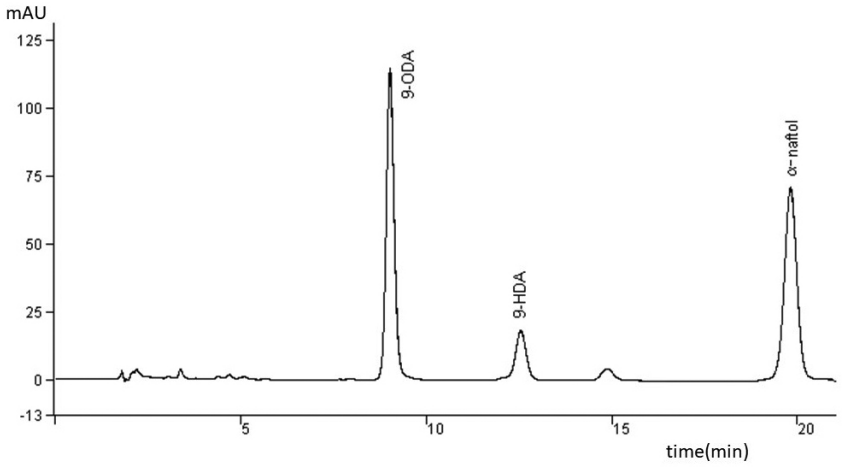

Figure 1. Chromatogram of extract of the mandibular gland of the Africanized queen by absconding for temperature (inducing the increase of temperature), using the mobile phase solution of methanol and acidified water pH 2.5 in the proportions 50:50, v/v.

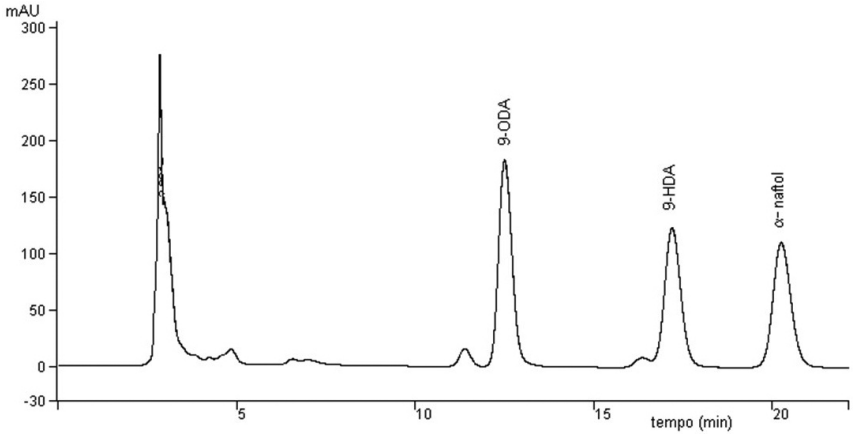

Figure 2. Chromatogram of extract of the mandibular gland of the Africanized queen by absconding for lack of food, using as mobile phase solution of methanol and acidified water pH 2.5 in the proportions 50:50, v/v.

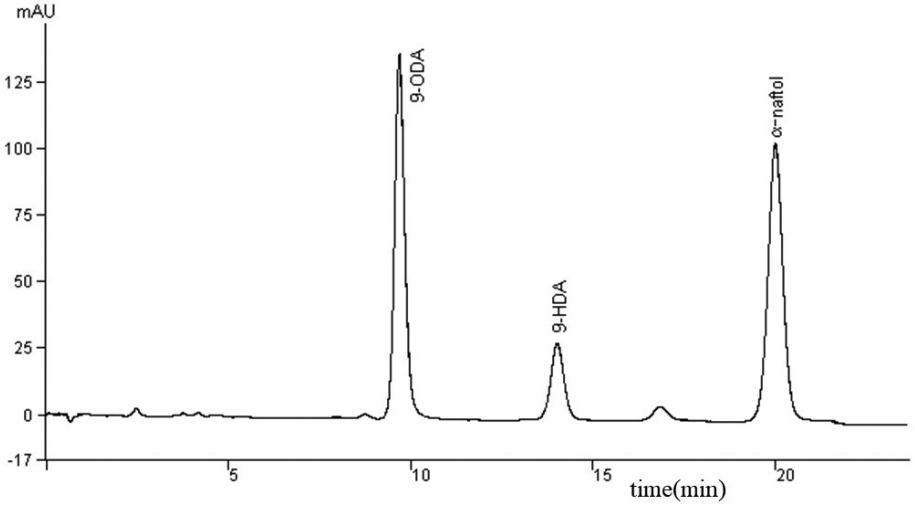

Figure 3. Chromatogram of extract of the mandibular gland of the Africanized reproductive queen, using as mobile phase solution of methanol and acidified water pH 2.5 in the proportions 50:50, v/v.

comparing them with reproductive Africanized (average of 9-HDA = 179.4 μg bee^{-1} and 9-ODA = 10.63 μg bee^{-1}).

After verifying the differences in quantities of pheromones between breeds studied, detected differences in quantities of pheromones 9-ODA and 9-HDA (on average) in Africanized queens reproductive and reproductive queens

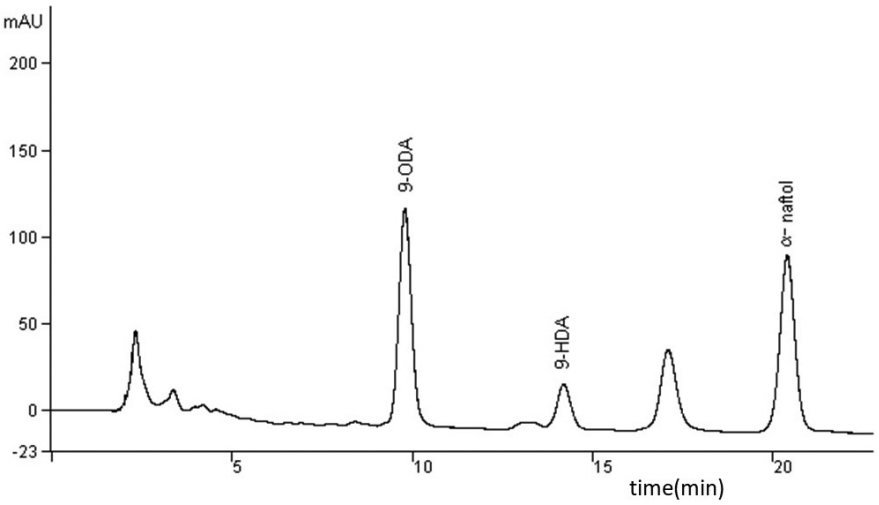

Figure 4. Chromatogram of extract of the mandibular gland of the Africanized virgin queen with only 1 day old, using as mobile phase solution of methanol and acidified water pH 2.5 in the proportions 50:50, v/v.

Table 4. Analysis of queen pheromone components of Africanized bees (*Apis mellifera* L.) and European carniolan bees (*Apis mellifera carnica*). Average quantity of pheromones on the types of queens

		Pheromone / (μg bee^{-1})				
Pheromone	Type of queen	V Min	V Max	X	SD	n
9-ODA	Africanized reproductive queen	0.38	54.27	10.63	6.42	8
	absconding by temperature Africanized queen	0.86	11.12	4.30	3.41	3
	Africanized virgin queen	4.56	11.24	6.56	1.25	5
	absconding by lack of food queen	1.17	3.72	2.60	0.75	3
	European carniolan virgin queen	66.38	69.80	67.63	1.09	3
9-HDA	European carniolan virgin queen	60.2	63.25	61.76	0.88	3
	Africanized reproductive queen	1.89	294.48	179.40	61.49	8
	absconding by temperature Africanized queen	43.66	157.30	107.39	33.53	3
	africanized virgin queen	0.95	3.54	2.08	0.42	5
	absconding by lack of food Africanized queen	18.92	211.91	109.95	55.98	3

VMin: minimum pheromone value; VMax: maximum pheromone value; X: average value; SD: standard deviation, n: number of samples; 9-ODA: (*E*)-9-oxodec-2-enoic acid; 9-HDA: (*E*)-9-hydroxydec-2-enoic acid.

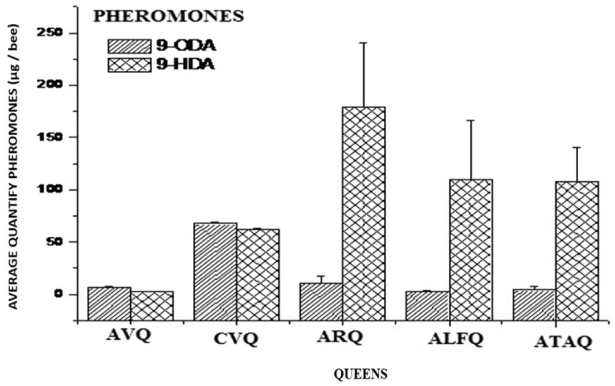

Figure 5. Analysis of queen pheromone components of Africanized bees (*Apis mellifera* L.) and European carniolan (*Apis mellifera carnica*). The average quantity of pheromones (μg bee^{-1}) on the types of queens. Types of queens tested: AVQ Africanized virgin queen, CVQ: European carniolan virgin queen, ARQ: Africanized reproductive queen, ALFQ: absconding by lack of food queen, ATAQ: absconding by temperature Africanized queen. Median and standard deviation.

behavior of Africanized who had abandoned for lack of food and high temperatures (41.8 ºC). These two factors analyzed (food and temperature), we see that the 9-HDA (reproductive = 179.40 μg bee^{-1}, food = 109.95 μg bee^{-1} and temperature = 107.39 μg bee^{-1}) is high in relation to quantities of 9-ODA (reproductive = 10.60 μg bee^{-1}, feed = 2.60 μg bee^{-1} and temperature = 4.30 μg bee^{-1}) in bees (Table 4 and Figure 5). The average quantities of 9-HDA in reproductive behavior of Africanized absconding bees are reduced compared to the quantities of 9-HDA in reproductive Africanized queens.

According to the results, a racial and reproductive difference were related to the average quantities of pheromones 9-ODA and 9-HDA. In relation to the pheromones concentration in virgin queens, it was observed that virgin queens European carniolan (Germany) presented

67.63 to 61.76 μg of 9-ODA and 9-HDA respectively, and Africanized queens virgin (Brazil) showed 6.56 μg of 9-ODA and 2.08 μg of 9-HDA. Differences were also found in previous studies with virgin queens Africanized and European (*Apis mellifera*) from other regions.[32,33] The literature also reported a queen equivalent concentration using synthetic formulations, where 9-ODA was 150 μg and 9-HDA 55 μg.[34]

Comparing the quantity of pheromones of the European carniolan reproductive queens (15.97 μg 9-ODA and 7.22 μg 9-HDA) and reproductive Africanized queens (10.63 μg 9-ODA and 179.40 μg 9-HDA) in the presented study with the reproductive Africanized (100 μg 9-ODA and 39 μg 9-HDA) and reproductive European (200 μg 9-ODA and 83 μg 9-HDA) is possible to see the notable difference values, showing the environmental influence into the pheromones individual content.[33]

Both differences in quantities of pheromones are linked to racial differences, and reproductive effects such as including secretions depends on the age and "status" of queen physiological (impregnated or not), time of day and season.[33,34] In general, the production of pheromones is higher after 6 days of adult life of the queen, reaching up to 200 μg in a queen in less than 18 months of age.[34,35] The accentuated variation in the patterns of errors for Africanized normal reproductive queens or swarms presented in our results could be also attributed to a wide variation in the age of the reproductive species, which did not occur with all virgin queens (Figure 5).

Winston[35] noted that virgin queens with less than 2 days old produced between 7 to 9 μg of 9-ODA once virgin queens with 5 to 10 days of age produced 108 to 133 μg. In the same study, the authors observed that fertilized queens in position less than 18 months of age, produced from 100 to 200 μg. Old queen in position, showed reduced production of 9-ODA.[35] According to Winston,[35] this could be attributed to the replacement of workers by the queen. In laying queen, the 9-HDA is produced in quantities considerably, less than the 9-ODA, about 5 μg *per* queen.[35] One of the functions of 9-ODA and 9-HDA is the inhibition of the development and creation of a queen, which prevents the reproduction replacement of the queen.[33,36]

In the swarming and absconding behavior, queens use 9-ODA and 9HDA pheromones to agglomerate the workers and stabilize the swarm. Abiotic and biotic factors, such as feeding, temperature, and queen size can affect pheromone content and thus influence its diffusion throughout the swarm.[34] Therefore, the pheromone content varied with increasing sample number (Figure 5). Thus, the pheromone content will be different between individuals. Literature studies[34,35] had demonstrated large variations, which are in accordance with those observed here in the present study.

The lower concentration of 9-ODA levels in Africanized bees found in this study can be attributed, among other factors, to the higher quantities of swarming process (absconding) that ocurred in Ribeirão Preto and even Mossoró. As the role of queen pheromones is a very important factor for the cohesion and maintenance of the colony, the low levels of 9-ODA would destabilize the colony. Thus, any stress or disturbance caused by environmental factors (mainly temperature) would allow circumvention of the population or even result in the constant replacement of the queens in the colony.

The literature[14-17] indicates that 9-ODA and 9-HDA pheromones play critical roles in swarming. Bees are rarely grouped only with 9-ODA content. When 9-ODA and 9-HDA are together it is observed stable clustering, wich suggests that 9-ODA acts as an attractant pheromone and 9-HDA as a colony stabilizer. Furthermore, adding a mixture of synthetic 9-ODA and 9-HDA pheromones attracted and stabilized the queenless swarm, which demonstrated the pheromones importance in the swarming process.

In contrast, swarms without queens are less attracted to the 9-ODA or 9-ODA. This was demonstrated by the study of Free[36] where a mixture of synthetic pheromones 9-ODA or 9-HDA was evaluated to the swarms attract and colony stabilize, without the bee queen. However, the higher attraction associated with the initial 9-ODA could be a reflection of its greater volatility, while the greater persistence of 9-HDA can help prolong the effect of clustering, 9-HDA can act to prolong the clustering and stabilize them.[36]

In summary, although the absolute amounts of the "queen substance" 9-ODA were similar among the three evaluated groups, the proportions of 9-ODA decreased with increasing reproductive quality. However, there does not exclude the possibility that other pheromone signals exist in the honeybee colony, but these would have to arise from other semi chemicals.

Conclusions

The methodology used in this study proved to be suitable for quantification and determination of pheromones 9-ODA and 9-HDA. The results obtained in this work when compared to the literature, show that there are differences in quantities and proportions of 9-ODA and 9-HDA in several races because of genetic variability. The low amount of 9-ODA and a large quantity of 9-HDA in Africanized queens assessed in this study may explain the large numbers swarming for absconding and may reflect greater adaptation

to the activities of our race with high rates of the swarming process existing mainly in northeastern Brazil, as these two pheromones contribute to stabilization and cohesion of swarms.

The pheromones tested (9-ODA and 9-HDA) are directly related to the swarming process (absconding) since a decrease or an increase in both factors analyzed cause output weight of individuals (lack of food and temperature) in natural or artificial conditions. So, the present study contributes to a better understanding of the contribution of pheromone to many honeybees processes, being a potential tool for other pheromone studies.

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Author Contributions

All authors contributed significantly and equally to this manuscript.

References

1. Slessor, K. N.; Winston, M. L.; Le Conte, Y.; *J. Chem. Ecol.* **2005**, *31*, 2731. [Crossref]
2. Slessor, K. N.; Kaminski, L. A; King, G. G. S.; Borden, J. H.; Winston, M. L.; *Nature* **1988**, *332*, 354. [Crossref]
3. Naumann, K.; Winston, N. L.; Slessor, K. N.; Prestiwich, G. D.; Webster, F. X.; *Behav. Ecol. Sociobiol.* **1991**, *29*, 321. [Crossref]
4. Naumann, K.; Winston, M. L.; Slessor, K. N.; Prestwich, G. D.; Latli, B.; *Can. Entomol.* **1992**, *124*, 917. [Crossref]
5. Seeley, T. D.; *Behav. Ecol. Sociobiol.* **1979**, *5*, 391. [Crossref]
6. Naumann, K.; Winston, M. L.; Wybern, M. H.; Slessor, K. N.; *J. Econ. Entomol.* **1990**, *83*, 1271. [Crossref]
7. Naumann, K.; Winston, M. L.; Slessor, K. N.; *J. Insect Behav.* **1993**, *6*, 211. [Crossref]
8. Pankiw, T.; *Apidologie* **2004**, *35*, 217. [Crossref]
9. Beggs, K. T.; Glendining, K. A.; Marechal, N. M.; Vergoz, V.; Nakamura, I.; Slessor, K. N.; Mercer, A. R.; *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 2460. [Crossref]
10. Brockmann, A.; Dietz, D.; Spaethe, J., Tautz, J.; *J. Chem. Ecol.* **2006**, *32*, 657. [Crossref]
11. Plettner, E.; Slessor, K. N.; Winston, M. L.; Oliver J. E.; *Science* **1996**, *271*, 1851. [Crossref]
12. Meikle, W. G; Weiss, M.; *Apidologie* **2016**, *47*, 14. [Crossref]
13. Meikle, W. G.; Barg, A.; Weiss, M.; *Apidologie* **2022**, *53*, 51. [Crossref]
14. Butler, C. G.; Fairey, E. M.; *J. Apic. Res.* **1964**, *3*, 65. [Crossref]
15. Butler, C. G.; Simpson, J.; *Proc. R. Entomol. Soc.* **1967**, *42*, 149. [Crossref]
16. Morse, R. A.; Boch, R.; *Ann. Entomol. Soc. Am.* **1971**, *64*, 1414. [Crossref]
17. Boch, R.; Shearer, D. A.; Young, J. C.; *J. Chem. Ecol.* **1975**, *1*, 133. [Crossref]
18. Winston, M. L.; *The Biology of the Honey Bee*; Harvard University Press: Cambridge, Massachusetts, 1987.
19. Strauss, K.; Scharpenberg, H.; Crewe, R. M.; *Behav. Ecol. Sociobiol.* **2008**, *62*, 1523. [Crossref]
20. Mori, K.; *Bioorg. Med. Chem.* **2007**, *15*, 7505. [Crossref]
21. Trulin, M.; Rajchard, J.; *Vet. Med.* **2011**, *6*, 265. [Crossref]
22. Tan, K.; Yang, M.; Wang, Z.; Radlof, S.; Pirk, C. W. W.; *Comp. Physiol. A* **2012**, *198*, 319. [Crossref]
23. Yusuf, A. A.; Pirk, C. W. W.; Crewe, R. M.; *Apidologie* **2015**, *46*, 559. [Crossref]
24. Niu, D. F.; Pirk, C. W. W.; Zheng, H.-Q.; *Apidologie* **2016**, *47*, 515. [Crossref]
25. Princen, S. A.; Oliveira, R. C.; Ernst, U. R.; Millar, J. G.; Van Zweden, J. S.; Wenseleers, T.; *Proc. R. Soc. B* **2019**, *286*, 517. [Crossref]
26. Grozinger, C. M.; Richards, J.; Mattila, H. R.; *Apidologie* **2014**, *45*, 327. [Crossref]
27. Koshio, S.; Almeida-Muradian, L. B.; *Quim. Nova* **2003**, *26*, 670. [Crossref]
28. Baier, T.; Neuwirth, E.; *Comput. Stat.* **2007**, *22*, 91. [Crossref]
29. Free, J. B.; *A Organização Social das Abelhas Apis*; Edusp: São Paulo, Brazil, 1980, p. 79.
30. Pettis, J. S.; Winston, M. L.; Collins, A. M.; *Insectes Soc.* **1995**, *42*, 113. [Crossref]
31. Carvalho, C. A. L.; Bento, J. M. S.; Marchini, L. C.; *Feromônios de Insetos: Biologia, Química e Aplicação*, 2nd ed.; Holos: Ribeirão Preto, Brazil, 2001, p. 83.
32. Almeida, G. F.: *Fatores que Interferem no Comportamento Enxameatório de Abelhas Africanizadas*; PhD Thesis, Universidade de São Paulo, Ribeirão Preto, 2008. [Link] accessed in July 2023
33. Pankiw, T.; Winston, M. L.; Plettner, E.; Slessor, K.; *J. Chem. Ecol.* **1996**, *22*, 605. [Crossref]
34. Slessor, K. N.; Kaminski, L. A.; King, G. G. S.; Winston, M. L.; *J. Chem. Ecol.* **1990**, *16*, 851. [Crossref]
35. Winston, M. L.; *A Biologia da Abelha*; Magister: Porto Alegre, 2003, p. 273.
36. Free, J. B.; *Pheromones of Social Bees*; Bee Research Unit. Cornell University Press: London, UK, 1987.

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