



# Role of juvenile hormone in oogenesis, chemical profile, and behavior of the wasp *Mischocyttarus consimilis* (Vespidae: Polistinae)

Erika Fernandes Neves<sup>1,2</sup> · Thiago dos Santos Montagna<sup>1,2</sup> · Kamylla Balbuena Michelutti<sup>2,3</sup> · Sidnei Eduardo Lima-Junior<sup>3</sup> · Claudia Andréa Lima Cardoso<sup>3</sup> · William Fernando Antonialli-Junior<sup>1,2,3</sup>

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## Abstract

In social insects, juvenile hormone (JH) affects the degree of ovarian development, reproductive status, and temporal polyethism in workers. JH also contributes to determining the epicuticular chemical composition, which differentiates the castes of queens and workers. However, a few studies have evaluated the action of JH on behavioral ontogeny, cuticular chemical profile, and oocyte length and width, especially in social wasps of independent foundation. Therefore, the following hypotheses were tested: (i) topical application of JH changes the behavioral ontogeny of newly emerged workers; and (ii) changes might be detected in the cuticular chemical composition and oocyte length and width of newly emerged females receiving topical application of JH. The treatment consisted of application of JH, at a concentration of 25  $\mu\text{g}\cdot\mu\text{L}^{-1}$  in acetone, to 1-day-old *Mischocyttarus consimilis* workers. The application of JH to newly emerged *M. consimilis* females significantly altered oocyte length and width, with effects on behavioral ontogeny and the cuticular chemical compounds signaling these parameters in the colony. No effects of the solvent on female physiology were observed, reinforcing that the observed changes were due to the specific effects of JH.

**Keywords** Foraging · Aggressiveness · Cuticular hydrocarbons · Independent foundation · Social wasps

## Introduction

The reproductive division of labor is one of the remarkable features among social insects. In this model of division, one or a few females may be responsible for laying

eggs, while the rest of the colony works to maintain the resources necessary for the success of the group, involving feeding, cleaning, caring for the immature, and colony defense (Wilson 1971). Species of independent foundation build simple nests without a protective envelope, founded by one or a few females (Gadagkar 1991; Jeane 1991). A feature shared among these species is slight or absent morphological differentiation between queens and workers (Robinson and Vargo 1997). Furthermore, the workers do not lose the ability to reproduce (Gadagkar et al. 1991). However, due to greater access to food and more aggressive behavior, the queens maintain a high level of ovarian development and avoid high-risk tasks that have high energy costs (Queller and Strassmann 1989; Röseler 1991; O'Donnell 1998; Torres et al. 2012).

In social insects, the division of labor is mediated, among other factors, by juvenile hormone (JH), which modulates the physiology and behavior of queens and workers (Robinson and Vargo 1997). In *Apis mellifera* bees, JH is known to modulate caste determination, with its level being higher in critical steps during the development of queen larvae (Rembold et al. 1992). The JH level also modulates extranidal

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✉ Kamylla Balbuena Michelutti  
kamylla\_michelutti@yahoo.com.br

Erika Fernandes Neves  
erika\_snakes@yahoo.com.br

William Fernando Antonialli-Junior  
williamantonialli@yahoo.com.br

<sup>1</sup> Programa de Pós-Graduação Em Entomologia e Conservação da Biodiversidade, Universidade Federal da Grande Dourados, Dourados, MS, Brazil

<sup>2</sup> Laboratório de Ecologia Comportamental (LABECO), Universidade Estadual de Mato Grosso do Sul, Dourados, MS, Brazil

<sup>3</sup> Programa de Pós-Graduação Em Recursos Naturais, Universidade Estadual de Mato Grosso do Sul, Dourados, MS, Brazil

activities in *Apis* workers (Robinson 1992). In the newly emerged individuals, the level of JH in the hemolymph is low, while its synthesis by the corpora allata gradually increases with age, leading to changes in the development of intranidal tasks, culminating in foraging (Huang et al. 1991).

The topical application of JH alters its level in the hemolymph, affecting the age polyethism. This mechanism of task specialization has been observed for the leaf-cutting ant *Acromyrmex octospinosus* (Reich 1793) (Norman et al. 2016) and *A. mellifera* (Sullivan et al. 2000), where the application of JH in young workers advanced the foraging age. In social wasps, although the effects of JH have been less investigated, studies with wasps of the genus *Polybia* have indicated that JH is responsible for the production of new foragers, accelerating temporal polyethism, defined as the modification of tasks with increasing age (Wilson 1985; O'Donnell and Jeanne 1993; O'Donnell 1998). Even in species of independent foundation, such as *Polistes canadensis*, increase of the JH titer anticipates implementation of the guard task and increases the number of foragers (Giray et al. 2005). In colonies of *Polistes fuscatus*, increase of the JH titer was found to modulate sexual receptivity, promoting the development of ovaries, but negatively affected female life expectancy (Walton et al. 2020). However, there is a need for further studies concerning the effects of JH in social wasps. In *Ropalidia marginata*, the Indian paper wasp, changes in the JH titer did not lead to altered temporal polyethism (Agrahari and Gadagkar 2003). Evidence suggests that the JH titer is equally important for social wasps and bees. The work of Montagna et al. (2015) evidenced that the JH titer may even be decisive in the determination of castes in social wasps of independent foundation, in this case being, at least in part, pre-imaginal.

In addition to JH, cuticular hydrocarbons (CHCs) are also important in modulating and mediating behaviors in colonies of social insects, constituting part of their chemical communication (Hölldobler and Wilson 1990; Billen 2006). The qualitative and quantitative variation of these compounds present in the epicuticle of insects signals a variety of information to other individuals, enabling cohesion of the colonies (Blomquist and Bagnères 2010). Studies have shown a relationship between the JH titer and the CHCs' composition, reflected in differential abundances of these compounds between workers and queens (Bonavita-Cougourdan et al. 1991; Sledge et al. 2001). Studies including those of Torres (2014); Soares et al. (2014); and Oi et al. (2019) have shown that for females of different species of social wasps, there is a relationship between the CHCs' composition and physiological condition. Oliveira et al. (2017) found a correlation among reproductive status, JH titer, and CHC composition in females of *Vespa vulgaris*. Studies with colonies of *Polistes dominula* (Sledge et al. 2001, 2004) and

*Polistes metricus* (Judd et al. 2010) also showed a relationship between JH levels and the CHCs' composition.

However, although several studies have identified a relationship between JH levels and CHCs composition, only a single study has investigated the effects of JH on behavior, physiology, and CHCs' composition (Kelstrup et al. 2017). The latter study was carried out with a species of social wasp in a temperate climate, reinforcing the need for further studies elsewhere. Therefore, the present work investigates the effect of topical application of JH on behavioral ontogeny, oocyte length and width, and CHCs in newly emerged workers of the species *Mischocyttarus consimilis*, a social wasp from a tropical climate region. Two hypotheses were tested: (i) there is significant alteration of the oocyte length and width and behavioral ontogeny of newly emerged workers receiving topical application of JH; and (ii) these modifications lead to a significant change in the CHCs.

## Materials and methods

### Collection of individuals and treatments

The study involved the experimental manipulation of 11 colonies of the eusocial wasp *Mischocyttarus consimilis*, employing a total of 98 females in the phase of production of workers (Keeping 2002), under natural conditions in rural areas of the municipality of Dourados, Mato Grosso do Sul State, Brazil (22°13'16" S, 54°48'20" W).

Newly emerged 1-day-old females were collected from their nests using pincers, marked with nontoxic ink on a right leg and then subjected to the different procedures. Thirty-five workers received application of JH (Hormone III Code J2000, Sigma-Aldrich, São Paulo, Brazil), using acetone as solvent, while 35 workers received application of acetone alone, enabling evaluation of the effects of the application of JH, in relation to the solvent. Twenty-eight workers were used as controls, without receiving any type of treatment, and were submitted to the same analyses employed for the treatment groups.

Females that received JH treatment were subjected to a single application of JH at a concentration of 25  $\mu\text{g}\cdot\mu\text{L}^{-1}$ , following the methodology proposed by Montagna et al. (2015). The application was performed with 1-day-old females, because at this age, the wasps of this species have not yet acquired the colonial signature (Neves et al. 2012). In the treatment with the solvent, the females were subjected to a single application of 1  $\mu\text{L}$  of acetone, at the same age. For the behavioral observations, the females submitted to the treatments were divided into two groups. The first group, with ages from 1 to 5 days, was considered young, while the second group consisted of females with ages between

6 and 12 days, considered to be older individuals (Torres et al. 2011).

### Effects of topical application of JH on oocyte length and width and behavior

Following the treatment with JH, the death/disappearance rates were recorded during the entire period of the experiment, noting the number of wasps that exited the colony every day after the applications. All the marked females were monitored for 12 days, 4 h per day (9:00–11:00 am and 2:00–4:00 pm), between September 2013 and September 2015. The period of 12 days was chosen, because it allowed the *M. consimilis* workers to develop all the intranidal and extranidal behaviors of the ontogeny of their behavioral repertoire (Torres et al. 2011). The behavioral repertoires were evaluated using the focal animal method (Del Claro 2010), based on the behavioral acts described by Torres et al. (2011). The observations were divided into sessions of 1 h, during the peak of activities described for *M. consimilis* (Torres et al. 2011), recording the number of behaviors exhibited by each marked individual.

For both young and old females, the behaviors quantified during 12 days were grouped into three categories, based on the study of Bruyndonckx et al. (2006): 1—aggressive acts (butt given, butt received, dispute for food, and request for food); 2—inactivity (remaining still); 3—foraging (foraging for nectar, water, and wood pulp, and unsuccessful foraging). The inactivity behavior was observed as a response to the application of JH, due to changes in oocyte length and width. Larger ovaries reduce the foraging behavior, so the wasp becomes more immobile and leaves the nest less frequently (Montagna et al. 2015). Regarding foraging, JH might alter the temporal polyethism, leading females submitted to JH treatment to begin foraging earlier (Sullivan et al. 2000; Giray et al. 2005).

Since the JH treatment led to physiological changes, the level of aggressiveness was evaluated. Alterations in ovary development may cause wasps to become more or less aggressive, compared to control wasps (Röseler et al. 1984; Giray et al. 2005). The foraging frequencies of the females were also recorded. Work with social insects has shown that application of JH promotes earlier initiation of foraging, with the insects presenting anticipating behavioral ontogeny (O'Donnel and Jeanne 1993). For the same reason, the frequency of inactivity was noted, since changes in oocyte length and width affect the performance of this behavior. Twelve days after the JH treatment, the wasps were collected using pincers.

For analysis of ovarian development, the gasters were dissected in a Petri dish containing saline solution. Determination of the average length of the six largest oocytes was used to obtain the individual ovarian development index (Giray

et al. 2005), with measurements made using a stereomicroscope (Stemi 2000C, Carl Zeiss, Oberkochen, Germany) fitted with a micrometric eyepiece.

### Effect of topical application of JH on cuticular hydrocarbons determined by gas chromatography–mass spectrometry

Gas chromatography coupled with mass spectrometry was used to analyze the CHCs of the thorax of each collected female, after dissection of the gaster for evaluation of the ovaries. Each sample was incubated for 2 min in 2 mL of hexane, in a glass flask. The solution resulting from the extraction was dried under an exhaust hood and stored at  $-20\text{ }^{\circ}\text{C}$  for a maximum of 30 days. For chromatographic analysis, the extracts were solubilized in 200  $\mu\text{L}$  of hexane.

The samples (1  $\mu\text{L}$ ) were analyzed using a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) coupled to a mass detector (GCMS Ultra 2010, Shimadzu, Kyoto, Japan). The chromatograph was fitted with a fused silica DB-5 capillary column (5% phenyl-dimethylpolysiloxane, 30 m length  $\times$  0.25 mm diameter  $\times$  0.25  $\mu\text{m}$  film thickness, J & W, Folsom, California, USA). Helium gas (99.999%) was used as the mobile phase, at a flow rate of 1.0  $\text{mL}\cdot\text{min}^{-1}$  (in splitless injection mode). The heating program started at 150  $^{\circ}\text{C}$ , followed by a ramp to 300  $^{\circ}\text{C}$ , at 3  $^{\circ}\text{C}\cdot\text{min}^{-1}$ , and holding at 300  $^{\circ}\text{C}$  for 10 min. The injector temperature was set at 250  $^{\circ}\text{C}$ , while the detector and transfer line temperatures were set at 300  $^{\circ}\text{C}$ . The mass spectrometer was operated using electron impact ionization voltage of 70 eV, mass range from  $m/z$  45 to 600, and scan interval of 0.3 s.

Identification of the compounds was performed using the calculated retention index (Van den Dool and Kratz 1963), according to the following equation:

$$\text{LRI} = \frac{R_t X - R_t N}{R_t N_{+1} - R_t N} + 100.n.$$

LRI: linear retention index.

$R_t X$ : component retention time.

$R_t N$ : retention time of the N alkane with retention time prior to the X component.

$R_t N_{+1}$ : retention time of the N alkane with retention time after the X component.

n: number of carbon atoms of alkane N.

The numbers of carbon atoms in the compounds were determined using a series of linear alkanes ( $\text{C}_7$ – $\text{C}_{40}$ , purity  $\geq 95\%$ , Sigma-Aldrich) and comparison with the literature (Bonavita-Courgourdan et al. 1991; Howard 2001; Howard et al. 2001; Abdalla et al. 2003; Zhu et al. 2006; Bonckaert et al. 2012; Costanzi et al. 2013; Weiss et al. 2015) or with mass spectral data from the equipment library (NIST 21 and Wiley 229). The major compounds

were considered to be those with relative percentage area greater than 4%.

## Statistical analysis

The data for the degree of ovarian development in the females were submitted to analysis of variance (ANOVA), using a logarithmic function to homogenize the data variance (Bussab and Morretin 2002). For analysis of the differences between the groups, the Tukey post hoc test was applied (Hair et al. 2009). The effect of the application of JH on the behavioral ontogeny of the females was analyzed using the nonparametric chi-square test ( $X^2$ ), with a significance level of  $p < 0.05$  (Siegel and Castellan-Junior 2006). The same test was applied to the relative frequencies of performance of aggressive behavior (typical of dominants) and foraging (typical of workers), since foraging frequency is expected to be higher in older females (Torres et al. 2012).

Discriminant analysis was used to analyze the effects of the topical applications of the treatments on CHCs, in comparison to the control group. The relative abundances of the chromatogram peaks were used to identify any significant differences between the CHCs of the females in the acetone, JH, and control groups (Quinn and Keough 2005). In this analysis,  $p \leq 0.05$  was considered significant.

## Results

### Effects of topical application of JH on oocyte length and width and behavior

Of the total of 98 females, 32 individuals had their behavioral ontogeny monitored during 12 days. The mortality/disappearance rates were 71% of females treated with JH, 68.5% of females treated with acetone, and 60.7% of females that did not receive any treatment (control).

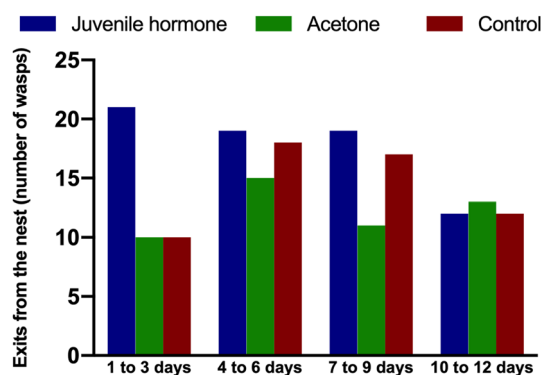
Table 1 shows the average length and width measurements of the oocytes. The results revealed significant differences among the oocyte lengths for the control, acetone, and JH group females (ANOVA,  $F = 39.53$ ,  $p = 0.03$ ). Application of the Tukey test revealed that the JH group females had significantly larger oocytes ( $p < 0.05$ ), compared to the control group.

**Table 1** Measurements of oocyte length and width for the *Mischocyttarus consimilis* females submitted to different treatments

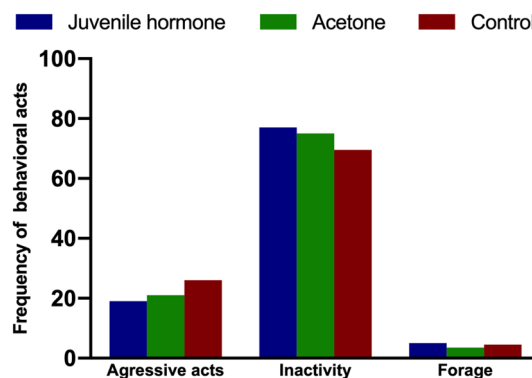
Treatments	Length (mm)	Width (mm)
Control ( $n = 10$ )	$1.16 \pm 0.33$	$0.75 \pm 0.15$
Acetone ( $n = 9$ )	$1.68 \pm 1.41$	$0.74 \pm 0.52$
Juvenile hormone ( $n = 11$ )	$1.93 \pm 0.73$	$1.00 \pm 0.25$

The foraging frequencies showed that the wasps treated with JH started to forage within 3 days after emergence, whereas the wasps of the acetone and control groups went out to forage more frequently from the 4th day after emergence (Fig. 1). The chi-square analysis revealed that for the number of foraging events within 3 days following emergence, there were significant differences between the JH and control groups ( $X^2 = 3.90$ ,  $p = 0.04$ ), as well as between the JH and acetone groups ( $X^2 = 3.90$ ;  $p = 0.04$ ). No difference was observed between the control and acetone groups ( $p > 0.05$ ). For the older wasps, there were no significant differences in foraging frequency among the groups ( $p > 0.05$ ).

Figure 2 shows the behavioral categories that were analyzed. Chi-square analysis showed no significant differences in the frequency of aggressive behavior between the JH and control groups ( $X^2 = 1.14$ ,  $p = 0.19$ ), the acetone and control groups ( $X^2 = 0.40$ ,  $p = 0.53$ ), and the JH and acetone groups ( $X^2 = 0.19$ ,  $p = 0.66$ ). Furthermore, there were no significant differences in the level of inactivity between the JH and control groups ( $X^2 = 0.32$ ,  $p = 0.57$ ), the acetone and control groups ( $X^2 = 0.17$ ,  $p = 0.68$ ), and the JH and acetone groups ( $X^2 = 0.02$ ,  $p = 0.89$ ). A similar trend was



**Fig. 1** Numbers of foraging exits from the nest



**Fig. 2** Relative frequencies of behaviors, divided into three behavioral categories

observed for foraging frequency, comparing the JH and control groups ( $X^2=0.02$ ,  $p=0.89$ ), the acetone and control groups ( $X^2=0.04$ ,  $p=0.85$ ), and the JH and acetone groups ( $X^2=0.11$ ,  $p=0.74$ ).

Figure 3 shows the frequencies with which the wasps performed foraging in search of resources. Significant differences between the JH and control wasps were observed for foraging prey ( $X^2=16.58$ ,  $p<0.01$ ), nectar ( $X^2=6.84$ ,  $p<0.01$ ), and water ( $X^2=8.72$ ,  $p<0.01$ ). The acetone and control groups showed no significant differences in the frequencies of foraging for any resource (prey:  $X^2=0.45$ ,  $p=0.50$ ; nectar:  $X^2=0.00$ ,  $p=1.00$ ; water:  $X^2=1.10$ ,  $p=0.29$ ; wood pulp:  $X^2=0.00$ ,  $p=1.00$ ). With the exception of wood pulp ( $X^2=0.43$ ,  $p=0.51$ ), significant differences were found between the foraging frequencies of the JH and acetone groups (prey:  $X^2=22.06$ ,  $p<0.01$ ; nectar:  $X^2=6.84$ ,  $p<0.01$ ; water:  $X^2=15.51$ ,  $p<0.01$ ).

### Analysis of the effect of topical application of JH on cuticular hydrocarbons using gas chromatography-mass spectrometry

The different treatments resulted in qualitative and quantitative variations of the CHCs (Table 2, Fig. 4). The percentage of branched alkanes was higher than that of linear alkanes, with the treatments using JH and acetone causing no change in the percentages of compounds of the two categories, compared to the control. However, the application of JH reduced the number of branched alkanes. About 80% of the compounds were found in all three groups of females. Four compounds were unique to the JH treatment, two were only found in samples from the acetone treatment, and two were exclusive to the control samples (Table 2).

Major compounds found in the three groups were 3-methylheneicosane, heptacosane, 3-methylheptacosane, 3-methylnonacosane, triacontane, and 13, 17-dimethylhentriacontane. Nonacosane was a major compound for the JH treatment (Table 2). Discriminant analysis revealed

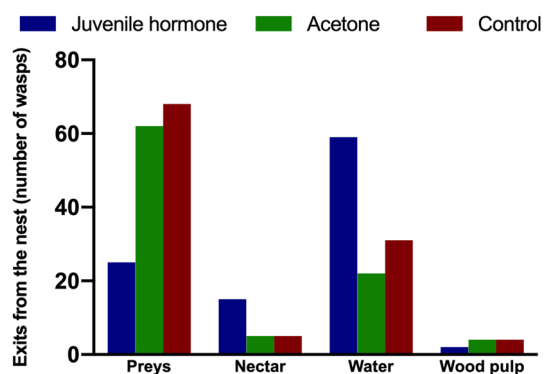


Fig. 3 Relative foraging frequencies for different items

differences in the CHCs profiles among the three treatments (Wilks' lambda=0.000,  $F=49.029$ ,  $p=0.000$ ). The first canonical root explained 99% of the results, while the first and second canonical roots together explained 100% of the results (Fig. 5). The statistical analysis indicated ten peaks as being most significant for separation of the treatments (Fig. 5, Table 3).

## Discussion

The results demonstrated that topical application of JH to newly emerged *M. consimilis* females acted to modify oocyte length and width and ontogeny of the behavioral repertoire, in agreement with previous work (Giray et al. 2005). The treatment with JH also altered the chemical composition of the epicuticle of *M. consimilis*. These findings corroborated the observation that application of JH in workers of *V. vulgaris* led to the occurrence of CHCs' signals similar to those produced by the queen, suggesting that these signals were under the control of JH (Oliveira et al. 2017).

The oocytes from the females treated with JH were larger than those from the control females, leading to significant behavioral alterations. Previous studies found that topical application of JH could induce physiological modification, accompanied by behavioral changes, in the wasp species *Belonogaster longitarsus* (Kelstrup et al. 2017) and *Polistes fuscatus* (Walton et al. 2020). In the ant species *Dinoponera quadriceps*, Norman et al. (2019) observed alteration of reproductive physiology after application of JH, although no change in dominance was noted. In *Vespa vulgaris*, application of JH did not alter ovary development, but it was demonstrated that *V. vulgaris* queen fertility and signals production were both under the control of JH (Oliveira et al. 2017).

Aggressiveness, inactivity, and foraging are behaviors that can indicate behavioral changes due to physiological modifications, since females with larger oocytes tend to have higher hierarchical rank, becoming more aggressive and inactive, and performing less foraging. In *B. longitarsus*, increase of the hemolymph JH titer was not related to dominance behaviors Kelstrup et al. (2017), similar to the present findings. The application of JH was more important for modulating ovarian development and the CHCs profile. In *M. consimilis* colonies, subordinate females foraged more frequently than dominant females that displayed higher aggression (Torres et al. 2012).

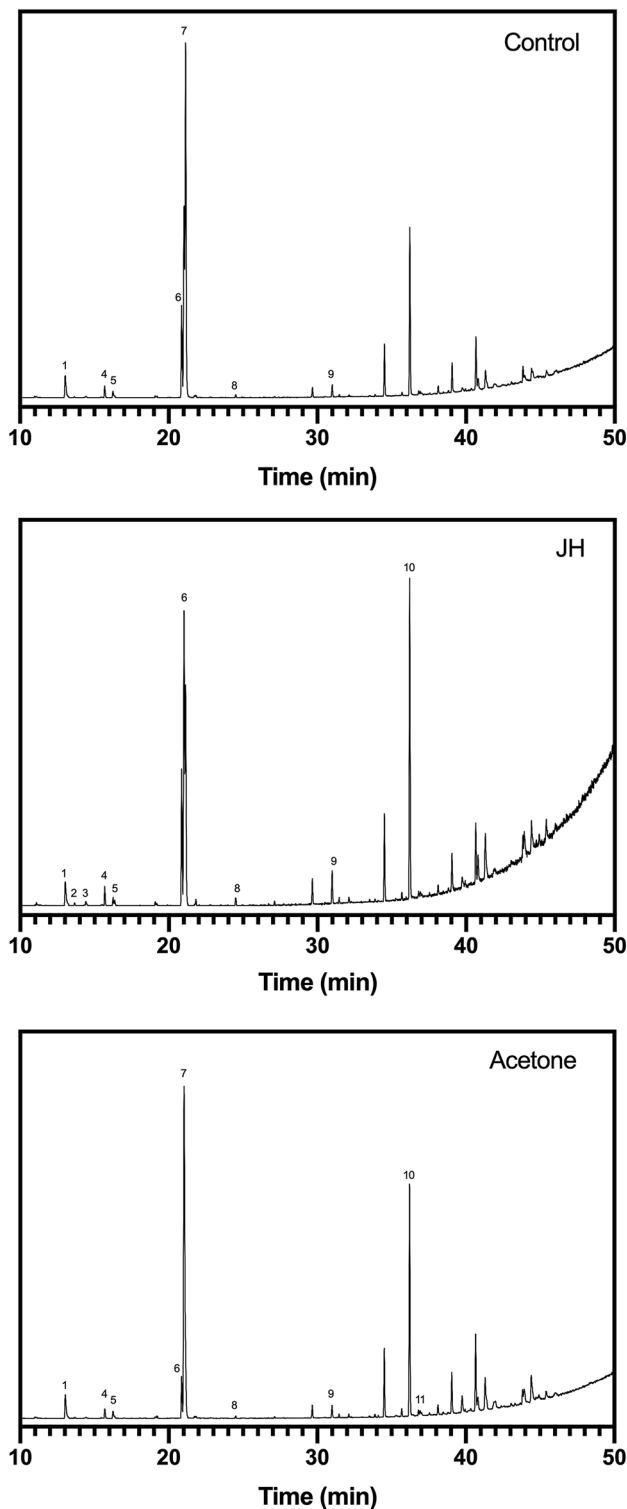
Analysis of the items foraged by *M. consimilis* showed that the wasps treated with JH reduced foraging for prey, presenting preference for the foraging of water and nectar. This behavior might reflect changes in their physiology, since larger oocytes influence the hierarchical rank in colonies (O'Donnell 1998). Dominant females forage less for prey,

**Table 2** Relative abundances (mean  $\pm$  SD) of cuticular hydrocarbons from *Mischocyttarus consimilis* wasps submitted to different treatments

Compound	Calculated index	Juvenile hormone (%)	Acetone (%)	Control (%)
NI	1753	Tr	–	–
Octadecane	1800	Tr	–	Tr
3-Methyloctadecane	1875	5.80 $\pm$ 2.90*	2.50 $\pm$ 2.27	4.07 $\pm$ 3.40
Nonadecane	1900	Tr	–	–
9-Methylnonadecane	1929	Tr	0.15 $\pm$ 0.29	0.18 $\pm$ 0.42
3-Methylnonadecane	1973	0.11 $\pm$ 0.32	Tr	0.12 $\pm$ 0.33
1-Eicosene	1997	Tr	Tr	0.14 $\pm$ 0.25
Eicosane	2000	0.15 $\pm$ 0.19	Tr	0.17 $\pm$ 0.23
1-Heneicosene	2097	0.01 $\pm$ 0.04	0.04 $\pm$ 0.11	0.12 $\pm$ 0.14
Heneicosane	2100	0.14 $\pm$ 0.15	0.11 $\pm$ 0.16	0.10 $\pm$ 0.18
2-Methylheneicosane	2162	1.22 $\pm$ 3.07	0.91 $\pm$ 1.29	1.69 $\pm$ 2.86
3-Methylheneicosane	2169	6.56 $\pm$ 13.40*	31.40 $\pm$ 23.10*	20.40 $\pm$ 18.40*
Docosane	2200	0.31 $\pm$ 0.17	0.15 $\pm$ 0.18	0.22 $\pm$ 0.22
Tricosane	2300	0.18 $\pm$ 0.17	0.10 $\pm$ 0.14	0.47 $\pm$ 0.26
Tetracosane	2400	0.18 $\pm$ 0.16	Tr	0.15 $\pm$ 0.15
Pentacosane	2500	0.81 $\pm$ 0.38	0.76 $\pm$ 0.33	1.55 $\pm$ 0.64
5-Methylpentacosane	2552	1.70 $\pm$ 0.94	1.26 $\pm$ 0.82	2.11 $\pm$ 1.18
3-Methylpentacosane	2570	0.12 $\pm$ 0.16	Tr	0.21 $\pm$ 0.24
Hexacosane	2600	0.37 $\pm$ 0.55	1.22 $\pm$ 2.90	0.24 $\pm$ 0.12
2-Methylhexacosane	2654	0.85 $\pm$ 2.38	1.04 $\pm$ 2.69	0.14 $\pm$ 0.14
NI	2668	Tr	Tr	0.20 $\pm$ 0.19
NI	2678	–	Tr	–
Heptacosane	2700	5.91 $\pm$ 2.42*	4.43 $\pm$ 2.53	6.76 $\pm$ 2.13*
9-Methylheptacosane	2729	–	–	Tr
5-Methylheptacosane	2748	0.33 $\pm$ 0.26	0.32 $\pm$ 0.26	0.36 $\pm$ 0.21
3-Methylheptacosane	2772	36.27 $\pm$ 9.60*	23.83 $\pm$ 6.97*	29.95 $\pm$ 7.83*
Octacosane	2800	0.42 $\pm$ 0.33	0.22 $\pm$ 0.21	0.51 $\pm$ 0.57
NI	2803	Tr	Tr	0.14 $\pm$ 0.25
14-Methyloctacosane	2831	–	–	Tr
2-Methyloctacosane	2858	1.23 $\pm$ 0.29	0.85 $\pm$ 0.35	1.02 $\pm$ 0.62
Nonacosane	2900	5.01 $\pm$ 2.30*	3.79 $\pm$ 1.84	3.54 $\pm$ 1.74
9-, 11-, 13-, 15-Methylnonacosane	2930	0.78 $\pm$ 0.83	1.13 $\pm$ 1.43	0.87 $\pm$ 0.95
7-Methylnonacosane	2940	–	Tr	–
9,15-Dimethylnonacosane	2957	–	0.23 $\pm$ 0.37	0.14 $\pm$ 0.37
3-Methylnonacosane	2974	12.57 $\pm$ 5.74*	8.74 $\pm$ 3.99*	6.18 $\pm$ 4.08*
5,9-Dimethylnonacosane	2989	1.94 $\pm$ 0.94	1.83 $\pm$ 0.63	2.01 $\pm$ 0.74
Triacontane	3000	4.11 $\pm$ 1.02*	4.82 $\pm$ 2.16*	5.63 $\pm$ 2.35*
14-Methyltriacontane	3034	–	0.52 $\pm$ 0.90	0.59 $\pm$ 1.16
10,14-Dimethyltriacontane	3058	1.61 $\pm$ 4.55	–	–
3-Methyltriacontane	3075	–	0.17 $\pm$ 0.29	0.16 $\pm$ 0.43
Hentriacontane	3100	–	Tr	–
11-, 13-Methylhentriacontane	3122	0.96 $\pm$ 0.88	1.79 $\pm$ 1.54	3.65 $\pm$ 1.86
11-Methylhentriacontane	3130	1.66 $\pm$ 1.32	1.24 $\pm$ 1.11	0.72 $\pm$ 1.33
13,17-Dimethylhentriacontane	3153	6.40 $\pm$ 2.52*	4.64 $\pm$ 2.40*	4.32 $\pm$ 2.90*
3-Methylhentriacontane	3177	Tr	0.26 $\pm$ 0.28	0.11 $\pm$ 0.28
Dotriacontane	3200	1.39 $\pm$ 0.92	1.39 $\pm$ 1.06	1.08 $\pm$ 0.86
NI	3416	0.65 $\pm$ 1.84	–	–

SD standard deviation, NI not identified, Tr trace (<0.1%). Tr compounds were those identified in the samples with relative abundance equal to or less than 0.1%.

\*Major compounds



**Fig. 4** Discriminant analysis dispersion diagram, showing the two canonical roots for differentiation of *Mischocyttarus consimilis*

avoiding higher risks and energy-intensive tasks (Ross and Matthews 1991; O'Donnell 1998; Torres et al. 2011, 2012), corroborating the present findings, since the *M. consimilis*

females treated with JH foraged for materials that were less costly in terms of energy and risk.

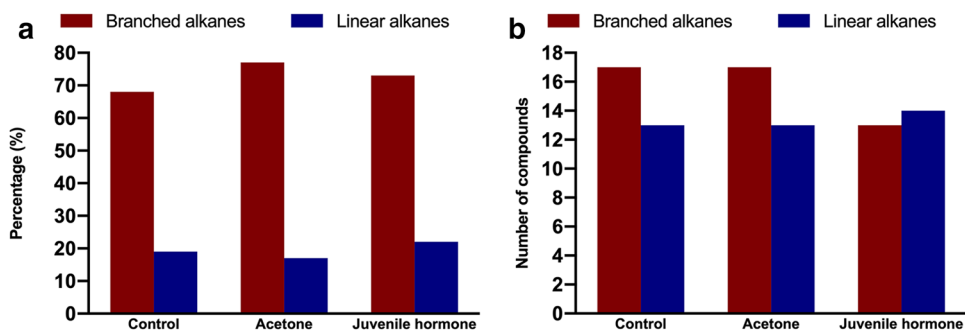
The application of JH also changed the ontogeny of the behavioral repertoire. Newly emerged females treated with JH began foraging earlier and displayed a higher frequency of foraging during the first 3 days following emergence (Fig. 1). Similar features were described for newly emerged *A. mellifera* bees (Robinson and Ratnieks 1987) and *P. canadensis* wasps (Giray et al. 2005), where the application of JH advanced the foraging activities among the workers. Therefore, the effect of JH in modulating and advancing foraging varies among different species of social Hymenoptera, based on different levels of sociability.

Following the treatments with JH and acetone, a high percentage of the females disappeared from the colonies. A possible explanation was death due to handling, marking, or toxic effects of the solvent. An additional possibility was related to changes in oocyte length and width and behavior of the females, with reduced tolerance of other queen-like individuals. Some individuals may have been expelled for not fitting in the hierarchical ranking, or may have left their colonies to create new ones (Gianotti and Machado 1999; Zara and Balestieri 2000).

Acetone, the solvent used in this study, did not have any effects on oocyte length and width or behavior, as reported previously (Tibbetts et al. 2018; c; Jatsch and Ruther 2021). Therefore, the observed effects could be attributed to the treatment with JH. Nonetheless, other studies suggested that acetone might affect the performance of *A. mellifera* (Paes de Oliveira and Cruz Landim 2001; Pinto et al. 2002), indicating the need for further investigations concerning the effects of solvents in social insects. The JH treatment altered the cuticular chemical signature (Fig. 5), which could be explained, at least in part, by changes in oocyte length and width and/or behavior (Oliveira et al. 2017), since these compounds mediate interactions within the colony, signaling the hierarchical rank (Sledge et al. 2004; Monnin et al. 1998). In the ant *Myrmicaria eumenooides*, treatment with JH also led to modification of the chemical signature of workers performing intranidal tasks, which then resembled the profile for the foragers (Lengyel et al. 2007). Kelstrup et al. (2017) found a clear association among JH, ovarian status, and the proportions of CHCs classes in females of *B. longitarsus*. Oliveira et al. (2017) demonstrated that JH treatment caused the CHCs profile of *V. vulgaris* workers to become similar to that of queens (Fig. 6).

Methyl-branched compounds were the most abundant, in terms of both percentage and number (Table 2). Branched alkanes have been shown to be present at high percentages in *M. consimilis* (Soares et al. 2017; Michelutti et al. 2017), *Mischocyttarus cassununga* (Murakami et al. 2015), *Mischocyttarus bertonii* and *Mischocyttarus latior* (Soares et al.

**Fig. 5** **a** Percentage and **b** number of compounds identified in the epicuticle of *Mischocyttarus consimilis*



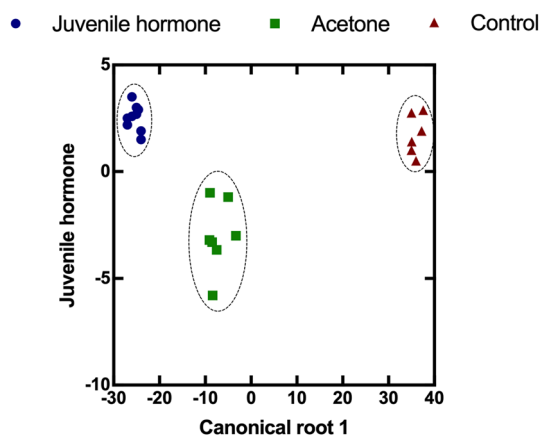
**Table 3** Discriminant analysis results, highlighting the 11 most significant compounds identified from the epicuticle of *Mischocyttarus consimilis*

Compound	Root 1	Root 2
Octadecane	4.495	0.036
3-Methyloctadecane	- 11.212	0.919
Nonadecane	- 6.299	1.151
x-Methylnonadecane	7.847	0.917
3-Methylnonadecane	- 13.256	1.193
1-Eicosene	- 19.305	- 0.829
2-Methylheneicosane	- 10.956	0.049
3-Methylheneicosane	8.182	0.977
Tricosane	12.657	- 0.430
5-Methylpentacosane	3.893	0.291
3-Methylpentacosane	12.466	0.036
3-Methylheptacosane	0.556	1.651
Octadecane	- 13.506	0.448
7-Methylnonadecane	- 1.617	0.139

The root 1 and root 2 are the X and Y axes in the scatter plot

2017), and other species of social wasps such as *P. dominula* (Bonavita-Cougourdan et al. 1991) and *Polybia occidentalis* (Singer et al. 1998). The application of JH promoted changes in the epicuticular composition, especially branched alkanes (Table 3), with the number of compounds decreasing, compared to the control treatment. Branched alkanes were found to be associated with development of the ovaries in *B. longitarsus*, reinforcing that they are the main compounds responsible for modulating female wasp colonies (Kelstrup et al. 2017; Dani et al. 2001).

Nonacosane was the major compound identified in females treated with JH (Table 2). A further four compounds occurred exclusively in females that received JH application, while two compounds (nonadecane and 10, 14-dimethyltriacontane) were absent in these individuals. In other work, it was found that the most abundant compounds in *V. vulgaris* workers treated with JH were long-chain linear alkanes, 3-methyl branched alkanes, and other monomethyl



**Fig. 6** Representative chromatograms of cuticular hydrocarbons in samples from *Mischocyttarus consimilis* wasps, indicating (\*) 11 compounds that were identified: (1) unknown; (2) nonadecane; (3) 9-methylnonadecane; (4) 3-methylnonadecane; (5) 1-eicosene; (6) 1-heneicosene; (7) 3-methylheneicosane; (8) tricosane; (9) 3-methylpentacosane; (10) 3-methylheptacosane; (11) octacosane

and dimethyl branched compounds (Oliveira et al. 2017). The compounds nonacosane and 3-methylnonacosane, abundant in *M. consimilis* treated with JH, are considered queen pheromones that are known to suppress worker reproduction (Oliveira et al. 2017). Nonacosane was also the major epicuticular component identified in queens of the Saxon wasp *Dolichovespula saxonica* (Van Zweden et al. 2013).

## Conclusions

Treatment of newly emerged *M. consimilis* females with JH led to morphological alteration, which was reflected by changes in both behavioral ontogeny and the chemical signature of the epicuticle, especially in relation to compounds with essential signaling roles in the colony. The findings bring new information concerning a little investigated wasp species from a tropical climate, showing that physiological and behavioral responses were associated with the effect



of juvenile hormone. Further studies with different species of tropical wasps are necessary to understand whether the responses observed in the present study may also apply to other species.

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## Declarations

**Conflict of interest** The authors have no competing interests.

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