Chemical signatures in the developmental stages of *Protopolybia exigua*

E.R.S. Silva[1], K.B. Michelutti[2], W.F. Antonialli-Junior[2], M. Batistote[1] and C.A.L. Cardoso[1]

[1]Centro de Pesquisa em Biodiversidade,
Universidade Estadual de Mato Grosso do Sul, Dourados, MS, Brasil
[2]Laboratório de Ecologia Comportamental,
Universidade Estadual de Mato Grosso do Sul, Dourados, MS, Brasil

Corresponding author: C.A.L. Cardoso
E-mail: claudia@uems.br

Genet. Mol. Res. 15 (1): gmr.15017586
Received September 3, 2015
Accepted November 13, 2015
Published March 28, 2016
DOI http://dx.doi.org/10.4238/gmr.15017586

ABSTRACT. The chemical signature of social insects is very important for communication, and specific signs of each colony and its individuals are acquired throughout their development. This chemical signature can also be related to the composition of the materials employed during nest construction. Furthermore, the venom also contains important chemicals required for the maintenance of wasp colonies. Therefore, the objective of this study was to evaluate the chemical composition of the wasp *Protopolybia exigua* along its different development stages, including the nest substrate and the venom of adult worker at different ages. To achieve this, gas chromatography coupled to mass spectrometry was used. The results show that the chemical cuticular compounds present in different stages of development, and in the nest of *P. exigua*, are qualitatively similar, but quantitatively different, demonstrating that these compounds can be used as signals for the identification of co-specific individuals within the colonies. However, there was no significant variation in these compounds between different colonies, which may be related to the parental level between the colonies and due to them sharing the same resources. The

non-polar compounds of the venom vary significantly according to the age of the workers, probably due to their different roles within the colony, and there was a clear increase in the complexity of the compounds as the wasps aged. Older wasps perform higher-risk activities, such as foraging, and therefore need to make more use of venom.

Key words: Cuticular hydrocarbon; Ontogeny; Temporal polyethism; Gas chromatography

INTRODUCTION

Protopolybia exigua (Saussure, 1854) (Hymenoptera, Polistinae) is a social wasp of the Epiponini tribe. These social wasps maintain their cohesive colonies by means of a complex communication system involving various signals (acoustic, sound, visual, tactile), with chemical signals being the most effective (Krasnec and Breed, 2013). When chemical signals are involved in intraspecific communication, they are called pheromones, and in interspecific interactions, they are called allele chemicals (Richard and Hunt, 2013). The signals responsible for chemical communication are involved in the coordination of basic activities for the maintenance of colonies, such as defense, recruitment, and foraging (Billen and Morgan, 1998).

Pheromones can be found in the cuticles of insects. Several studies have indicated that the compounds making up insect cuticles are basically hydrocarbons, particularly linear alkanes, branched alkanes, and alkenes (Olaniran et al., 2013). These compounds provide specific recognition signals for each colony and even for each individual, indicating, for example, an individual's age and role within the colony (Blomquist and Bagnères, 2010). In this context, the nest that houses the colonies performs a fundamental role in the recognition system, and its composition is similar to that of the individuals it contains (Espelie and Hermann, 1990; Sumana et al., 2005). The chemical profile of the colony may be related to the composition of the plant materials used for its construction, along with associated gland secretions. The interactions between individuals and the nest are important to obtain the chemical profile of the colony (Fortunato et al., 2004; Neves et al., 2013). The factors that enable variation in the chemical composition of cuticles can highlight the nesting environment or the individual's stage of development. Eggs contain hydrocarbons and as insects develop, there is increased complexity in the composition of these compounds (Espelie and Hermann, 1990). This variation may also be related to the recognition of each stage, since the workers need to recognize specific developmental stages in order to feed their larvae (Torres et al., 2013).

Venom is also important for the maintenance of wasp colonies. Its primary function is to capture prey and to act quickly in defense, especially against vertebrates and social species (Macalintal and Starr, 1996). In general, venom is composed of proteins and peptides, ora complex mixture of low molecular weight compounds (Palma, 2006). More specifically in social wasps, the compounds making up venom are used in an alarm mechanism and are excreted to produce stereotyped reactions, which function to recruit workers and accelerate the movements of attack in the colony. During behavioral ontogeny, the workers perform a subset of tasks at a given point in their life, and can change activity in response to interactions with the environment and with other members of the colony. The tasks of the young workers involve less risk as they occur inside the nest. As the wasps age, they start to realize that the environment outside the nest is riskier than that of the inside (Torres et al., 2012, 2013). Studies with *P. exigua* have explored aspects of their basic biology (Rocha et al., 2009) and some information about the polar composition of their venom

(Mendes et al., 2005; Mendes and Palma, 2006). This study evaluated the chemical compounds present inside the nest and in the wasp cuticles throughout different stages of development, as well as the composition of worker venom according to insect age.

MATERIAL AND METHODS

Collection and material selection

Three colonies of *P. exigua* in the post-emergent phase were collected in Dourados, MS, Brazil (22°20'23"S; 55°33'46"W). The population was taken to the laboratory, and stored in a freezer at -20°C until further use. During this period, samples of different developmental stages were weighed and cataloged before the extraction and chemical analyses. Eggs, larvae, and pupae were measured with the aid of a magnifying glass Leica S6D model coupled to an ocular micrometer. The larval instars were analyzed as described previously (Hunt et al., 2007). To analyze the chemical composition of each nest, a sample of 1 cm^2 of the nest central cells was used for the extraction. To analyze the venom, the ages of the workers were determined in accordance with the methods described by Torres et al. (2013), with some adaptation. The workers were divided into three categories: young, intermediate, and old. For each age category, the contents of the venom glands of three workers were used per colony.

Extraction

The cuticle and nest constituents of each sample were extracted with 2 and 4 mL hexane (HPLC grade, Tedia), respectively, for 2 min. After filtration, the solvent from each sample was removed in an exhaustion chapel. Each extract was dissolved in 100 µL hexane for chromatographic analysis. Hexane was analyzed by chromatography under the same conditions as the samples to assess possible contamination. The glands were burst and their contents submerged in 1 mL hexane (HPLC grade, Tedia) for 2 min. Next, the solvent from each sample was removed in an exhaustion chapel. Each extract was dissolved in 100 µL hexane (HPLC grade, Tedia) for chromatographic analysis.

Gas chromatography with mass spectrometer detector (GC-MS)

Analyses were performed on a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) with a mass spectrometer detector (GC-MS Ultra 2010) using a fused silica capillary column DB-5 (60 m length x 0.25 mm inside diameter x 0.25 mm thick film). The analysis conditions were: helium (99.999%) as the carrier gas with a flow rate of 1.0 mL/min, injection of 1 µL in the splitless mode. Conditions for the cuticle were as follows: initially, the oven temperature was kept at 100°C and was increased to 300°C at a rate of 3°C / min, and then remained at 300°C for 20 min. Conditions for the nest were as follows: initially, the oven temperature was kept at 150°C and was increased to 300°C at a rate of 3°C / min, and then remained at 300°C for 10 min. The injector, detector, and transfer line were kept at 280°C. The parameters included scanning MS voltage electron impact ionization of 70 eV and range of mass 45-800 m/z. Identification of compounds was performed using the calculated retention index (Van den Dool and Kratz, 1963) and using a mixture of linear alkanes (C_{14}-C_{36}, Sigma Aldrich with a purity ≥90%). The sample retention index was compared to the values reported in the literature (Lange et al., 1989; Grunshaw et al., 1990;

Bonavita-Cougourdan et al., 1991; Brown et al., 1991) associated with the interpretation of mass spectra obtained from the samples, and compared to available databases (NIST21 and WILEY229).

The compounds present in the samples at each stage of development, venom and in the nest are reported as the percentage relative abundance. For this study, compounds were considered in a minority if the relative abundance was less than 2%, intermediate if the abundance was 3-6.99%, and in the majority between 7 and 23%.

Statistical analysis

The relative abundance of compounds obtained of development stage and nest by GC were analyzed by the tests using the discriminant function of the SYSTAT 12 software (San Jose, CA, USA).

RESULTS

Cuticular chemical profiles in different stages of development and nest

GC coupled to MS (GC-MS) analysis of developmental stages and of the nest detected 49 peaks (Figure 1). All of these compounds were present in the samples from all colonies, nests, and from all stages of development (Table 1). These peaks lie between tetradecane and hexatriacontane, and 41 peaks were identified in the samples representing 95-98% relative abundance, with eight being unidentified and representing 2-5% (Table 1).

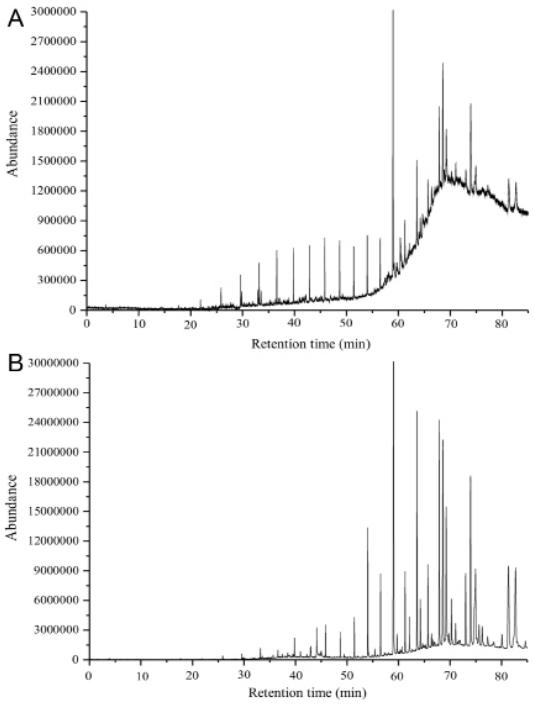

Figure 1. Representative chromatograms of the adult individual (**A**) and the nest (**B**) of *Protopolybia exigua.*

Table 1. Comparison of linear alkanes and branched alkanes identified at different stages of development and in the nest by GC-MS.

Index*	Compounds	Mean (%) ± SD**				
		Eggs	Larvae	Pupae	Adults	Nest
1400	Tetradecane	0.42 ± 0.02	0.07 ± 0.00	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
1500	Pentadecane	0.21 ± 0.02	1.18 ± 0.05	0.41 ± 0.03	0.02 ± 0.01	0.01 ± 0.01
1600	Hexadecane	0.17 ± 0.00	0.68 ± 0.00	0.57 ± 0.29	0.20 ± 0.05	0.06 ± 0.03
1700	Heptadecane	0.22 ± 0.04	0.61 ± 0.01	0.65 ± 0.10	0.43 ± 0.01	0.09 ± 0.03
1802	Octadecane	1.95 ± 0.02	0.71 ± 0.06	1.22 ± 0.33	0.55 ± 0.03	0.19 ± 0.07
1874	3-methyloctadecane	5.36 ± 0.02	1.67 ± 0.05	0.53 ± 0.00	0.04 ± 0.01	0.07 ± 0.01
1901	Nonadecane	0.85 ± 0.03	0.26 ± 0.01	1.42 ± 0.23	0.62 ± 0.02	0.14 ± 0.03
1965	4-methylnonadecane	0.26 ± 0.01	0.03 ± 0.01	0.06 ± 0.03	0.04 ± 0.00	0.13 ± 0.03
2000	Eicosane	0.30 ± 0.01	0.70 ± 0.06	1.72 ± 0.33	0.70 ± 0.04	0.37 ± 0.06
2051	5-methyleicosane	0.19 ± 0.01	0.04 ± 0.00	0.03 ± 0.01	0.10 ± 0.05	0.01 ± 0.01
2078	3-methyileicosane	0.33 ± 0.02	0.10 ± 0.01	0.12 ± 0.03	0.18 ± 0.01	0.08 ±0.03
2103	Heneicosane	0.37 ± 0.02	0.18 ±0.00	1.63 ± 0.10	0.74 ± 0.04	0.24 ± 0.07
2206	Docosane	0.25 ± 0.01	0.28 ± 0.02	1.81 ± 0.58	0.79 ± 0.01	0.64 ± 0.08
2299	Tricosane	0.31 ± 0.03	0.64 ± 0.01	2.54 ± 0.26	0.73 ± 0.02	0.48 ± 0.20
2333	9-methyltricosane	0.73 ± 0.01	0.10 ± 0.02	0.05 ± 0.00	0.02 ± 0.01	0.07 ± 0.01
2400	Tetracosane	0.80 ± 0.02	0.78 ± 0.04	1.73 ± 0.02	0.68 ± 0.05	0.79 ± 0.03
2498	Pentacosane	1.52 ± 0.01	7.86 ± 1.07	5.39 ± 0.14	0.80 ± 0.02	2.74 ± 0.36
2555	5-methylpentacosane	0.57 ± 0.01	4.78 ± 0.63	3.06 ± 0.06	0.06 ± 0.03	0.17 ± 0.03
2599	Hexacosane	4.60 ± 0.02	2.13 ± 0.04	1.87 ± 0.45	0.76 ± 0.02	1.73 ± 0.36
2698	Heptacosane	17.76 ± 0.02	19.36 ± 0.27	11.59 ± 0.20	4.33 ± 0.10	14.06 ± 0.02
2730	9-methylheptacosane	0.20 ± 0.01	0.28 ± 0.03	0.41 ± 0.03	0.44 ± 0.21	0.63 ± 0.13
2801	Octacosane	3.35 ± 0.01	1.84 ± 0.04	1.24± 0.10	1.09 ± 0.39	1.90 ± 0.07
2832	10-methyloctacosane	11.77 ± 0.02	6.16 ± 0.22	1.34 ± 0.09	0.79 ± 0.03	0.96 ± 0.03
2898	Nonacosane	9.42 ± 0.01	7.35 ± 0.16	3.17 ± 0.07	4.11 ± 0.07	6.40 ± 2.92
2929	9-methylnonacosane	0.20 ± 0.01	3.62 ± 0.04	1.96 ± 0.20	2.68 ± 0.05	1.62 ± 0.38
2999	Triacontane	6.20 ± 0.02	1.56 ± 0.02	3.23 ± 0.20	3.52 ± 0.03	2.31 ± 0.01
3100	Hentriacontane	11.73 ± 0.01	3.91 ± 0.52	5.94 ± 0.12	5.45 ± 0.94	7.36 ± 0.05
3130	9-methylhentriacontane	3.25 ± 0.01	14.64 ± 0.89	17.21 ± 0.30	7.17 ± 0.99	10.33 ± 2.10
3154	7-methylhentriacontane	2.66 ± 0.01	3.84 ± 0.20	13.28 ± 0.17	1.20 ± 1.81	6.63 ± 1.25
3199	Dontriacontane	2.72 ± 0.01	1.19 ± 0.01	0.39 ± 0.11	4.41 ± 0.42	2.08 ± 0.48
3245	5-methyldontriacontane	0.90 ± 0.03	1.66 ± 0.18	0.64 ± 0.01	5.12 ±0.42	0.42 ± 0.31
3275	3-methyldotriacontane	0.04 ± 0.01	1.66 ± 0.03	0.17 ± 0.21	7.07 ± 0.13	2.54 ± 0.05
3298	Tritriacontane	3.91 ± 0.02	2.93 ± 0.01	5.23 ± 0.39	7.44 ± 0.04	8.09 ± 0.95
3324	11-methyltritriacontane	0.22 ± 0.01	4.37 ± 0.14	2.27 ± 012	8.24 ± 0.03	6.94 ± 1.80
3351	5-methyltritriacontane	0.19 ± 0.03	1.26 ± 0.12	0.02 ± 0.00	2.93 ± 0.06	1.26 ± 0.49
3356	4-methyltritriacontane	0.22 ± 0.03	0.30 ± 0.00	0.02 ± 0.00	1.05 ±0.03	0.60 ± 0.60
3400	Tetratriacontane	0.27 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	7.61 ±2.49	0.08 ± 0.00
3466	4-methyltetratriacontane	0.17 ± 0.01	0.06 ± 0.01	0.02 ± 0.00	5.57 ± 0.08	0.67 ± 0.11
3494	Pentatriacontane	0.34 ± 0.03	0.07 ± 0.01	1.44 ± 0.61	4.45 ±0.12	6.63 ± 0.14
3543	7-methylpentatriacontane	0.08 ± 0.02	0.05 ± 0.01	0.17 ± 0.01	4.88 ± 0.14	8.23 ± 1.96
3594	Hexatriacontane	0.13 ± 0.02	0.06 ± 0.01	0.01 ± 0.01	0.44 ± 0.06	0.46 ± 0.10

*Calculated index: Van den Dool and Kratz, 1963. **Means represent N = 30; SD = standard deviation.

Analysis of developmental stages and nests revealed the relative standard deviation of the same colony to be less than 1%. In different colonies at the egg stage, and of larva, and pupae, the relative standard deviation was less than 3%, and in the adult stage and nest it was less than 10%

(Table 1). Thus, these results indicate that there were no significant chemical differences between the sample profiles of different colonies (Wilk's lambda: 0.985, F: 0.014 and P = 0.999; Figure 2).

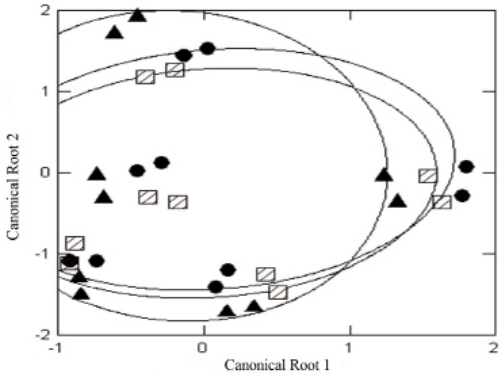

Figure 2. Discriminant analysis employing all the compounds described between the different colonies of *Protopolybia exigua*. Colony 1 = circles, Colony 2 = squares, Colony 3 = triangles, N = 10.

Discriminant analysis between developmental stages and the nest shows that there are significant differences between the chemical profiles of all stages of development and the nest (Wilk's lambda: 0.000, F: 587.635 and P < 0.001). The first root explained 76 and 24% of the second root (Figure 3).

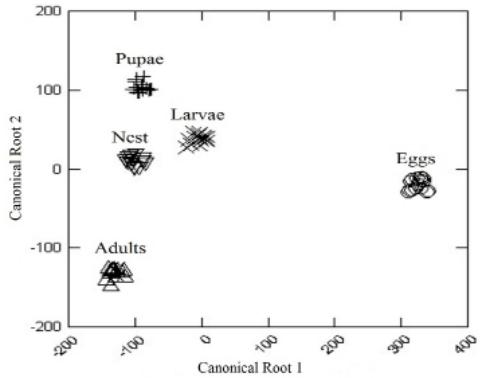

Figure 3. Discriminant analysis using all the compounds described in the developmental stages and nests in *Protopolybia exigua*, N = 30.

Quantitative differences between groups were observed between samples, as all the compounds are present in all stages (Table 1). Of all compounds identified across the developmental stages and in the nest, 22 were linear alkanes and 19 were branched alkanes. The total linear alkanes exhibited the highest abundance, which ranged from 48 to 67% and the total branched alkanes ranged from 26 to 47%. There was a decline in the number of linear alkanes between the stages of eggs and larvae, but they remained virtually constant from larvae to adults, with an abundance of around 50% (Figure 4).

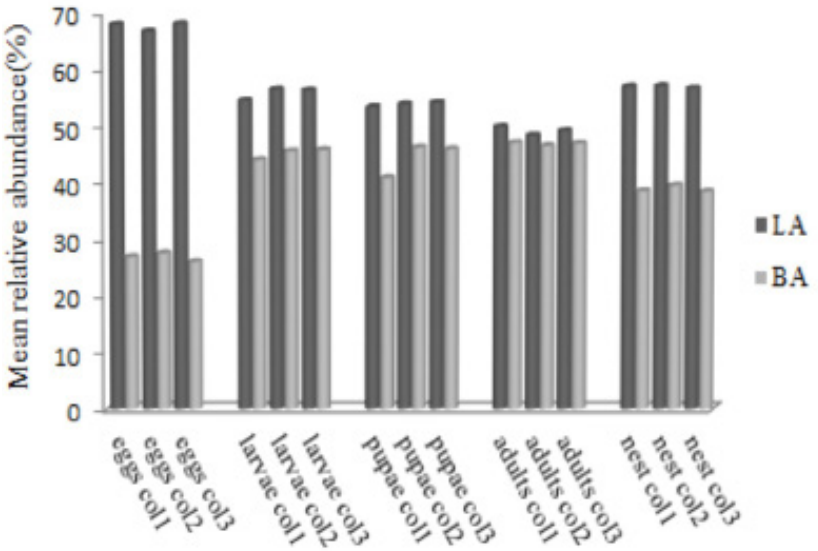

Figure 4. Percentage in total abundance of linear alkanes (LA) and branched alkanes (BA) separated by colonies of *Protopolybia exigua*. col1 = colony 1; col2 = colony 2; col3 = colony 3.

In eggs, the major compounds included heptacosane, nonacosane, hentriacontane, and 10-methyloctacosane, and in larvae the major compounds were pentacosane, heptacosane, nonacosane, and 9-methyllhentriacontane. In pupae, the major compounds were heptacosane, 9-methylhentriacontane, and 7-methylhentriacontane. In adults, the major compounds were 9-methylhentriacontane, tritriacontane, 11-methyltritriacontane, and tetratriacontane. In nests, the major compounds included heptacosane, hentriacontane, 9-methylhentriacontane, tritriacontane, and 7-methylpentatriacontane. The nest compounds were qualitatively and quantitatively similar to those identified in the adult insects (Table 1).

Variation in the composition of nonpolar compounds in venom

In the venom samples analyzed, 85 peaks were obtained, from which 35 compounds were identified. These compounds represented more than 80% of the total abundance in the samples. There were quantitative and qualitative differences in venom between workers of different ages (Table 2). The compounds identified were linear alkanes, branched alkanes, alkenes, and esters (Table 2). These compounds vary according to the age of the worker, and a high number of alkanes have been identified in the venom of younger workers, alkenes were identified in higher number of the intermediate workers, and esters were identified only in old workers. With increasing age, there was an increase in both the number of compounds identified in the venom and also in the appearance of different classes of compounds. The major compounds in the venom of young workers were heptacosane, nonacosane, 11-methylnonacosane, and 3-methylnonacosane. These alkanes, with the exception of heptacosane, were in the majority in insects of all ages. In the intermediate age group, the main compound identified was 1-octacosene. In old workers, the main compound identified was 9-methylnonanoate (Table 2).

Table 2. Chemical composition of polar compounds in the venom of *Protopolybia exigua* at different ages identified by GC-MS.

Index*	Compounds	Mean (%) ± SD**		
		Young	Intermediary	Old
1900	Nonadecane	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01
1932	9-methylnonadecane	0.51 ± 0.01	-	0.09 ± 0.01
1982	2,3-dimethylnonadecane	0.56 ± 0.01	0.39 ± 0.01	0.16 ± 0.01
1998	Eicosane	1.48 ± 0.02	-	0.11 ± 0.01
2100	Heneicosane	-	0.1 ± 0.01	0.14 ± 0.01
2149	9 ethyloctadecenoate	-	-	0.16 ± 0.01
2171	9-methylnonanoate	-	-	20.13 ± 0.08
2199	Docosane	-	0.17 ± 0.01	0.25 ± 0.01
2275	1-tricosene	-	-	0.83 ± 0.01
2300	Tricosane	-	0.22 ± 0.01	1.7 ± 0.01
2385	1-tetracosene	-	0.16 ± 0.01	0.34 ± 0.01
2400	Tetracosane	-	0.18 ± 0.01	0.23 ± 0.01
2433	11-methyltetracosane	-	0.09 ± 0.01	-
2487	1-pentacosene	-	0.24 ± 0.01	0.15 ± 0.01
2501	Pentacosane	5.33 ± 0.03	0.97 ± 0.01	0.87 ± 0.01
2575	3-methylpentacosane	-	0.12 ± 0.01	0.2 ± 0.01
2601	Hexacosane	1.77 ± 0.02	0.5 ± 0.01	0.31 ± 0.01
2699	Heptacosane	13.03 ± 0.03	5.77 ± 0.07	3.62 ± 0.02
2732	11,15-dimethylheptacosane	3.10 ± 0.02	0.43 ± 0.01	0.32 ± 0.01
2742	11-methylheptacosane	-	-	0.13 ± 0.01
2751	5-dimethylheptacosane	-	0.33 ± 0.01	0.32 ± 0.01
2775	1-octacosene	2.87 ± 0.01	11.02 ± 0.06	3.89 ± 0.02
2801	Octacosane	2.86 ± 0.02	1.69 ± 0.02	0.62 ± 0.01
2832	11-methyloctacosane	0.90 ± 0.01	0.56 ± 0.01	0.14 ± 0.01
2837	10-methyloctacosane	4.53 ± 0.02	0.42 ± 0.01	6.46 ± 0.03
2860	6-methyloctacosane	-	0.70 ± 0.01	0.30 ± 0.01
2875	3-methyloctacosane	-	0.57 ± 0.01	0.35 ± 0.01
2900	Nonacosane	13.15 ± 0.06	11.51 ± 0.06	7.98 ± 0.05
2932	11-methylnonacosane	11.04 ± 0.03	22.59 ± 0.08	7.81 ± 0.05
2952	6-methylnonacosane	-	1.10 ± 0.01	0.82 ± 0.01
2966	2-methylnonacosane	-	1.13 ± 0.01	2.23 ± 0.01
2975	3-methylnonacosane	11.50 ± 0.05	18.8 ± 0.08	13.21 ± 0.05
2984	1-triacontene	2.16 ± 0.02	0.94 ± 0.01	0.46 ± 0.01
3000	Triacontane	5.63 ± 0.04	0.83 ± 0.01	3.47 ± 0.02
Total	Identified	80.54	81.67	77.92
	Unidentified	19.46	18.36	22.08

*Calculated index: Van den Dool and Kratz, 1963. **Means represent N = 9; SD = standard deviation.

DISCUSSION

Cuticular chemical profiles of different stages of development and nest

Comparing the variation in hydrocarbon content between the colonies revealed that adults have greater variation in hydrocarbon composition in relation to more immature colonies. Cotoneschi et al. (2007) observed a greater difference in the cuticular signature between adults of the same colony than in the larvae of different colonies. Brown et al. (1991), studying *Vespula germanica*, also reported that the quantitative composition of chemicals among adults of different colonies was higher when compared to the variation between individuals of the same colony. One of

the explanations for the similar composition of the colonies observed in the present study is that they are nested in nearby locations and may have a degree of kinship, as previously suggested by Klahn and Gamboa, (1983) for *Polistes fuscatus*. This was also described by Dapporto et al. (2004) in *P. dominula*, who noted that similarity of the cuticular chemical composition is higher among nested colonies in nearby regions. Another factor that could influence this similarity is the use of the same resources for colony maintenance, as suggested by Cotoneschi et al. (2007) in a study on *P. dominula.*

The most important compounds for distinguishing between groups were alkanes (tetradecane and nonadecane) when present in a low abundance in the samples. These data differ from those reported by Cotoneschi et al. (2007), who concluded that major compounds were responsible for distinguishing the different stages of development of the *P. dominula.* The highest abundance of linear alkanes was found in the egg stage, which may indicate a need for waterproofing. According to the findings from previous studies (Gibbs, 1998, 2002), the main function of linear alkanes is to prevent water loss through dehydration at the expense of branched alkanes, which function in chemical signaling (Brown et al., 1991; Gamboa et al., 1996), although these findings have not yet been fully clarified (Tannure-Nascimento et al., 2007).

Vespula vulgaris eggs were found to contain the same compounds identified in the present study (Bonckaert et al., 2012). In the larvae of *P. dominula*, the linear alkanes heptacosane, nonacosane, triacontane, and hentriacontane were dominant, which are sex flags from the larval stage (Cotoneschi et al., 2009). Regarding the chemical composition of pupae in *V. germanica*, Brown et al. (1991) reported that heptacosane, nonacosane, and hentriacontane were most abundant at this stage. Gamboa et al. (1996) identified branched alkanes, which are 15-methylhentriacontane and 13, 15, 17 methyltritriacontane signals, that are responsible for recognition in adults of *P. fuscatus* colonies. Across the development stages, there was an increase in the abundance of branched alkanes with carbon chain than 31, especially in adults and in nest material (Table 1). Brown et al. (1991) studied the wasp *V. germanica* and identified alkanes with branched carbon chains that were shorter in the immature stages and longer in the adults, in which they contained a higher abundance of compounds with carbon chains 29-37 carbons long. Compounds with shorter carbon chain (heptacosane-hentriacontane) were the most abundant in immature stages, and longer carbon chains (hentriacontane) were most abumdant in adults (Table 1).

The present results are consistent with those reported by Cotoneschi et al. (2007) between larvae and adult wasp *P. dominula*. In fact, the differences in the chemical profile observed between immature stages, including larvae, and adults may be explained by the difference between these alimentation stages, since the larvae feed on more protein while adults feed on more carbohydrate (nectar) (Cotoneschi et al., 2007). This may have occurred because the pupa does not receive food, and therefore, it must utilize the reserves that accumulated during the larval stage (Brown et al., 1991).

In a study by Espelie and Hermann (1990), the chemical profiles of *Polistes annularis* adults were similar to those of the nest. The colonial profile is only defined in adults after a few days or at times of emergency (Lorenzi et al., 2004), since these individuals rub their gaster on the nest surface and in some cases, acquire and transfer compounds that influence the characterization of the chemical profile (Neves et al., 2013). Therefore, there is a clear relationship between the chemical profile of the nest and the members of the colony, especially of adults (Klahn and Gamboa, 1983; Espelie and Hermann, 1990; Brown et al., 1991; Sumana et al., 2005; Cotoneschi et al., 2007; Neves et al., 2013).

Variation in the composition of non-polar compounds of venom

This is the first study that investigates the composition of the non-polar portion of *P. exigua* venom. Some studies have indicated that volatile compounds are responsible for alarm function (Bruschini et al., 2008). Esters are compounds that may make up the alarm pheromones found in the venom of social wasps (Fortunato et al., 2004). Esters in wasp venom have been identified as a defense strategy following disturbances in colonies (Veith et al., 1984). Fortunato et al. (2004) identified some esters and alkanes in the venom of wasps of the *Ropalidia genre*. Linear alkanes were identified in *Vespa orientalis* colonies (Saslavasky and Ishay, 1973). Linear alkanes and alkenes have been identified in *Polybioides raphisgatra* (Sledge et al., 1999). In a study by Dani et al. (1998) using venom from seven different species of the Stenogastrinae subfamily, hydrocarbon chains containing between 11 and 17 carbons were identified. Uçkan et al. (2006), in a study on *Pimpla turionellae*, reported that there are gradual changes in the chemical profile of venom with age in the female and that these changes are probably related to the different tasks insects undertake throughtout their behavioral development.

Changes in the chemical composition of venom are probably associated with the function of the female within the colony. Studies by Zara and Balestieri (2000), and Torres et al. (2012), using wasps of the genus *Polistes*, have shown that there is a time polyethism in the worker caste, and that there must be a relationship between the age of the worker and the role it undertakes with in the colony. Older workers work more effectively in foraging activities and in colony defense, tasks that involve greater energy expenditure and have higher mortality risks, thus exposing the wasp to predators (Zara and Balestieri, 2000; Torres et al., 2012).

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors thank Orlando T. Silveira for assisting with the identification of species; Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT); Coordenação Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a Master's fellowship to E.R.S. Silva; W.F. Antonialli-Junior and C.A.L. Cardoso acknowledge research grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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