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Analysis of cuticular chemical profiles of *Latrodectus geometricus* (Araneae: Theridiidae) females and juveniles using GC×GC/qMS

Análise do perfil químico cuticular de fêmeas e imaturos de *Latrodectus geometricus* (Araneae: Theridiidae) usando GC×GC/qMS

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Abstract

Communication in spiders can occur by several mechanisms, especially chemical cues, and cuticular hydrocarbons (CHCs) play an important role in intraspecific recognition. Several techniques have been used to evaluate CHCs in spiders. Here, rapid-scanning two-dimensional gas chromatography with quadrupole mass spectrometry (GC×GC/qMS) was employed to assess the CHCs of L. geometricus females and juveniles of different ages. Samples of adult females' cephalothorax, abdomen and legs, were used to assess whether there is variation in CHCs between body parts. To assess whether there is variation between ages, juveniles of 5 and 20 days post-emergence were evaluated. The results demonstrate that there is variation in CHCs of different body parts of females, with abdomen presenting greater number of compounds, as well as between adults and juveniles. Branched alkanes represented the majority of compounds in all samples, followed by linear alkanes. Alkenes were only present in adult's abdomen and cephalothorax. The compounds 10-methyloctacosane and 14-methyloctacosane; and 10-methyltriacontane and 14-methyltriacontane showed the same retention time in ^{1}D and were separated in ^{2}D. According to the results, rapid-scanning GC×GC/qMS can be considered a reliable technique, since it was possible to identify the spider's CHCs, and detect and separate two cases of chromatographic coelution.
Keywords: *Gas chromatography. Spider. Communication. Mass spectrometry.*

Resumo

A comunicação em aranhas pode ocorrer por vários mecanismos, especialmente sinais químicos, e os hidrocarbonetos cuticulares (CHCs) desempenham papel fundamental no reconhecimento intraespecífico. Várias técnicas têm sido utilizadas para avaliar os CHCs em aranhas. Aqui, a Cromatografia bidimensional de varredura rápida com espectrometria de massa quadrupolo foi empregada para avaliar o perfil de CHCs de fêmeas de L. geometricus e imaturos de diferentes idades. Amostras do cefalotórax, abdome e pernas de fêmeas foram usadas para avaliar se há variação em CHCs entre as partes. Para avaliar se há variação entre idades, foram usados imaturos de 5 e 20 dias pós-emergência. Os resultados demonstram que há variação em CHCs de diferentes partes do corpo, com abdome apresentando o maior número de compostos, bem como entre adultos e imaturos. Alcanos ramificados representaram a maioria dos compostos em todas as amostras, seguidos por alcanos lineares. Alcenos só estavam presentes no abdome e cefalotórax das adultas. Os compostos 10-metiloctacosano e 14-metiloctacosano; e 10-metiltriacontano e 14-metiltriacontano tiveram o mesmo tempo de retenção em ^{1}D e foram separados em ^{2}D. De acordo com os resultados, a técnica pode ser considerada confiável, já que foi possível identificar os CHCs da aranha, e detectar e separar dois casos de coeluição cromatográfica.
Palavras-chave: *Cromatografia gasosa. Aranha. Comunicação. Espetrometria de massas.*

1 Introduction

Communication in spiders can occur by several mechanisms, such as visual, sound and chemical cues. Pheromones play an important role as they serve as signals for reproduction, recognition of conspecifics and among relatives, warning of danger, territory marking, signs of dominance, state of health and even reproductive status (ANDRADE; KASUMOVIC, 2005; BARUFFALDI; ANDRADE, 2015; JOHANSSON; JONES, 2007; PROUVOST et al., 1999; STOLTZ et al., 2007; UETZ; ROBERTS, 2002; UHL, 2013; WYATT, 2003).

Some non-volatile compounds, present on spider's body or silk can also act as pheromones, being called contact pheromones. Cuticular Hydrocarbons (CHCs), are types of contact pheromones related to intraspecific recognition, male choice and courtship behavior, changes in pheromone emissions by mated females and mediation of aggressive behavior (BARUFFALDI; ANDRADE, 2015; BARUFFALDI; COSTA, 2010, 2014; GASKETT, 2007; GRINSTED et al., 2011; GUIMARÃES et al., 2016; POURIÉ; TRABALON, 1999; POURIÉ et al., 2005; PROUVOST et al., 1999; TRABALON; ASSI-BESSÉKON, 2008; TRABALON et al., 1996, 1997).

Several techniques have been used to evaluate the cuticular chemical composition in spiders and other arthropod's species, especially in social insects. Fourier Transformed Infrared Photoacoustic Spectroscopy (FTIR-PAS) has been used to assess qualitative variations in these cuticular chemical compounds. This technique has been proven to be reliable for assessing intraspecific variation of cuticular chemical composition in ants, social wasps and spiders (ANTONIALLI-JUNIOR et al., 2007, 2008; GUIMARÃES et al., 2016; NEVES et al., 2012, 2013; TORRES et al., 2014). The most usual techniques employed in qualitative and quantitative evaluation of cuticular composition are Gas Chromatography with Flame Ionization Detection (GC-FID) (POURIÉ; TRABALON, 1999, 2001; POURIÉ et al., 2005; TRABALON, 2011; TRABALON et al., 1996, 1997, 1998) or Gas Chromatography coupled to Mass Spectrometric detection (GC-MS) (GRINSTED et al., 2011; GUIMARÃES et al., 2016; PROUVOST et al., 1999; TRABALON et al., 1996, 2005).

Considerable research has been dedicated to the combination of independent techniques with the aim of strengthening the resolving power of chromatographic systems. The satisfactory separation of a complex sample requires a higher peak capacity, being indicated the use of comprehensive two-dimensional gas chromatography (GC×GC), a relatively new technique (MONDELLO et al., 2008). In GC×GC, two orthogonal mechanisms are used to separate the constituents of the sample within a single analysis, based on the application of two GC columns with different stationary phases connected in series, with a transfer device, defined as modulator. The function of the modulator is to isolate, re-concentrate, and introduce small portions of the primary column effluent onto a secondary column, continuously. The time required to complete this process is defined as the modulation period (MONDELLO et al., 2008).

Complex mixtures can present coelution of compounds that might not be detected by the usual techniques, thus GC×GC can provide satisfactory analytical results for the analysis of many complex samples, since it provides better separation and identification of compound classes (ISAACMAN et al., 2012). Recently, it has been used to analyze cadaveric Volatile Organic Compounds (VOCs), which are used to establish the Post-Mortem Interval (PMI) in medico–legal investigations (BALA; SHARMA, 2016; DEKEIRSSCHIETER et al., 2012). This technique has also been used to identify the sexual pheromone components of the defoliator insect *Ectropis grisescens* which, according to the authors, could be useful in developing alternative management tools for the pest (MA et al., 2016). Ralston-Hooper et al. (2008) used GCxGC to compare metabolite profiles of an invertebrate species and established that the technique was able to detect differences, which could be helpful in ecotoxicological studies. However, to our knowledge, this is the first study employing this technique to assess cuticular chemical composition.

The species studied here, *Latrodectus geometricus, p*resents an hourglass in the ventral face, usually orange, and is known as "hourglass spider" or "brown widow spider". These spiders are widespread across all continents except in polar regions, and are considered synanthropic (COSTELLO; DAANE, 1998; GARB et al., 2004; LOTZ, 1994; MÜLLER, 1993). The species first description was in 1841, however, its place of origin is uncertain because by that time its occurrence had already been documented in Africa and South America (GARB et al., 2004). Despite possessing one of the venoms with higher toxicity within the genus (MCCRONE, 1964; VINCENT et al., 2008) these spiders are not aggressive and accidents usually happen when they are compressed. Thus, although they are cosmopolitan, studies with this species are scarce, probably because there are few cases of accidents with humans (LEVI et al., 2001). Few studies focus on aspects of basic biology and natural history of this species, such as Liu et al. (2009) regarding embryonic development, Segoli et al. (2008) on sexual cannibalism, Guimarães et al. (2012) regarding the influence of aggregation behavior on life expectancy of juveniles and Guimarães et al. (2016) studying the role of cuticular compounds as mediators of interactions between adults and juveniles. Considering that cuticular chemical profiles are a complex mixture and, as such, might present coelution of compounds that may not be resolved by the most common techniques, in this study rapid-scanning GC×GC/qMS was employed to assess the cuticular chemical composition of *L. geometricus* females' body parts and juveniles of different ages.

2 Material and Methods

Adult females of *L. geometricus* were collected in Dourados, State of Mato Grosso do Sul. Eggsacs were used for species identification because they have a peculiar conformation, spherical and covered with conical protuberances, which, according to Abalos (1962), facilitates their identification. Females with their eggsacs were individually transferred to laboratory in 500 ml containers. Relative humidity was controlled by moistened cotton placed at the bottom of each container, as a way to preserve silk elasticity (EDMONDS; VOLLRATH, 1992; WORK; MOROSOFF, 1982; WORK; YOUNG, 1987). Spiders were fed with *Tenebrio molitor* larvae offered twice a week

GC×GC/qMS analysis

In order to assess whether there is variation in cuticular chemical composition between the different body parts of *L. geometricus* adult females, samples of cephalothorax, abdomen and legs were evaluated by GC×GC/qMS. To assess whether the technique can detect cuticular composition variations between juveniles of different ages as found by GUIMARÃES et al. (2016), juveniles of 5 and 20 days post-emergence were evaluated.

The cuticular constituents of each sample were extracted by immersing each sample in a glass container with 1 ml hexane (Tedia, HPLC grade) for 10 minutes, using ultrasonic bath. Then, the solvent was dried under fume hood and each extract was solubilized in 200 µl of hexane for chromatographic analysis. Samples were analyzed in triplicate, with coefficient of variation of less than 5%.

GC×GC/qMS analyses were carried out on a Shimadzu GC×GC/qMS system consisting of a GC2010 gas chromatograph and a QP2010 plus quadrupole mass spectrometer (Shimadzu Corp., TYO, Japan). The system was provided with a loop-type modulator (Zoex Corp., TX, USA) cooled with liquid nitrogen and with the hot jet pulse time set at 500 ms (300°C) with modulation times of 5 s. Initial temperature of the ^{1}D column was 80°C reaching 280°C at a rate of 4°C min^{-1}, remaining at this temperature for 15 min, totaling 65 min. Carrier gas helium (99.999%) at a flow rate of 1mL min^{-1}; 1 µL of injection volume, split ratio (1:20). The ^{2}D column was a DB-17 (50% phenyl methylpolysiloxane, Agilent Technologies, CA, USA) analytical column (2.15 m × 0.18 mm × 0.18 µm) and the analyses conditions were the same as above described for the ^{1}D. The temperatures of the injector, transfer line and detector were maintained at 280 °C. The MS scan parameters included electron impact ionization voltage of 70 eV, a mass range of 50 to 650 Daltons and a scan interval of 0.5 s. The hexane was analyzed in the same conditions of samples.

The retention index (RI) was calculated employing a linear C_7-C_{40} alkane mixture analyzed using identical GC×GC/qMS conditions. The RI was calculated for each compound of the sample according to the equation below (1). The RI values obtained by linear equation were compared with the values of literature.

$$\mathrm{RI} = 100x\left[n + (N - n)\frac{\mathrm{tr(unknow)} - \mathrm{tr}(n)}{\mathrm{tr}(N) - \mathrm{tr}(n)}\right] \quad (1)$$

Where **RI** is the Krafts index, **n** is the number of carbon atoms of the lower n-alkanes, **N** is the number of carbon atoms of the higher n-alkanes and **tr** is the retention time.

Data acquired were processed using GC Image software (2.2b1; Zoex Corp., TX, USA). Compounds were tentatively identified by comparing their RI to those reported in literature (BONAVITA-COUGOURDAN et al., 1991; BROWN et al., 1991; MICHELUTTI et al., 2017) and the mass spectra obtained were compared with NIST21 and WILEY229 databases. An Identity Spectrum Match factor above 800 resulting from the NIST and WILEY was determined to be acceptable for positive identification.

3 Results

The analyses of the samples detected 50 peaks in total, from heptadecane to 17-, 13-, 15-, 11-methylpentatriacontane (Table 1). Forty-one peaks were present in abdomen samples, 22 in cephalothorax, 13 in legs, and 8 in the juvenile's samples of both ages (Table 1). All peaks present in adult's cephalothorax and legs, and 20 days post-emergence juvenile's samples were identified, representing 100% of relative abundance, as well as 38 peaks present in adult's abdomen samples and 7 in juveniles of 5 days post-emergence, representing 99% and 80.5% respectively (Table 1).

Table 1 - Means of the relative percentual area of cuticular compounds, obtained by rapid-scanning GC×GC/qMS, of the different body parts and juveniles of *Latrodectus geometricus*.

Compounds	Nomenclature	1D (min)	2D (s)	1D+2D (min)	Samples/Volume (%)					Calculated Index	Literature Index
					Cephalothorax	Abdomen	Legs	Juvenile 5 days	Juvenile 20 days		
C_{17}	*n*-heptadecane	24.00	1.65	24.03	-	0.1	-	-	-	1704	1700
C_{18}	*n*-octadecane	24.58	1.65	24.61	-	0.3	-	-	-	1796	1800
C_{19}	*n*-nonadecane	27.08	1.68	27.11	-	0.1	-	-	-	1894	1900
Unknown	Unknown	28.58	1.65	28.61	-	0.1	-	-	-	1924	
10-MeC_{19}	10-methylnonadecane	29.08	1.77	29.11	-	1.9	-	-	-	1945	1942
2-MeC_{19}	2-methylnonadecane	29.83	1.74	29.86	-	14.1	-	-	-	1967	1962
C_{20}	*n*-eicosane	30.17	1.65	30.19	-	2.3	-	-	-	1999	2000
Unknown	Unknown	31.92	1.71	31.95	-	0.8	-	-	-	2067	
C_{21}:1	1-heneicosene	32.50	1.74	32.53	-	0.5	-	-	-	2093	2098
C_{21}	*n*-heneicosane	32.67	1.71	32.70	-	0.6	-	-	-	2100	2100
9-MeC_{21}	9-methylheneicosane	33.25	1.68	33.28	-	0.3	-	-	-	2137	2139
2-MeC_{21}	2-methylheneicosane	33.67	1.77	34.28	4.0	51.0	8.7	-	-	2173	2167
C_{22}	*n*-docosane	34.08	1.74	34.61	3.0	9.6	4.9	-	-	2199	2200
C_{23}	*n*-tricosane	37.25	1.92	37.28	-	0.4	-	-	-	2301	2300
11-,9-MeC_{23}	11-,9-methyltricosane	37.42	1.89	37.45	-	0.3	-	-	-	2332	2338
3-MeC_{23}	3-methyltricosane	38.33	1.77	38.36	-	0.6	-	-	-	2367	2367
C_{24}	*n*-tetracosane	39.17	1.74	39.20	-	0.3	-	-	-	2409	2400
8-MeC_{24}	8-methyltetracosane	39.42	1.98	39.45	0.8	0.1	-	-	-	2422	
Z-7-C_{25}:1	(Z)-7-pentacosene	40.33	2.01	40.94	1.2	-	-	-	-	2479	2476
C_{25}	*n*-pentacosane	40.92	1.62	41.53	1.3	0.7	-	-	-	2500	2500
Unknown	Unknown	41.50	1.62	41.86	-	0.3	-	-	-	2542	
2-MeC_{25}	2-methylpentacosane	41.83	1.65	42.28	-	0.1	-	-	-	2560	2563
3-MeC_{25}	3-methylpentacosane	42.25	1.65	42.78	-	0.2	-	-	-	2570	2572
C_{26}	*n*-hexacosane	42.75	1.65	44.45	-	0.2	-	-	-	2600	2600
Z-9-C_{27}:1	(Z)-9-heptacosene	44.42	2.19	44.61	-	0.6	-	-	-	2680	2675
C_{27}	*n*-heptacosane	44.58	1.68	45.19	-	0.4	-	-	-	2691	2700
13-MeC_{27}	13-methylheptacosane	45.17	1.68	46.36	-	0.2	-	-	-	2732	2731
C_{28}	*n*-octacosane	46.33	1.71	46.86	1.0	0.2	-	1.7	-	2795	2800

to be continued...

Table 1 -Continued

Compounds	Nomenclature	1D (min)	2D (s)	1D+2D (min)	Samples/Volume (%)					Calculated Index	Literature Index
					Cephalothorax	Abdomen	Legs	Juvenile 5 days	Juvenile 20 days		
10-MeC$_{28}$	10-methyloctacosane	46.83	1.68	48.03	-	0.3	-	-	-	2830	2829
14-MeC$_{28}$	14-methyloctacosane	46.83	1.92	48.53	-	-	-	0.6	6.7	2830	2829
C$_{29}$	*n*-nonacosane	48.00	1.74	49.20	4.4	0.4	4.1	0.70	6.2	2900	2900
13-MeC$_{29}$	13-methylnonacosane	48.50	1.74	49.78	6.3	1.2	7.5	1.10	6.8	2930	2931
7,17-diMeC$_{29}$	7,17-dimethylnonacosane	49.17	1.74	50.20	4.5	0.9	5.7	-	11.0	2970	2973
C$_{30}$	*n*-triacontane	49.75	1.74	50.70	-	0.1	-	-	-	3005	3000
14-MeC$_{30}$	10-methyltriacontane	50.67	1.77	51.63	2.0	0.3	2.2	-	-	3031	3032
10-MeC$_{30}$	14-methyltriacontane	50.67	2.01	51.86	-	-	-	41.7	-	3031	3032
3-MeC$_{30}$	3-methyltriacontane	51.00	1.80	52.4	3.4	0.3	1.6	-	-	3071	3073
C$_{31}$	*n*-hentriacontane	51.58	2.61	54.45	17.5	4.5	15.0	-	20.0	3103	3100
15-, 13-MeC$_{31}$	15-, 13-methylhentriacontane	51.83	1.89	56.12	10.2	1.4	13.4	-	17.6	3139	3132
3-MeC$_{31}$	3-methylhentriacontane	52.42	1.92	56.87	14.9	1.7	13.3	-	19.1	3178	3174
C$_{32}$	*n*-dotriacontane	52.75	1.92		1.1	-	-	-	-	3200	3200
11-MeC$_{32}$	11-methyldotriacontane	53.25	1.95		0.9	-	1.2	-	-	3236	3232
2-MeC$_{32}$	2-methyldotriacontane	53.83	2.13		3.1	0.2	-	-	-	3266	3262
C$_{33}$	*n*-tritriacontane	54.25	4.47		-	-	-	7.3	-	3303	3300
11-,13-, 15-, 17-MeC$_{33}$	11-,13-, 15-, 17-methyltritriacontane	54.42	2.04		2.5	0.3	-	-	-	3329	3332
C$_{34}$	*n*-tetratriacontane	55.33	2.13		1.2	-	-	-	-	3397	3400
13-,15-MeC$_{34}$	13-,15-methyltetratriacontane	56.08	2.19		5.7	1.0	9.6	-	-	3424	3425
C$_{35}$	*n*-pentatriacontane	56.83	2.25		8.2	1.3	12.8	27.4	12.6	3502	3500
17-, 13-, 15-, 11-MeC$_{35}$	17-, 13-, 15-, 11-methylpentatriacontane	57.33	2.28		0.7	-	-	-	-	3532	3530
Unknown	Unknown	62.00	4.22		-	-	-	19.5	-	3962	
Total of identified compounds					100.0	99.0	100.0	80.5	100.0		
Linear alkanes					37.7	21.5	36.8	37.1	38.8		
Branched alkanes					61.1	76.4	63.2	43.4	61.2		
Alkenes					1.2	1.1	0.0	0.0	0.0		
Unknown					0.0	1.2	0.0	19.5	0.0		
Total of compounds					100	100.2	100	100	100		

For all samples, branched alkanes represented the majority of compounds with 61.1% of relative abundance in adult's cephalothorax samples, 76.4% in abdomen, 63.2% in legs, 43.4% in juveniles of 5 days post-emergence and 61.2% in juveniles of 20 days post-emergence, while linear alkanes represented 37.7%, 21.5%, 36.8%, 37.1% and 38.8% respectively. Alkenes were only present in adult's cephalothorax and abdomen samples (Figure 1). The chromatograms for all types of samples are shown in Figures 2-6.

Figure 1 - Percentage abundance of Linear alkanes, Branched alkanes and Alkenes from cuticular chemical profiles of adults' body parts and juveniles of *Latrodectus geometricus*

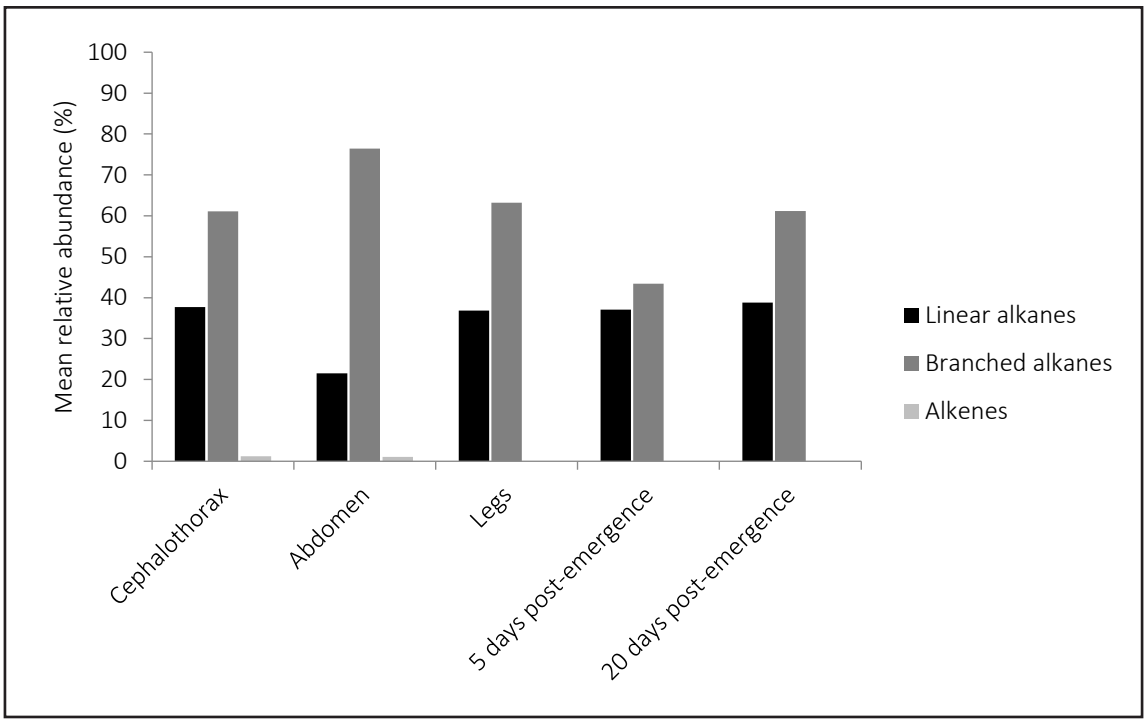


Figure 2 Bi-dimensional chromatogram of the cuticular chemical composition of cephalotorax of *Latrodectus geometricus* adult females

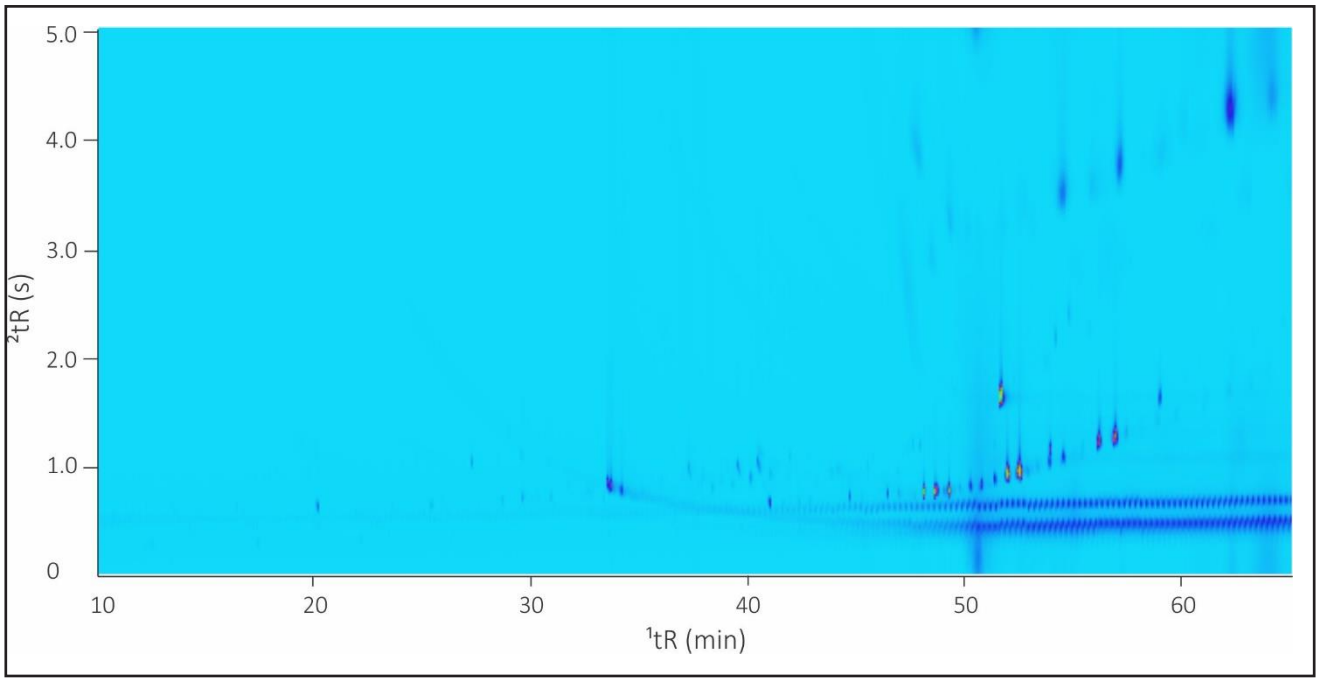


Figure 3 Bi-dimensional chromatogram of the cuticular chemical composition of abdomen of *Latrodectus geometricus* adult females

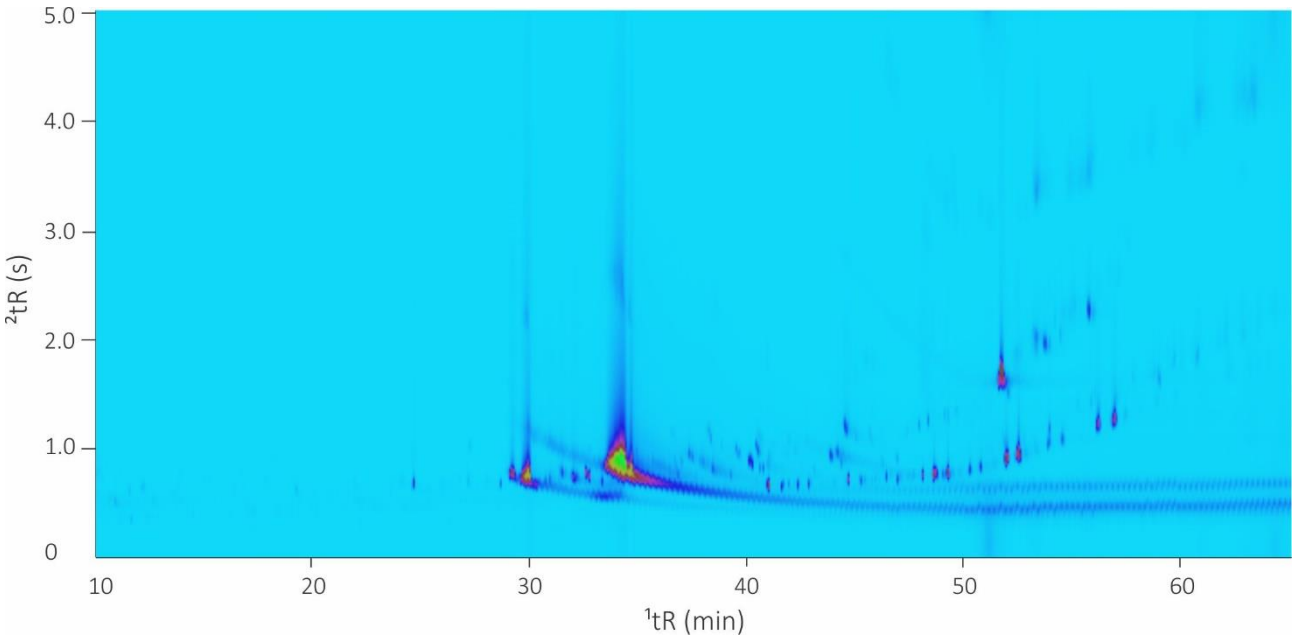
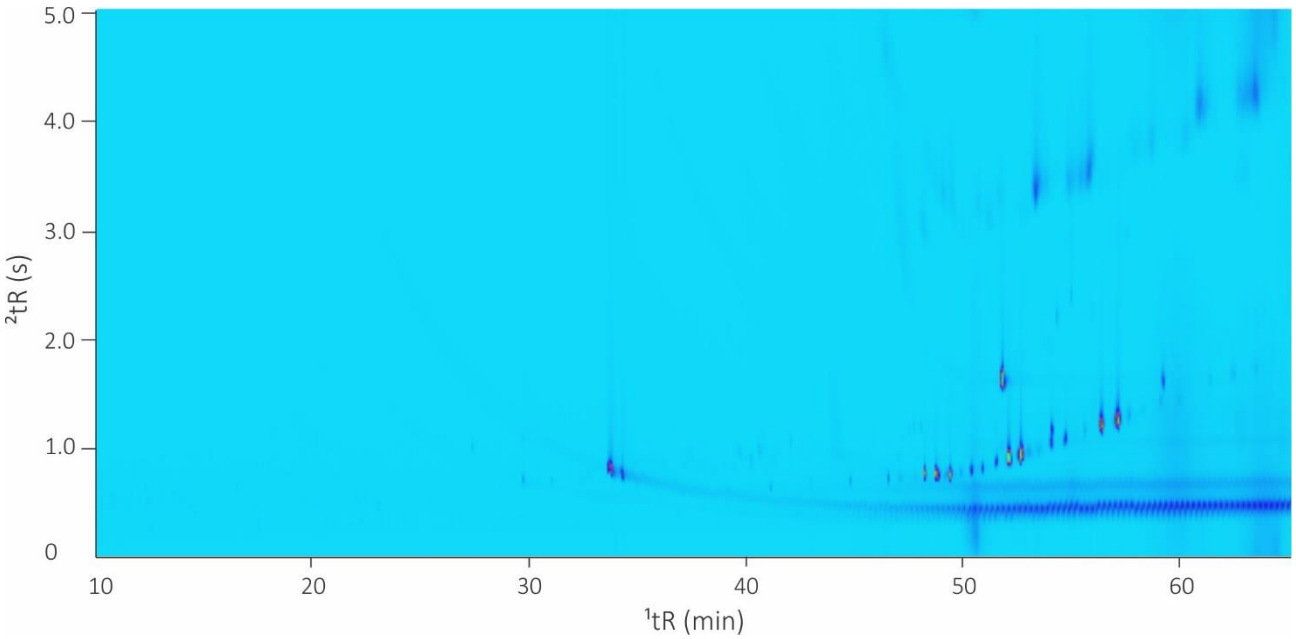


Figure 4 Bi-dimensional chromatogram of the cuticular chemical composition of legs of *Latrodectus geometricus* adult females



It was found two cases of compounds that seemed to co-elute in the first dimension and were completely separated in the ^{2}D (Table 1). It is possible to observe two methyl alkanes that had the same $^{1}t_{R}$ 46.83 min and presented the following retention times in the ^{2}D: 10-methyloctacosane ($^{2}t_{R}$ 1.68 s) and 14-methyloctacosane ($^{2}t_{R}$ 1.92 s); and the separation of two compounds that elute at $^{1}t_{R}$ 50.67 min and presented the following retention times in the ^{2}D: 10-methyltriacontane ($^{2}t_{R}$ 1.77 s) and 14-methyltriacontane ($^{2}t_{R}$ 2.01 s).

In adult's cephalothorax, the major compounds included hentriacontane, 3-methylhentriacontane, 15-,13-methylhentriacontane and pentatriacontane. In adult's legs, the major compounds were hentriacontane, 15-, 13-methylhentriacontane, 3-methylhentriacontane and pentatriacontane, while in abdomen the major compound was 2-methylheneicosane. In juveniles of 5 days post-emergence the major compounds were 10-methyltriacontane and pentatriacontane, while in juveniles of 20 days post-emergence the major compounds were hentriacontane, 3-methylhentriacontane and 15-, 13-methylhentriacontane (Table 1).

Figure 5 Bi-dimensional chromatogram of the cuticular chemical composition of *Latrodectus geometricus* juveniles of 5 days

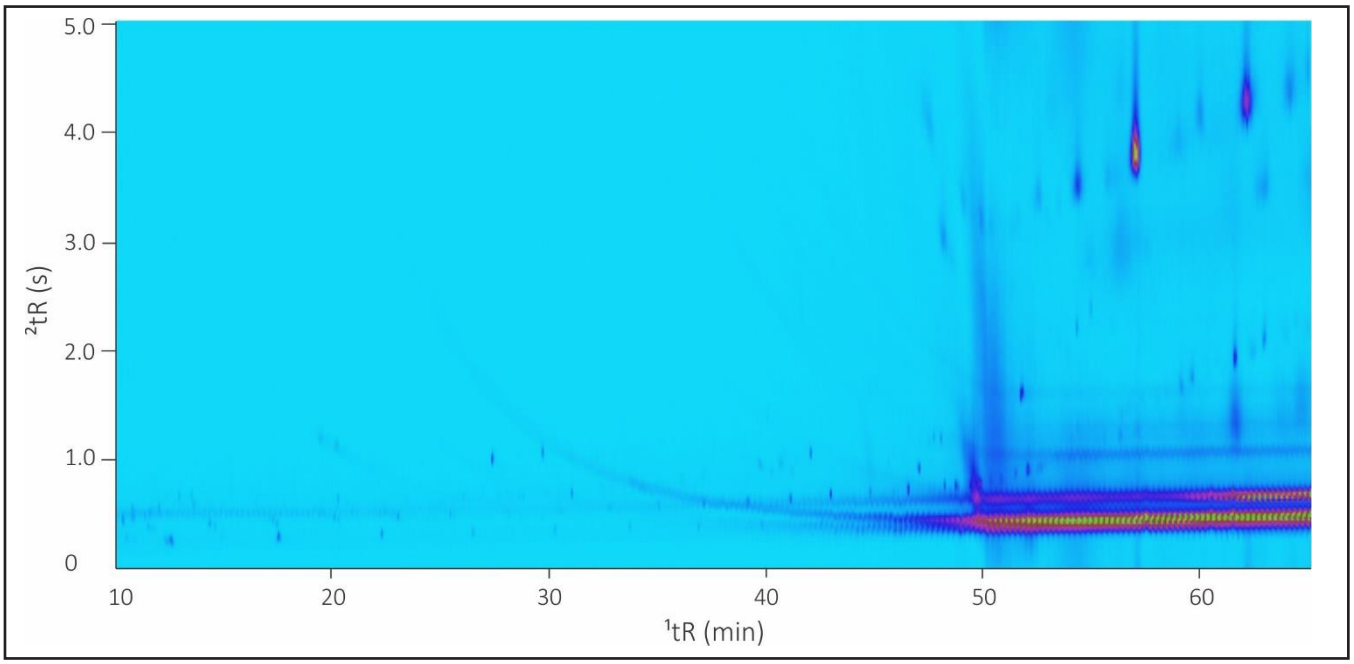
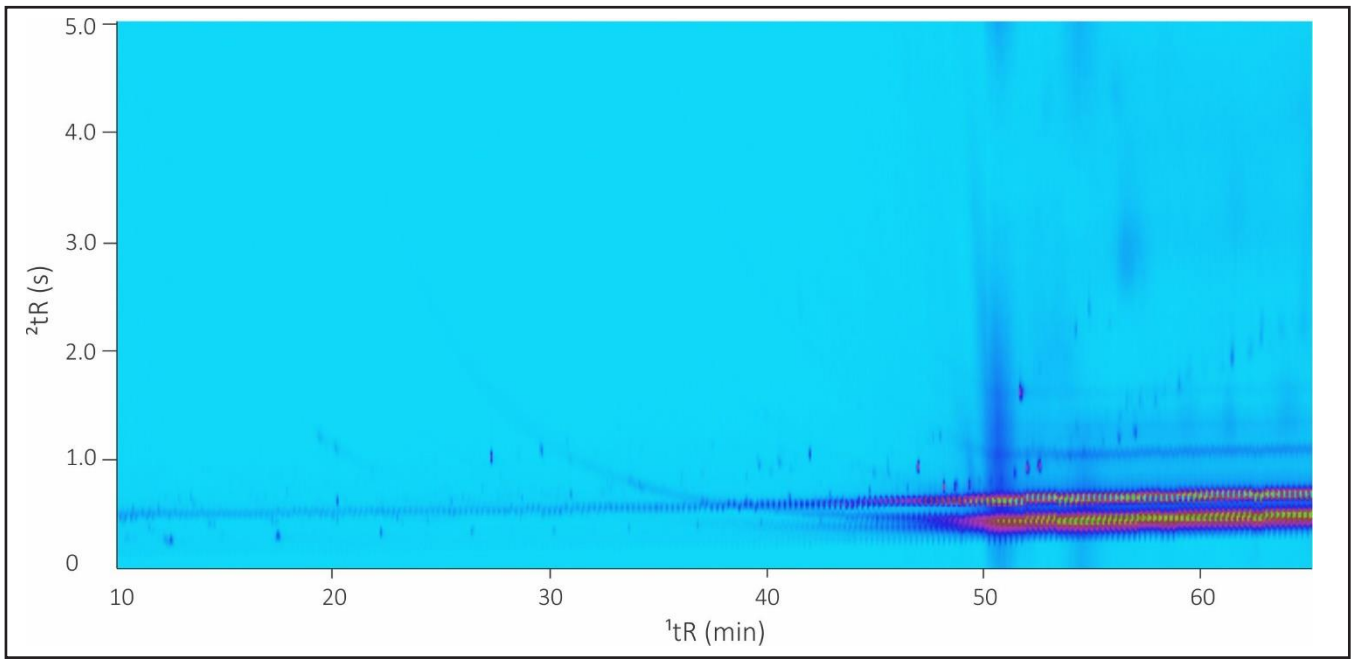


Figure 6 Bi-dimensional chromatogram of the cuticular chemical composition of *Latrodectus geometricus* juveniles of 20 days



4 Discussion

Comparing the variation in cuticular chemical compounds between *L. geometricus* adult's body parts it is possible to notice that the abdomen has greater number of compounds in relation to cephalothorax and legs. Guimarães et al. (2016) assessing the cuticular chemical profile of the same species by FTIR-PAS found that the readings of the compounds from all body parts have the same peaks, however in the abdomen these peaks showed greater intensity. These results may be explained by the fact that FTIR-PAS only detects vibrations of molecular chemical groups, and as such, it is capable of identifying and distinguishing molecular radicals and some types of chemical bonds, which could explain the presence of the same peaks in all body parts, despite the qualitative differences found by GC×GC/qMS, since some of the peaks might be composed of the same chemical groups.

The fact that females' abdomen present greater number of compounds than the other body parts can be related to the presence of spinnerets, organs responsible for producing the silk threads, and ovaries. Some specific substances, called sex pheromones appear to be strongly associated with the female's silk, since it can trigger male's courtship behavior (FOELIX, 2011). Indeed, experiments demonstrate that sealing the female's spinnerets with wax, thus preventing the deposition of silk on the ground, causes males to fail to court (DONDALE; HEDGEKAR, 1973). Thus, it is possible that during silk manipulation, either during courtship behavior or web production, some of the silk compounds stay adhered to the abdomen's cuticle. In addition, males' ability to discriminate between silk threads from virgin and mated females has been reported in many studies (e.g. ANAVA; LUBIN, 1993; ANDRADE; KASUMOVIC, 2005; BARUFFALDI; COSTA, 2010; ROBERTS; UETZ, 2005), supporting the hypothesis that the physiological stage of ovaries present on the abdomen of females might influence on CHC composition. In the juveniles cases the lower number of compounds relative to all body parts of the adults is likely because their chemical signatures are not complete yet, since they undergo several molts until reaching adulthood. Indeed, it is well known that cuticular composition changes as the juveniles develop, especially around the period of dispersion (GRINSTED et al., 2011; TRABALON et al., 1996).

Table 1 show the separation of compounds that seemed to co-elute in the first dimension and were completely separated in the ^{2}D. The separation of two compounds that elute at $^{1}t_{R}$ 50.67 min and presented the following retention times in the ^{2}D: 10-methyltriacontane ($^{2}t_{R}$ 1.77 s) and 14-methyltriacontane ($^{2}t_{R}$ 2.01 s) with distinct molecular mass. It is possible to observe two compounds that had the same $^{1}t_{R}$ 46.83 min and presented the following retention times in the ^{2}D: 10-methyloctacosane ($^{2}t_{R}$ 1.68 s) and 14-methyloctacosane ($^{2}t_{R}$ 1.92 s) and the mass spectra of these compounds show base peak and molecular ion characteristic of both compounds. This type of situation is an example of coelution and demonstrates the selectivity and peak capacity added to the system as a different interaction mechanism among analytes and stationary phase provided by the second dimension column. The mass spectra deconvolution software may be employed and may provide separation among two or more compounds (ÖZÇIMEN; KARAOSMANOĞLU, 2004). This can represent a great implication on the results presented on studies that assessed cuticular chemical composition in both spiders and other arthropods, since the number of compounds described can be greater than the ones found by the usual chromatographic techniques. In this sense, species that presented a relatively large number of cuticular compounds in previous studies, such as the spider *Anelosimus eximius* and the paper wasp *Mischocyttarus consimilis*, might actually have an even greater number of compounds (MICHELUTTI et al., 2017; PASQUET et al., 1997).

GC×GC/qMS analysis of the samples detected 50 peaks in total, from heptadecane to 17-, 13-, 15-, 11-methylpentatriacontane with branched alkanes representing the majority of compounds in all samples, similar to the cuticular chemical profile of the subsocial spider *Stegodyphus lineatus*, assessed by GC-MS, which was characterized by 38 hydrocarbons peaks, belonging to 3 different classes: linear alkanes and mono- and di-methyl–branched alkanes with chain lengths ranging between C_{23} and C_{35}; with branched alkanes also representing the majority of compounds (GRINSTED et al., 2011). On the other hand, total extract of *Tegenaria atrica* female, also assessed by GC-MS, is composed of a mixture of 64 saturated hydrocarbons, 7 methyl-esters, 4 acids and 2 unknown compounds, with the majority of compounds being linear alkanes (TRABALON et al., 1996). This difference might be explained by the fact that nestmate recognition in social insects most commonly depends on branched alkanes and alkenes as recognition cues, while linear alkanes have little influence on nestmate recognition (DANI et al., 2001, 2005; VAN ZWEDEN et al., 2009). Thus, branched alkanes might play a more significant role in subsocial spider species or the ones with extended aggregation periods, such as *S. lineatus* and *L. geometricus* respectively, than in solitary species with short aggregation period such as *T. atrica*. Interestingly, a case of subsociality in the genus *Latrodectus* has been recently described in the Brazilian species *Latrodectus* cf. *curacaviensis* (BERTANI et al., 2008).

4 Conclusions

According to the results discussed in this study it is possible to infer that there is variation in cuticular chemical composition between the different body parts of adult females of the spider *L. geometricus*, with the abdomen presenting a greater number of compounds, as well as between adults and juveniles since cuticular chemical profile of all adults' body parts showed greater number of compounds than both juvenile's samples. Also, this is the first study using GC×GC/qMS to assess cuticular chemical profiles, and according to the results, it can be considered a reliable technique to assess these type of matrices, since it was possible to identify the majority of compounds present in the spider's cuticle, in addition to detect and separate two cases of coelution of compounds. Thus, it can be inferred that the use of this technique is advantageous in assessing species whose cuticular chemical profiles are more complex and therefore could be underestimated by most usual techniques.

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