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**Original article**

# Chemical profile from the head of *Vespa velutina* and *V. crabro*

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**Abstract –** *Vespa velutina* and *V. crabro* are eusocial insects in which chemical communication is decisive for social interactions. *V. velutina* was accidentally introduced in 2004 in France and subsequently in northern Spain in 2010. It is an invasive species that severely affects the beekeeping sector. The hornet autochthonous *V. crabro* with a similar ecological niche is captured in bait traps used to control *V. velutina* populations. Insect cuticle and head structures had an important role in chemical communication so that this research approaches for the first time chemical compounds extracted from the heads of *V. velutina* and *V. crabro*. Chemical compounds were profiled using GC/MS. The main compounds identified were carbohydrates, fatty acids, and hydrocarbons. The chemical profile of both species was compared and also an intrinsic differentiation was made between queens and workers in *V. velutina*. Chemometric techniques (PCA and LSD) were used to achieve this goal.

**chemical compounds / cuticular compounds / *Vespa velutina* / *Vespa crabro* / GC/MS**

## 1. INTRODUCTION

*Vespa velutina nigrithorax* du Buysson, 1905 is a social species of invasive hornet that was accidentally introduced into southwest France (Arca et al. 2015; Monceau et al. 2014). In a few years, this invasion moved to other European countries. In the Iberian Peninsula was first recorded in 2010. Its expansion across Europe, as well as its impacts on the environment, was widely studied (Arca et al. 2015; Monceau et al. 2014; Rodríguez-Flores et al. 2019). *Vespa velutina* is a predator of some important pollinators such as *Apis mellifera*, Linnaeus, 1758, causing environmental and socio-economic impacts. The voracious appetite for the honeybee has turned the species into the target of the study of several research works whose main objective is to give information on how to develop an effective control method (Monceau et al. 2014). Bait-trapping is one of the most used methods for capturing founder queens in spring and autumn (Rodríguez-Flores et al. 2019; Rojas-Nossa et al. 2018). However, this method is not specific and could affect the biodiversity of the local entomofauna if it is used in an uncontrollable and erroneous way (Rodríguez-Flores et al. 2019). *Vespa crabro*, Linnaeus, 1758, the European hornet, is one of the affected species captured in the bait traps. *V. crabro* and *V. velutina* have overlapping ecological niches and could be potential competitors (Monceau et al. 2015; Cini et al. 2018). Both species are eusocial insects being this behavior the highest level of the social relationship between animals (Wilson 1971; Smith and Szathmary 1997). In eusocial species,

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colonies are composed of thousands of individuals, so individual recognition and regulation of social interaction are essential to maintain the stability of colonies. Many studies stood out the important role of chemical communication ruled by chemical signals (semiochemicals) in this interaction (Wilson and Bossert 1963; Ali and Morgan 1990; Batra 1980; Cappa et al. 2019; Dani 2006; Keller and Nonacs 1993; Maisonasse et al. 2010; Pain 1973; Richard and Hunt 2013; Wyatt 2017). Within the semiochemicals, pheromones are the most known chemical signals used in the communication between individuals of the same species (Karlson and Lüscher 1959; Ali and Morgan 1990; Wyatt 2017). However, other semiochemicals can be hydrocarbons that cover the insect cuticle and are important in social recognition of bees, ants, termites, and wasps (Couto et al. 2017; Kaib et al. 2000; Richard and Hunt 2013; Ruther et al. 2002).

Hornets distinguish their nest mates from members of other colonies through receptors located in some parts of the body like the antennae that recognize a broad spectrum of cuticular chemicals such as hydrocarbons (CHCs) (Dani et al. 2001; Dani 2006). The cuticle compounds are predominantly endogenous in origin and allow recognition through a stored recognition mechanism (Wyatt 2017). In addition, there are exogenous signals from nest material and/or food that correspond to chemical substances absorbed by the cuticle helping to recognize the specific smell of the colony (Hölldobler and Michener 1980). The cuticle is covered by a layer with waxy substances that is made up of a mixture of lipids and that provide waterproofing (Gévar et al. 2017; Richard and Hunt 2013; Singer 1998). The lipid composition can contain normal and branched saturated and unsaturated hydrocarbons, free fatty acids, free alcohols, alkyl esters, glycerides, sterols, and aldehydes (Richard and Hunt 2013; Singer 1998). Some CHCs could be methyl-branched alkanes of the cuticle, but also other long-chain compounds or different combinations of these chemicals (Richard and Hunt 2013). In addition, CHCs also serve as contact pheromones for inter- and intra-specific recognition. Females of *V. crabro* and *Polistes dominulus*, Latreille, 1802, produce short-distance contact sex pheromones in their cuticular hydrocarbons from their antennal glands (Batra 1980; Wen et al. 2017).

Knowledge about chemical signals in insects and their role in social communication are of great interest in the scientific community due to these chemicals help to understand relationships among individuals and tasks. Also, semiochemicals can be interesting as a control method of pest species. Currently, the most widely strategy for pest control is the use of conventional pesticides that are different chemicals from those found in nature and involve environmental risks. Thus, the development of ecofriendly alternatives is a priority in society (Howse 2006). Some studies focus the management of pest in the use of microbial pesticides (bacteria, fungi, viruses, etc.) (Bartolomé et al. 2020; Dalmon et al. 2019; Mazzei et al. 2019; Yang et al. 2020); while other focus on the possible use of semiochemicals such as pheromones or other chemicals (Couto et al. 2014; Monceau et al. 2014; Wen et al. 2017).

Cuticular compounds and other chemical compounds such as pheromones in hornets are distributed throughout the body and their functions are associated with many aspects of colony organization (Richard and Hunt 2013). Recently, it has been shown that visual and olfactory cues can mediate various types of social recognition (Couto et al. 2017; Wang et al. 2014). Some important sensory channels in recognition and communication are found in the head area of hornets. *V. velutina* can perceive chemical signals in which cuticular hydrocarbons participate through the olfactory sensor located in the antennas (Couto et al. 2017). In Hymenoptera, there are a series of glands connected to the oral cavity, which constitute the salivary system, formed by the thoracic salivary glands, the mandible, and the hypopharyngeal glands. In *V. velutina*, the mandibular glands could be a potential source of sex pheromones (Monceau et al. 2014), although these can also provide alarm pheromones as in *Vespula* spp. (Cheng et al. 2017). Although there are some studies on cuticular hydrocarbons (CHCs) in *V. velutina* (Couto et al. 2017; Gévar et al. 2017; Wen et al. 2017; Pérez-de-Heredia et al. 2017), this work aims to contribute to the study of chemical compounds extracted from the

heads of *V. velutina* and *V. crabro* and show the differences and similarities between both species and castes of *V. velutina*.

## 2. MATERIAL AND METHODS

### 2.1. Procedure to capture *V. velutina* and *V. crabro* specimens

The capture of *V. velutina* and *V. crabro* individuals was carried out during the spring period of 2018 from Northwest of the Iberian Peninsula. Ten hornets of *V. velutina* were collected alive with the aid of beekeepers and kept individually. Hornets were classified as females, five founding queens, and five workers. The specimens were frozen and stored by separated at −20 °C in the laboratory until analysis. Eleven individuals of *V. crabro* were captured by beekeepers and were subjected to the same laboratory procedure. Regarding this species of hornet, all the individuals were classified as workers. For the identification of the samples and the discrimination between queens and workers, both the description made by Monceau et al. (2014) and the sampling period were considered.

### 2.2. *V. velutina* and *V. crabro* head extraction

Prior to and during the extraction, the individuals were kept on ice. After separation of the hornet heads, these were homogenized at room temperature with a high-speed vibration milling (Pulverisette 23, Fritsch) at a frequency of 50 Hz, for 10 min. Each head was extracted in 400 μL of methanol and 200 μL of decanoic acid (250 ng/μL; internal standard), according to the method adapted from the previously described work (Maisonasse et al. 2010). After centrifuging at 4000 rpm for 20 min at 4 °C, the supernatant was collected and concentrated under a nitrogen stream. The samples were then derivatized with 10 μL of bistrimethylsilyltrifluoroacetamide (BSTFA), mixtured, and left at room temperature for 40 min. The final samples were then diluted in 150 μL of hexane and analyzed by gas chromatography-mass spectrometry (GC-MS) using 1 μL as the injection volume.

### 2.3. Gas chromatography-mass spectrometry procedure

Gas chromatographic analysis was performed using a Perkin Elmer system with a Clarus® 580 GC module and a Clarus® SQ 8 S MS module, equipped with DB-5MS fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific, Inc.). The oven temperature was programmed as 100–200 °C at 13.4 °C/min, then held isothermal for 1.65 min and subsequently at 3.4 °C/min up to 250 °C, and finally held isothermal for 4.9 min; injector and detector temperatures, 280 °C. The transfer line temperature was 280 °C; ion source temperature, 230 °C; carrier gas, helium, adjusted to a linear velocity of 1.45 mL/min; splitless; ionization energy, 70 eV; scan range, 35–600 u; scan time, 1 s. Data acquisition was performed using the software Turbomass (software version 6.1.0, Perkin Elmer, Shelton, CT, USA). The identification of compounds was assigned by comparison of their retention times and GC-MS spectra from a commercial MS database (NIST 2011 mass spectral library). Quantification of the identified compounds was done based on the internal standard method.

### 2.4. Statistical analysis

Concentration values were analyzed statistically with IBM SPSS Statistics Base 23.0 software and STATGRAPHICS Centurion XVI.I. A principal component analysis (PCA) has been performed to simplify and describe the data set and correlate the identified compounds according to species. To differentiate between workers and queens, a multiple-sample comparison was performed by caste of each species. The method used to discriminate among the means was Fisher's least significant difference (LSD) procedure.

## 3. RESULTS

### 3.1. Chemical compounds profile of *V. velutina*

The chemical profile isolated from each individual head is a combination of no more than 11 compounds. Within all batch of samples analyzed,

it was possible to identify 19 compounds in *V. velutina*'s head (Table I). The most frequent were the carbohydrates glucofuranoside; D-(-)-tagatofuranose; D-(+)-trehalose; and L-(-)-sorbofuranose followed by the carboxylic acid D-gluconic acid and the fatty acids, oleic acid and 9,12,15-octadecatrienoic acid. These chemical compounds presented a concentration greater than 100 ng/mL. The rest of the chemical compounds identified in lower concentrations were the carbohydrates β-D-(+)-mannopyranose; β-D-glucopyranose; β-D-(+)-talopyranose; D-(+)-turanose; α-D-glucopyranose, glycerol-3-phosphate, the fatty acids octadecanoic acid and hexadecanoic acid, the aldonolactone d-gluconic acid ,δ-lactone, the prostaglandins prosta-5,13-dien-1-oic acid, 9,11,15-trihydroxy-, ion(1-), (5Z,9α,11α,13E,15S) or 5-trans-PGF2α (hereinafter), the hydrocarbons octadecane, 3-ethyl-5-(2-ethylbutyl)- and the sterol ethyl iso-allocholate.

In addition, the frequency in which these compounds occurred in the studied samples was considered. The following compounds L-(-)-sorbofuranose; glucofuranoside; oleic acid; hexadecanoic acid; D-(-)-tagatofuranose; and octadecanoic acid were identified in more than 50% of the samples.

### 3.2. Chemical compounds profile of *V. crabro*

The chemical profile of *V. crabro* revealed the presence of 17 compounds (Table I). However, as in *V. velutina*, the chemical profile diverges between specimens, with each individual presenting no more than 15 compounds. As in *V. velutina*, the compounds with a concentration greater than 100 ng/mL were carbohydrates D-(-)-tagatofuranose, glucofuranoside; L-(-)-sorbofuranose; 1,5-anhydro-D-sorbitol; β-D-(+)-talopyranose; 3-α-mannobiose; D-(-)-ribofuranose; 2-α-mannobiose; and the fatty acids oleic acid; hexadecanoic acid; 9-octadecenoic acid (Z)-; and octadecanoic acid. Glycerol-3-phosphate, the carbohydrates D-glucose and β-D-(+)-mannopyranose, the amine 9-octadecenamide, (Z)-, and the hydrocarbon 1-docosene were found in lesser concentrations.

Regarding the frequency, the chemical compounds that appeared in more than 50% of the samples were D-(-)-tagatofuranose; L-(-)-sorbofuranose; glucofuranoside; oleic acid; 9-octadecenoic acid (Z)-; β-D-(+)-talopyranose; hexadecanoic acid; and octadecanoic acid.

### 3.3. Comparison between the two species

A total of 27 chemical compounds were identified between both species. Both species had 9 chemical compounds in common: Glucofuranoside; D-(-)-tagatofuranose; L-(-)-sorbofuranose; β-D-(+)-mannopyranose; and β-D-(+)-talopyranose such carbohydrates; oleic acid; octadecanoic acid; and hexadecanoic acid such fatty acids and the glycerol-3-phosphate. On the other hand, 18 compounds were different between the chemical profiles of both species. *V. velutina* presented 10 compounds that were not identified in *V. crabro*, while the autochthonous species presented 8 compounds that did not appear in the invasive species (Table I).

A PCA has been carried out in the first place to reduce the dimension of the data. At the same time, it has allowed to correlate the identified chemical compounds based on the samples. This procedure obtained a number of linear combinations of the 27 variables (identified chemical compounds) extracting 8 components which accounted 89.1% of the variability in the original data. Table II shows the summary of the PCA, where the percentage of variance and accumulated for each of the components extracted is observed, together with the table of the main weights for each variable included in this analysis. The variables that most influenced the first component were 1,5-anhydro-D-sorbitol; D-(-)-ribofuranose; 9-octadecenoic acid, (Z); D-(-)-tagatofuranose; L-(-)-sorbofuranose; glucofuranoside, β-D-(+)-talopyranose, D-gluconic acid; β-D-glucopyranose Glycerol-3-phosphate; β-D-(+)-mannopyranose; and D-(+)-turanose. The heaviest values in the second component were octadecanoic acid; oleic acid; hexadecanoic acid; 3-α-mannobiose and 2-α-mannobiose, 9,12,15-octadecatrienoic acid; ethyl iso-allocholate; 5-trans-PGF2α; α-D-glucopyranose; and octadecane, 3-ethyl-5-(2-ethylbutyl)-.

**Table I.** Main compounds identified in both hornet species

| Chemical compounds identified | *V. velutina* | | | *V. crabro* | | |
|---|---|---|---|---|---|---|
| | RT | ng/uL | SD | RT | ng/uL | SD |
| Decanoic acid (standard) | 5.5 | 250.0 | 0.0 | 5.5 | 250.0 | 0.0 |
| 9,12,15-octadecatrienoic acid | 6.0 | 125.3 | 27.3 | - | - | - |
| Glycerol-3-phosphate | 8.1 | 90.5 | 17.8 | 8.4 | 84.5 | 25.3 |
| L-(-)-Sorbofuranose | 8.4 | 136.4 | 115.0 | 8.4 | 341.9 | 685.6 |
| D-(-)-Tagatofuranose | 8.5 | 254.2 | 243.4 | 8.5 | 530.4 | 1099.0 |
| Glucofuranoside | 8.8 | 275.4 | 270.9 | 8.8 | 468.2 | 930.0 |
| d-Gluconic acid, δ-lactone | 9.3 | 49.2 | 7.8 | - | - | - |
| β-D-(+)-Mannopyranose | 9.3 | 98.3 | 36.7 | 9.4 | 52.4 | 72.6 |
| β-D-(+)-Talopyranose | 9.5 | 85.5 | 75.6 | 9.5 | 210.7 | 350.0 |
| α-D-Glucopyranose | 10.4 | 33.4 | 26.9 | - | - | - |
| D-Glucose | - | - | - | 10.4 | 80.0 | 98.5 |
| β-D-Glucopyranose | 10.5 | 90.4 | 41.6 | - | - | - |
| D-Gluconic acid | 10.8 | 246.5 | 70.1 | - | - | - |
| Hexadecanoic acid | 11.6 | 51.9 | 22.7 | 11.5 | 130.3 | 57.1 |
| 9-Octadecenoic acid (Z)- | - | - | - | 12.4 | 128.6 | 148.0 |
| Ethyl iso-allocholate | 12.4 | 18.1 | 18.7 | - | - | - |
| Oleic acid | 14.5 | 175.0 | 156.9 | 14.5 | 247.6 | 155.8 |
| Octadecanoic acid | 15.0 | 52.9 | 34.1 | 14.9 | 114.8 | 57.4 |
| 9-Octadecenamide, (Z)- | - | - | - | 17.2 | 76.9 | 48.2 |
| Octadecane, 3-ethyl-5-(2-ethylbutyl)- | 19.4 | 25.5 | 19.6 | - | - | - |
| 1-Docosene | - | - | - | 19.4 | 25.4 | 1.7 |
| 5-trans-PGFα | 21.5 | 25.9 | 21.9 | - | - | - |
| D-(-)-Ribofuranose | - | - | - | 21.7 | 144.4 | 89.5 |
| 3-α-Mannobiose | - | - | - | 22.2 | 173.1 | 77.0 |
| 1,5-Anhydro-D-sorbitol | - | - | - | 22.3 | 222.3 | 122.5 |
| D-(+)-Turanose | 24.7 | 38.9 | 3.5 | - | - | - |
| D-(+)-Trehalose | 24.9 | 196.1 | 151.6 | - | - | - |
| 2-α-Mannobiose | - | - | - | 24.9 | 142.9 | 115.2 |

*RT*, retention time; *SD*, **s** tandard deviation; *ng/uL*, final mean concentration based on the standard decanoic acid

The first two components have been represented in Figure 1, both of which explain 44% of the data. The variables included in this analysis (Figure 1a) and the samples of both species (Figure 1b) can be seen represented and separated respectively in this space. With the exception of a *V. crabro* sample, which appears isolated on the right flank, the *V. velutina* samples tend to be placed towards the lower part while those of *V. crabro* towards the upper part. In this sense, *V. crabro* samples are mainly influenced by the compounds 1,5-anhydro-D-sorbitol; D-(-)-ribofuranose; 9-octadecenoic acid, (Z); D-(-)-tagatofuranose; L-(-)-sorbofuranose; glucofuranoside, β-D-(+)-talopyranose; octadecanoic acid; oleic acid; hexadecanoic acid; 3-α-mannobiose; and 2-α-mannobiose, while D-gluconic acid; β-D-glucopyranose Glycerol-3-phosphate; β-D-(+)-mannopyranose; D-(+)-turanose; 9,12,15-octadecatrienoic acid; ethyl iso-allocholate; 5-trans-PGF2a; α-D-glucopyranose; and octadecane, 3-ethyl-5-(2-ethylbutyl)-

**Table II.** Summary of the PCA analysis and the contribution of the variables in each component

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC 6 | PC 7 | PC 8 |
|---|---|---|---|---|---|---|---|---|
| **Eigenvalue** | 6.4 | 5.5 | 3.7 | 2.9 | 1.7 | 1.5 | 1.3 | 1.0 |
| **Percent of variance** | 23.7 | 20.3 | 13.9 | 10.8 | 6.2 | 5.7 | 4.8 | 3.9 |
| **Cumulative percentage** | 23.7 | 44.0 | 57.8 | 68.7 | 74.8 | 80.5 | 85.3 | **89.1** |
| | | | **Table of weights** | | | | | |
| **Variables** | **PC 1** | **PC 2** | **PC 3** | **PC 4** | **PC 5** | **PC 6** | **PC 7** | **PC 8** |
| D-Gluconic acid | 0.135 | 0.099 | 0.352 | 0.227 | 0.243 | −0.073 | 0.097 | 0.119 |
| β-D-Glucopyranose | 0.127 | 0.065 | 0.366 | 0.194 | −0.173 | −0.302 | 0.100 | 0.164 |
| Glycerol-3-phosphate | 0.117 | 0.230 | 0.171 | 0.183 | 0.028 | 0.418 | −0.019 | −0.028 |
| β-D-(+)-Mannopyranose | 0.115 | 0.097 | 0.303 | 0.177 | −0.422 | 0.012 | 0.054 | −0.064 |
| D-(+)-Turanose | 0.101 | 0.076 | 0.297 | 0.191 | 0.360 | −0.038 | 0.090 | 0.061 |
| Oleic acid | 0.099 | 0.382 | 0.016 | 0.159 | 0.061 | −0.018 | 0.075 | 0.112 |
| Octadecanoic acid | 0.070 | 0.403 | −0.071 | 0.083 | −0.010 | 0.030 | 0.061 | 0.027 |
| d-Gluconic acid, δ-lactone | 0.066 | −0.008 | 0.151 | 0.024 | 0.536 | 0.136 | −0.148 | −0.199 |
| D-(+)-Trehalose | 0.059 | −0.020 | 0.156 | 0.012 | −0.426 | −0.334 | −0.060 | 0.053 |
| Hexadecanoic acid | 0.049 | 0.352 | −0.202 | 0.145 | 0.012 | −0.150 | 0.067 | −0.071 |
| D-Glucose | 0.043 | 0.163 | −0.008 | 0.061 | −0.334 | 0.589 | −0.078 | −0.277 |
| 9,12,15-octadecatrienoic acid | 0.037 | −0.136 | −0.049 | −0.160 | 0.002 | 0.174 | 0.666 | 0.172 |
| Octadecane, 3-ethyl-5-(2-ethylbutyl)- | 0.024 | −0.181 | −0.166 | 0.148 | −0.001 | 0.125 | 0.524 | 0.179 |
| 9-Octadecenamide, (Z)- | 0.014 | 0.035 | −0.077 | −0.122 | 0.005 | 0.127 | −0.397 | 0.714 |
| 3-α-Mannobiose | 0.011 | 0.347 | −0.257 | −0.034 | −0.053 | 0.036 | 0.024 | 0.009 |
| α-D-Glucopyranose | −0.002 | −0.169 | −0.238 | 0.458 | −0.003 | −0.026 | −0.082 | 0.010 |
| 5-trans-PGFα | −0.003 | −0.168 | −0.238 | 0.459 | −0.003 | −0.026 | −0.080 | 0.011 |
| 1-Docosene | −0.004 | 0.127 | −0.163 | −0.094 | 0.084 | −0.367 | 0.062 | −0.439 |
| Ethyl iso-allocholate | −0.004 | −0.163 | −0.236 | 0.462 | −0.003 | −0.026 | −0.075 | 0.016 |
| 2-α-Mannobiose | −0.008 | 0.326 | −0.269 | −0.046 | 0.050 | −0.130 | 0.055 | 0.164 |
| 1,5-Anhydro-D-sorbitol | −0.331 | 0.187 | −0.021 | 0.021 | 0.036 | −0.043 | 0.078 | 0.080 |
| D-(-)-Ribofuranose | −0.336 | 0.205 | −0.042 | 0.015 | 0.035 | −0.048 | 0.082 | 0.113 |
| 9-Octadecenoic acid (Z)- | −0.351 | −0.022 | 0.105 | −0.045 | 0.015 | 0.010 | −0.074 | 0.009 |
| D-(-)-Tagatofuranose | −0.364 | 0.035 | 0.170 | 0.053 | −0.037 | 0.011 | 0.041 | −0.017 |
| L-(-)-Sorbofuranose | −0.370 | 0.026 | 0.139 | 0.101 | −0.032 | 0.026 | 0.036 | −0.018 |
| Glucofuranoside | −0.375 | −0.008 | 0.070 | 0.127 | 0.016 | 0.042 | 0.008 | −0.032 |
| β-D-(+)-Talopyranose | −0.380 | 0.017 | 0.065 | 0.109 | −0.019 | 0.047 | 0.027 | −0.005 |

*PC*, principal component

were the variables that explain the differentiation of the *V. velutina* samples.

### 3.4. Differentiation between workers and queens of *V. velutina*

The chemical compounds in the samples were studied according to whether they belonged to workers or queens (Table III). Thirteen compounds were common among queens and workers of *V. velutina*. Five compounds were only presented in the workers: α-D-glucopyranose; D-(+)-trehalose; ethyl iso-allocholate; glycerol-3-phosphate; and 5-trans-PGF2alpha. The fatty acid 9,12,15-octadecatrienoic acid was only identified in queens.

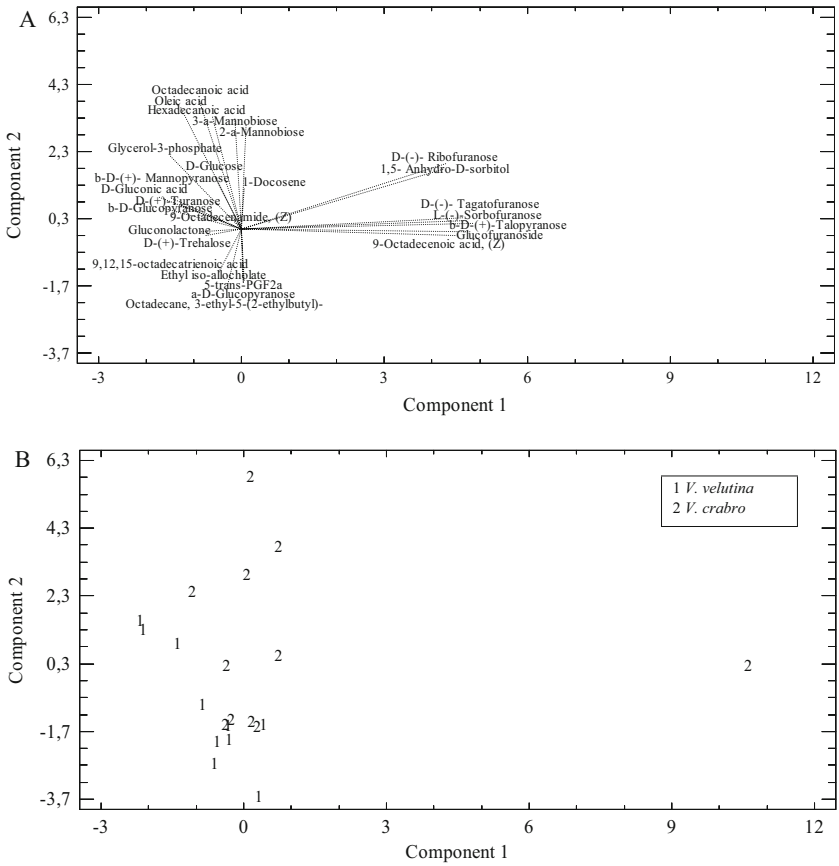


**Figure 1** Representation of the first two components of the PCA. **a** Chemical compounds identified and their correlations between them. **b** Samples based on the location of the compounds identified in both species.

No significant differences were found between the common compounds according to the type of caste. However, some compounds had a higher frequency in the samples and a higher mean content. Hexadecanoic and octadecanoic acid, and oleic acid were found at higher frequencies (in more than 80% of the samples) and with a higher average content in worker individuals (Table III). It also is worth noting the carbohydrates β-D-(+)-talopyranose and β-D-glucopyranose present in more than 60% and in higher mean content in the workers.

## 4. DISCUSSION

The understanding of the systems of recognition is important in the study of pest species like *V. velutina*. Research efforts on chemicals from *V. velutina* are necessary to improve knowledge on social communication and later to develop strategies for population control. Compounds of diverse nature were identified in the heads of *V. velutina* and *V. crabro*, among them, carbohydrates, fatty acids, and hydrocarbons (alkanes). Some compounds come from the cuticle but other are related to the hypopharyngeal gland. The broad nature of these compounds identified in this work also implies that they could perform various functions. Recognition between nestmates, sexual communication, or being alarm compounds are among the most prominent functions of this type of compound in the literature. The presence of some of these compounds, especially the of CHCs, serves as a personalized chemical signature and would be useful to

**Table III.** Statistical description for each compound based on the two castes (workers and queens) in *V. velutina*

| Chemical compounds | Caste | Mean | Maximum | Minimum | SD | Frequency (%) |
|---|---|---|---|---|---|---|
| 9,12,15-octadecatrienoic acid | Workers | - | - | - | - | - |
| | Queens | 50.1 | 144.6 | 0.0 | 70.0 | 40.0 |
| Glycerol-3-phosphate | Workers | 36.2 | 103.1 | 0.0 | 50.4 | 40.0 |
| | Queens | - | - | - | - | - |
| L-(-)-Sorbofuranose | Workers | 83.8 | 215.2 | 18.5 | 81.4 | 100.0 |
| | Queens | 134.4 | 361.7 | 0.0 | 149.5 | 60.0 |
| D-(-)-Tagatofuranose | Workers | 72.5 | 323.6 | 0.0 | 140.7 | 60.0 |
| | Queens | 181.7 | 599.1 | 0.0 | 269.0 | 40.0 |
| α-D-Glucofuranoside, methyl | Workers | 160.2 | 600.0 | 0.0 | 251.4 | 60.0 |
| | Queens | 170.3 | 647.1 | 0.0 | 272.0 | 60.0 |
| d-Gluconic acid, δ-lactone | Workers | 8.7 | 43.7 | 0.0 | 19.5 | 20.0 |
| | Queens | 10.9 | 54.6 | 0.0 | 24.4 | 20.0 |
| β-D-(+)-Mannopyranose | Workers | 31.6 | 94.1 | 0.0 | 44.5 | 40.0 |
| | Queens | 27.4 | 137.0 | 0.0 | 61.3 | 20.0 |
| β-D-(+)-Talopyranose | Workers | 38.7 | 152.7 | 0.0 | 64.8 | 60.0 |
| | Queens | 29.7 | 148.6 | 0.0 | 66.5 | 20.0 |
| α-D-Glucopyranose | Workers | 13.4 | 52.4 | 0.0 | 22.7 | 40.0 |
| | Queens | - | - | - | - | - |
| β-D-Glucopyranose | Workers | 48.2 | 129.1 | 0.0 | 54.1 | 60.0 |
| | Queens | 24.1 | 120.7 | 0.0 | 54.0 | 20.0 |
| D-Gluconic acid | Workers | 114.4 | 300.8 | 0.0 | 157.0 | 40.0 |
| | Queens | 33.5 | 167.4 | 0.0 | 74.8 | 20.0 |
| Hexadecanoic acid | Workers | 49.9 | 73.5 | 18.5 | 24.8 | 100.0 |
| | Queens | 12.4 | 61.8 | 0.0 | 27.7 | 20.0 |
| Ethyl iso-allocholate | Workers | 7.2 | 31.3 | 0.0 | 13.6 | 40.0 |
| | Queens | - | - | - | - | - |
| Oleic acid | Workers | 155.1 | 339.6 | 16.9 | 166.8 | 100.0 |
| | Queens | 54.8 | 274.2 | 0.0 | 122.6 | 20.0 |
| Octadecanoic acid | Workers | 40.3 | 90.0 | 0.0 | 40.5 | 80.0 |
| | Queens | 12.6 | 62.8 | 0.0 | 28.1 | 20.0 |
| Octadecane, 3-ethyl-5-(2-ethylbutyl)- | Workers | 6.1 | 23.4 | 0.0 | 10.2 | 40.0 |
| | Queens | 9.2 | 46.0 | 0.0 | 20.6 | 20.0 |
| D-(+)-Turanose | Workers | 8.3 | 41.3 | 0.0 | 18.5 | 20.0 |
| | Queens | 7.3 | 36.4 | 0.0 | 16.3 | 20.0 |
| D-(+)-Trehalose | Workers | 78.4 | 303.3 | 0.0 | 131.5 | 40.0 |
| | Queens | - | - | - | - | - |

differentiate both species in the search of new control strategy. These can play an important role like recognition cues in social insects (Cervo et al. 2015; Richard and Hunt 2013).

Many of the identified compounds in this study were carbohydrates. Carbohydrates can be components located on cell surfaces and play an important role in the molecular

adhesion of insects, as well as in cellular communication (Betz 2010; Reitz et al. 2015). They are generally in bound form as polysaccharides or glycoconjugates, such as glycoproteins, glycolipids, and proteoglycans. In aqueous solution are in cyclic form as a mixture of various stereoisomeric forms: α and β anomers. Carbohydrates as a result of an analysis of chemical profiles in insects have been seen in the tarsal secretions of the desert locust, detailing anomers of arabinose, xylose, glucose, galactose, pyranose, and furanose (Reitz et al. 2015). Other carbohydrates, as L-(-)-sorbofuranose, could be part of other larger molecules such as spiroacetals. Some spiroacetals are insect pheromones and are very widespread in nature as volatile constituents (Francke and Kitching 2001; Martin et al. 1996).

Other abundant compounds were fatty acids, other carboxylic acids, and hydrocarbons. These compounds probably belonged to the enormous variety of lipid compounds of the cuticle of both species. Cuticular lipids have a protective function, but these are also involved in the communication of many insect species (Richard and Hunt 2013). The carboxylic acids are also compounds that come from the glandular secretion in Vespidae. For example, hexadecanoic, octadecadienoic, and octadecenoic acids are generic molecules that have already been identified in other Vespidae species as the main carboxylic acids in *P. dominulus* and *Polistes sulcifer*, Zimmermann, 1930 (Dani et al. 1995) or defensive pheromones from the mandibular gland in *P. dominulus* (Fortunato et al. 2001). In other insects, the occurrence of gluconic acid in the defensive secretions of species of cockroaches has also been seen (Dateo and Roth 1967). As in this study, this carboxylic acid has also been found to be interrelated with a lactone in cockroaches (Farine et al. 2000) and termites (Hamilton et al. 2011), converting to gluconolactone.

In both species, fatty acids have been found but specifically 9,12,15-octadecatrienoic acid (linolenic acid) has been identified in *V. velutina* and 9-octadecenoic acid (Z)- (oleic acid isomer) in *V. crabro*. Some researchers indicated that the most important recognition pheromones were fatty acids, such as oleic acid, hexadecanoic acid, and octadecanoic acid (Dani et al. 1995; Dani 2006) with can be well detected by olfaction/gustation. Furthermore, these fatty acids and some of their esters can act as both pheromones and kairomones, as has been shown in the brood of honeybees (Trouiller et al. 1991) and in the eggs of European grape vine moth (Gabel and Thiéry 1996). Another function attributed to fatty acids is as precursors in the biosynthesis of some pheromones, such as linoleic acid (an omega-fatty acid like linolenic acid found in *V. velutina* samples). This may function as a precursor on the biosynthetic pathway of male sex pheromone in the parasitic wasp *Nasonia vitripennis*, Walker, 1836 (Blaul et al. 2014; Ruther et al. 2009). In the case of *V. velutina*, 4-oxo-octanoic acid and 4-oxo-decanoic acid were identified as sexual pheromones (Wen et al. 2017). These are derived from the octanoic fatty acid. In addition, fatty acids can also be pheromones from the mandibular glands. This is the case of the composition of the mandibular pheromones in other hymenopterans, such as the honeybee, where (E)-9-oxodec-2-enoic acid (9-ODA) and 9-hydroxydec-2-enoic acid (9-HDA) were detected as unsaturated fatty acid and perform a functional role within the colony (Keeling et al. 2001; Maisonasse et al. 2010; Plettner et al. 1997).

The hydrocarbons compounds are remarkable constituents of the cuticle in insects and were related to the post-pharyngeal gland (Ruther et al. 2002). Cuticular hydrocarbons are particularly attractive candidates as recognition cues (Dani et al. 1995; Kaib et al. 2000). Also, some eusocial insects like bumblebees (Krieger et al. 2006) and halictine bees (Ayasse et al. 1999) can produce sex pheromones from their cuticular hydrocarbons. With regard to *V. velutina*, diverse hydrocarbons with a chain length from 21 to 35 carbon atoms, n-alkanes, mono- and dimethyl-alkanes, and alkenes were identified in the literature (Dani et al. 2001). Some of these compounds differ between queens and workers (Gévar et al. 2017). In *V. crabro*, a greater number of compounds were identified (Ruther et al. 2002). In this work, octadecane, 3-ethyl-5- (2-ethylbutyl)- characterized the samples of

*V. velutina*, while 1-docosene those of *V. crabro*. It is important to mention that other hydrocarbons such as (octacosane; 17-Pentatriacontene; heptacosane; tetratetracontane; and 1-dimethyl (prop-2-enyl) octadecane) have been identified in the *V. velutina* samples; however, since each of them appears in a single individual, they have not been considered to characterize.

Finally, the rest of the identified compounds can be precursors in routes of synthesis of volatile or semi-volatile compounds of semiochemicals. Long-chain amides were reported as potential chemical messengers in insects (Calvello et al. 2003), concretely 9-octadecenamide, (Z)- (or oleamide) could be an endogenous ligand that is associated with a chemosensory specific protein in insects such as locusts or in gypsy moth (Ban et al. 2003; Nardella et al. 2015). In *V. velutina* samples, some sterol-type compounds have been found, such as ethyl iso-allocholate, which is a bioactive compound studied for its antimicrobial, diuretic, and anti-inflammatory activity (Singariya et al. 2012). Furthermore, the 5-trans-PGF$\alpha$ is a prostaglandin, a compound related to pheromones. The presence of prostaglandins in insects was first reported by Destephano et al. (1974), who described the biosynthesis of prostaglandin E (PGE) in reproductive tissues of mated female crickets and its role as a primer pheromone to help initiate the events that occur in the female after copulation. This compound was mentioned by Hagan and Brady (1982) in cabbage looper.

The compounds found in the samples were diverse respect to the castes and the species. Eighteen different compounds were identified between both species. *V. velutina* presented 4 carbohydrates not present in *V. crabro*, the compound 9,12,15-octadecatrienoic acid as the exclusive fatty acid, octadecane, 3-ethyl-5- (2-ethylbutyl)- as a hydrocarbon, in addition to other compounds not found in *V. crabro* such as a carboxylic acid (D-Gluconic acid), an aldonolactone (d-Gluconic acid, δ-lactone), a prostaglandin (5-trans-PGF$\alpha$), and a sterol (ethyl iso-allocholate). While *V. crabro* presented 4 different carbohydrates, the fatty acid 9-Octadecenoic acid (Z)-, 1-Docosene as the exclusive hydrocarbon, and the amide (9-Octadecenamide, (Z)-), and anhydro sugar (1,5-Anhydro-D-sorbitol) compounds. These chemical compounds have been essential in differentiating both species using the PCA statistical procedure. Regarding the differentiation between workers and queens of the *V. velutina* samples, this work highlights the presence of the 9,12,15-octadecatrienoic acid fatty acid identified only in queens. The workers presented 5 exclusive compounds already mentioned in previous paragraphs and some compounds in higher frequency and concentration.

According to Thiéry et al. (2018), although the pheromones are species-specific, their composition may vary within species due to population history; thus, its taxonomic value can be questioned. However, the main composition of the respective identified profiles provides valuable information to find components that serve as control methods for *V. velutina*. Some of these identified chemical compounds could be useful as attractors for selective capture since they could be recognition molecules, or they could deter by being alarm compounds. In this way, the ecological impact on the biodiversity of insects or arachnids could be avoided (Rodríguez-Flores et al. 2019). Although it is important to continue increasing the number of samples throughout the territory and the seasons, this work can be considered as a contribution to the scarce existing literature in the study of the chemical composition of *V. velutina* and *V. crabro*. The chemical description made is a contribution to the study of the differentiation between both species. *V. velutina*, increasingly common in our ecosystems, is one of the depopulation factors in hives, so the study of biology and the differentiation between castes are important when it comes to combating it. This differentiating is useful to capture the foundress queens being an effective method in decreasing their populations.

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## AUTHOR CONTRIBUTION

Experiment and material preparation and data collection were carried out by MSRF and SF. The data were analyzed by MSRF, SF, and MCS. MSRF, SF, OE, MCS, and MVB read and approved the final manuscript.

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## DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## DECLARATIONS

**Ethics approval** The study does not require ethics approval.

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

**Profil chimique de la tête de *Vespa velutina* et *V. crabro*.**

**Composés chimiques / Composés cuticulaires / *Vespa velutina* / *Vespa crabro* / GC/MS.**

**Das chemische Profil des Kopfes von *Vespa velutina* und *V. crabro*.**

**Chemische Stoffe / Kutikulakomponenten / *Vespa velutina / Vespa crabro* / GC/MS**

## REFERENCES

Ali, M., Morgan, E. D. (1990) Chemical communication in insect communities: a guide to insect pheromones with special emphasis on social insects. Biol. Rev. 65(3), 227-247

Arca, M., Mougel, F., Guillemaud, T., Dupas, S., Rome, Q., et al (2015) Reconstructing the invasion and the demographic history of the yellow-legged hornet, *Vespa velutina*, in Europe. Biol. Invasions 17(8), 2357-2371

Ayasse, M., Engels, W., Lübke, G., Taghizadeh, T., Francke, W. (1999) Mating expenditures reduced via female sex pheromone modulation in the primitively eusocial halictine bee, *Lasioglossum* (*Evylaeus*) *malachurum* (Hymenoptera: Halictidae). Behav. Ecol. Sociobiol. 45(2), 95-106

Ban, L., Scaloni, A., Brandazza, A., Angeli, S., Zhang, L., Yan, Y., Pelosi, P. (2003) Chemosensory proteins of *Locusta migratoria*. Insect Mol. Biol. 12(2), 125-134

Bartolomé, C., Buendía-Abad, M., Benito, M., Sobrino, B., Amigo, J., Carracedo, A., et al (2020). Longitudinal analysis on parasite diversity in honeybee colonies: new taxa, high frequency of mixed infections and seasonal patterns of variation. Sci. Rep. 10(1), 1-9

Batra, S. W. (1980) Sexual behavior and pheromones of the European hornet, *Vespa crabro* germana (Hymenoptera: Vespidae). J. Kansas Entomol. Soc. 53, 461-469

Betz, O. (2010) Adhesive exocrine glands in insects: morphology, ultrastructure, and adhesive secretion. In: Byern, J., Grunwald, I. (Eds.), Biological Adhesive Systems. From Nature to Technical and Medical Application. Springer, Heidelberg, Germany, pp. 111-152

Blaul, B. Steinbauer, R., Merkl, P., Merkl, R., Tschochner, H., Ruther, J. (2014) Oleic acid is a precursor of linoleic acid and the male sex pheromone in *Nasonia vitripennis*. Insect Biochem. Mol. Biol. 51, 33-40

Calvello, M., Guerra, N., Brandazza, A., D'ambrosio, C., Scaloni, A. et al (2003) Soluble proteins of chemical communication in the social wasp *Polistes dominulus*. Cell. Mol. Life Sci. 60(9), 1933-1943

Cappa, F., Cini, A., Pepiciello, I., Petrocelli, I., Inghilesi, A.F., Anfora, G., Cervo, R et al (2019) Female volatiles as sex attractants in the invasive population of *Vespa velutina nigrithorax*. J. Insect Physiol. 119, 103952

Cervo, R., Cini, A., Turillazzi, S. (2015) Visual recognition in social wasps. In: Aquiloni, L., Tricarico, E. (Eds) Social recognition in invertebrates, Springer International Publishing Switzerland, pp. 125-145

Cheng, Y. N., Wen, P., Dong, S. H., Tan, K., Nieh, J. C. (2017) Poison and alarm: the Asian hornet *Vespa velutina* uses sting venom volatiles as an alarm pheromone. J. Exp. Biol. 220(4), 645-651

Cini, A., Cappa, F., Petrocelli, I., Pepiciello, I., Bortolotti, L., Cervo, R. et al (2018) Competition between the native and the introduced hornets *Vespa crabro* and

*Vespa velutina*: a comparison of potentially relevant life-history traits. Ecol. Entomol. 43(3), 351-362

Couto, A., Monceau, K., Bonnard, O., Thiéry, D., Sandoz, J. C. (2014) Olfactory attraction of the hornet *Vespa velutina* to honeybee colony odors and pheromones. PLoS One 9(12), e115943

Couto, A., Mitra, A., Thiéry, D., Marion-Poll, F., Sandoz, J. C. (2017) Hornets have it: A conserved olfactory subsystem for social recognition in hymenoptera?. Front. Neuroanat. 11, 48

Dalmon, A., Gayral, P., Decante, D., Klopp, C., Bigot, D., Thomasson, M., Le Conte, Y., et al (2019). Viruses in the invasive hornet *Vespa velutina*. Viruses, 11(11), 1041

Dani, F. R. (2006) Cuticular lipids as semiochemicals in paper wasps and other social insects. Ann. Zool. Fenn. 43, 500-514.

Dani, F. R, Morgan, E. D, Turillazzi, S. (1995) Chemical analysis of sternal gland secretion of paper wasp *Polistes dominulus* (Christ) and its social parasite *Polistes sulcifer* (Zimmermann) (Hymenoptera: *Vespidae*). J. Chem. Ecol. 21(11), 1709-1718

Dani, F. R., Jones, G. R., Destri, S., Spencer, S. H., Turillazzi, S. (2001) Deciphering the recognition signature within the cuticular chemical profile of paper wasps. Anim. Behav. 62(1), 165-171

Dateo, G. P., Roth, L. M. (1967) Occurrence of gluconic acid and 2-hexenal in the defensive secretions of three species of Eurycotis (Blattaria: Blattidae: Polyzosteriinae). Ann. Entomol. Soc. Am. 60(5), 1025-1030

Destephano, D. B., Brady, U. E., Lovins, R. E. (1974) Synthesis of prostaglandin by reproductive tissue of the male house cricket, *Acheta domesticus*. Prostaglandins 6(1), 71-79

Farine, J. P., Everaerts, C., Abed, D., Brossut, R. (2000) Production, regeneration and biochemical precursors of the major components of the defensive secretion of *Eurycotis floridana* (Dictyoptera, Polyzosteriinae). Insect Biochem. Mol. 30(7), 601-608

Fortunato, A., Maile, R., Turillazzi, S., Morgan, E. D., Moneti, G., Jones, G. R., Pieraccini, G. (2001) Defensive role of secretion of ectal mandibular glands of the wasp *Polistes dominulus*. J. Chem. Ecol. 27(3), 569-579

Francke, W., Kitching, W. (2001) Spiroacetals in insects. Curr. Org. Chem. 5(2), 233-251

Gabel, B., Thiéry, D. (1996). Oviposition response ofLobesia botrana females to long-chain free fatty acids and esters from its eggs. J. Chem. Ecol. 22(1), 161-171

Gévar, J., Bagnères, A. G., Christidès, J. P., Darrouzet, E. (2017) Chemical heterogeneity in inbred European population of the invasive hornet *Vespa velutina nigrithorax*. J. Chem. Ecol. 43(8), 763-77

Hagan, D. V., Brady, U. E. (1982). Prostaglandins in the cabbage looper, *Trichoplusia ni*. J. Insect Physiol. 28(9), 761-765

Hamilton, C., Lay, F., Bulmer, M. S. (2011) Subterranean termite prophylactic secretions and external antifungal defenses. J. Insect Physiol. 57(9), 1259-1266

Hölldobler, B., Michener, C. D. (1980). Mechanisms of identification and discrimination in social Hymenoptera. In: Markl, H. (ed) Evolution of social behaviour: hypotheses and empirical tests. Berlin, pp 35-58

Howse, P. (2006). Feromonas de insectos y su uso en el control de plagas. Davinci Continental, Barcelona

Kaib, M., Eisermann, B., Schoeters, E., Billen, J., Franke, S., Francke, W. (2000) Task-related variation of postpharyngeal and cuticular hydrocarbon compositions in the ant *Myrmicaria eumenoides*. J. Comp. Physiol. A. 186(10), 939-948

Karlson, P., Lüscher, M. (1959) 'Pheromones': a new term for a class of biologically active substances. Nature, 183(4653), 55-56

Keeling, C. I., Otis, G. W., Hadisoesilo, S., Slessor, K. N. (2001) Mandibular gland component analysis in the head extracts of *Apis cerana* and *Apis nigrocincta*. Apidologie, 32(3), 243-252

Keller, L., Nonacs, P. (1993) The role of queen pheromones in social insects: queen control or queen signal?. Anim. Behav. 45(4), 787-794

Krieger, G. M., Duchateau, M. J., Van Doorn, A., Ibarra, F., Francke, W., Ayasse, M. (2006) Identification of queen sex pheromone components of the bumblebee *Bombus terrestris*. J. Chem. Ecol. 32(2), 453

Maisonasse, A., Alaux, C., Beslay, D., Crauser, D., Plettner, E., Le Conte, Y. (2010) New insights into honey bee (*Apis mellifera*) pheromone communication. Is the queen mandibular pheromone alone in colony regulation? Front. Zool. 7, 18-26

Martin, A., Salazar, J.A., Suárez, E. (1996) Synthesis of chiral spiroacetals from carbohydrates. T. JOC. 61(12), 3999-4006

Mazzei, M., Cilia, G., Forzan, M., Lavazza, A., Mutinelli, F., Felicioli, A. (2019). Detection of replicative Kashmir bee virus and Black queen cell virus in Asian hornet *Vespa velutina* (Lepelieter 1836) in Italy. Sci. Rep. 9(1), 1-9

Monceau, K., Bonnard, O., Thiéry, D. (2014) *Vespa velutina*: a new invasive predator of honeybees in Europe. J. Pest. Sci. 87(1), 1-16

Monceau, K., Maher, N., Bonnard, O., Thiéry, D. (2015). Evaluation of competition between a native and an invasive hornet species: do seasonal phenologies overlap?. B Entomol Res. 105(4), 462

Nardella, J., Terrado, M., Honson, N. S., Plettner, E. (2015) Endogenous fatty acids in olfactory hairs influence pheromone binding protein structure and function in *Lymantria dispar*. Arch. Biochem. Biophys. 579, 73-84

Pain, J. (1973) Pheromones and hymenoptera. Bee World. 54(1), 11-24

Pérez-de-Heredia, I., Darrouzet, E., Goldarazena, A., Romón, P., Iturrondobeitia, J. C. (2017). Differentiating between gynes and workers in the invasive hornet

*Vespa velutina* (Hymenoptera, Vespidae) in Europe. J. Hymenopt. Res. 60, 119

Plettner, E., Otis, G. W., Wimalaratne, P. D. C., Winston, M. L., Slessor, K. N., Pankiw, T., Punchihewa, P. W. K. (1997) Species-and caste-determined mandibular gland signals in honeybees (*Apis*). J. Chem. Ecol. 23(2), 363-377

Reitz, M., Gerhardt, H., Schmitt, C., Betz, O., Albert, K., Lämmerhofer, M. (2015) Analysis of chemical profiles of insect adhesion secretions by gas chromatography–mass spectrometry. Anal. Chim. Acta 854, 47-60

Richard, F. J., Hunt, J. H. (2013) Intracolony chemical communication in social insects. Insect. Soc. 60(3), 275-291

Rodríguez-Flores, M. S., Seijo-Rodríguez, A., Escuredo, O., Seijo-Coello, M. C. (2019) Spreading of *Vespa velutina* in northwestern Spain: influence of elevation and meteorological factors and effect of bait trapping on target and non-target living organisms. J. Pest. Sci. 92(2):557-565

Rojas-Nossa, S. V., Novoa, N., Serrano, A., Calviño-Cancela, M. (2018). Performance of baited traps used as control tools for the invasive hornet *Vespa velutina* and their impact on non-target insects. Apidologie, 49(6), 872-885

Ruther, J., Sieben, S., Schricker, B. (2002) Nestmate recognition in social wasps: manipulation of hydrocarbon profiles induces aggression in the European hornet. Naturwissenschaften 89(3), 111-114

Ruther, J., Matschke, M., Garbe, L. A., Steiner, S. (2009) Quantity matters: male sex pheromone signals mate quality in the parasitic wasp *Nasonia vitripennis*. Proc. R. Soc. B 276(1671), 3303-3310

Singariya, P., Kumar, P., Mourya, K. K. (2012) Isolation of some new steroids and evaluation of bio-activity of *Cenchrus ciliaris*. Int. J. Res. Pharm. Sci. 3, 678-684

Singer, T. L. (1998). Roles of hydrocarbons in the recognition systems of insects. Am. Zool. 38(2), 394-405

Smith, J. M., Szathmary, E. (1997) The major transitions in evolution, first ed. Oxford University Press, New York

Thiéry, D., Bonnard, O., Riquier, L., de Revel, G., Monceau, K. (2018) An alarm pheromone in the venom gland of *Vespa velutina*: evidence revisited from the european invasive population. Entomol. Gen. 38(2), 145-156

Trouiller, J., Arnold, G., Le Conte, Y., Masson, C., Chappe, B. (1991). Temporal pheromonal and kairomonal secretion in the brood of honeybees. Naturwissenschaften. 78(8), 368-370

Wang, Z. W. Chen, G. Tan, K. (2014) Both olfactory and visual cues promote the hornet *Vespa velutina* to locate its honeybee prey *Apis cerana*. Insectes Soc. 61 (1), 67-70

Wen, P., Cheng, Y. N., Dong, S. H., Wang, Z. W., Tan, K., Nieh, J. C. (2017) The sex pheromone of a globally invasive honey bee predator, the Asian eusocial hornet, *Vespa velutina*. Sci. Rep. 7(1), 12956

Wilson, E. O. (1971). The insect societies. Cambridge, Harvard University Press

Wilson, E. O., Bossert, W. H. (1963). Chemical communication among animals. Recent Prog. Horm. Res. 19, 673.

Wyatt, T. (2017) Pheromones and other Chemical Communication in Animals. In: Squire L. R., (ed) Encyclopedia of neuroscience. Academic Press, Oxford, pp 611-616.

Yang, S., Gayral, P., Zhao, H., Wu, Y., Jiang, X., Wu, Y., Deng, S. et al (2020). Occurrence and molecular phylogeny of honey bee viruses in Vespids. Viruses, 12(1), 6