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RESEARCH ARTICLE

Comparison of the cuticular profiles of several dung beetles used as host carriers by the phoretic mite *Macrocheles saceri* (Acari: Mesostigmata)

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Abstract

Macrochelid mites have developed phoretic interactions with coprophilous arthropods in order to disperse and colonize new substrate. *Macrocheles saceri* (Acari: Mesostigmata) is a mite phoretically specialized on the dung beetles of the *Scarabaeus* genus. A previous study has demonstrated that *M. saceri* can discriminate among available hosts and showed host preferences using the host cuticular material. In this present study, we have compared the cuticular chemicals including the volatiles present among several species of *Scarabaeus* and *Bubas* genera. By comparing the host chemical profiles to the host preferences, several hypotheses regarding the role of some of these volatile chemicals in host discrimination based on attractiveness and repellency, are proposed.

Keywords: Cuticle, dung beetle, phoresy, Macrocheles, aliphatic alkanes, aliphatic alcohols

Introduction

Arthropod cuticle is composed by glycoproteins, glycosamines, and scleroproteins secreted by the epidermis cells underneath. The cuticle itself is covered by a layer of lipid secretions constantly renewed (Lockey, 1985). These lipids included polar cuticular compounds, including esters, glycerides, fatty acids, sterols, alcohols, and aldehydes, volatiles as well as apolar chemical compounds, mainly represented by hydrocarbons (alkenes and alkanes). Cuticular compounds are synthetized in insects by specialized ectodermic cells namely "the oenocytes", localized between epidermis cells and the basal membrane (Diehl, 1975; Raccaud-Schoeller, 1980). Once synthetized by the oenocytes, the cuticular compounds are transported by hemolymphatic lipophorines (Katase & Chino, 1984; Gu et al., 1995; Van der Horst et al., 1993). Cuticular lipids can intervene in insect interactions, playing a role as sexual pheromone (Dillwith et al., 1981; Antony & Jallon, 1982; Trabalon et al., 2006), as aggregation pheromone (Bartelt & Jackson, 1984; Byers, 1989), as host recognition signal for parasitic interactions (Howard et al., 1990; Dettner & Liepert, 1994) or in commensal interactions (Niogret et al. 2006). Phoretic interactions are among the most important interactions necessary for the survival of small organisms that are limited in their dispersal capabilities. It has been described as an association between poorly moving small organisms (the phoretic) and highly mobile and often bigger organisms (the host carrier). The phoretic organism would climb on its host carrier when the substrate on which they developed start decaying, and would leave its host carrier when it has reached a new habitat or

substrate suitable for the development of the phoretic organism (Athias-Binche 1994; Niogret et al. 2004). Phoresy allow small organism to exploit substrates (e.g. dung pads, carrions etc) that would have been out of reach otherwise (Schwarz & Koulianos 1998). The coprophilous mite *Macrocheles saceri* (Acari: Mesostigmata) seem to be exclusively phoretic of dung beetles of the genus *Scarabaeus* (Coleptera: Scarabaeidae), and rarely found on other Coleopteran species (Krantz 1991). *Macrocheles* mites use olfactory cues from their environment (Niogret et al. 2004) and kairomonal signals based on the cuticular compounds to recognize a suitable host (Niogret et al. 2006).

The goal of this study is to compare the cuticular composition of some putative Coleopteran hosts used by the phoretic mite *M. saceri*, to understand which chemical compounds could potentially play a role in the host discrimination towards particular *Scarabaeus* species.

Materials and Methods

Insect capture

Dung beetles *Scarabaeus sacer* and *S. cicatricosus* were collected in the Maamora forest in Morocco using pitfall traps of standard design baited with fresh cattle dung (Lobo *et al*. 1988; Veiga *et al*. 1989).

Scarabaeus laticollis and *Bubas bison* and *B. bubalus* were capture in South of France using the same trapping system. Insects were brought alive in our laboratory for further analysis.

Solvent extract and analysis

Cuticular compounds have been extracted on the five dung beetle species: *Scarabaeus sacer* (*n* = 7), *S. cicatricosus* (*n* = 9), *S. laticollis* (*n* = 9), *Bubas bubalus* (*n* = 5) and *B. bison* (*n* = 6). No significant qualitative and quantitative differences have been observed between both *Bubas* species. *Bubas* species are not considered a true host for *M. saceri* as it has never been reported carrying the specialist mite. Therefore, data of both *Bubas* species were combined together to form an external group '*Bubas*' to be compared with the various species of *Scarabaeus*.

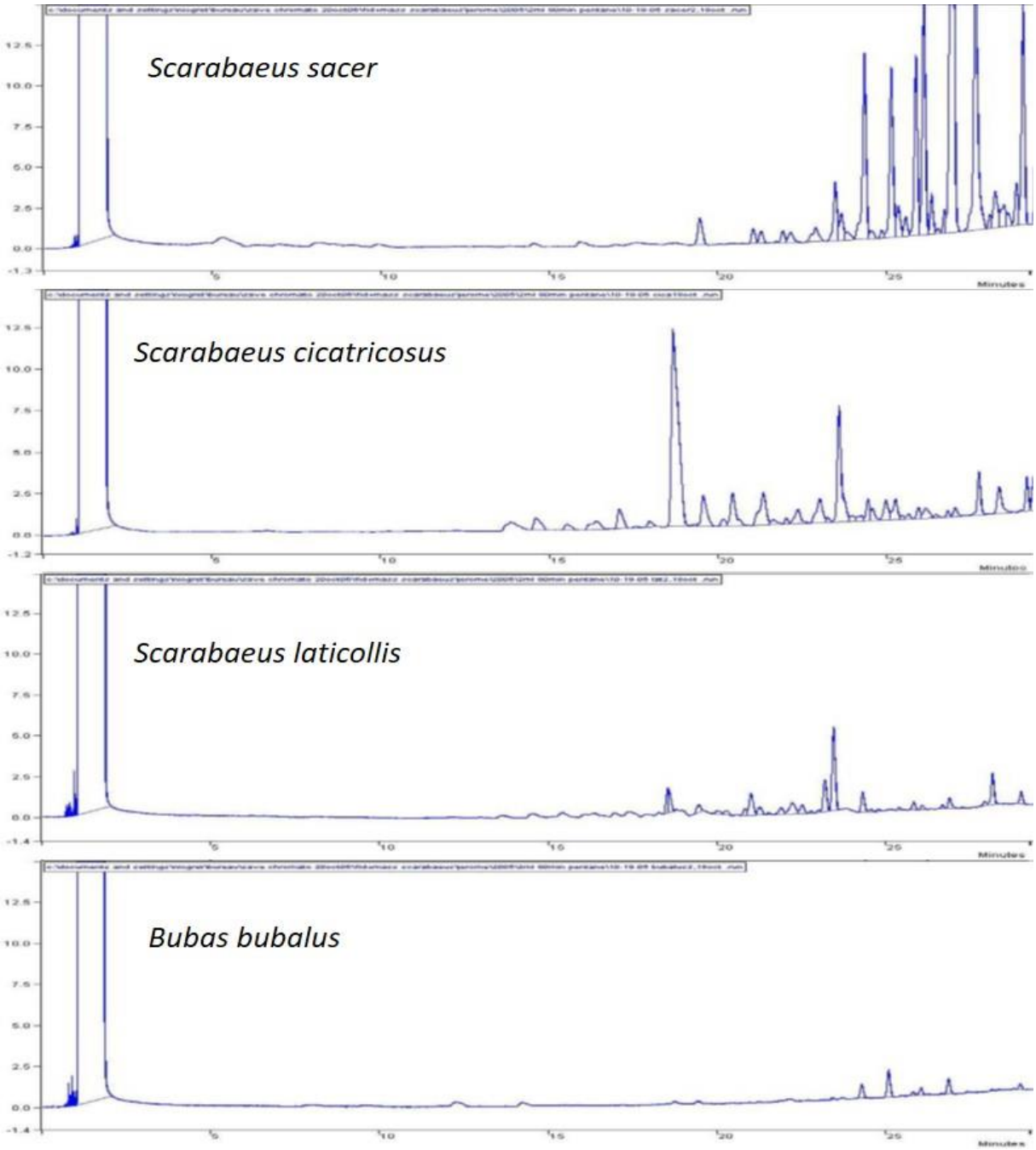
The ventral surface (abdomen and thorax) of the insects were soaked into 2 mL of pentane for 90 minutes. Cuticular extracts were then stored at –32 °C until analysis. Some of the extracts are concentrated under a nitrogen flow before injecting 2 µL into a gas-chromatograph Varian® CP-3800 equipped with a 30 m capillary column WCOT CP-Sil-8CB (Chrompack®, Middelburg, The Netherland) (30 m; 0.25 Ø; 1 mL/min flow) coupled with an ion trap mass spectrometer Varian® Saturn 2000 (injector 1177 at 270°C; with 70eV). Quantitative analysis has been done using a GC-FID Varian® Star 3380 equipped with an injector 1177 at 270 °C, detector at 280 °C, and a capillary column WCOT CP-Sil-5CB (25 m; 0,32 mm). Similar temperature program has been used on both instruments: 5 min isotherm at 5 at 200 °C, then by 3 °C/min to 270 °C, and plateau at 270 °C for one minute. Cuticular volatile compounds have been identified by their respective retention indexes (RI). Quantification has been done using Star Chromatography Software®. Chemical compounds have been identified by their Retention Index (RI).

The comparison of the relative quantity of each of compounds identified from various beetle species has analyzed by Kruskall Wallis and Mann Whitney non-parametric tests (Statistica® 6.0 package).

Results and Discussion

Chromatographic analysis of the cuticular extracts revealed qualitative and quantitative differences in the chemical profiles of the various dung beetle species (Figure 1). Most of the compounds belonged to alkanes and alcohol functional groups.

Figure 1: Representative chromatograms of cuticular profiles for *Scarabaeus sacer, S. cicatricosus, S. laticollis* et *Bubas bubalus*. Left axis in millivolts, mV.



The comparison of the chemical profiles from *Scarabaeus* species (*S. sacer*, *S. cicatricosus* and *S. laticollis*) and from *Bubas* species (*B. bubalus* and *B. bison*) has underlined 45 cuticular compounds. Among those chemicals, only 6 were found in common to the three species of *Scarabaeus* (RI= 2564-2602; 2648 and 2676) (Table 1).

Table 1: Relative quantity (%) of cuticular compounds identified by their Retention Indexes from the *Scarabaeus* and *Bubas* species (average ± standard deviation). RI = Retention Index.

RI	*S. sacer*	*S. cicatricosus*	*S. laticollis*	*Bubas* spp.
2300	0.0 ± 0.0	1.34 ± 1.41	0.92 ± 0.44	0.83 ± 0.48
2334	0.0 ± 0.0	0,68 ± 0,15	1.50 ± 0.76	0.0 ±0.0
2372	0.0 ± 0.0	0.0 ± 0.0	2.36 ± 0.29	0.0 ± 0.0
2379	0.0 ± 0.0	0.0 ± 0.0	2.42 ± 1.19	0.0 ± 0.0
2400	0.0 ± 0.0	1.48 ± 0.85	1.83 ± 1.11	0.0 ± 0.0
2386	0.0 ± 0.0	0.0 ± 0.0	2.14 ± 1.31	0.0 ± 0.0
2433	0.0 ± 0.0	0.52 ± 0.26	1.34 ± 1.05	0.0 ± 0.0
2462	0.0 ± 0.0	13.6 ± 3.56	13.93±5.30	0.0 ± 0.0
2474	0.0 ± 0.0	11.6 ± 2.98	0.0 ± 0.0	0.0 ± 0.0
2488	0.0 ± 0.0	0.0 ± 0.0	1.67 ± 0.27	0.0 ± 0.0
2500	1.41 ± 0.48	7.24 ± 3.65	7.47 ± 2.64	1.46 ± 0.54
2515	0.0 ± 0.0	1.12 ± 1.21	2.21 ± 0.62	0.0 ± 0.0
2533	0.0 ± 0.0	5.55 ± 2.16	5.49 ± 5.91	0.0 ± 0.0
2550	0.0 ± 0.0	0.0 ± 0.0	1.22 ± 0.70	0.0 ± 0.0
2564	0.66 ± 0.11	1.99 ± 0.80	3.48 ± 1.38	0.0 ± 0.0
2574	0.84 ± 0.16	4.04 ± 1.76	4.67 ± 2.65	0.0 ± 0.0
2580	0.0 ± 0.0	0.49 ± 0.18	2.07 ± 1.90	0.0 ± 0.0
2602	0.71 ± 0.31	1.08 ± 0.46	2.09 ± 0.55	0.0 ± 0.0
2603	0.0 ± 0.0	0.0 ± 0.0	1.62 ± 0.26	0.18 ± 0.49
2606	0.82 ± 0.09	1.92 ± 0.40	4.20 ± 1.84	0.0 ± 0.0
2628	0.0 ± 0.0	0.0 ± 0.0	1.77 ± 0.54	0.0 ± 0.0
2634	0.0 ± 0.0	1.67 ± 1.14	1.16 ± 0.58	0.30 ± 0.23
2648	1.25 ± 0.10	3.25 ± 2.00	0.87 ± 0.33	0.0 ± 0.0
2642	0.0 ± 0.0	0.44 ± 0.27	2.87 ± 2.29	0.0 ± 0.0
2663	2.98 ± 0.61	9.92 ± 2.24	10.09±5.13	1.21 ± 0.39
2676	6.47 ± 6.44	1.54 ± 0.46	0.94 ± 0.16	0.0 ± 0.0
2684	0.53 ± 0.29	0.30 ± 0.15	0.0 ± 0.0	0.0 ± 0.0
2688	0.53 ± 0.18	1.01 ± 0.43	0.0 ± 0.0	0.0 ± 0.0
2700	8.18 ± 2.73	2.53 ± 0.95	4.43 ± 1.64	11.22 ± 4.01
2705	0.45 ± 0.04	0.87 ± 0.20	0.0 ± 0.0	0.0 ± 0.0
2713	0.30 ± 0.13	1.46 ± 0.51	0.0 ± 0.0	0.0 ± 0.0
2731	6.29 ± 1.44	2.67 ± 0.52	0.88 ± 0.28	25.21 ± 9.84
2744	1.37 ± 0.11	0.46 ± 0.11	0.0 ± 0.0	1.96 ± 0.67
2747	1.29 ± 0.69	0.77 ± 0.34	0.40 ± 0.22	0.54 ± 0.51
2761	6.12 ± 2.01	1.19 ± 0.37	0.80 ± 0.37	2.32 ± 2.00
2773	10.18 ± 2.08	1.73 ± 0.65	1.02 ± 0.14	8.19 ± 5.15
2778	1.59 ± 0.33	0.51 ± 0.31	0.34 ± 0.08	1.21 ± 2.60
2800	0.62 ± 0.18	0.49 ± 0.18	0.79 ± 0.29	1.22 ± 0.38
2812	23.26 ± 5.91	2.99 ± 2.25	1.81 ± 0.74	21.87 ± 9.19
2831	15.05 ± 4.48	3.66 ± 0.42	1.21 ± 0.73	3.96 ± 4.60
2837	0.66 ± 0.27	1.53 ± 1.58	0.0 ± 0.0	0.33 ± 1.10
2862	1.79 ± 0.93	3.47 ± 0.93	2.02 ± 1.90	2.73 ± 1.90
2869	1.28 ± 0.58	0.41 ± 0.26	0.71 ± 0.19	1.43 ± 0.32
2900	1.82 ± 0.27	1.59 ± 1.56	0.0 ± 0.0	3.25 ± 2.45

Some of these compounds could be responsible for the attraction of *M. saceri* towards the *Scarabaeus* species since these chemicals were absent in the chemical profiles of both unattractive *Bubas* species (*Bubas* species are not a host carrier for *M. saceri*). Among these compounds present in the *Scarabaeus* genus, but absent in *Bubas* species, only RI=2676, a C27 linear alkane was quantitatively correlated ($H = 31.09$; $p < 0.0001$) with the host preferences of *M. saceri* observed by Niogret et al. (2006) in the following order *S. sacer* > *S. cicatricosus* > *S. laticollis* > non host. Similarly, some compounds (RI=2684, 2688, 2705, and 2713) were present in both *S. sacer* and *S. cicatricosus,* but absent from the poorly attractive *S. laticollis*. These volatile chemicals could also play a role in the attractiveness of the mite towards its usual host carriers. However only RI=2684 was significantly in higher quantity in *S. sacer* than in *S. cicatricosus* ($z = - 2.17$; $p<0.05$), which made him the most interesting candidate chemical responsible for attraction.

Potential repellant effect of some cuticular chemicals of host carriers could also explain the discrimination of some hosts by *M. saceri*. Five chemical compounds were exclusively present in *S. laticollis* (RI=2372, 2386, 2488, 2550, and 2628) or present in both poorly and unattractive beetles *S. laticollis* and *Bubas* sp. (RI=2603). Therefore, these chemicals could be responsible for a potential repellent effect and for the lack of attractiveness of these beetles compare to the favorite hosts *S. sacer* and *S. cicatricosus*.

Our chromatographic analysis showed the nature of the cuticular compounds susceptible to play a kairomonal role in the host-seeking and phoretic behaviors of the specialist mite *M. saceri*. Choice tests among the various cuticular extracts have confirmed the fact that the active chemicals triggering the host preferences are indeed present in these specific cuticular extracts (Niogret et al. 2006). These chemicals were used by the mite to discriminate among their host carriers to preferably choose the *Scarabaeus* species over other potential available hosts. All these compounds were alkanes and/or aliphatic alcohol between 20 to 29 carbons. It is likely that these chemicals found in common to the species of the *Scarabaeus* genus but absent from *Bubas* species were contributing to the discrimination and the selection among the hosts. These cuticular volatile compounds, once identified using an appropriate MS library, should be further tested for their potential attractive and repellant effects in an olfactometer or appropriate experimental setup.

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