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Epicuticular factors involved in host recognition for the aphid parasitoid Aphidius rhopalosiphi

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Abstract: In insect parasitoids, fitness is dependent on the host finding and recognition abilities of the female. Host recognition cues have been described for various host-parasitoid systems, but are still under investigation in aphid parasitoids. Our study aimed to clarify the respective role of physical and chemical cues in recognition of the aphid cuticle. Shed aphid exuviae were used as an elicitor in order to avoid any influence of color, movement, or volatiles present in a living aphid. We assessed the effect of chemical and heat treatments on the texture of the cuticle by using scanning electron microscopy and tested the recognition of treated cuticles by the parasitoid. We showed that recognition cues of the cuticle can be removed chemically (using combined treatments with n-hexane and methanol). Moreover, heat treatment destroyed the physical texture of the cuticle without significantly reducing parasitoid recognition. In a second step, we showed that epicuticular extracts deposited on pieces of paper triggered female attack behavior. First results concerning the chemical composition of the active extract are presented. This study shows that chemical compounds extractable by organic solvents mediate cuticle recognition by aphid parasitoids.

Keywords: Cuticular kairomones – chemical cues – oviposition behavior – aphidiinae – *Sitobion avenae* – cuticular hydrocarbons – wax esters.

INTRODUCTION

In insect parasitoids, fitness depends to a considerable extent on the host recognition capabilities of the adult female. The host recognition process involves both physical and chemical factors (Godfray, 1994). Host body size (Shirota *et al*., 1983; Kouamé and Mackauer, 1991), host cuticle texture (Arthur, 1981), shape (Vinson, 1985), and color (Ankersmit *et al*., 1981, 1986; Michaud and Mackauer, 1994, 1995; Battaglia *et al*., 2000), as well as host movements (Arthur, 1981; Mackauer *et al*., 1996; Dippel and Hilker, 1998) act as cues triggering the attack behavior of female parasitoids. It has also been frequently reported that host recognition is mediated by semiochemicals (Strand and Vinson, 1982; Grasswitz and Paine, 1992; Battaglia *et al*., 1993).

Several authors have pointed out the role of epicuticular factors in host recognition by aphidiine wasps (Pennacchio *et al*., 1994). For example, in three aphid parasitoid species, females are not able to distinguish between host and nonhost species at a short distance, and an antennal contact is needed for recognition at the species level (Le Ralec *et al*., 2005). Oviposition attempts of aphid parasitoids toward host exuviae have been occasionally observed (Michaud, personal communication; Outreman, personal communication). Exuviae are useful biological material in behavioral analyses because visual factors like movement, color, and global shape are excluded as well as semiochemicals secreted by living aphids. Moreover, from the perspective of chemical analysis, only cuticular compounds are extracted from exuviae without any contaminants from the internal body (Grasswitz, 1998). Exuviae from *Acyrthosiphon pisum* elicit attacks by *Aphidius ervi* even when coarsely crushed (Battaglia *et al*., 2000). This response is still observed under red light, indicating that visual cues are not involved. Nevertheless, the attack behavior is not observed if the parasitoid is prevented from touching the exuviae with its antennae. Battaglia *et al*. (2000) evoked the "possibility that a cuticular compound, which functions as recognition kairomone," occurs in the cornicle secretion and on the cuticle of exuviae. When antennal contact is required for host recognition, nonvolatile chemical compounds are likely to be involved, and a role for the cuticle texture must be considered (Godfray, 1994). Moreover, *Aphidius* wasps present both chemo- and mechanoreceptors on the antennae (Battaglia *et al*., 2002). Scanning electron micrographs of aphid epicuticle show the occurrence of wax secretion in a bloom covering (Retnakaran *et al*., 1979; Pope, 1983). Besides its waterproofing function (Pope, 1983), the epicuticular wax layer can also play a key role in communication. Hydrocarbons of the wax layer of the insect cuticle may be involved in, e.g., species, gender, and nestmate recognition, as well as in chemical mimicry (Howard and Blomquist, 2005). Therefore, the integration of both chemical and physical factors is a tentative explanation for the mechanism of host recognition by aphidiine parasitoids.

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In order to assess the respective roles of cuticular wax semiochemicals and/or texture in parasitoid host recognition, exuviae were treated in various ways. In the first step, potential semiochemicals were washed off with solvents. During the second step, cuticle texture was altered by using a heat treatment. The effect of the solvent extraction on cuticular surface texture was studied by scanning electron microscopy (SEM), and the consequences of heat treatment and solvent extraction on parasitoid behavior were analyzed. To further assess the role of chemical cues, we observed the behavior of the parasitoid towards a cuticular extract. Finally, the chemical composition of the extract was investigated by coupled gas chromatography-mass spectrometry (GC-MS).

Our biological model was the aphid-parasitoid system, *Sitobion avenae Fabricius-Aphidius rhopalosiphi* De Stefany Perez. *A. rhopalosiphi* is one of the most abundant Aphidiinae species in cereal fields in North Europe (Jones, 1972; Stilmant, 1997) and has shown potential for biological control of aphids (Levie *et al*., 2005).

METHODS AND MATERIALS

Insect rearing

A. rhopalosiphi individuals were collected in winter wheat fields near Louvain-la-Neuve, Belgium, in summer 2000, and reared on *S. avenae* maintained on winter wheat (*Triticum aestivum* cv. Windsor). Colonies of both *A. rhopalosiphi* and *S. avenae* were kept in the laboratory under the following conditions: 19.5 ± 0.6°C, 40-50% relative humidity, and 16:8 hr L:D photoperiod. Parasitoids were reared on synchronized L2 aphid colonies. In a framed wooden cage (50 x 50 x 30 cm) with fine mesh on the sides, pairs of parasitoids (one pair per 50 aphids) were released for 24 hr. Ten days later, mummies were removed from wheat with a scalpel and kept in Petri dishes in groups of 50 individuals each. To avoid possible influences of hunger or mate-searching behavior (Michaud, 1994), emergent females were given access to food (honey-water) and males. All females were naive (no access to host) when tested.

Collection of exuviae

Sheets of paper were placed for 1-3 d under aphid colonies of mixed age. Exuviae falling from the colony were collected and carefully separated from dead aphids and waste material. They were weighed with a 0.1-mg-precision balance (Ohaus explorer).

Experiment 1: Effect of solvent washing of the exuviae

Exuviae were washed in methanol (SoxtM) or hexane (SoxH) for 4 hr by using a soxhlet. Exuviae were recovered and allowed to dry for 24 hr before use in behavioral assays. Unwashed exuviae were used as controls. In a second part of the experiment, cuticular compounds present on the surface of exuviae were extracted (1) for 4 hr at room temperature with only one solvent, methanol (M), or n-hexane (H), or (2) sequentially for 1.5 hr by two successive solvents-n-hexane followed by methanol (H + M), or methanol followed by n-hexane (M + H), and finally (3) for 4 hr with a mixture of n-hexane/methanol (ratio 1:2 v/v). During the extraction process, exuviae were held in a glass bottle and shaken at 100 rpm. In all cases, 1 mg exuviae was added to 10 ml solvent.

Experiment 2: Effect of heat treatment of the exuviae

Exuviae were heated for 16 hr in an oven at 80°C. At the same time, a control batch was kept at room temperature. Needles and grips were washed in n-hexane and methanol prior to use in each treatment to avoid chemical transfer between specimens. Exuviae of the same experiment came from the same collection.

Scanning electron microscopy method

In order to investigate the surface of aphid epicuticle, exuviae were studied by two different SEM methods. First, exuviae were sputter-coated with fine gold and directly observed in a Philips XL20 SEM (INRA, Rennes). Second, using a SEM (Oxford CT1500 cryosystem) at the microbiology laboratory (MBLA unit, UCL), specimens were flash frozen (-212°C) in liquid nitrogen under vacuum for cryo-SEM, transferred to the preparation chamber, and then to the SEM chamber where the frozen samples were sublimated (-80°C) to remove ice particles. Specimens were viewed under 2-5 kV at -190°C to -170°C (SEM Phillips XL20). To normalize the observations, all illustrations reported here represent the surface of wing buds of a "fourth instar exuvia" (between L4 and adult instar of the aphid). To ensure that observed differences were not due to individual variation, at least 20 exuviae were observed for each treatment.

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Behavioral assays

Glass Petri dishes (diam. 5 cm, height 1.5 cm, Schott®) were prepared by washing them with ethanol and deionized water and placed on a light table (2500 lx). Sixteen exuviae per dish were regularly disposed 1 cm apart in four rows of four exuviae each. Exuviae were maintained within a droplet of deionized water that dried at room temperature for 30 min before use. One parasitoid female was released at the center of the Petri dish and observed for 15 min. The behavioral items noted were "encounter with exuviae" (ENC), "antennal contact on exuviae" (ANT), and "abdominal bending towards exuviae" (ABD). In order to minimize variability, each set of experiments was completed during the same working day. Between 20 and 30 females were tested per treatment, each female being tested against one substrate only. The temperature during experiments was 23 ± 1°C.

Experiment 3: Assay of the cuticular extract activity

Exuviae were extracted as described above with a mixture of n-hexane/methanol (1:2, v/v) for 15 min. The extract was filtered and concentrated using a rotavapor (R110, Buchi, Switzerland) to 2 ml. The extract was deposed at the bottom edge of a filter paper (Whatman No. 1,3 x 3 cm) held vertically, the solvent moving up the paper by absorption. The paper was allowed to dry at room temperature for 2 hr before the behavioral test. Small pieces (1 x 1 mm) were cut from the filter paper by using a scalpel parallel to the front of migration. Sixteen pieces of paper were deposited in a glass Petri dish, and a behavioral test was conducted as described above except that the tests stopped after 5 min. New scalpel blades and clean grips were used for each treatment.

Chemical analysis of the cuticular extract of exuviae

Exuviae (20 mg) were extracted with an n-hexane/methanol (1:2, v/v) mixture for 15 min (as described above). After evaporation of the solvent in a rotavapor, the crude extract was dissolved in 2 ml of n-hexane/methanol, and 1 µl was analyzed by GC-MS. The GC-MS investigations were performed on a Hewlett Packard HP 5989 Mass Spectrometer coupled with an HP 6890N gas chromatograph equipped with an HP-5 (cross-linked 5% phenylmethylpolysiloxane) column (30 m x 0.25 mm I.D.; film thickness 0.25 µm). The operating conditions were fixed as follows: split-splitless injector at 280°C; carrier gas, helium at 1 ml min^{-1}; temperature program, from 50°C to 300°C at 15°C min^{-1}, then at 300°C for 25 min. The mass spectra were recorded in the electron impact mode at 70 eV (source temperature, 230°C; scanned mass range, 35 to 700 amu). The detected peaks were identified by their retention time data and their characteristic fragmentation patterns. The mass spectra of the compounds were also compared with those of the NBS75K.L and Wiley275K.L computer MS Libraries. Four batches of 20 mg exuviae were analyzed by the described method.

Statistical analysis

Data were analyzed using SAS System (SAS Institute, Cary, NC, 1999). PROC UNIVARIATE was first used to test the distribution of all behavioral observations. Data were log transformed in case of significant deviation from normality. To compare the number of behavioral events, variance analyses were performed using PROC GLM with Scheffe's multiple comparison method. To represent the proportion of exuviae rejected for oviposition after antennal analysis, a rejection ratio (Rej) was computed as follows: 1 (ABD/ ANT) (where ABD = number of abdominal bendings, ANT = number of antennal contacts). As each female rejected a proportion of exuviae after antennal contact, this ratio was calculated for each female. Since these data cannot be normalized by any transformation, the ratios were analyzed by using the Kruskal-Wallis test with multiple comparisons following Siegel and Castellan (1988). The effect of cuticular extract was compared to the control using chi-square tests.

RESULTS

Experiment 1: Chemical treatments

SEM observations revealed that the cuticular surface of the control exuviae is coated by a bloom of epicuticular waxes (Figure 1). The surface became smooth after soxhlet extraction (Figure 1), whereas the ornamentation remained intact when methanol was used on its own at room temperature (Figure 2). The SEM studies revealed no differences between individual exuviae that received the same treatment.

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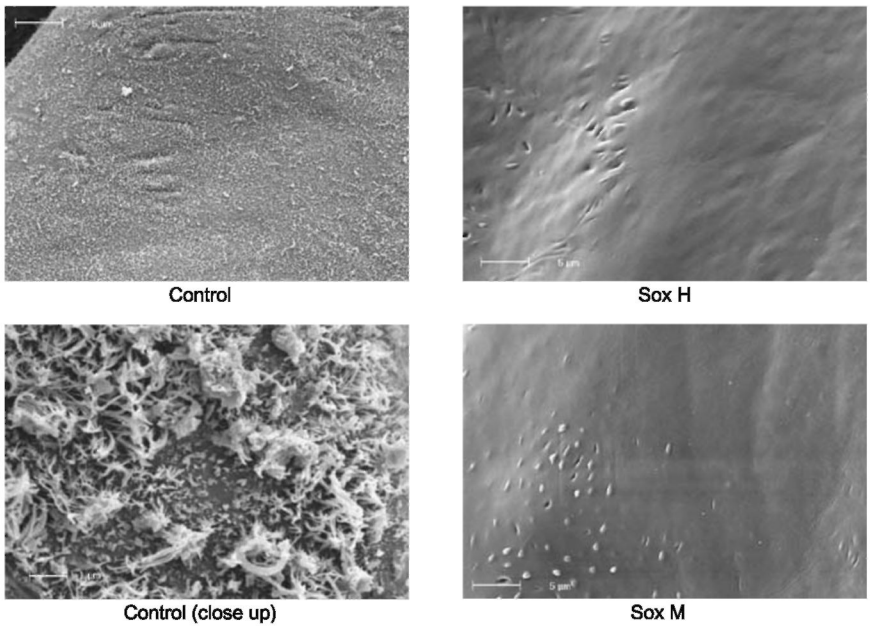
***Fig. 1**. Cuticular surface of the wing bud of fourth instar exuviae after chemical treatments in Soxhlet apparatus. Sox H = exuviae treated with n-hexane in soxhlet, Sox M = exuviae treated with methanol in soxhlet.*



Analysis of variance showed a significant effect of washing exuviae with hot solvents on the behavioral response of the parasitoid (Table 1). The number of encounters ($F_{2,66}$ = 21.98; P < 0.001), antennal contacts ($F_{2,66}$ = 35.50; P < 0.001), and abdominal bendings ($F_{2,66}$ = 14.99; P < 0.001) were clearly reduced by chemical treatment. The rejection ratio followed the same trend with a higher level of rejection after chemical treatment. The rejection ratio could not be tested statistically due to the high number of females that made no antennal contact. In this experiment, extraction with methanol inhibited recognition stronger than the n-hexane extraction.

At room temperature, solvent extraction had no significant effect on exuviae recognition (Table 2). However, successive chemical treatments with two solvents had a significant effect on host acceptance compared to control. The number of abdominal bendings towards exuviae was strongly reduced when both solvents were used sequentially or in a 1:2 mixture ($F_{5,108}$ = 19.57; P < 0.001). Again, the rejection ratio followed the same trend with a strong increase in rejection for treatments employing both solvents.

***Table 1**. Mean Number of Behavioral Items Observed on soxhlet-extracted exuviae.*

	N	**Enc**	**Ant**	**Abd**	**Rejection ratio**
Sox M	24	9.3^{b} (1.5)	1.8^{c} (0.6)	0.17^{c} (0.09)	0.91
Sox H	22	14.6^{b} (2.3)	4.8^{b} (1.0)	1.18^{b} (0.3)	0.76
Control	23	34.8^{a} (4.2)	21.4^{a} (2.9)	6.87^{a} (1.6)	0.61

Standard errors are given in brackets. Means of the same column sharing the same letter are not significantly different (a = 0.05). Enc = encounter, Ant = antennal contact, Abd = abdominal bending, Sox H = exuviae treated with n-hexane in soxhlet, Sox M = exuviae treated with methanol in soxhlet.

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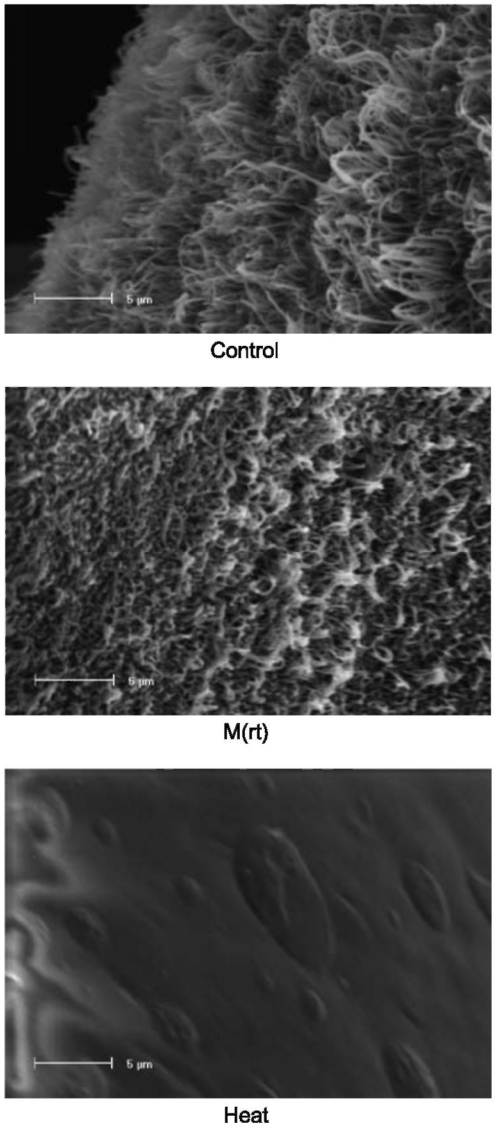
***Fig. 2**. Cuticular surface of the wing bud of fourth instar exuviae after chemical treatment at room temperature or after heat treatment. M(rt) = exuviae treated with methanol at room temperature, Heat = exuviae heated in drying oven at 80°C.*



Experiment 2: Heat treatment

Heating exuviae up to 80°C caused a "melting" of the epicuticular waxes that led to a "smoothing" of their typical microstructures. Indeed, when heated, the lipid surface constituents became amorphous with some spots that we considered as accumulated material (Figure 2). Heat treatment did not significantly affect the behavioral responses of the parasitoids ($F_{1,36}$ = 0.04; P = 0.847 for encounter, $F_{1,36}$ = 0.41; P = 0.526 for antennal contact, $F_{1,36}$ = 2.00; P = 0.166 for abdominal bending) (Table 3).

Experiment 3: ***Activity of the cuticular extract***

The parasitoids were stimulated to attack pieces of filter paper containing the cuticular extract (Figure 3). More than 75% of the females (N = 20) showed repeated abdominal bendings towards the extract. Only one female (N = 17) showed a single abdominal bending event on the control paper. Both the top (χ_2^2= 28.3, P < 0.001) and the bottom (χ_2^2 = 204.0, P < 0.001) portion of the filter paper had a significant effect on parasitoid behavior compared to the control filter paper.

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***Table 2**. Mean number of behavioral items observed on exuviae treated with Solvents at Room Temperature.*

	N	Enc	Ant	Abd	Rejection ratio
H + M	29	24.4^{b} (2.4)	11.4^{d} (1.6)	1.0^{b} (0.4)	0.91^{A}
M + H	10	16.0^{b} (2.0)	6.4^{cd} (1.1)	0.6^{b} (0.2)	0.91^{A}
[HM]	15	34.0^{ab} (4.3)	13.2^{bcd} (2.6)	0.3^{b} (0.2)	0.97^{A}
Hrt	18	34.2^{ab} (4.1)	23.6^{b} (2.8)	9.5^{a} (1.7)	0.60^{B}
Mrt	17	33.9^{ab} (3.9)	26.2^{b} (3.3)	11.4^{a} (2.2)	0.57^{B}
Control	20	51.1^{a} (4.3)	41.1^{a} (3.8)	15.4^{a} (2.1)	0.63^{B}

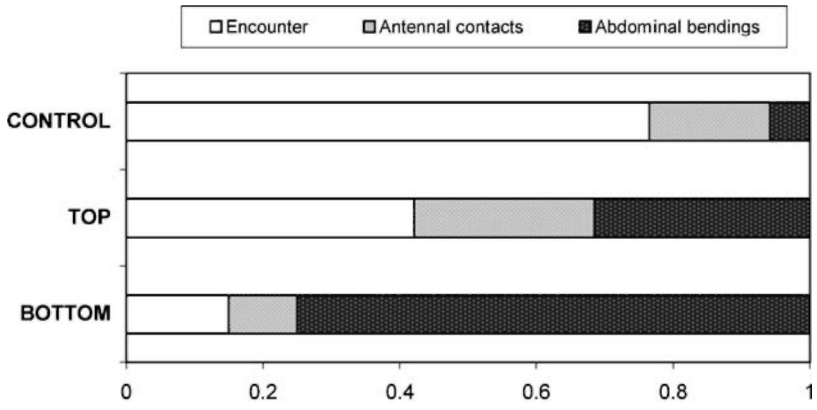
Standard errors are given in brackets. Means of the same column sharing the same letter are not significantly different (α = 0.05). Enc = encounter, Ant = antennal contact, Abd = abdominal bending. H + M = exuviae sequentially treated with n-hexane and then methanol, M + H = exuviae sequentially treated with methanol and then n-hexane, [HM] = exuviae treated with a n-hexane/ methanol (1:2) mixture, Hrt = exuviae treated with n-hexane, Mrt = exuviae treated with methanol.

***Table 3**. Mean Number of Behavioral Items Observed on Heated and Control Exuviae.*

	N	Enc	Ant	Abd	Rejection ratio
Heat	18	31.8^{a} (5.8)	20.9^{a} (3.9)	6.4^{a} (1.3)	0.69^{A}
Control	20	32.2^{a} (4.7)	23.4^{a} (3.6)	9.3^{a} (1.5)	0.60^{A}

Standard errors are given in brackets. Means of the same column sharing the same letter are not significantly different (α = 0.05). Enc = encounter, Ant = antennal contact, Abd = abdominal bending. Heat = exuviae heated in drying oven.

***Fig. 3**. Behavioral response of the females to pieces of paper covered with the cuticular extract. Encounter = proportion of females that did not react when encounter the extract, Antennal contacts = proportion of females that showed antennal drumming, Abdominal bendings = proportion of females that showed abdominal bendings. "Top" and "bottom" refer to the part of the filter paper that received the cuticular extract, the front of migration of the extract being from bottom to top. "Control" refers to filter paper that received pure solvent.*



Chemical composition of the cuticular extract

The cuticular extract composition consists of compounds belonging to four major classes of compounds: approximately 20 hydrocarbons, 3 wax esters, 2 alcohols, and 2 aldehydes. The list of the detected compounds is presented in Table 4. The hydrocarbon fraction consists of a homologous series of n-alkanes (C25 to C31), branched monomethyl alkanes (X-MeC25 to X-MeC31), and one dimethyl alkane (11,13-DiMeC29). The wax esters detected at the end of the run (Table 4) were medium-chain fatty acids (C16) esterified to long-chain alcohols (C18 to C22). The extract is also characterized by the occurrence of two long-chain aldehydes (C28 and C30). The identification of the octacosanal (C28) has been confirmed by comparison with the mass spectra of synthesized compounds kindly provided by Prof. Morris (Horticulture and Food Research Institute of New Zealand, Auckland, New Zealand).

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***Table 4**. Cuticular Compounds Identified in Hexane/Methanol Extracts.*

RT (min)	Compound	Diagnostic MS ions	Area % (mean ± SE)[a]
15.89	n-Pentacosane	352	3.08 ± 0.12
16.21	2-Methylpentacosane	351, 323	0.55 ± 0.20
16.27	3-Methylpentacosane	351, 337	0.31 ± 0.01
16.40	n-Hexacosane	366	0.70 ± 0.02
16.72	2-Methylhexacosane[b]	-	0.17 ± 0.06
16.91	Heptacosane	380	11.22 ± 0.09
17.07	11-Methylheptacosane[b]	-	0.33 ± 0.03
17.16	5-Methylheptacosane	337, 85	0.25 ± 0.02
17.27	3-Methylheptacosane	365	0.21 ± 0.03
17.41	n-Octacosane	394	0.61 ± 0.02
17.96	n-Nonacosane	408	10.99 ± 0.33
18.15	11-Methylnonacosane	407, 168, 280	1.06 ± 0.03
18.26	5-Methylnonacosane	365, 85	0.12 ± 0.06
18.32	11,13-Dimethylnonacosane	168, 224, 239, 295	0.23 ± 0.05
18.58	n-Triacontane	422	0.67 ± 0.11
18.79	11-Methyltriacontane[b]	-	0.33 ± 0.11
18.89	Octacosanal	408, 390	0.92 ± 0.07
19.30	n-Hentriacontane	436	4.57 ± 0.19
19.38	Unidentified alcohol	-	14.61 ± 0.70
19.54	11-Methylhentriacontane	168, 308	1.02 ± 0.12
20.59	Triacontanal	418	1.27 ± 0.24
21.11	Unidentified hydrocarbon	-	0.92 ± 0.04
21.27	Unidentified alcohol	-	13.57 ± 0.66
24.83	Hexadecanoic acid octadecyl ester	257, 508	2.50 ± 0.12
29.20	Hexadecanoic acid eicosanyl ester	257, 536	20.97 ± 0.56
33.94	Trihexanoin[c]	285, 383, 99	5.32 ± 0.80
35.49	Hexadecanoic acid docosanyl ester	257, 564	3.51 ± 0.09

RT = retention time.
100 % = total area of detected peaks.
[a] Means and standard errors calculated of four cuticular extracts.
[b] Methyl position inferred from Kovats index only.
[c] Tentative interpretation.

DISCUSSION

This study used aphid exuviae in order to better understand the role played by semiochemicals and/or texture associated with wax secretions in host recognition by the parasitoid *A. rhopalosiphi*. The results confirm that epicuticular factors are involved in host recognition. When these factors are chemically removed, the exuviae lose their activity in terms of eliciting responses from the parasitoid. Secondly, the results show that the tactile recognition of the epicuticular wax layer by the parasitoid is not involved in host recognition. The microstructure of the epicuticular wax layer is destroyed by the heat treatment, but these exuviae retain their activity for the parasitoid. We observed that the wax layer was still

present on the heated cuticle, although its microstructure was destroyed. However, soxhlet extraction took away epicuticular waxes. The behavioral study revealed significant activity when the wax layer was still present regardless of its physical state. Rather, the kairomones embedded within epicuticular waxes seem to play a role in recognition of the host cuticle. The parasitoid's response was lost when the host cuticle received specific chemical treatments. First, hot chemical treatment was efficient, using n-hexane or methanol. Second, at room temperature, the solvents had to be combined to destroy the recognition response. Long extraction time (4 hr) in the soxhlet apparatus led to complete extraction of epicuticular constituents, some of which were not fully extracted at room temperature. With simple macerations at room temperature, two solvents of different polarities (hexane and methanol) were necessary for complete extraction of the active factor. At the 1:2 ratio, n-hexane and methanol are fully miscible and form a mixture that can be considered as a novel solvent with novel properties, such as e.g., polarity and boiling point. The use of the two solvents, sequentially or in mixture, enabled us to extract the active compounds from the cuticle. This suggests that the activity is due to several compounds with different polarities, rather than to a single compound. Moreover, heat treatment of the exuviae reduced the parasitoid's response but did not completely inactivate the cuticle. This suggests that the kairomonal activity of the exuviae is due more to a (mixture of) contact chemical(s) rather than to a mixture of short-range volatiles, as these would certainly almost be destroyed or removed by heating. Finally, the presence of cuticular kairomones was unequivocally proven by the parasitoid's responses towards pieces of paper impregnated by the extract. This represents the first direct evidence of the occurrence of cuticular kairomone(s) that elicit aphid parasitoid attacks. The results also show that a rough fractionation of the extract can be made by using the absorption capacity of the filter paper, since the bottom of the filter elicits a stronger reaction of the parasitoid than the top.

Hydrocarbons are known to be commonly involved in insect communication (Chapman, 1998). For example, chemical mimicry can often be attributed to cuticular hydrocarbons of similar composition (Dettner and Liepert, 1994; Liepert and Dettner, 1996; Allan *et al*., 2002). Methylene chloride extract of the cuticle of *S. avenae* revealed n-alkanes ranging from 23 to 35 carbon atoms and several methyl-branched homologues (Hebanowska *et al*., 1989). However, these results were obtained by extracting whole aphids for 2 weeks, and contamination from internal body contents cannot be excluded (Grasswitz, 1998). In our study, the compounds extracted by the hexane/methanol mixture were not exclusively hydrocarbons. Wax esters as well as long-chain alcohols were also present in the extract. The cuticular extract also contained two aldehydes that are not often reported in studies on insect cuticles (Howard and Lord, 2003) and, to our knowledge, never on aphid cuticles. Moreover, we cannot exclude that other molecules such as sugars were extracted from the cuticle but not detected by the GC-MS technique. In further studies, the extract will be fractionated, and the different fractions will be assayed for recognition activity in order to identify the active compounds by a biologically guided chemical analysis.

In the experiments presented here, all oviposition attempts on exuviae were preceded by antennal contacts. This is not the case when a parasitoid faces a living aphid (Battaglia *et al*., 1993). *A. rhopalosiphi* usually starts its oviposition sequence on aphids without antennal contact, approaching the host with the antennae bending backwards (van Baaren *et al*., 2004). The differences in the behavioral sequence may be due to the nature of the stimulus involved. Both short-range (color, movement) and contact (kairomones) cues are present on the aphid, whereas on the cuticle, the chemical contact kairomones are the only remaining cues. These kairomones can be recognized antennal contact and also during ovipositor contact. The ovipositor of the Aphidiinae consists of three pairs of valvulae (Le Ralec and Rabasse, 1988; Le Ralec *et al*., 2001). At rest, the valvulae 1 and 2 are protected inside the third ones. During the stinging, the third valvulae weigh upon the host cuticle and separate from each other allowing the penetration of the valvulae 1 and 2. The third valvulae have been shown to wear both mechano- and chemoreceptors that could receive information from the cuticle of the host (Le Ralec and Rabasse, 1988). This means that the parasitoid may use two sequential tools to acquire information about the cuticular chemical cues of its host.

It has been shown that host feces or host secretions may play a role in the host-searching process and act as cues for location of host colonies (Weseloh, 1981). With regard to Aphidiine wasps, several authors have demonstrated a role for aphid honeydew in the host-location process (Budenberg, 1990). A similar mechanism seems to be involved in the recognition of exuviae: chemical traces inform the parasitoid of the host presence. If exuviae recognition by the parasitoid is advantageous to host location, the ecological significance of the attacks of exuviae is unclear. Indeed, the attack of an exuvia should be costly in time and energy for the parasitoid, and this behavior should vanish by natural selection. A possible explanation could come from the "Neo-Hopkins principle" (Jaenike, 1983; Corbet, 1985): the response to cuticular compounds could come from the chemical environment experienced by the parasitoid at the emergence from the mummy. This conditioned chemosensory responsiveness can influence the host-searching and host-recognition behaviors of the adult. This effect on host location has already been shown for *A. rhopalosiphi* (van Emden *et al*., 2002) but remains to be tested at the host-recognition level. From an applied point of view, the identified chemical stimulus could provide opportunities to manipulate parasitoid behavior in order to enhance oviposition in artificial rearing systems (Battaglia *et al*., 1995).

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