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## Characterization of the cuticular surface wax pores and the waxy particles of the dustywing, *Semidalis flinti* (Neuroptera: Coniopterygidae)

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### Abstract

The adult dustywing, *Semidalis flinti* Meinander (Neuroptera: Coniopterygidae), begins producing circular-shaped waxy particles after eclosion. The waxy material, which forms the particles, is extruded from individual pores found in clusters on the abdomen. Pores also are present in two rows of three pores on the frontalis and two pores on the first segment of each antennae. The pores have a rosette-like appearance and each pore extrudes dual waxy ribbons. As each ribbon extends a short distance out of the pore, it begins to curl back on itself until the end makes contact with the ribbon. The curled end then breaks free from the extruding ribbon to form the circular waxy particles with fluted edges approximately 2.75- $\mu$ m diameter. The adults use the particles to cover all parts of their body except for their eyes and appear to lightly coat their antennae. The lipid portion of the particles consists largely of free fatty acids, almost exclusively the 24-carbon fatty acid, tetracosanoic acid. Minor lipid classes are hydrocarbons, fatty alcohols and unidentified material.

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### 1. Introduction

Dustywings (Neuroptera: Coniopterygidae) are minute (1–3 mm) predators of small arthropods such as aphids, scale insects, whiteflies and mites. Most species are typically found in trees and

shrubs, where their prey resides, although a few species occur on herbaceous vegetation (Meinander, 1972). The common name derives from their covering of a whitish, powdery exudation. Because of their small size and dusty appearance, they are often mistaken for whiteflies (Arnett, 1993). Many whitefly species also produce waxes from pores on their body as immatures and as adults (Byrne and Hadley, 1988). The adult whitefly produces such copious amounts of the waxy particles that

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they become scattered over their surroundings, possibly to camouflage their eggs (in particular the giant whitefly, *Aleurodicus dugesii* (Nelson et al., 1999), and nymphs, and producing a grayish appearance on the surfaces of heavily infested leaves. Thus, both whiteflies and dustywings appear white due to the coating of waxy particles.

Insects are known to utilize chemical mimicry (Stowe, 1988) or camouflage (Howard, 1993) in order to effectively approach other species in either a mutually beneficial or a predatory interaction. For example, the cuticular hydrocarbons of the carpenter ant, *Camponotus modoc* Wheeler, and the larvae of the obligate predator, *Microdon piperi* Knab, are the same and apparently enable the larval predator to live in the ant brood without being detected (Howard et al., 1990). An example of camouflage has been termed 'wolf-in-sheep's clothing' where the predator lacewing, *Chrysopa slossonae* Banks, covers itself with wax of its prey, the woolly alder aphid, *Prociphilus tessellatus* Fitch, to avoid being detected by ants tending the aphids (Eisner et al., 1978).

The waxy particles of a number of species of whiteflies are composed of a mixture of long-chain *n*-alcohols and *n*-aldehydes with a predominant chain length, depending on the species, of 30, 32 or 34 carbons (Buckner et al., 1994; Nelson et al., 1994, 1997, 1998, 1999). These waxy particles were distinct from the lipids covering the cuticular surface, which were true wax esters. To our knowledge, waxy particles produced by dustywings have not been previously investigated. In this article, we describe the chemistry of the waxy material covering the surface of the dustywing *Semidalis flinti* Meinander (Neuroptera: Coniopterygidae), indigenous to California, Texas and Mexico (Meinander, 1990) and in Arizona (unpublished). Larvae of this species were observed feeding on *Bemisia tabaci* (Gennadius) (strain B) [= *Bemisia argentifolii* Bellows and Perring] infesting various trees and shrubs in southeastern California and Arizona in areas surrounding agricultural areas and in urban areas, where whiteflies spend time between crop seasons. The species has attracted interest as a predator of the economically-damaging *B. tabaci* (Hoelmer et al., 1998). We also describe the morphology of the pores producing the waxy particles, and how the waxy particles appear to be formed.

## 2. Materials and methods

### 2.1. Insects

The dustywings were collected from tea roses (cultivar 'opening night') near Phoenix, AZ, in 1996 and 1997, and cultured on *B. tabaci*, biotype B, reared on cotton. New individuals collected from roses were added to the culture, each subsequent year. Dustywing larvae and adults were placed in glass vials and shipped live overnight to Fargo, ND, for analysis. These insects were placed in clean glass vials and held overnight (18 h) to obtain lipid samples with a minimal amount of contaminants such as phthalate and paraffin. The adults were chilled and removed from the vials for extraction of surface lipids. The material deposited in the vials was removed with chloroform in order to determine the composition of particles shed from the insects, which were assumed to contain relatively little, if any, cuticular surface lipids.

### 2.2. Electron microscopy

Waxed, hydrated insects were examined by scanning electron microscopy at low accelerating voltages without fixation or dehydration. Other samples were frozen and then sputter coated with gold/palladium prior to examination. Wax pores were examined following treatment with hexane and/or chloroform (CHCl<sub>3</sub>) to remove the wax. These samples were then dehydrated in acidified 2,2-dimethoxypropane (DMP), critical point dried using CO<sub>2</sub> as the transitional fluid and then sputter coated. Scanning electron microscopy (SEM) was conducted using a JEOL JSM 6300 microscope. For transmission electron microscopy (TEM), the insects were fixed in 2.5% glutaraldehyde (Millonig's phosphate buffer pH 7.4), post-fixed in buffered 2% osmium tetroxide, dehydrated in a graded series of acetone, enbloc stained in 70% acetone saturated with uranyl acetate, and embedded in epon-araldite for ultrathin sectioning on an RMC ultramicrotome. Sections were examined and photographed using a JEOL JEM 100CX transmission electron microscope.

### 2.3. Lipid extraction

All glassware, glass wool and metal pins, etc., were rinsed with CHCl<sub>3</sub> before use. All plastics,

hand lotion, etc. were avoided. Ice-chilled insects or insects (10–20 per sample; 3 samples) killed by freezing were placed in a glass champagne column fitted with a plug of glass wool and initially rinsed with 5–8 ml of hexane for 1.5 min. This solvent rinse removed hydrocarbons and wax esters but the majority of the external lipids were found in a subsequent rinse with 5 ml of  $\text{CHCl}_3$  for 0.5 min. As a result of these observations, a rinse with 6–8 ml of  $\text{CHCl}_3$  for 1 min was chosen as the usual extraction procedure.

#### 2.4. Chromatography and structural identification

An indication of the lipid classes present was determined by thin-layer chromatography (TLC). A portion of the  $\text{CHCl}_3$  extract and standards were spotted on high-performance silica gel plates with hexane/diethyl ether/formic acid (80:20:1 v/v) as the developing solvent. The locations of the lipid bands were visualized by charring plates after spraying with a solution of 5% sulfuric acid in 95% ethanol, allowing the ethanol to evaporate, heating at 150 °C for 10 min, and then at 250 °C for 10 to 20 min.

Samples were analyzed in total as previously described (Nelson et al., 1994) by GC-MS on a Hewlett-Packard quadrupole system equipped with an autosampler and a temperature and pressure programmable cool on-column injection port. The injection port was attached to a 1 m retention gap connected to a 12 m $\times$ 0.2 mm capillary column of cross-linked dimethyl silicone Ultra 1 (H-P). The column temperature was programmed from 150 to 320 °C at 4°/min and then held for a total time of 200 min to elute any diacylglycerols and wax esters. All the hydrocarbons had eluted within 60 min. The mass range scanned was 50–800 amu with a new scan every 1.1 s. The alcohol components were analyzed as their acetate derivatives, and both the alcohol and fatty acid components as their trimethylsilyl (TMS) derivatives. Acetate esters were prepared by dissolving a portion of the total sample in pyridine, adding acetic anhydride, and allowing to react overnight as described (Nelson et al., 1994). The trimethylsilyl ether derivatives were prepared by dissolving a portion of the total sample in benzene, then adding dimethylformamide (DMF), gently mixing and then adding *N,O*-bis(trimethylsilyl)acetamide (BSA) to give a ratio of benzene:DMF:BSA of 20:60:20. The reac-

tion vial was capped, heated for 70 min at 75 °C, cooled and immediately analyzed by GC-MS.

#### 2.5. Quantification of GC-MS data

The total ion current (TIC) data were analyzed using a computer spreadsheet program in which the dose response was adjusted using a 3-component standard curve prepared using acetate ester, wax ester and *n*-alkane standards. The equation component from 0 to 3.1 ng was linear, the next equation component was polynomial from 3.1 to 100 ng, and the third component was again linear over 100 ng. The formula in the spreadsheet selected the equation component to be used based on the peak area of the total ion current of the GC-MS peak being measured to calculate the femtomoles and/or nanograms that the peak represented. The quantity response for the acetate and TMS derivatives was determined by analyzing the TMS derivatives of a mixture of standard fatty acids and the acetate and TMS derivatives of standard alcohols. Their responses were related to the responses of the acetate ester, wax ester and *n*-alkanes in the standard mixture run on the GC-MS system before and after the samples.

### 3. Results

#### 3.1. Biology

Upon eclosion the dustywing adult begins to form waxy particles, and with a grooming behavior with its front and hind pairs of legs (prothoracic and metathoracic tibia), covers all parts of its body with the particles except for the eyes (Fig. 1a and b). Relatively fewer particles are found hanging on the hairs of the antennae. Whether the adult is deliberately grooming particles onto the antennae, or they get there accidentally is open to speculation. The waxy particles are produced by clusters of wax pores located on the lateral sides of each segment of the abdomen (Fig. 2a). The particles are also produced by single pores located on the outer top edge of each scape, by rows of 3 pores located on the head above each scape (above the antennal suture) and by single pores located on each side of the midline of the frons (Fig. 2b).

The waxy particles are extruded from rosette-shaped pores (Fig. 3a and b). Each pore extrudes dual ribbons of material with fluted edges (Fig.

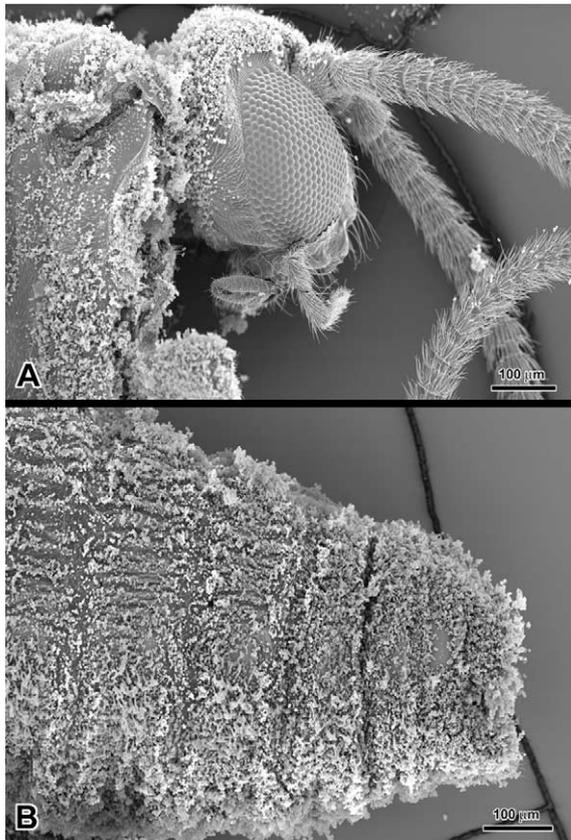


Fig. 1. SEM of *S. flinti* adult. Waxy particles are found: (a) covering the head and thorax, except for the eyes; (b) completely covering the abdomen.

4a). The end of each ribbon curls back and makes contact with the elongating ribbon forming a circle, which breaks free to produce the particles. The particles are circular, approximately 2.75- $\mu\text{m}$  outside diameter and 1.5- $\mu\text{m}$  inside diameter and resemble the rim of an automobile wheel with fluted edges (Fig. 4b). A cross section through a wax-producing pore shows the pore penetrating the cuticle, as well as a large area of material, presumably the waxy material to form the particles, at the base (Fig. 5).

### 3.2. Thin-layer chromatography

The total lipids extracted from the waxy material shed by the insects and from the cuticular surface were analyzed by silica gel HPTLC, and the lipid bands were visualized by charring (data not shown). Two intense bands corresponding to

hydrocarbons and free fatty acids were present. Barely visible bands corresponding to alcohols, diacylglycerols and monoacylglycerols also were present. All samples showed a band at the origin, which was not characterized further. The total lipid sample was then analyzed, underivatized, by GC-MS, to determine if minor amounts of wax esters and aldehydes were present in the samples.

### 3.3. GC-MS chromatography and distribution of lipid classes

The GC-MS chromatogram depicts the chromatographic profile obtained from analysis of a total extract of the external surface lipids (Fig. 6). A similar GC-MS profile was obtained for the

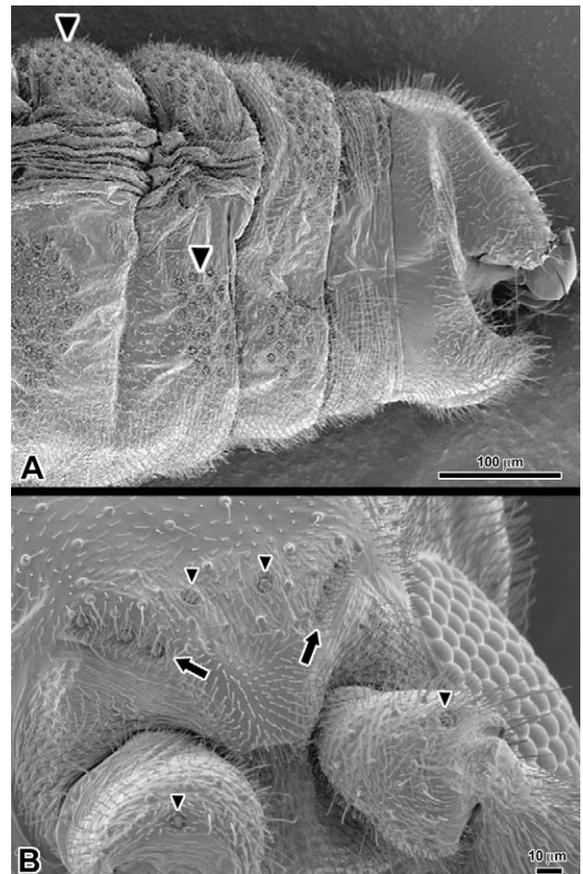


Fig. 2. SEM of *S. flinti* adult male after surface lipids were removed with chloroform: (a) the abdomen has clusters of wax-producing pores; (b) the frontal portion of the head where the antennae join showing wax-producing pores on the scape and the frons.

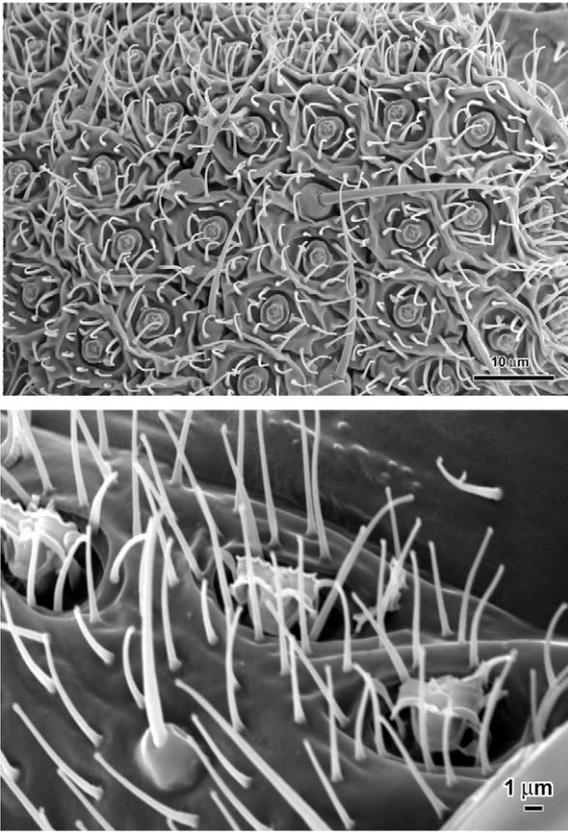


Fig. 3. (a) A close-up view of part of a cluster of pores showing their 'rosette' shape; (b) a side view of waxy ribbons beginning to extrude from the rosette-shaped pores.

waxy material shed from the adults in the vials in which they were held. The largest peak was that due to the fatty acid tetracosanoic acid. The percentage distribution of the lipid classes analyzed by GC-MS showed that free fatty acids were the major lipid class in total surface lipids of both males and females, and in the waxy material shed by them in the vials (Fig. 7). The unknown components were largely diacylglycerols. The similarity in the composition of the lipid classes obtained from the insect vs. those obtained from the vial indicated that there was not a separate lipid class present on the cuticular surface in detectable amounts. Total lipids were 2159 ng/female and 2186 ng/male (Table 1).

### 3.3.1. Fatty acids

Free fatty acids were 55–60% of the lipids (Fig. 7). The major fatty acid in all samples was

tetracosanoic acid, identified by GC-MS both as the free acid and as the TMS derivative. Tetracosanoic acid was 57% of surface lipid fatty acids and approximately 43% of fatty acids deposited in the vials (Table 2). Uric acid was also present in the waxy material in the vials but was considered to be an excretory product and was not included in the percentages. No uric acid was detected in the external surface lipids. If expressed as a percent of the fatty acids found in the vials, the amount of uric acid ranged from 1 to 23%. Fatty acids ranged in chain length from tetradecanoic to octacosanoic. Two methyl-branched fatty acids, with carbon backbones of 14 and 16 carbons, and the unsaturated fatty acids 16:1, 18:1 and 18:2, were also present. Also identified were three hydroxy fatty acids with carbon backbones of 21, 23 and 24 carbons. Quantities of fatty acids from females and males were 1228 and 1302 ng/insect, respec-

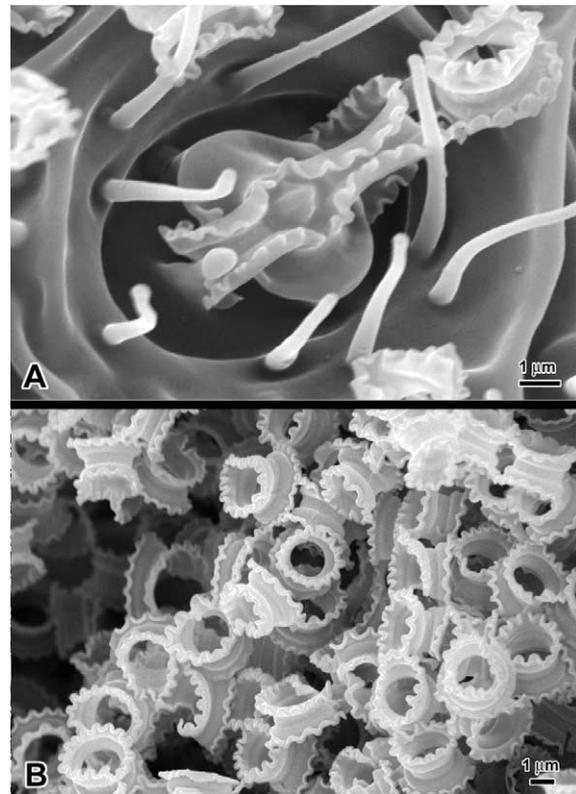


Fig. 4. (a) The circular waxy particles are formed as the ends of the elongating ribbons curl back on the ribbon and break off; (b) the waxy particles with their shape resembling an automobile wheel-rim with fluted edges and without spokes.

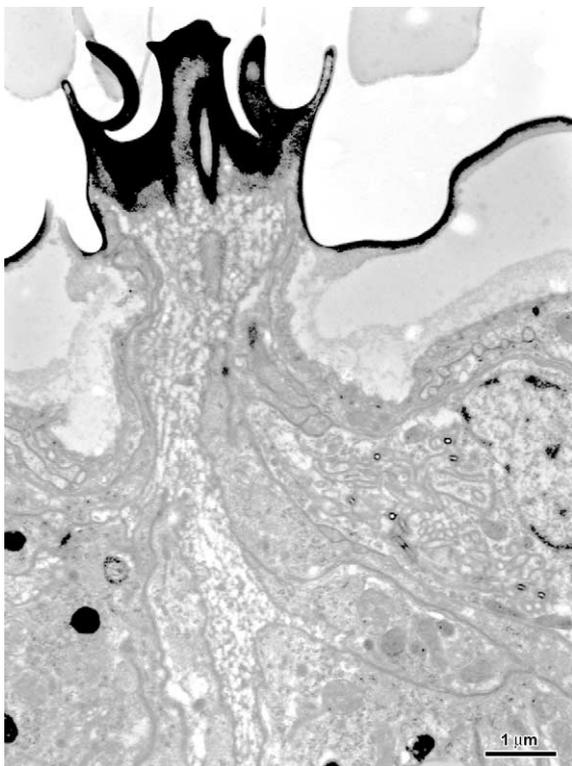


Fig. 5. Cross-section through a pore penetrating the cuticle and with a large vessel of material presumably used to form the waxy ribbons.

tively, and from the vials, the values were 369 and 285 ng/insect, respectively (Table 1).

### 3.3.2. Hydrocarbons

Hydrocarbons were approximately 20% of the external lipids (Fig. 7). The majority of the hydrocarbons were methyl-branched alkanes, including mono-, di-, tri- and tetramethyl-branched. The dominant methylalkanes were a series of heptacosanes, which ranged in amount from 58.2% of the hydrocarbons in the female vial to 66.5% of the hydrocarbons on males (Table 3); i.e. 3,7-dimethyl-, 3,7,11-trimethyl- and 3,7,11,15-tetramethylheptacosane. The major methyl-branched alkane in both males and females, and in their holding vials, was 3,7,11-trimethylheptacosane. This was a unique composition of hydrocarbons. Of the total female hydrocarbons, 81% had a methyl branch on carbon 2, 3 and 4; 75% had a methyl branch on carbon 3. There were only minor amounts of *n*-alkanes, terminally branched monomethylalkanes, and no internally branched mono-, di-, tri- or

tetramethyl-branched alkanes were detected. The absence of these structures was fortuitous because their GC-MS elutions tend to overlap with those of the major methylalkanes present. For example, 3,7-dimethylheptacosane would elute immediately after *n*-octacosane; 3,7,11-trimethylheptacosane would overlap with internally branched monomethyloctacosanes and 3,7,11,15-trimethylheptacosane would overlap with internally branched dimethyloctacosanes and/or with terminally branched monomethyloctacosanes. The amount of overlap or resolution possible would depend on the position(s) of the methyl branches in the internally branched methylalkanes.

The structures and mass spectra of the methyl-branched heptacosane series (27-carbon backbone) is depicted in Fig. 8. Interpretation of a mass spectrum is strengthened by comparison with other mass spectra of the methylalkanes in the series with additional methyl branches, i.e. Fig. 8a, b and c, as well as with the mass spectrum of a methylalkane with an elongated carbon backbone, 4,8,12-trimethyloctacosane (28-carbon backbone) (Fig. 8d). Fig. 8a has the expected fragment ions for 3,7-dimethylheptacosane with ions of the expected intensities. The intense ion pair at  $m/z$  308:309 was expected since the 22-carbon secondary ion fragment does not contain a methyl branch and would tend to undergo a rearrangement to lose a hydrogen forming a significant amount of the ion at  $m/z$  308. When a third methyl branch is present, as in 3,7,11-trimethylheptacosane, the major fragmentation occurs around the second methyl branch point (Fig. 8b). This resulted in ions of lower relative intensity at  $m/z$  252:253 for the 18-carbon secondary ion fragment compared to the intensity of ions at  $m/z$  308:309 for the corresponding 22-carbon fragment of the dimethylalkane (Fig. 8a). When a fourth methyl branch is present, as in 3,7,11,15-tetramethylheptacosane, the relative intensity of the fragment ions is comparable for all fragmentations (Fig. 8c).

Elongation of the carbon chain by one carbon forms a methyl-branched alkane with an even-numbered carbon backbone, e.g. 4,8,12-trimethyloctacosane (Fig. 8d). Biosynthesis of such an even-numbered carbon backbone compound would necessarily require propionate as the primer molecule. Therefore, the first methyl branch occurs on carbon 4 (or 6, 8, etc.) rather than on carbon 3, 5, 7, etc. A methyl branch on carbon 4 forms an additional diagnostic fragment ion at  $m/z$  70,

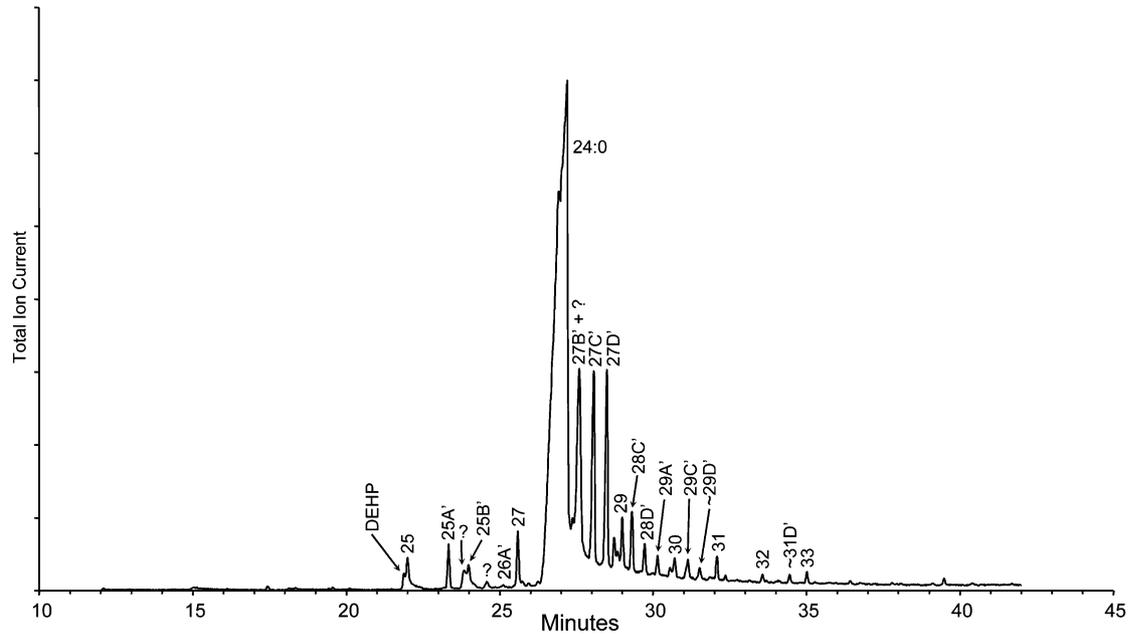


Fig. 6. GC-MS of the total external lipids from adults of *S. flinti*. The numbers indicate the number of carbon atoms in the backbone of the molecule. The letters A, B, C and D indicate one, two, three or four methyl branches, respectively, in the hydrocarbons. The 'prime' mark indicates that one of the methyl branches is near the end of the molecule, on carbon 2, 3 or 4. Tetracosanoic acid is designated as 24:0 according to standard fatty acid abbreviation. DEHP is the common laboratory contaminant, bis(2-ethylhexyl)phthalate.

apparent if the mass spectrum is sufficiently intense (Fig. 8d). In this case, the intensity was moderate and the ion at  $m/z$  70 was not as intense

as the ion at  $m/z$  69. However, it was of comparable intensity to the ion at  $m/z$  56 and this was considered to be verification of a methyl branch

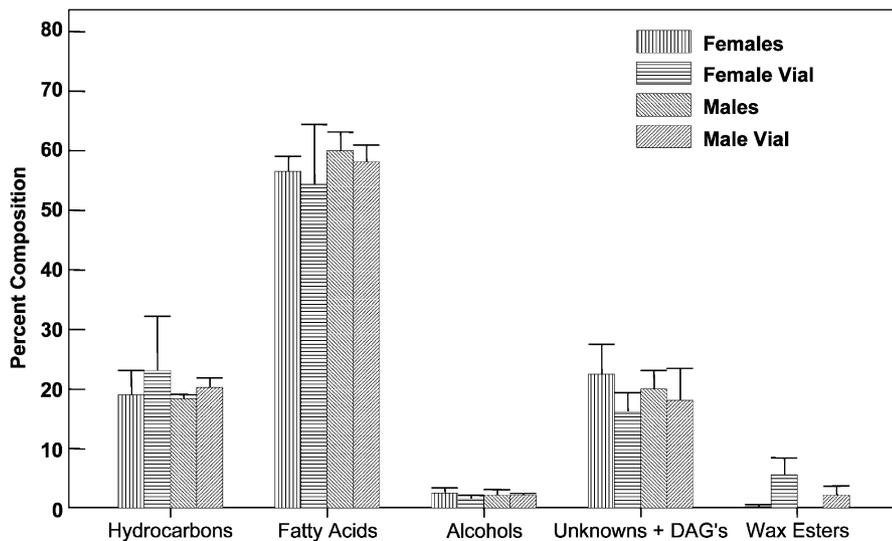


Fig. 7. Percentage composition of the lipid classes in the lipid extracts of the total adult female and male external lipids, and in the lipids coating the walls of the glass vials in which they had been held for 18 h. The 'unknowns' were mainly diacylglycerols. Error bars are indicated ( $n=3$ ).

Table 1

Nanograms per adult ( $\pm$ S.D.) of waxy material extracted from the cuticular surface and of waxy material shed over an 18-h period in the glass holding vial

Lipid class <sup>a</sup>	Female	Female vial	Male	Male vial
Hydrocarbons	407 $\pm$ 49	143 $\pm$ 34	394 $\pm$ 45	100 $\pm$ 23
Fatty acids	1228 $\pm$ 85	369 $\pm$ 135	1302 $\pm$ 214	285 $\pm$ 57
Alcohols	52 $\pm$ 18	10 $\pm$ 5	44 $\pm$ 12	10 $\pm$ 2
Unknowns	502 $\pm$ 148	107 $\pm$ 23	447 $\pm$ 89	84 $\pm$ 12
Wax esters	11 $\pm$ 4	36 $\pm$ 12	trace	11 $\pm$ 8
Totals	2159 $\pm$ 209	664 $\pm$ 121	2186 $\pm$ 309	490 $\pm$ 80

<sup>a</sup> Lipid classes were determined by GC-MS; fatty acids, alcohols and unknowns as their TMS derivatives. Unknowns also included diacylglycerols but do not include uric acid, which was only found in the vials. There was 65 ng/insect of uric acid in the female vials and 50 ng/insect in the male vials ( $n=3$ ).

on carbon 4. In a methylalkane with no methyl branch on carbon 4, the intensity of  $m/z$  70 would be intermediate between that of the ions at  $m/z$  56 and 84 (Tissot et al., 2001). Other fragmenta-

tions around the methyl branch points are analogous to those described above (Fig. 8a, b and c) and provide further support for the validity of the interpretation of the mass spectra.

Table 2

Percent composition ( $\pm$ S.D.) of the TMS derivatives of the free fatty acids from the cuticular surface of adult males and females, and of the free fatty acids from waxy particles that were dislodged from them into the vial

GC-MS peak <sup>a</sup>	Percent composition <sup>b</sup>				Acid identification <sup>c</sup>
	Female	Female vial	Male	Male vial	
14	1.1 $\pm$ 0.1	2.1 $\pm$ 0.2	1.3 $\pm$ 0.1	2.3 $\pm$ 0.1	Tetradecanoic
14A	–	0.4 $\pm$ 0.2	–	0.7 $\pm$ 0.2	<i>x</i> -Methyltetradecanoic
15	0.4	1.2	0.5 $\pm$ 0.1	1.3 $\pm$ 0.2	Pentadecanoic
16:1	–	1.1 $\pm$ 0.4	0.3	0.7 $\pm$ 0.3	Pentadecenoic
16	3.7 $\pm$ 0.9	8.9 $\pm$ 1.0	4.0 $\pm$ 0.4	7.7 $\pm$ 1.4	Hexadecanoic
16A	<i>t</i>	0.4 $\pm$ 0.2	–	–	<i>x</i> -Methylhexadecanoic
17	0.5 $\pm$ 0.4	1.4 $\pm$ 0.2	0.6 $\pm$ 0.1	1.5 $\pm$ 0.2	Heptadecanoic
18:2	0.8 $\pm$ 0.3	1.7 $\pm$ 0.3	1.0 $\pm$ 0.2	1.6 $\pm$ 0.2	Octadecadienoic
18:1	1.1	6.1 $\pm$ 0.7	1.2 $\pm$ 0.2	2.9 $\pm$ 0.2	Octadecenoic
18	6.3 $\pm$ 2.3	11.3 $\pm$ 2.1	6.4 $\pm$ 0.7	10.6 $\pm$ 2.6	Octadecanoic
19	0.1	0.8 $\pm$ 0.4	0.2	0.5 $\pm$ 0.1	Nonadecanoic
20	0.3 $\pm$ 0.1	1.0 $\pm$ 0.2	0.3	1.3 $\pm$ 0.3	Icosanoic
22	1.0	0.9 $\pm$ 0.4	0.6 $\pm$ 0.1	1.2 $\pm$ 0.3	Docosanoic
23	0.6 $\pm$ 0.2	1.0 $\pm$ 0.2	0.6 $\pm$ 0.1	0.9 $\pm$ 0.2	Tricosanoic
21-OH	4.1 $\pm$ 0.3	3.4 $\pm$ 0.1	4.3 $\pm$ 0.3	3.9 $\pm$ 0.4	14-Hydroxyhenicosanoic
24	57.3 $\pm$ 4.2	42.2 $\pm$ 3.4	56.1 $\pm$ 1.8	43.7 $\pm$ 0.8	Tetracosanoic
25	1.2 $\pm$ 0.1	0.8 $\pm$ 0.4	1.2 $\pm$ 0.1	1.2 $\pm$ 0.3	Pentacosanoic
23-OH	4.0 $\pm$ 0.3	3.2 $\pm$ 0.3	3.9 $\pm$ 0.1	3.7 $\pm$ 0.4	13- to 16-Hydroxytetracosanoics
24-OH	3.9 $\pm$ 0.7	2.2 $\pm$ 0.4	5.2 $\pm$ 0.1	3.3 $\pm$ 0.5	16- and 17-Hydroxytricosanoics
26	11.1 $\pm$ 0.1	7.8 $\pm$ 0.4	9.7 $\pm$ 0.3	8.8 $\pm$ 0.7	Hexacosanoic
28	2.4 $\pm$ 0.3	2.3 $\pm$ 0.1	2.4 $\pm$ 0.3	2.1 $\pm$ 0.3	Octacosanoic

<sup>a</sup> The GC-MS peaks are listed in their order of elution. Minor components may not be visible in Fig. 6. The number is the number of carbon atoms in the backbone (carbon chain) of the molecule.

<sup>b</sup> Percentage composition was calculated from the integrated area data, adjusted for system response, from GC-MS analysis and rounded to the nearest tenth. The amount of trace components, less than 0.2%, are indicated by '*t*'. Where no standard deviation is given, it was less than 0.1 when rounded ( $n=3$ ).

<sup>c</sup> Structural identifications of the hydroxy acids were from analysis of mass spectral data of the TMS derivatives obtained by GC-MS. Methyl branch and double bond positions cannot be determined from these data.

Table 3

Percent composition ( $\pm$ S.D.) of the hydrocarbons from the cuticular surface of adult males and females, and of the hydrocarbons from waxy particles that were dislodged from them into the vial

GC-MS peak <sup>a</sup>	Percent composition <sup>b</sup>				Identification <sup>c</sup>
	Female	Female vial	Male	Male vial	
23	<i>t</i>	0.4 $\pm$ 0.4	0.2	0.3	Tricosane
24	–	0.6 $\pm$ 0.8	<i>t</i>	0.3	Tetracosane
25	1.4 $\pm$ 0.2	1.7 $\pm$ 0.5	2.5 $\pm$ 0.2	2.9 $\pm$ 0.3	Pentacosane
25A'	1.4 $\pm$ 0.1	1.4 $\pm$ 0.4	3.3 $\pm$ 0.2	4.4 $\pm$ 0.9	3-Methylpentacosane
26	–	0.8 $\pm$ 0.8	–	–	Hexacosane
25B'	0.5 $\pm$ 0.5	1.0 $\pm$ 0.3	1.6 $\pm$ 0.1	1.5 $\pm$ 0.1	3,7-Dimethylpentacosane
26A'	–	0.2 $\pm$ 0.2	–	–	2- and/or 4-Methylhexacosane
26A'	0.3 $\pm$ 0.1	0.3 $\pm$ 0.3	0.4	0.4	3-Methylhexacosane
27	6.6 $\pm$ 0.8	9.2 $\pm$ 1.7	3.8 $\pm$ 0.2	4.4 $\pm$ 0.4	Heptacosane
26B'	0.2 $\pm$ 0.2	0.6 $\pm$ 0.2	0.5	0.4	?
26C'	0.4 $\pm$ 0.1	0.8 $\pm$ 0.4	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	?
28	–	1.5 $\pm$ 1.1	–	–	Octacosane
27B'	26.5 $\pm$ 2.3	21.2 $\pm$ 4.0	20.8 $\pm$ 0.2	19.3 $\pm$ 1.5	3,7-Dimethylheptacosane
27C'	32.8 $\pm$ 3.1	31.6 $\pm$ 3.4	24.8 $\pm$ 1.2	23.9 $\pm$ 1.3	3,7,11-Trimethylheptacosane
27D'	4.4 $\pm$ 0.9	5.4 $\pm$ 1.4	20.9 $\pm$ 0.8	19.1 $\pm$ 0.3	3,7,11,15-Tetramethylheptacosane
29	5.0 $\pm$ 0.7	3.9 $\pm$ 0.2	3.9 $\pm$ 0.1	3.5 $\pm$ 0.1	Nonacosane
28C'	4.9 $\pm$ 0.5	4.5 $\pm$ 0.5	3.9 $\pm$ 0.1	4.5 $\pm$ 0.4	4,8,12-Trimethyloctacosane
28D'	0.2 $\pm$ 0.3	0.8 $\pm$ 0.3	1.9 $\pm$ 0.1	2.1 $\pm$ 0.3	4,8,12,16-Tetramethyloctacosane
29A'	1.6 $\pm$ 0.4	1.4	1.9 $\pm$ 0.1	2.0 $\pm$ 0.3	3-Methylnonacosane
30	0.7 $\pm$ 0.2	0.5 $\pm$ 0.2	0.6 $\pm$ 0.1	0.9 $\pm$ 0.2	Triacontane
29B'	3.0 $\pm$ 0.4	3.2	2.0 $\pm$ 0.1	2.0	3,7-Dimethylnonacosane
29C'	3.5 $\pm$ 0.1	3.6 $\pm$ 0.2	2.1	2.2	3,7,11-Trimethylnonacosane
30A'	1.3 $\pm$ 0.2	1.0 $\pm$ 0.1	1.2 $\pm$ 0.0	1.3 $\pm$ 0.1	?
31	1.6 $\pm$ 0.2	1.2 $\pm$ 0.3	1.4 $\pm$ 0.1	1.3 $\pm$ 0.1	Hentriacontane
30C'	0.6	0.8 $\pm$ 0.1	0.4	0.3	4,8,12-Trimethyltriacontane
32	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.8 $\pm$ 0.2	Dotriacontane
32A	0.6	0.7 $\pm$ 0.2	–	0.3 $\pm$ 0.1	?
31C'	0.5 $\pm$ 0.1	0.6 $\pm$ 0.2	0.6	0.9 $\pm$ 0.2	?
33	1.0 $\pm$ 0.2	0.4 $\pm$ 0.1	0.6	0.5 $\pm$ 0.1	Tritriacontane

<sup>a</sup> The GC-MS peaks are listed in their order of elution. Minor components may not be visible in Fig. 6. The number is the number of carbon atoms in the backbone (carbon chain) of the molecule. The letters indicate the number and approximate positions of the methyl branch(es). The letters A, B, C and D indicate one, two, three and four methyl branches, respectively. The 'prime' mark indicates that one of the methyl branches is near the end of the molecule, on carbon 2, 3 or 4. There were indications of trace amounts of unidentified methyl-branched alkanes eluting on the leading and tailing edges of the *n*-alkanes; these would be expected to be 2,*x*- and/or 4,*x*- on the leading edge, and 3,*x*-dimethylalkanes on the tailing edge.

<sup>b</sup> Percent composition was calculated from the integrated area data, adjusted for system response, from GC-MS analysis and rounded to the nearest tenth. The amount of trace components, less than 0.2%, are indicated by '*t*'. Where no standard deviation is given, it was less than 0.1 when rounded ( $n=3$ ).

<sup>c</sup> Structural identifications were from analysis of mass spectral data obtained by GC-MS. Structures which were only indicated by their elution position, but which were present in amounts insufficient to obtain a definitive mass spectrum, are indicated by '?'.

### 3.3.3. Fatty alcohols

Fatty alcohols were minor components of the exterior lipids and constituted approximately 2%

of the exterior lipids obtained from both males and females, and from the vials in which they were held (Fig. 7). They ranged in chain length

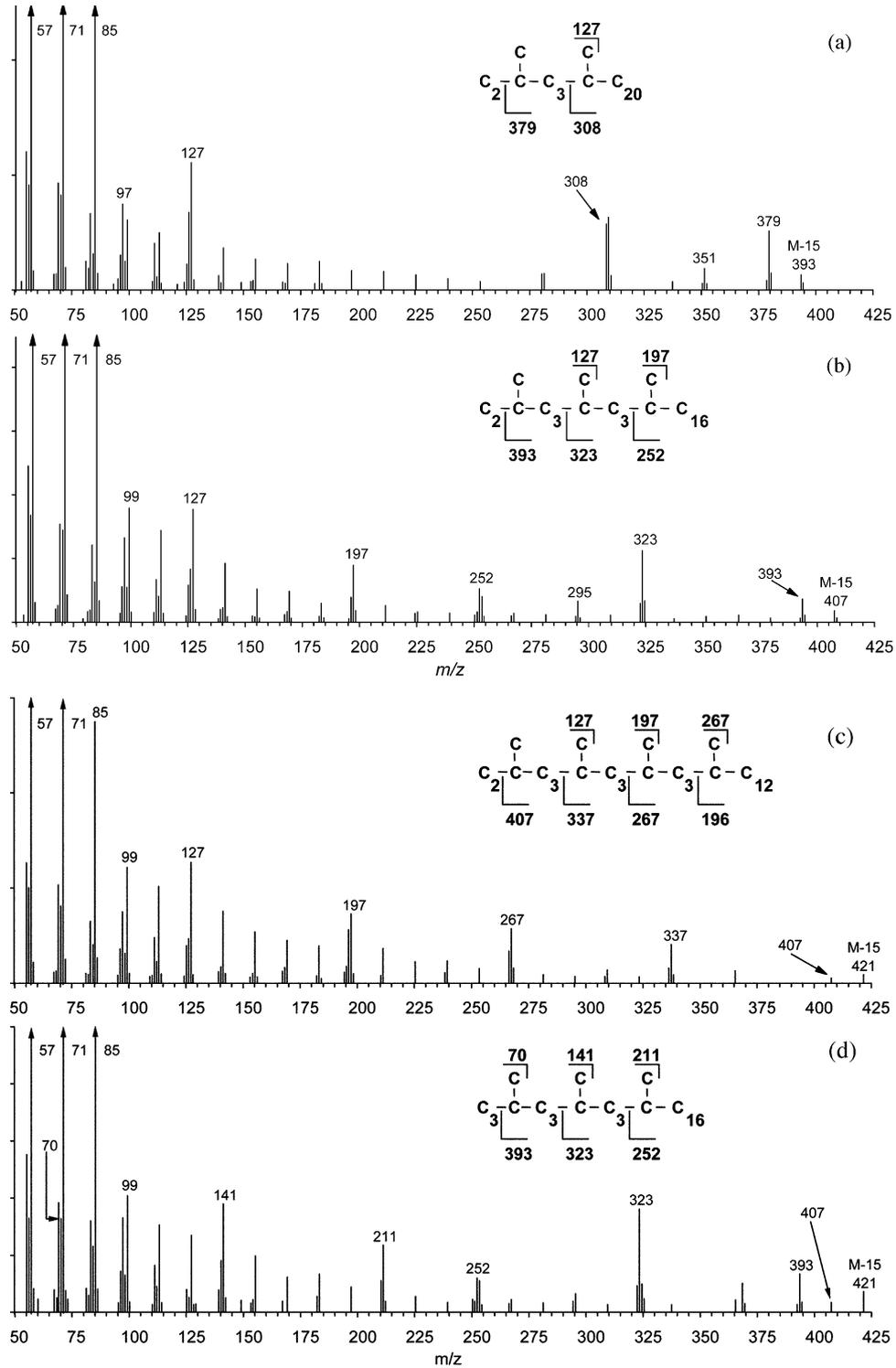


Fig. 8. GC-MS mass spectra of the series of methyl-branched heptacosanes: (a) Peak 27B', 3,7-dimethylheptacosane; (b) Peak 27C', 3,7,11-trimethylheptacosane; (c) Peak 27D', 3,7,11,15-tetramethylheptacosane; and (d) of an even carbon-numbered homologue, peak 28C', 4,8,12-trimethyloctacosane.

Table 4

Percent composition ( $\pm$ S.D.) of alcohols from the cuticular surface of adult males and females and from waxy particles that were dislodged from them into the vial

Relative alcohol carbon no. <sup>a</sup>	Percent composition <sup>b</sup>				Identification <sup>c</sup>
	Female	Female vial	Male	Male vial	
16	0.9 $\pm$ 0.2	3.2 $\pm$ 0.7	2.0 $\pm$ 0.8	2.6 $\pm$ 1.1	Hexadecanol
18	1.2 $\pm$ 0.2	5.6 $\pm$ 0.9	1.8 $\pm$ 0.8	3.1 $\pm$ 0.8	Octadecanol
19.8	3.0 $\pm$ 0.4	8.0 $\pm$ 1.5	5.1 $\pm$ 0.8	6.3 $\pm$ 2.1	8-Hydroxyhenicosane
21.8	15.6 $\pm$ 5.0	17.0 $\pm$ 4.7	11.2 $\pm$ 0.5	37.8 $\pm$ 19.2	9- and 8-Hydroxytricosanes**
22.9	14.0 $\pm$ 6.1	5.0 $\pm$ 3.6	12.2 $\pm$ 1.3	10.0 $\pm$ 1.5	9- and 8*-Hydroxytricosanes**
24.9	55.8 $\pm$ 10.6	0.0	60.5 $\pm$ 2.5	18.3 $\pm$ 21.8	9- and 8-Hydroxypentacosanes
28	3.0 $\pm$ 0.7	32.0 $\pm$ 1.8	0.0	7.0 $\pm$ 0.7	Octacosanol
30	0.4 $\pm$ 0.6	9.3 $\pm$ 2.2	0.0	3.1 $\pm$ 1.0	Triacontanol
32	0.0	2.2 $\pm$ 3.2	0.0	4.4 $\pm$ 0.7	Dotriacontanol
34	6.0 $\pm$ 1.1	17.7 $\pm$ 6.0	7.2 $\pm$ 1.8	7.5 $\pm$ 0.1	Tetratriacontanol

<sup>a</sup> Carbon number of the alcohols analyzed as their TMS derivatives relative to *n*-alcohols analyzed as their TMS derivatives.

<sup>b</sup> Percent composition was calculated from the integrated area data, adjusted for system response, from GC-MS analysis and rounded to the nearest 0.1 ( $n=3$ ). Where no standard deviation is given, it was less than 0.1 when rounded.

<sup>c</sup> Structural identifications were from analysis of mass spectral data obtained by GC-MS analysis of TMS derivatives. A '\*' indicates the major isomer. \*\*Structures of peaks with relative alcohol carbon numbers of 21.8 and 22.9 are tentative as they had identical mass spectra but were separated by 1.1 carbon equivalents.

from C16 (hexadecanol) to C34 (tetratriacontanol) (Table 4). Secondary alcohols, approximately 89% of the alcohols from males and females, with carbon backbones of 21, 23 and 25 carbons, were characterized (Table 4). The major secondary alcohols were identified from the mass spectrum of their trimethylsilyl derivatives as a mixture of 8- and 9-hydroxypentacosanes.

#### 3.3.4. Wax esters

Wax esters from the insects varied from a trace amount in male lipids to 5% in lipids from the female vials (Fig. 7). The composition was variable but consisted of even carbon-numbered wax esters from 34 to 44 carbons. All wax ester peaks were a mixture of isomers, all with 16- and 18-carbon fatty acid moieties; in the case of the 40- and 42-carbon wax esters, a 20-carbon fatty acid moiety also was present. The two major wax esters from the females consisted of 40 carbons (29 $\pm$ 16%) and 42 carbons (53 $\pm$ 27%). No unsaturated wax esters were detected from the insects themselves. However, the wax esters recovered from the vials in which the females had been held ranged from 34 to 46 carbons and had an 18:1 fatty acid moiety in wax esters with carbon numbers of 40, 42, 44 and 46. The unsaturated wax esters accounted for approximately 25% of the

total wax esters from the female vial. The wax esters in vials in which the males had been held were of similar composition but in lesser amounts. No branched or hydroxy acids or alcohols, or secondary alcohols were detected in the wax esters.

## 4. Discussion

Both whiteflies and *S. flinti* form waxy particles with which they groom and give themselves a white appearance. The Aleyrodidae whitefly family is divided into Aleyrodinae and Aleurodicinae. The particles of the Aleyrodinae studied (see Nelson et al., 1997) are produced by pores on abdominal wax plates, which cover a major portion of the abdomen (Domenichini, 1981; Byrne and Hadley, 1988; Nelson et al., 2000a). As the filaments are extruded, the ends curl to form a hook. The adult then rakes its tibia across the wax plates breaking off the extruding filaments forming semi-circular particles, which are caught on the hairs and spines. In the few Aleurodicinae studied, the filaments do not curl and when broken off by the tibia form short pieces of the filaments (Freeman et al., 1997; Nelson et al., 1999, 2000a,b). The wax-producing pores, and occasionally the waxy material, of a number of other insects have been described (Foldi, 1983; Foldi and Cassier, 1985;

Foldi and Pearce, 1985; Foldi, 1991; Foldi and Lamdin, 1995; Locke, 1974; Waku and Foldi, 1984).

All adult whiteflies studied have waxy particles that are composed mainly of a mixture of a long-chain alcohol and a long-chain aldehyde (Buckner et al., 1994; Nelson et al., 1994, 1997, 1998, 1999, 2000b). This report on *S. flinti*, a predator feeding on whiteflies, demonstrates the presence of waxy particles composed mainly of free fatty acids with one major component, tetracosanoic acid. We know of no reports of exterior lipid particles or cuticular surface lipids where the major components are free fatty acids. Usually the presence of free fatty acids would be suspected as being due to contamination from internal lipids during the extraction of the external lipids. However, we know of no reports where fatty acids larger than 18 carbons are the major fatty acid in the internal lipids of arthropods. Also, the fact that the lipids in the vials had a similar composition of all lipid classes indicates that internal lipids/fatty acids were not extracted from the insects. We did not detect the presence of long-chain aldehydes, found in all whiteflies studied, or of acetate esters which have been reported for two species of whiteflies, *Aleyrodes singularis* Danzig (Nelson et al., 1998), and the giant whitefly, *Aleurodicus dugesii* Cockerell (Nelson et al., 1999, 2000a).

However, other classes of polar lipids can be found on the exterior lipids of a number of insect species (see review by Buckner, 1993). The adult female scale insect, *Drosicha corpulenta* Kuwana, secreted a white waxy material, which consisted of partially curled particles (Hashimoto and Kitao-ka, 1982). The waxy material consisted of 18% hydrocarbons and 82% wax esters of which the major ester was hexacosanyl hexacosanoate. Frequently, studies are conducted on the larval stages rather than on the adults. For example, both the chemistry of the waxy material (93% *n*-alcohols; the major component hexacosan-1-ol) and the wax-producing pores were characterized in larvae of the red-backed alder sawfly, *Eriocampa ovata* Linnaeus (Percy et al., 1983). However, although the structures of the wax pores were described, the structure of the flocculent white tufts of waxy material was not presented. Larvae of the whitefly, *Metaleurodicus griseus* (Dozier) produces a flocculent material composed largely of the true wax ester, triacontanyl triacontanoate, and from the published photographs, some of this material may

occur on the adult (Mason et al., 1991). Larvae of the cochineal insect, *Dactylopius confusus* (Cockerell), produced a fluffy material characterized as a keto-wax ester, 15-oxotetracontanyl 11-oxotriacontanoate (Meinwald et al., 1975).

Frequently found as major components on insects are hydrocarbons (Blomquist et al., 1987; Lockey, 1988; Nelson, 1993; Nelson and Blomquist, 1995). However, hydrocarbons were a very minor lipid class in cuticular lipids of all whiteflies studied, but were second in abundance in the exterior lipids of the dustywing reported herein. Wax esters were not a component of the waxy particles in the adult whiteflies (see citations above). However, in the external lipids of the nymphs and exuvial structures of the whitefly, *B. tabaci*, the major lipid class was wax esters (84%), with lesser amounts of hydrocarbons, aldehydes and alcohols (Buckner et al., 1999). Wax esters appear to be a minor component of the external lipids of the dustywing, but may be associated with material deposited in the vials.

The function of the dustywing waxy particles is unknown, but some possible biological activity may be indicated from studies of other insects. Adults of *A. singularis* cover their immature stages with waxy particles and these were observed to cover the parasitoids, *Encarsia sophia* (Girault and Dodd) [= *Encarsia transvena* (Timberlake)] and *E. inaron*, and interfere with parasitoid movement (Guershon and Gerling, 1994). *E. inaron*, a parasitoid of nymphs covered with waxy particles, spent time in cleaning its antennae and the tip of its ovipositor. *E. sophia*, a parasitoid of whitefly nymphs not covered with waxy particles, became coated with the waxy particles and then was unable to clean them off and frequently lost its balance.

Chemical mimicry (Stowe, 1988) and camouflage (Howard, 1993) are used by a species to approach another species in either a beneficial or a predatory interaction. Cuticular hydrocarbons of the carpenter ant, *C. modoc*, and the larvae of its obligate predator, *M. piperi*, are the same (Howard et al., 1990). Waxy materials produced by some species of prey insects are used by their predators to repel or camouflage the predator from attacks by predatory ants. Triacontanyl triacontanoate from the whitefly, *M. griseus*, is used by the predatory chrysopid larvae, *Ceraeochrysa cincta* Schneider to cover itself (Mason et al., 1991). 15-Oxotetracontanyl 13-oxodotriacontanoate from the woolly alder aphid *P. tessalatus* (Mein-

wald et al., 1975) is used by the predatory larvae of *C. slossonae* for camouflage (Eisner et al., 1978). This behavior of *C. slossonae* was termed ‘wolf-in-sheep’s clothing’ because the waxy covering enabled it to avoid being detected by ants tending the aphids. *C. slossonae* appears to be a specialist predator of *P. tessalatus* to the exclusion of other aphid species (Milbrath et al., 1993). Waxy material from the mealybug *Plotococcus eugeniae* Miller is used for cover by the predatory larvae of the chrysopid *C. cincta* (Eisner and Silberglied, 1988). The chrysopid *Chrysopodes collaris* (Schneider) and the syrphid *Ocyptamus parvicornis* Loew also may make use of the waxy material from the mealybug. Because of their use in a variety of camouflage material, these larval chrysopids are known as ‘trash carriers’. Furthermore, studies of *S. flinti* behavior and biology may suggest a function for the production of its waxy particles.

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