

The surface wax composition of the exuviae and adults of *Aleyrodes singularis*

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Abstract

Long-chain aldehydes, alcohols, hydrocarbons and wax esters were major components of the external lipids of adult *Aleyrodes singularis*. In exuviae, acetate esters replaced the hydrocarbons as a major component. The major long-chain alcohol and aldehyde from adults were C32 and were essentially the exclusive components of the wax particles. The major alcohol from exuviae was C26 and the aldehydes were C26, C28, C30 and C32. The major acetate esters were C28 and C30 in both adults and exuviae. There were wax esters of similar carbon number in adults and exuviae although the exuviae had a greater amount of wax esters with unsaturated fatty acids. The fatty acid and alcohol composition of the wax esters differed markedly between adults and exuviae. Wax esters of adults had similar amounts of C16, C18, C20, C22 and C24 fatty acids while those from exuviae contained largely C16 and C18. The major alcohol in the wax esters of adults was C22 and those of exuviae were C26 and C28. The distribution of fatty acids and alcohols among wax esters of varying chain length also differed between adults and exuviae: in adults C22 was the major fatty acid found in the dominant wax ester, C44 and the C22 alcohol was the major alcohol and found in wax esters C42 and C44. In exuviae C16 and C18 were the major fatty acids found in most wax esters and a C28 alcohol was the major alcohol found in wax esters C44 and C46, the two dominant wax esters in exuviae. It was clear that the difference in chemistry of the wax esters between the adults and exuviae is not evident unless the acid and alcohol moieties are characterized. © 1998 Elsevier Science Inc. All rights reserved.

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1. Introduction

Many homopteran insects are characterized by copious amounts of wax on their cuticular surface and often by waxy particles on the surfaces they occupy [2,3,5,6]. Even though the term ‘wax’ is used to describe this material, the composition may not be that of a true wax ester, i.e. an ester of a long-chain alcohol with a long-chain acid. The major lipid components of these external waxes have been characterized as a true wax ester, triacontanyl triacontanoate, on the whitefly *Metaleurodicus griseus* [9], but as waxy particles com-

posed of approximately equal amounts of long-chain alcohols and aldehydes on the greenhouse whitefly, *Trialeurodes vaporariorum*, the sweetpotato whitefly, *Bemisia tabaci* and the silverleaf whitefly, *Bemisia argentifolii* [4,13]. True wax esters also were identified and were shown to be the major components of the cuticular surface lipids of *T. vaporariorum*, *B. tabaci* and *B. argentifolii*, not components of the waxy particles.

Immature stages of whiteflies are frequently the preferred prey for parasitoids and predators. It is conceivable that the latter use cuticular characteristics, such as the nature and composition of waxes, as cues determining host location and suitability. Therefore, information about these materials is of great interest. Indeed, well known agricultural pests such as *T. vaporariorum* and

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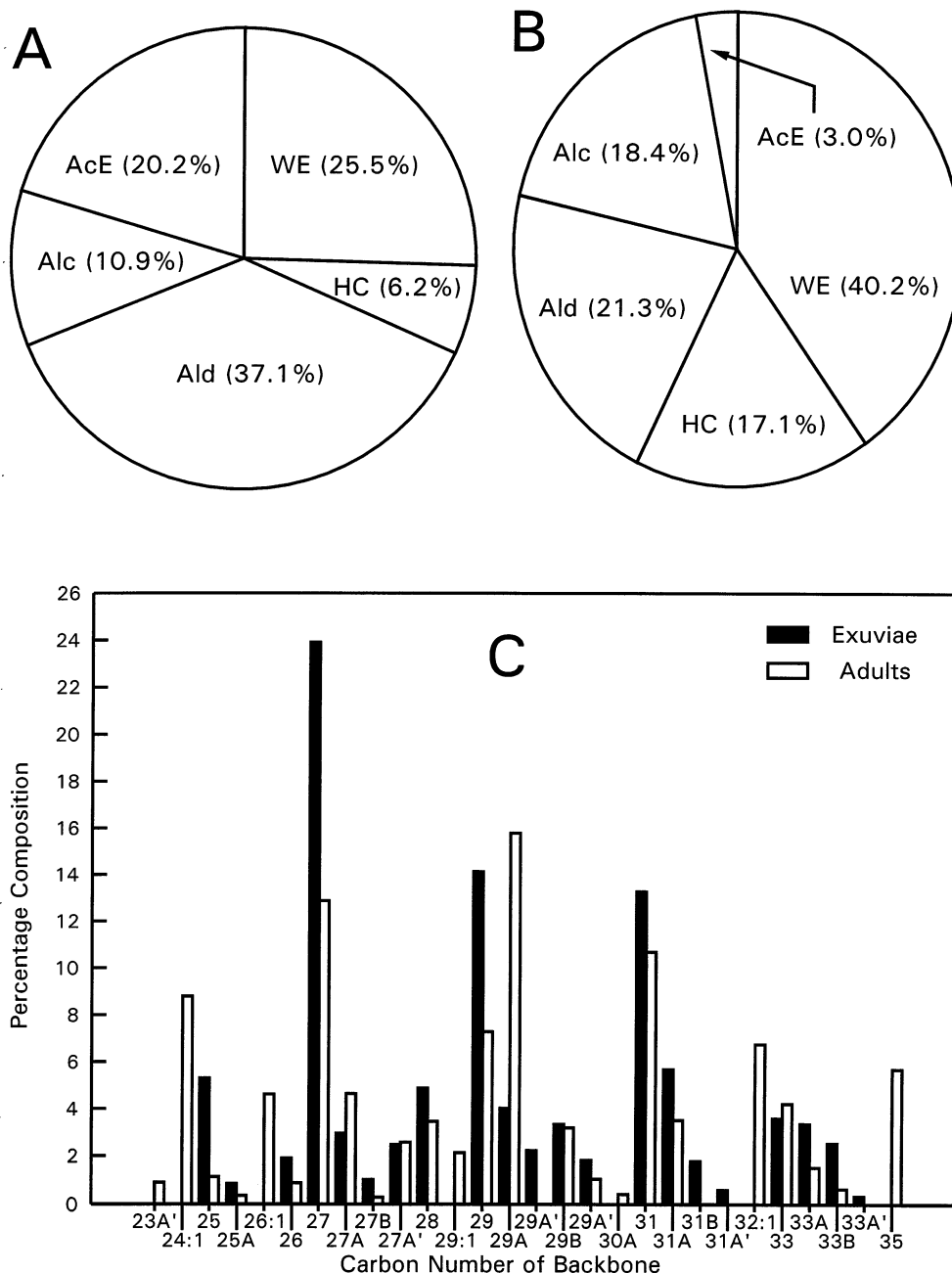


Fig. 2. The percentage distribution of the lipid classes extracted from exuviae (A) and the surface of adults (B) of *A. singularis*—Ald, aldehyde; Alc, alcohol; AcE, acetate ester; HC, hydrocarbon; WE, wax ester; and (C), the percentage distribution of hydrocarbons. The carbon number for the hydrocarbons only indicates the number of carbons in the backbone of the molecule. The letters A and B indicate one and two methyl branches, respectively, on the backbone. The letter, A', indicates one methyl branch near the end of the molecule. If the component eluted before a B component, it is a 2- or 4-methylalkane; if it eluted after a B component, it is a 3-methylalkane. Monoenes are indicated by the carbon number followed by ':1'.

2.2. Extraction procedures

Adults or exuviae (largely pupal exuviae) were placed in a champagne funnel and rinsed with 5–8 ml of hexane for 1.5 min. This removes hydrocarbons and wax esters from adults but does not dissolve all of the waxy particles [4,13]. Also, the hexane rinse did not remove all the hydrocarbons and wax esters from the

exuviae. Therefore, both adults and exuviae were subsequently rinsed with 4–6 ml of chloroform for an additional 30 s and the hexane and chloroform rinses were combined. The vials which had contained the adults were coated with waxy particles from the adults and these particles were removed with CHCl_3 to give a sample that represents the waxy particles produced by adults.

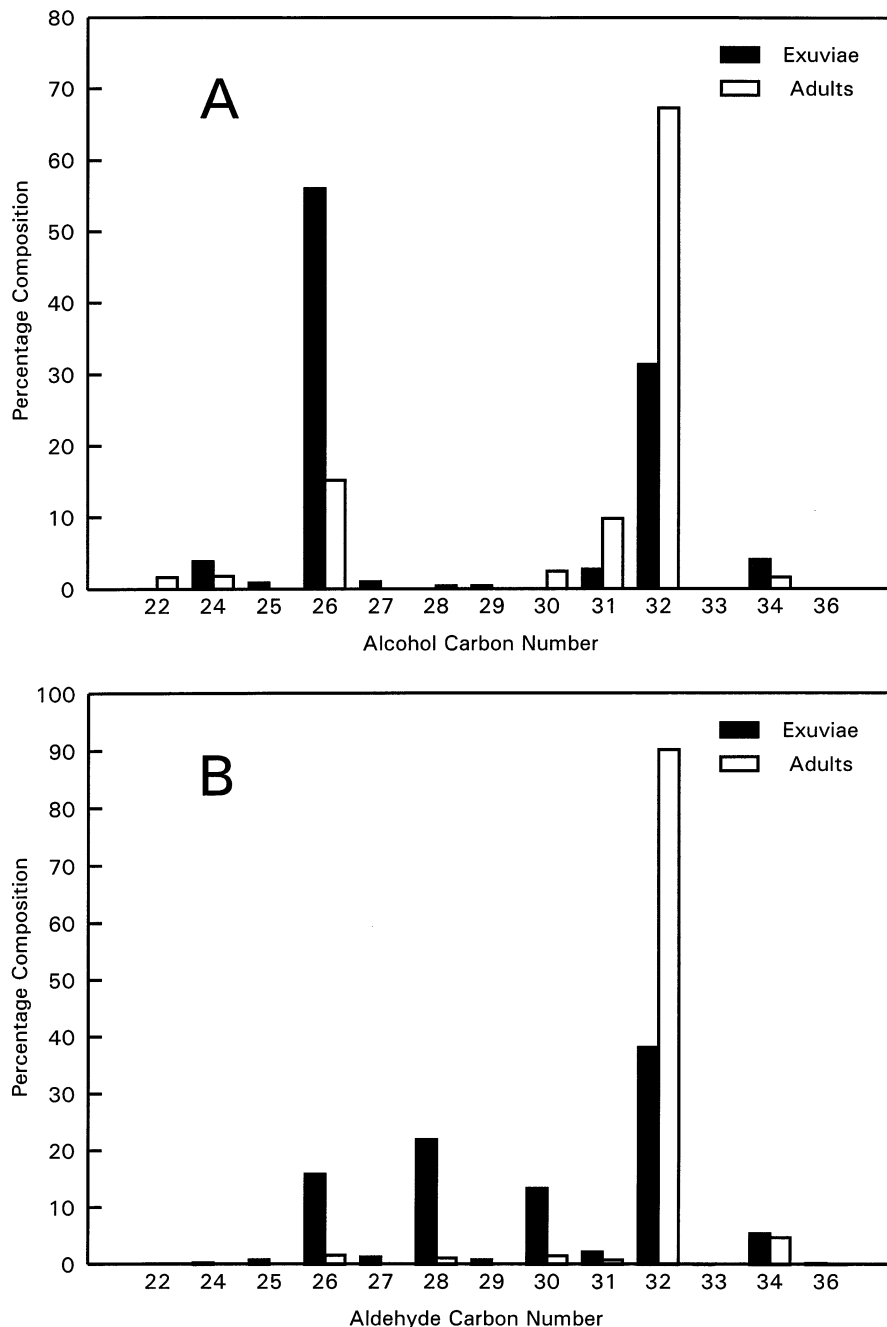


Fig. 3. The percentage composition of the long-chain alcohols (A) and long-chain aldehydes (B) extracted from exuviae and the surface of adults of *A. singularis*. The alcohols were analyzed as their acetate esters and the data corrected for the presence of the natural acetate esters present in these insects. The major alcohol and aldehyde components of the waxy particles produced by adults was C32.

2.3. Chromatography and structural identification

Lipid classes were separated by thin-layer chromatography (TLC). A portion of the 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 wax bands were visualized by charring plates previously sprayed with a solution of 5% concentrated sulfuric acid in 95% ethanol.

Samples were analyzed as previously described [13] by capillary gas chromatography-mass spectrometry (CGC-MS) on a Hewlett-Packard quadrupole system equipped with an autoinjector and a temperature and pressure programmable cool on-column injection port. The injection port was connected to a 1 m retention gap connected to a 12 m \times 0.2 mm capillary column of cross-linked dimethyl silicone Ultra 1. The column was programmed from 150 to 320° at 4° min⁻¹ and then held at 320° for about 200 min. The mass spectra were

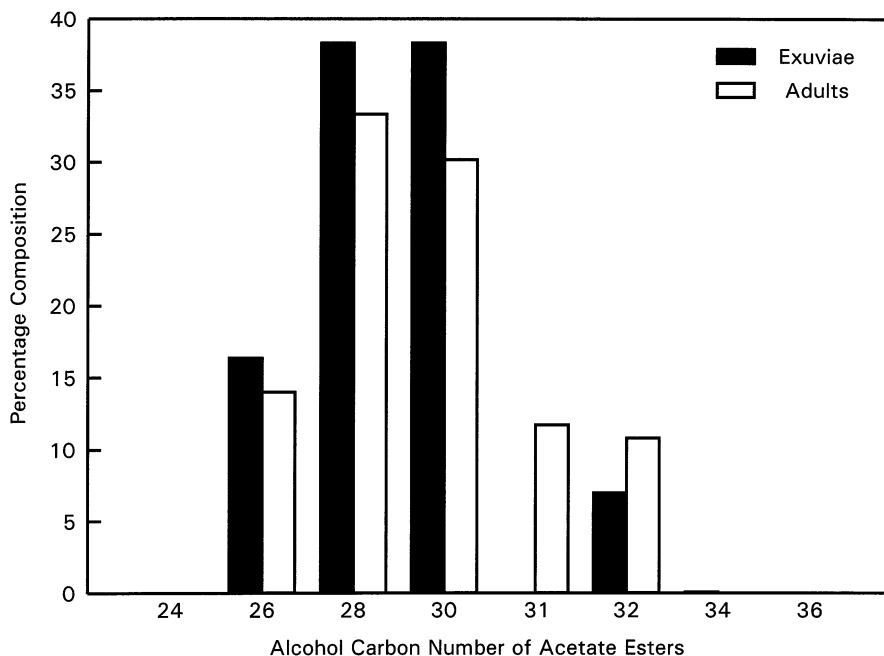


Fig. 4. The percentage composition of the natural acetate esters extracted from exuviae and the surface of adults of *A. singularis*.

interpreted as previously described [3,11,13]. Rapid deterioration of the CGC-MS system performance was not observed during analysis of these samples as had been previously observed with samples from *Trialeurodes* and *Bemisia*.

Routine separation of the total external lipids into lipid classes before analysis by CGC-MS was impractical because of the small quantities of material in the lipid samples and because of the instability of the aldehydes due to autoxidation. As a consequence, an aliquot of the samples was analyzed by CGC-MS as soon after extraction as possible to get the most reliable determination of the aldehyde and *n*-alkane components. An aliquot of the remaining sample was then used to prepare the acetate ester derivatives of the free alcohols and they were then analyzed by CGC-MS.

The total ion current data were analyzed using a computer spreadsheet in which the dose response was adjusted using a three-component standard curve prepared using *n*-alkane standards. The equation component from 0 to 3 ng was linear and the next two equation components were polynomial from 3 to 25 ng and from 25 to 100 ng. For each peak, the formula in the spreadsheet selected the appropriate equation component based on the total ion current of the CGC-MS peak and used it to calculate the mass of the peak in nanograms. The fatty acid moieties and the corresponding fatty alcohol moieties of the wax esters were estimated by measuring the intensity of the characteristic single ion corresponding to each fatty acid. For the saturated fatty acid moiety of a wax ester the characteristic ion formed corresponds to the protonated acid ion,

e.g. 18:0 has a molecular weight of 284 and the protonated acid ion is at *m/z* 285. Thus, cleavage of the ester bond was accompanied by the rearrangement of two hydrogen atoms to form a putative protonated acid ion. For the unsaturated fatty acid moieties of the wax esters the characteristic ion corresponds to the loss of a hydrogen from the fatty acid acyl group: e.g., 16:1 (*m/z* 236), 18:1 (*m/z* 264) and 20:1 (*m/z* 292). Once the fatty acid was known, the amount of the corresponding fatty alcohol moiety for each wax ester was also known. The intensities of the single ions were then expressed as a percentage of all the single ions for each individual wax ester. This percentage was multiplied by the amount of each wax ester determined from the total ion current to give the amount of fatty acid and fatty alcohol present in each wax ester.

3. Results

3.1. CGC-MS chromatography

Thin-layer chromatography of the total lipid extracts from both adults and exuviae showed major bands corresponding to wax esters, aldehydes and/or acetate esters and alcohols. Analysis of the total lipid extract by CGC-MS gave a total ion chromatogram showing that aldehydes, alcohols and naturally occurring acetate esters were present as well as wax esters and small amounts of hydrocarbons (Fig. 1). The major components from adults and exuviae were long-chain aldehydes and alcohols (alcohols usually decompose during

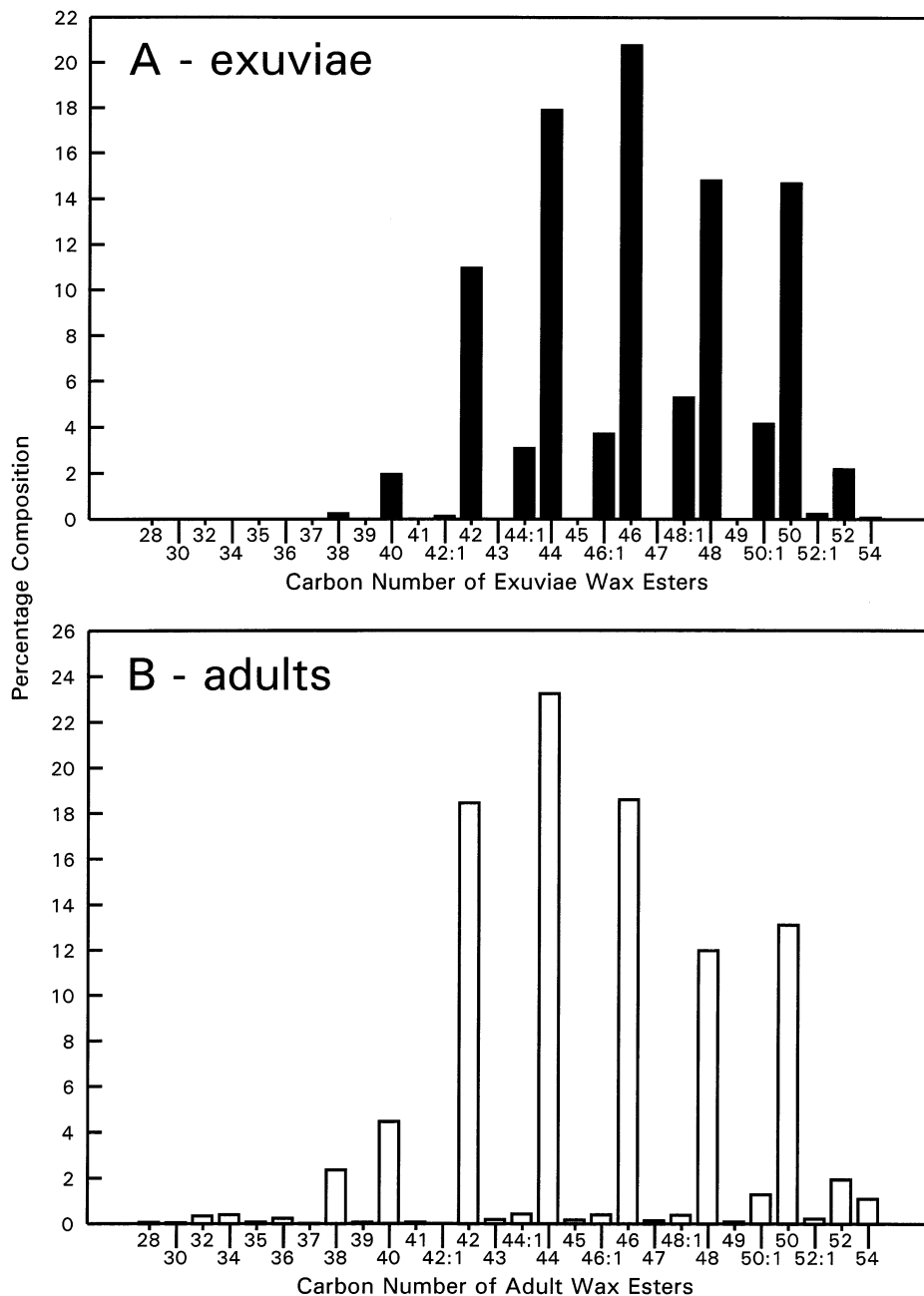


Fig. 5. The percentage composition of the wax esters extracted from exuviae (A) and the surface of adults (B) of *A. singularis*. The numbers on the x-axis indicated the number of carbon atoms of the wax ester. The carbon number followed by '1' indicates those wax esters in which the fatty acid moiety was monounsaturated; almost exclusively an 18:1 fatty acid.

analysis but some are present in the trace) and wax esters (Fig. 2). The free alcohols were subsequently converted to their acetate esters and analyzed by CGC-MS.

Acetate esters were a major lipid component from exuviae but were only a minor component from adults. Hydrocarbons were minor components in exuviae but a notable amount was present in adults (Fig. 2). Exuviae were further characterized by the presence of notable amounts of unsaturated wax esters. The exuviae lipids were estimated at $2453 \text{ ng insect}^{-1}$ and likely contain

lipids from both the inner and outer surfaces of the exuviae. The amount of external lipid on the adults was estimated at $189 \text{ ng insect}^{-1}$. This is a minimal estimate of their total external lipids since much of the waxy particles (aldehydes plus alcohols) produced by the adults are shed onto their surrounding surfaces, including the collection vial. Unsat. wax esters: Hex1165 = $81.06 + \text{CHCl}_3 = 19.51 = = = 100.57 \text{ ng/exuviae}$ for sample A. Hex1189 = $69.39 + \text{CHCl}_3 = 22.10 = = = 91.49 \text{ ng/exuviae}$ for sample B. Average = 96 ng/exuviae OR 15.4% of Total Wax Esters. 22% of unsatWE

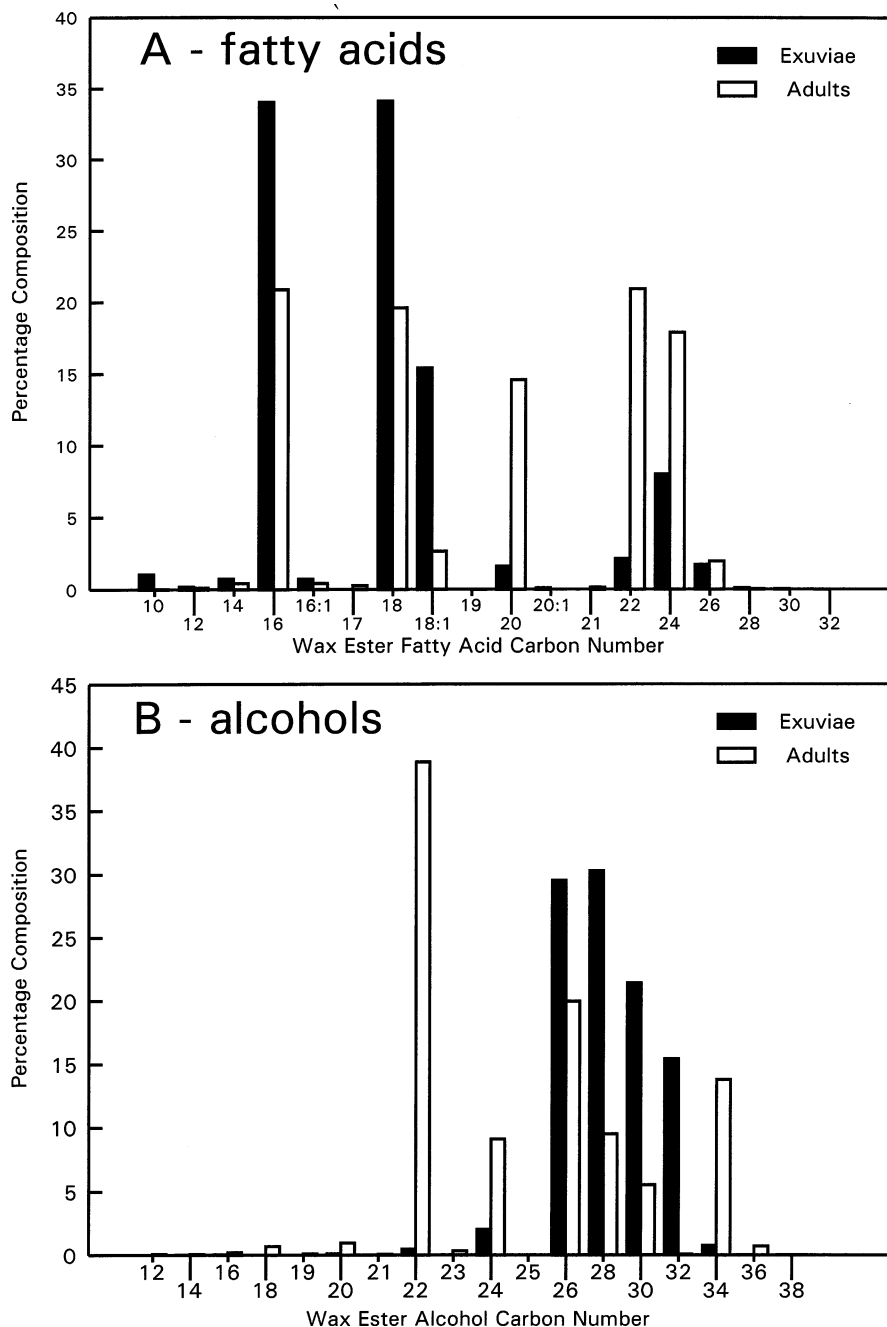


Fig. 6. The percentage composition of the fatty acid moieties (A) and the alcohol moieties (B) of the wax esters extracted from exuviae and the surface of adults of *A. singularis*.

extracted in CHCl_3 . FINAL ADULT VALUE FROM 'as-wsum.wk1' AB90 is 3% of WE's are unsaturated. FINAL EXUVIAE VALUE FROM 'asx-avgwe.wk1' G138 is 17% of WE's are unsaturated.

The individual components of each of the lipid classes were determined by CGC-MS and identified from their mass spectra. The chain lengths of the hydrocarbons, alcohols, aldehydes and acetate esters were similar. The percentage distribution of the hydrocarbon components shows that the *n*-alkanes (exuviae

67%; adults 46%) are the major hydrocarbon components. The chain length of the hydrocarbons ranged from 23A (23 carbon backbone with one methyl branch) to 35 (Fig. 2C). The adults were unique in that 22% of their hydrocarbons were *n*-alkenes; no alkenes were detected in exuviae. The A series of methyl-branched alkanes was a mixture of isomers with the methyl branch on C3, 11, 9 or sometimes 7. The A' series had the methyl branch on C3 except for the early eluting 29A' in adults where it was uncertain whether

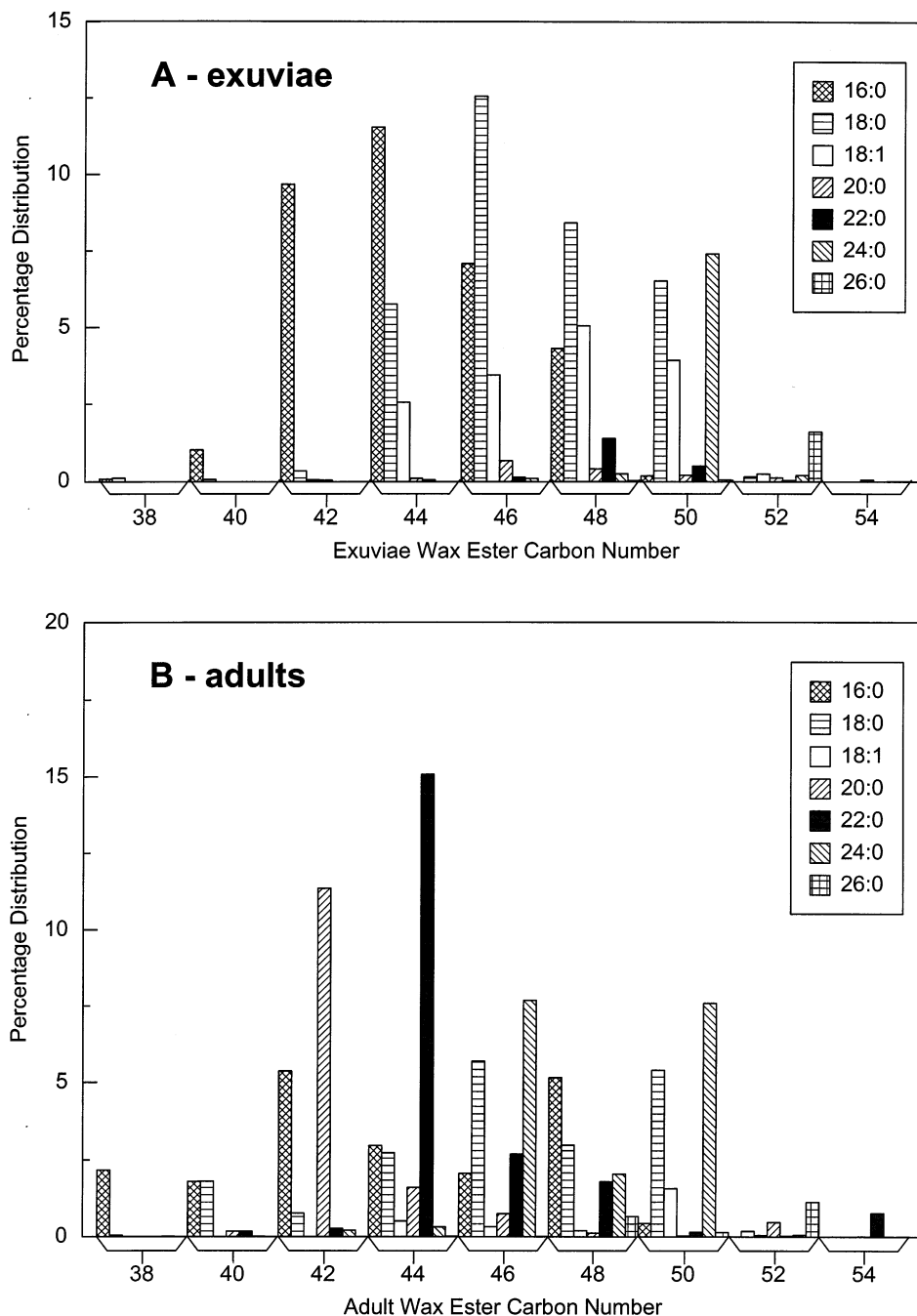


Fig. 7. The distribution of the fatty acid moieties in the wax esters from exuviae (A) and the surface of adults (B) of *A. singularis*. The seven fatty acids graphed represent 97% of the fatty acids found in the wax esters of the exuviae and 98% of those found in the wax esters of the adults.

the methyl branch was on C2 or 4. The B series had two methyl branches, usually on positions 9 and 13 and/or positions 11 and 15, except for 33B which was identified as 13,17-dimethyltrtriacontane.

Adult whiteflies produce waxy particles by scraping off the filaments with their hind legs as they are extruded from their wax glands. These particles cover the adults as well as the surfaces on which they are found. In other whiteflies, these waxy particles have been shown to consist of a mixture of a long-chain alcohol and a

long-chain aldehyde of 30, 32, or 34 carbons. In the case of *A. singularis*, the adults tend their nymphal and pupal stages by periodically coating them with waxy particles [7] and this would result in some contribution of lipid to the total surface lipid of the immatures. Waxy particles were obtained in two ways: (1) by brushing from the surface of immatures; and (2) from the surface of vials in which adults had been stored. CGC-MS analysis showed that particles from both sources consisted mainly of a mixture of 32-carbon alcohol and aldehyde.

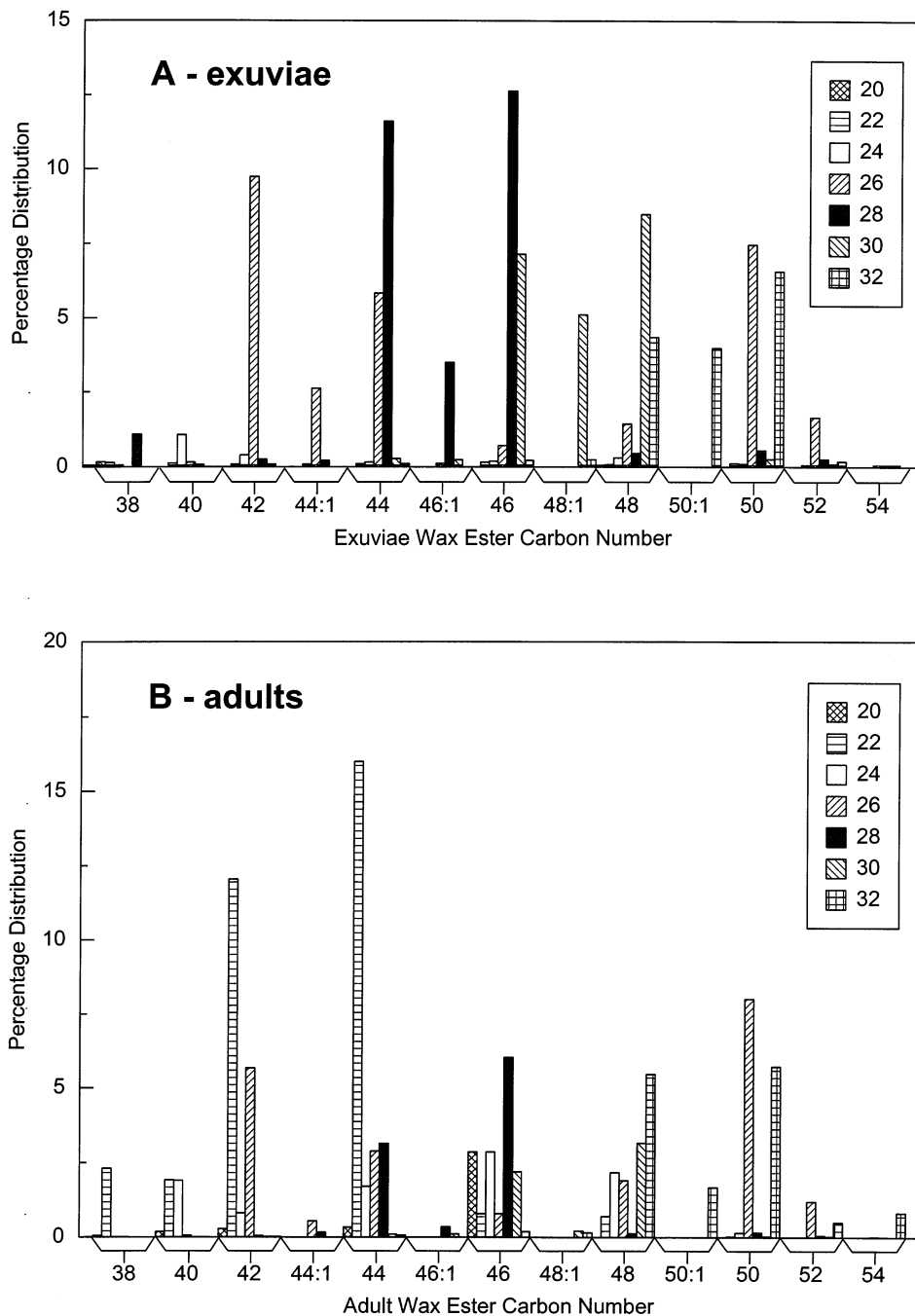


Fig. 8. The distribution of the alcohol moieties in the wax esters from exuviae (A) and the surface of adults (B) of *A. singularis*. The seven alcohols graphed represent 99% of the alcohols found in Adults: wfd-sum.wk1 @ F246 = 98%. Exuviae: asxavgfa.wk1 @ F181 = 97%. adult wax esters.

CGC-MS analysis of the total surface lipids of adults showed that both the major alcohol and aldehyde had 32 carbons (Fig. 3A and B). However, the major long-chain alcohol from exuviae was 26 carbons and the second most abundant component from exuviae was 32 carbons (Fig. 3A). The exuvial long-chain alcohols ranged in chain length from 24 to 34 carbons. Some or much of the C32 alcohol of the exuviae may be from contamination by adult waxy particles.

The major aldehyde from exuviae was 32 carbons with lesser amounts of C26, C28 and C30 (Fig. 3B). As

with the C32 alcohol, the C32 aldehyde was likely due to contamination by adult waxy particles. These results are reflective of the fact that in the adults, alcohols and aldehydes are components of the waxy particles but are not present to a significant extent in the cuticular surface lipids. However, the exuviae do have a complement of aldehydes in their surface lipids from 24 to 36 carbons. The profile indicates that the major component in exuviae may be C28, assuming the intensity of the C32 aldehyde is largely due to waxy particles deposited by the adults on the exuviae.

The surface lipids of both exuviae and adults contained acetate esters of the long-chain alcohols. The composition was similar for both exuviae and adults with acetate esters of the C28 and C30 alcohols being the major components (Fig. 4). However, whereas the acetate esters were a major component (20%; Fig. 2A) of the lipids of exuviae, they were only a minor component (3%; Fig. 2B) of the surface lipids of the adults.

The wax esters were the second most abundant class of lipids of the exuviae (26%) and the largest lipid class of the adults (40%) (Fig. 2). Exuviae: 78% of wax was found in hexane & 22% in subsequent CHCl₃. CGC-MS analysis detected wax esters from C38 to C54 in exuviae (Fig. 5A) and from C28 to C54 in adults (Fig. 5B). C44 was the major wax ester of adults and C46 was slightly greater than C44 in exuviae. Unsaturated wax esters also were present, 17 and 3% of the wax esters of exuviae and adults, respectively.

The fatty acid composition of the wax esters consisted largely of even-numbered acids from C10 to C32 (Fig. 6A). The major fatty acids of the wax esters of exuviae were C16 and C18 while those of adults consisted largely of even-numbered fatty acids of similar amounts from C16 to C24. However, unsaturated fatty acids, largely 18:1, made up about 17% of exuviae wax ester fatty acids; there were minor amounts of 16:1 and 20:1 detected.

The distribution of fatty acids among wax esters of varying chain length differed markedly between exuviae and adults. In exuviae, C16 was the major fatty acid moiety in wax esters C42 and C44. This was replaced by C18 fatty acid in wax esters C46 and C48 and by C24 fatty acid in wax ester C50 (Fig. 7A). However, in adults the major fatty acid moieties were C20 (78% of wax ester fatty acids) in wax ester C42, C22 (72% of wax ester fatty acids) in wax ester C44 and C24 in wax ester C46 (Fig. 7B).

The alcohol composition of the wax esters consisted largely of even-numbered *n*-alcohols from C26 to C32 for exuviae and from C22 to C34 for adults (Fig. 6B). The major alcohol components for exuviae were C26 (30%) and C28 (31%) whereas the major component for adults was C22 (39%).

The distribution of the alcohol moieties in the exuviae wax esters showed C26 was the major alcohol moiety in wax esters C42 and C50, C28 in wax esters C44 and C46 and C30 in wax ester C48 (Fig. 8A). The adult wax esters showed a markedly different distribution in which C22 was the major alcohol in wax esters C42 and C44, C28 in wax ester C46 and C32 in wax ester C48 (Fig. 8B). Interestingly, the composition of wax ester C50 was similar in exuviae and adults: C26 alcohol was the major component of wax ester C50 with a lesser amount of the C32 alcohol.

4. Discussion

The percentage composition of the lipid classes must be viewed in light of the fact that the amount of waxy particles are affected by the age and activity of the adult, bouncing of the adult during collection and bouncing of dead adults in the collection vials. An active adult and bouncing during collection would result in the dislodging and loss of waxy particles. The consequence is that the values for the aldehydes and alcohols due to the waxy particles must be considered as minimum values; this in turn would cause the values for the percentage composition of the other lipid classes to be increased.

Hydrocarbons are common components of insect surface lipids and frequently are a major component [1,12]. Methyl-branched alkanes are usually the major hydrocarbon components and may function as semiochemicals and also often are useful as taxonomic indicators [8,11]. Whiteflies appear to be somewhat unique in that hydrocarbons are relatively minor components of the surface lipids of adults of *T. vaporariorum*, *B. tabaci* and *B. argentifolii* and that methyl-branched alkanes are minor components of the hydrocarbons (Nelson and Buckner, unpublished). However, in the surface lipids of *A. singularis*, hydrocarbons represented 17% of adult and 6% of exuviae lipids. The lipids of the adults differed in several ways compared to those of the exuviae; they had a relatively high content of hydrocarbons, the major hydrocarbon component was 29A, a mixture of internally branched isomers of monomethyl-nonacosane and alkenes were present. No alkenes were detected in exuviae lipids and the major hydrocarbon component was the *n*-alkane, heptacosane.

The major aldehyde and alcohol of adults was C32 and represents the composition of the waxy particles produced by the adults and with which they cover themselves as well as the immature stages. The exuviae of the immature stages have a distribution of aldehydes from C24 to C36 and a distribution of alcohols from C24 to C34. We estimated that the major aldehyde was C28, assuming that the majority of the C32 found on the exuviae was from waxy particles produced by the adults and deposited on the exuviae. The major alcohol of the adults was C32 while those of exuviae were C26. Much of the C32 alcohol on the exuviae was probably due to waxy particles produced by the adults. Thus, the exuviae and the adults each have their unique composition of aldehydes and alcohols. The waxy particles of adult *T. vaporariorum* also were mostly C32 aldehyde and alcohol, while those of *B. tabaci* and *B. argentifolii* were C34 [4,13].

A. singularis is a whitefly that infests the weed, *Lactuca serriola*, belonging to the Compositae family.

This weed grows mainly on fallow fields and along road-sides. The adults of *A. singularis* had 3% of their external lipids as acetate esters but in the exuviae, 20% of the lipids were acetate esters. In both adults and exuviae, the chain length of the acetate esters ranged from C26 to C34 and the major components were C28 and C30. Acetate esters were not found in the surface lipids of adult *T. vaporariorum*, *B. tabaci* and *B. argentifolii* [4,13]. They also were not found in pupal exuviae of *B. tabaci* and *B. argentifolii*, but C26, C28 and C30 acetate esters were present in pupal exuviae of *T. vaporariorum* [10].

A major lipid class was the wax esters (WE) comprising 26% of exuviae and 40% of adult cuticular lipids. The exuviae were notable in that they contained a higher proportion (17%) of wax esters containing an unsaturated fatty acid than did the adults (3%). The unsaturated fatty acid was almost exclusively 18:1. Although the percentage composition of the wax esters was similar in adults and exuviae, the compositions of the fatty acid and alcohol moieties of the individual wax esters were markedly different between the adults and exuviae.

The major fatty acid in adult WE42 was C20 but in exuviae it was almost exclusively C16. In WE44 and WE46, the major fatty acids in adults were C22 and C24, respectively and in exuviae they were C16 and C18, respectively. The other wax esters also showed marked differences in the distribution of the fatty acid moieties between adults and exuviae.

As was anticipated from the marked differences in fatty acid distribution, there also were notable differences in the distribution of the alcohol moieties. Differences were most evident in WE42 and WE44 where C22 was the major alcohol in adults while in exuviae, WE42 contained almost exclusively C26 and WE44 contained mostly C28 and a lesser amount of C26.

It is apparent that there are notable differences between the surface chemistry of the adults and the exuviae even though on a gross level the lipid classes are very similar. This chemical signature may play a role in the interactions of parasites and predators with the different stages of whiteflies. It may also influence the particular whitefly species that a given parasite or

predator will attack. Our current research is directed toward determining the possible role of surface lipid components in the selection of whiteflies by predators.

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