Function and composition of cuticular hydrocarbons of stored-product insects

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Abstract

The main function of surface lipids is to minimise the loss of body water through the cuticle. For stored-product insects, living in extremely dry products and a desiccating environment, preservation of water supplies is crucial. The compounds commonly found in cuticular lipids belong to following classes: n-alkanes, n-alkanes 2- and 3-methylalkanes, primary and secondary alcohols, free fatty acids, and sterols. This paper presents a comparative analysis of chemical composition of hydrocarbons and their roles in stored-product insect life, and reviews the literature on this topic.

Introduction

The original habitats of stored-product insects were rodent burrows full of grain and seeds. When humans built store houses, the insects moved and modified their physiology to mild temperature and low humidity. This was achieved by appropriate adoption of cuticular lipids. These compounds (free and bounded) play an important role in insect physiology. The main function of free lipids is the reduction of water transpiration and the protect of insects from desiccation (Hadley 1981). Furthermore, lipid layers protect insects against infection from microorganisms and the absorption of insecticides (Blomquist et al. 1987). Some of the cuticular components are also involved in chemical communication between species; pheromones for aggregation, sex attraction, short-range mating stimulation and kairomones. They may act also as species and caste recognition cues.

The function of the cuticular waxes of stored-product insects prompted us to study their composition and to compare those in various species living in similar conditions. Recent developments in structural studies are presented in this paper as well.

Materials and Methods

Insects from laboratory breedings were extracted with methylene chloride. For outer lipids, adults or larvae were dipped in the solvent for 30 seconds and for internal waxes they were transferred to a new pool of solvent and left for 2 weeks. The solvent was removed under reduced pressure. The isolation of organic compound groups (group analysis) was performed by liquid-solid HPLC with gradient elution from eluent A-n-hexane to 100% of eluent B-diisopropyl ether. A light-scattering instrument was applied for detecting the fractions. A column (30 cm × 3 mm) packed with silica gel was used for separation (Separon SGX, 7 μm, Tessec Ltd, Praha, Czech Republic). Gas chromatography (GC) analyses were performed with a Varian 1400 gas chromatograph using 30 m × 0.23 mm fused silica capillary column with DB-1 liquid phase. The oven temperature was programmed from 150°C to 300°C at 2°C/minute. Argon was used as a carrier gas. Kovats' retention indexes were measured at temperatures adequate for k' values of the signals greater than 5 or with temperature programming. The error was ±1.0 unit. Field desorption fingerprinting of the fractions were performed with Varian MAT 711 mass spectrometer equipped with a combined FD-FI-EI ion source. GC/MS measurements were carried out with a VG Micromass 7070E (Dani 3800 GC) or VG Micromass 16F (Pye Unicam GC) mass spectrometer.

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Results and Discussion

Composition of free cuticular lipids of stored-product insects varies from species to species, being a chemical tool for taxonomy. Nevertheless, the following classes of chemical compounds can be found in cuticular lipids: alkanes, isoalkanes, alkenes, esters, glycerides, aldehydes, ketones, fatty acids and sterols. These complicated mixtures require a proper strategy for structural study. The first step of qualitative and quantitative analysis relies on suitable separation of the mixture into groups of the above-mentioned compounds.

The separation can be done by liquid-solid chromatography with gradient elution. Unfortunately, the compounds do not absorb in UV range and UV spectrophotometric detector cannot be applied. The refractive index detector, in turn, does not accept gradient elution. The only solution is the application of a mass detector with laser beam scattering—a selection which has been successfully used in different plant lipids analyses (Stolyhwo et al. 1985). Our separation of the cuticular lipids of *Tribolium confusum* is presented in Figure 1. All important classes of compounds represented in cuticular lipids are separated: (1) alkanes, (2) alkenes and alkadienes, (3) ester waxes, (4) triacylglycerols, (5) fatty acids and (6) sterols. HPLC analysis with gradient elution provides a method for fast screening of the classes of compounds in relation to habitat conditions. The collection of the fractions provides the mixtures of compounds for gas chromatography and mass spectrometry analyses.

The strategy of the structural study of the cuticular lipids separated into classes consisted of the following stages:

- field desorption mass spectrometric fingerprinting of the samples for molecular ions of the components;
- gas chromatographic analyses with Kovats' retention indexes measurement;
- recording of mass spectrum of GC separated single component (GC/MS);
- correlation of the structures with the results of biosynthesis e.g. of isoalkanes.

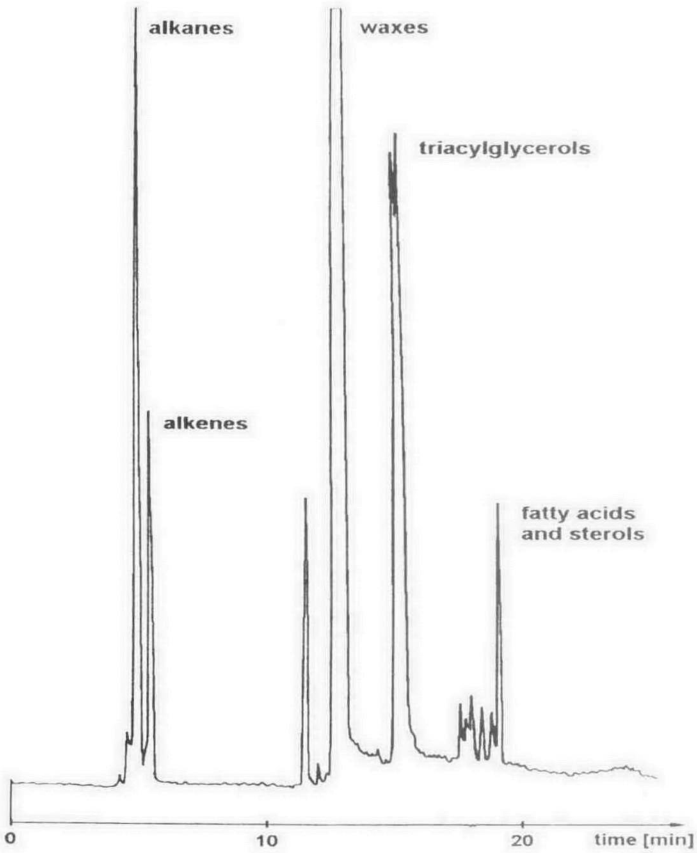

Fig. 1. HPLC group separation of cuticular lipids of *Tribolium confusum*. Gradient elution n-hexane-di-isopropylether, laser light scattering detector, silica gel column, 5μm.

The last step must be taken with precaution, because unusual positions of methyl branches are even found in nature (Gries et al. 1993).

Labelled substrate experiments showed (Chu and Blomquist 1980) that insects synthesise most of their hydrocarbons. Composition of these compounds is therefore an expression of genotype and as such is a taxonomic feature. The question arising is to what extent can habitat conditions modify the quantity and quality of cuticular waxes. For example, a dry season increases the wax blooms of the beetles belonging to the family Tenebrionidae, and the secretion stops when the relative humidity rises (Hadley 1979). The habitat of the species studied is warehouses with low humidity. Our results and literature data on cuticular hydrocarbons of stored-product pests are compiled in Tables 1–7. The insects studied belong (except one) to order Coleoptera (beetles). Most of them (eight species) represent Tenebrionidae, three species Curculionidae, two Dermestidae and Bruchidae, one Anobiidae, Bostrychidae and Cucujidae. One species belongs to the order Lepidoptera, family Pyralidae.

There is a great diversity of compounds found in cuticular waxes. Only n-alkanes occurred in all examined species, though in variable proportion. They are stated as continuous homologous series from nCl6 to nC35, and compounds of nC23, nC25, nC27 and nC29 are usually predominant and most abundant. Larvae of *Attagenus megatoma* and *Lasioderma serricorne* possess the most amount of n-alkanes (98 and 83% respectively). On the contrary, adults of *Lasioderma serricorne*, *Callosobruchus maculatus*, *Alphitobius bifasciatus* and *Acanthoscelides obtectus* have the least amounts of this fraction. Presence of monomethylalkanes (terminally and internally branched), dimethyl- and trimethylalkanes cause higher melting point of the lipid mixture. However, composition of these compounds varies among species, and larvae of *Attagenus megatoma* do not possess them at all (Tables 2, 3, 4 and 5).

n-Alkenes and n-alkadienes were stated only in a few species (Tables 6 and 7). Lockey (1976) and Jackson and Blomquist (1976) investigated the relationship between the hydrocarbon composition and the taxonomy of insects. Those early attempts concluded that most insect species had n-alkanes, monomethylalkanes and dimethylalkanes. But this finding did not build a solid base for taxonomy. The most pronounced results were obtained in chemotaxonomy of Tenebrionidae family (Crowson 1981). According to literature data (Lockey 1988), hydrocarbon composition is related to taxonomic grouping in such a way that closely related species, such as congeneric, tend to have qualitatively similar hydrocarbon mixtures but with sometimes different proportions. More distantly related species tend to have hydrocarbon mixtures which differ qualitatively and quantitatively. Among the species presented in Tables 1–7, *Tribolium castaneum* and *Tribolium confusum* have very similar hydrocarbon compositions (see Lockey 1988).

More or less similary qualitative and quantitative compositions were found in the following groups of species:

- Qualitative and quantitative great similarity
  Tribolium confusum T. castaneum
  Sitophilus oryzae S. zeamais
- Qualitative similarity
  Sitophilus oryzae S. zeamais S. granarius
  Tribolium confusum T. castaneum T. destructor
  Tenebrio molitor T. obscurus
  Acanthoscelides obtectus Callosobruchus maculatus
- Some similarity
  T. confusum T. castaneum T. destructor Blaps mucronata
  Anagasta kuehniella (larvae) *Lasioderma serricorne* (larvae) *Attagenus megatoma* (larvae)
- No similarity
  Rhyzopertha dominica Oryzaephilus surinamensis

A new approach in chemical ecology is the search for the roles of plant and insect cuticular lipids in insect- plant interactions. The behaviour of many herbivorous insects is affected by plant cuticular waxes and these compounds may play the role of attractants, stimulators of feeding or oviposition, and food deterrents. The main components of lipids on wheat grain surfaces are n-alkanes in the following proportions: n-pentacosane (C25), 3.6%; n-hexacosane (C26), 0.8%; n-heptacosane (C27), 23.8%; n-octacosane (C28), 3.9%; n-nonacosane (C29), 42.3%; n-triacontane (C30), 1.2%; n-hentriacontane (C31), 23.8%; n-tritriacontane (C33), 0.6%. It is worth emphasising that there are no other hydrocarbons on wheat grain. If we compare the composition of n-alkanes from grain and from grain feeding insects we can see the high correlation in the proportion of main components. Because grain cuticular lipids may play a major role in the main life processes of insects, e.g. finding food, oviposition and rate of population growth, it is very important to establish their composition and biological properties.

Table 1. n-alkanes in cuticular lipids of stored-product insects (% of total hydrocarbons).

Species	Number of carbon atoms																				Total amount [%]	References
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
Tribolium destructor										1.5	2.5	23.2	7.4	7.0		-					41.6	14,15
Tribolium destructor (larvae)										6.8	6.7	32.5	6.7	6.6		0.4					59.7	15
Tribolium castaneum										3.5	1.2	22.8	6.2	26.1	1.0	2,3	<0.1	<0.1			63.1	19
Tribolium confusum										0.6	0.2	23.3	0.5	26.1	1,1	0.5					61.7	19
Tenebrio molitor			<0.1	<0.1	0.2	<0.1	0.1	11.6	1.2	29.1	0.8	9.7	0.2	0.3							53.2	18
Tenebrio obscurus			<0.1	<0.1	<0.1	<0.1	<0.1	8.8	1.5	27.5	0.9	5.7	0.9	4.7	0.2						50.2	18
Blaps mucronata								1.6	1.5	15.4	4.2	14.1	2.6	1.7							41.1	21
Alphitophagus bifasciatus										0.3	0.8	8.6	3.1	8.5	1.7	0.4					23.4	20
Alphitobius diaperinus										2.0	0.6	15.4	5.2	11.6							34.8	20
Trogoderma granarium								2.2	1.1	5.2	1.5	9.8	1.4	9.7	0.5	2.5	0.5	0.5			34.9	9
Trogoderma granarium (larvae)												13.3	0.7	7.7		2.9		1.4			26.0	22
Attagenus megatoma (larvae)										1.6	0.5	20.6	0.6	16.7	0.5	21.1	0,9	32.1		4.7	98.3	1
Callobruchus maculatus												5.2	3.3	7.8		0.4					16.7	2
Acanthosceli-des obtectus										2.4	1.1	17.0	1.7	6.2		1.1					28.5	8
Lasioderma serricorne										0.2	0.2	3.7	0.6	1.0	0.2	1.6	0.3	0.9			8.7	3
Lasioderma serricorne (larvae)										0.3	0.3	2.5	12.2	8.9	2.1	11.1	2.5	37.2	1.0	5.4	83.5	3
Sitophilus oryzae								0.1		0.1		3.5	0.6	16.2	0.3	3.7		0.4			24.9	4
Sitophilus zeamais										0.1		3.6	0.7	17.0	0.3	2.8		0.4			24.9	4
Sitophilus granarius								0.3	0.1	2.9	0.3	15.3	0.3	15.3	0.3	3.5	0.2	0.5			39.7	4
Rhizopertha dominica												6.5	1.0	10.6	0.4	8.5		2.2		7.5	36.7	25
Oryzaephilus surinamensis	0.9	0.4	0.6	0.5	3.3	0.6	3.6	0.1	0.8	7.3	1.9	1.9	11.6	1.1	11.7	0.2	0.3				45.8	26
Anagasta kueniella (larvae)	1.4	1.3	1.5	0.8	1.0	1.2	1.4	12.6	2.5	25.2	3.4	11.6	2.6	8.5	1.2	1.9					78.1	13

Table 2. Composition of monomethyl-terminally branched alkanes in cuticular lipids of stored-product insects (% of total hydrocarbons).

Species	Number of carbon atoms												Total amount [%]	References
	24	25	26	27	28	29	30	31	32	33	34	35		
Tribolium destructor			0.4		20.3								20.7	14,15
Tribolium destructor (larvae)			0.5		4.2		0.1						4.8	15
Tribolium castaneum			1.5	0.4	7.9	0.3	2.7		0.1				12.9	19
Tribolium confusum			<0.1	0.1	7.6	0.4	2.9		<0.1				11.0	19
Tenebrio molitor			0.7		<0.1								0.7	18
Tenebrio obscurus			7.0		2.9								9.9	18
Blaps mucronata	0.8	0.3	9.7	2.3	13.8	0.5	1.3	0.2		0.9			29.8	21
Alphitophagus bifasciatus			0.3		16.6	1.6	5.3					0.2	24.0	20
Alphitobius diaperinus			0.4	1.1	8.7	1.3b 0.7	16.3	2.2c	0.3				30.0	20
Trogoderma granarium			2.2										2.2	9
Trogoderma granarium (larvae)					6.1		0.8						6.9	22
Attagenus megatoma (larvae)													0.0	4
Callosobruchus maculatus					7.3	1.7	33.0		3.5				45.5	2
Acanthoscelides obtectus			4.3		13.7								18.0	8
Lasioderma serricone			0.1	0.1	2.9	2.2	7.8						13.1	3
Lasioderma serricone (larvae)					1.8	0.1	0.9	0.1					2.9	3
Sitophilus oryzae							<0.1		<0.1				0.0	4
Sitophilus zeamais							<0.1		<0.1				0.0	4
Sitophilus granarius							0.3		0.2				0.5	4
Rhizoperta dominica					1.2								1.2	25
Oryzaephilus surinamensis													0.0	26
Anagasta kueniella (larvae)	0.5	0.5a	1.9		1.3								4.2	13

a/ 2-methyltetracosane b/ 2-methyloctacosane c/ 2-methyltriacontane

Table3. Composition of monomethyl-internally branched alkanes in cuticular lipids of stored-product insects (% of total hydrocarbons).

Species	Number of carbon atoms																									Total amount [%]	References
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46		
Tribolium destructor					0.7	1.2	13.0	5.2																		20.1	14,15
Tribolium destructor (larvae)					0.8	0.7	4.8	1.3	0.3																	7.9	15
Tribolium castaneum						0.6	14.4	2.2	0.2	0.1	0.2															17.7	19
Tribolium confusum						0.4	11.3	2.4	6.6	0.2	0.1															21.0	19
Tenebrio molitor	0.1	0.1	2.5	0.1	0.5		<0.1	<0.1	11.7	0.2	4.7	0.3	2.4													22.5	18
Tenebrio obscurus	<0.1	<0.1	2.1	0.1	0.5		0.9	0.7	1.4	0.7	2.4	0.2	1.0													10.0	18
Blaps mucronata			0.1	0.3	2.2	1.3	2.3	0.5	0.7	0.3	1.0	0.4	1.0	0.5	0.1											10.7	21
Alphitophagus bifasciatus						0.3	1.7	2.8	8.0	1.4	1.5	0.3	0.5	<0.1	<0.1											16.5	20
Alphitobius diaperinus					0.1	0.1	1.6	2.2	2.4	1.3	0.2	0.2	0.1	<0.1		1.0		1.1								10.3	20
Trogoderma granarium					4.0	1.8	32.9	2.8	9.1	1.6																52.2	9
Trogoderma granarium (larvae)						0.7	22.5	1.0	8.6	0.8	1.7		1.3													36.0	22
Attagenus megatoma (larvae)																										0.0	1
Callobruchus maculatus							3.2	1.6	17.3	1.9	1.4		0.2								0.4	0.1	1.0		0.5	27.6	2
Acanthosceli- des obtectus					1.4		11.8		6.0																	19.2	8
Lasioderma serricone						0.3	6.4	11.1	21.3	5.0	1.1	0.1			0.1											45.4	3
Lasioderma serricone (larvae)							1.7	1.1	3.9	0.4	0.5															7.6	3
Sitophilus oryzae											<0.1		0.1													0.1	4
Sitophilus zeamais											<0.1		0.1													0.1	4
Sitophilus granarius							0.3		0.4		0.5		0.2													1.4	4
Rhizoperta dominica							1.3				1.2		6.5		6.5											15.5	25
Oryzaephilus surinamensis							1.8						0.5		1.0				0.3							3.6	26
Anagasta kueniella (larvae)					0.5																					0.5	13

Table 4. Composition of dimethylalkanes in cuticular lipids of stored-product insects (% of total hydrocarbons).

Species	Number of carbon atoms																			Total amount [%]	References
	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45		
Tribolium destructor	1.3		13.5	1.3																16.1	14,15
Tribolium destructor (larvae)	0.8		1.6	0.4																2.8	15
Tribolium castaneum			2.5	0.5	0.7															3.7	19
Tribolium confusum			4.1	1.1	0.7															6.5	19
Tenebrio molitor	0.3	0.1	<0.1		1.7	0.9	1.4	0.1	3.2		0.1									7.8	18
Tenebrio obscurus	0.3	0.1		2.2	0.8	4.9	0.7	0.9	0.9		<0.1		<0.1	<0.1	<0.1					10.8	18
Blaps mucronata							4.7		3.9	1.4	4.4									14.4	21
Alphitophagus bifasciatus					2.2	1.3	1.7	0.7	0.7		0.3		0.6							7.5	20
Alphitobius diaperinus			1.8		3.5		0.9	0.5	0.8	0.5	5.3	2.1	3.1	1.4	3.7					23.6	20
Trogoderma granarium																				0.0	9
Trogoderma granarium (larvae)																				0.0	22
Attagenus megatoma (larvae)																				0.0	4
Callosobruchus macul.			2.2	1.0	28.5	1.4	2.7		0.5								1.5	0.1	2.2	40.1	2
Acanthoscelides obtectus			24.1	2.5	3.4															30.0	8
Lasioderma serricone			4.7	4.2	8.8	10.5	3.4	0.3	0.1											32.0	3
Lasioderma serricone (larvae)				0.1	1.8	0.2			1.1		0.9									4.1	3
Sitophilus oryzae									0.1		0.3									0.4	4
Sitophilus zeamais									0.2		0.3									0.5	4
Sitophilus granarius									0.5		0.2									0.7	4
Rhizoperta dominica							3.1		12.3		4.0									19.4	25
Oryzaephilus surinamensis											0.5		0.3		0.7					1.6	26
Anagasta kueniella (larvae)	1.7		0.7																	2.4	18

Table 5. Composition of trimethylalkanes in cuticular waxes of stored-product insects (% of total hydrocarbons).

Species	Number of carbon atoms						Total amount (%)	References
	31	32	33	34	35	36		
Acanthoscelides obtectus	2.5						2.5	8
Rhyzopertha dominica		0.4		2.6		5.7	8.7	27

Table 6. Composition of n-alkenes in cuticular waxes of stored-product insects (% of total hydrocarbons)

Species	Number of carbon atoms																							Total amount [%]	References
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45		
Tenebrio molitor	0.4	0.4	12.8		2.0		2.7		0.5		1.4		0.3				<0.1							20.5	18
Tenebrio obscurus	0.2	0.2	7.2		7.4		1.6	0.1	0.7		0.5		0.1				0.1							18.1	18
Alphitophagus bifasciatus					14.4	3.1	10.0		0.3															27.8	20
Trogoderma granarium (larvae)					4.0		13.0		5.0		1.0													23.0	22
Sitophilus oryzae							0.7	0.2	8.1	0.3	6.3	0.1	1.3		0.3						0.1		0.1	17.5	24
Sitophilus zeamais					0.1		2.4	0.2	6.3	0.2	4.2	0.1	1.0		0.1									14.6	4
Sitophilus granarius					0.4		2.2	0.6	14.0	0.5	7.9	0.1	0.6								0.1		0.1	26.5	4
Oryzaephilus surinamensis			0.4	0.9	13.5		12.0		3.9															30.7	26

Table 7. Composition of n-alkadienes in cuticular waxes of stored-product insects (% of total hydrocarbons)

Species	Number of carbon atoms																			Total amount [%]	References
	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47		
Trogoderma granarium (larvae)	2.4																			2.4	22
Sitophilus oryzae			0.2	0.2	7.8	0.7	22.5	0.8	14.3	0.3	2.3	0.1	0.4	0.2	1.0	0.1	0.4		0.1	51.4	23
Sitophilus zeamais			0.1	0.1	8.9	1.5	33.0	0.9	12.8	0.1	0.6		0.4		0.2					58.6	23
Sitophilus granarius			0.9	0.2	8.2	0.1	9.1	0.1	1.1		1.1	0.1	1.5	0.3	1.4	0.2	0.9		o.4	25.6	23

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