ELSEVIER

Comparative Biochemistry and Physiology Part B 123 (1999) 67–77

CBP

Hydrocarbons in adult *Chrysomela vigintipunctata* (Scopoli) (Coleoptera: Chrysomelidae)

N. Nikolova [a], T. Rezanka [b], B. Nikolova-Damyanova [c,*], P. Kalushkov [d]

[a] *Institute of Organic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[b] *Institute of Microbiology, Videnska 1083, 142 20 Prague, Czech Republic*
[c] *Chemistry of Natural Products Department, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, ul. Acad. G. Bonchev, bl. 9, 1113 Sofia, Bulgaria*
[d] *Institute of Zoology, Bulgarian Academy of Sciences, 1000 Sofia, Bulgaria*

Received 1 September 1998; received in revised form 14 January 1999; accepted 3 March 1999

Abstract

The hydrocarbon composition of the willow leaf beetle *Chrysomela vigintipunctata* (Scopoli) (Coleoptera: Chrysomelidae) is presented in this paper. Surface and internal hydrocarbons, total hydrocarbons of fed and starved insects, of the excrement and of the diet have been examined and compared. Normal chain-, terminally branched monomethyl-, internally branched monomethyl-, dimethyl-, trimethyl alkanes and only four alkenes have been identified in the insect. The chain-lengths varied in the interval C_{19} to C_{43}. Odd chain alkanes dominate. Surface hydrocarbons are characterised by the presence of longer-chain (C_{38},C_{39} and C_{41}) dimethyl- and trimethyl-alkanes. Dimethyl- and trimethyl alkanes with an unusually great number of methylene groups between the branch points have been detected. The excrement and the willow leaves have much simpler HC pattern: n-alkanes, terminally branched alkanes and alkenes. Based on the similarity between the HC pattern of the excrement and willow leaves it is assumed that the dietary HC are eliminated with the excrement.

Keywords: *Chrysomela vigintipunctata* (Scopoli); Adult insects; Surface hydrocarbons; Internal hydrocarbons; Total hydrocarbons; Unusual hydrocarbon structures; Excrement; Willow leaves; Diet; Gas chromatography–mass spectrometry

1. Introduction

In the last 20 years, insect lipids have been a subject of increasing attention and rigorous examination thus, recognising the fundamental role that they have in all cellular organisms. The major results obtained during that period were summarised and critically evaluated in two comprehensive volumes [16,27]. A major part of the investigations are directed to the great variety of hydrocarbons found in the insects. Their structure, biosynthesis, physiological and behavioural roles, as well as taxonomic and systematic utility have been intensively studied and reviewed [1,3,10,13,17].

It is well recognised at present that the examination of insect lipids and lipoidal compounds leads to a better understanding of their metabolism and their biological significance for these organisms. The results are also seen as a basis of novel strategies for the management of pest insects [26]. Undoubtedly, this is true in great extent for the leaf beetles or Chrysomelidae (Coleoptera). Many families and subfamilies of these pest insects are widely spread in Southeast Europe [8], some are known to cause serious damage to plants in Bulgaria. There is little information on the lipid composition of chrysomelids, however. The main interest so far has been concentrated on the chemical substances that play role in the selection of host plant, in mating and, particularly, in defence [2,7,9,15,18–23,25]. In con-

* Corresponding author. Tel.: +359-2-7134-136; fax: +359-2-700-225.
E-mail address: bmd@orgchm.bas.bg (B. Nikolova-Damyanova)

0305-0491/99/$ - see front matter
PII: S0305-0491(99)00042-5

Table 1
The design of the experiments for determination of HC in *C. vigintipunctata* reared in laboratory environment

Group	Number of insects	Treatment
I	1368	The insects were first soaked in ethanol to isolate the surface HC. Then the internal HC were isolated by the conventional way
II	160	The insect were fed for seven days with willow leaves. The total HC were isolated.
III	160	The insects were starved for three days. The total HC were isolated.

trast, only a few papers concern lipids [5] and hydrocarbons [4,6,12] in leaf beetles.

As a part of a broader programme on examination of chrysomelids of Bulgarian origin we examined the hydrocarbons of the beetle *Chrysomela vigintipunctata* (Scopoli) (Coleoptera: Chrysomelidae) which feeds on willow trees (*Salix* sp.).

The objective of the present work was to determine the composition of the surface and internal hydrocarbons (HC) and to monitor eventual effects caused by the diet. Therefore, the hydrocarbons in fed and starved insects, in the fed insect excrement and in the leaves of the host-plant have been determined and compared.

To the best of our knowledge, no data dealing with either HC or with lipids of the beetle *C. vigintipunctata* (Scopoli) have been reported as yet.

2. Experimental

Larvae of last instar and pupae of *C. vigintipunctata* (Scopoli) were collected in June 1995 near Sofia and reared to eclosion in laboratory environment.

The adult insects were separated into three groups and treated as shown in Table 1. The excrement of the fed insects were collected and examined separately for their HC content as were samples of the willow leaves used in the diet.

2.1. Chemicals and materials

All solvents and reagents were either analytical grade or HPLC grade. Light petroleum and acetone were used after preliminary distillation.

Docosane was purchased from Sigma (Sigma–Aldrich Chemie GmbH, Germany).

2.2. Extraction

2.2.1. Extraction of surface hydrocarbons

The insects (of the first group only, see Table 1) were soaked for 2 min in 10 ml of ethanol. The solvent was then evaporated in rotary evaporator and the residue was redissolved in dichloroethane to give 20 mg/ml solution.

2.2.2. Extraction of total hydrocarbons

The insects were homogenised in a blender for 2 min in 10 ml of ethanol and transferred to a round bottom flask. The total HC were extracted by refluxing the homogenate for 5–6 min with 20 ml dichloroethane-ethanol (1:1, v/v). The mixture was filtered (Filtrak 389) while still hot and the residue was suspended in fresh 20 ml of the same solvent mixture and extracted for 24 h (magnetic stirrer). After filtration the residue was extracted for another 24 h under the same conditions. The extracts were combined and the solvents were evaporated to dryness under nitrogen and redissolved in dichloroethane.

Exactly the same procedure was employed to extract the HC from the excrement and the willow leaves.

2.3. Isolation of the hydrocarbons

Each extract (10 mg solution in dichloroethane) was applied on a 20×20-cm home-made preparative glass plate (1.0 mm thickness of the silica gel layer, prewashed by two-fold development with hexane). Dodecane was applied alongside as a HC reference. The plate was developed with light petroleum-acetone, 100:6 (v/v) to a solvent front of 19 cm. Bands were detected by spraying the plate edges with 1% solution of 2,7-dichlorofluorescein in ethanol. Hydrocarbons migrated with the solvent front ($R_f \cong 1.0$) giving a clearly detectable band. The band was scrapped, transferred to a small glass column, and eluted with n-hexane. The solvent was evaporated under nitrogen and the residue was dissolved in hexane to give a 2% solution.

2.4. Gas chromatography–mass spectrometry (GC–MS) of HC

A HP 6890-5973 GC–MS performance system (Hewlett Packard, USA) with HP5973 MSD turbo pump and EI system for separation and identification of the HC was used. The samples were injected onto 60 m $\times$ 0.25 mm $\times$ 0.1 μ SPB-1 capillary column (Supelco, Czech Republic) by cool on-column injection under temperature programme (from 50°C for 5 min to 320°C at 10°C/min and held at the final temperature for 15 min. Hydrogen was the carrier gas at 0.52 ml/min. Mass spectra were recorded every 0.3 s at 70 eV. Identification of mass spectra was performed by one of the authors (T. Rezanka) and confirmed with help of a library (6th edition Wiley Library).

3. Results

The surface and internal HC, the total HC in fed and starved species of the leaf beetle *C. vigintipunctata* (Scopoli) (Coleoptera: Chrysomelidae), in the excrement of the fed insects and in the willow leaves, are presented in Table 2. One hundred and eighty five individual compounds are identified in total. Due to the extreme complexity of the mixture some components formed mixed peaks under the gas-chromatographic conditions employed. Hydrocarbons with chain-lengths shorter than C_{19} were detected in only traces amount. A typical GC–MS chromatogram (of the surface HC) is presented on Fig. 1.

The HC composition of the examined samples as distributed according to the structures, is shown on Fig. 2(A–F).

Internally branched dimethylalkanes (29.2% of the total) are most abundant in the surface HC (Fig. 2(A)) and 5,23-dimethyltritriacontane is the main individual compound (1.7%) detected in this HC class. The straight-chain alkanes (28.0%) are the second most abundant HC and nonacosane is the main representative (5.7%). The monomethylalkanes (18.3%) with few exceptions formed mixed peaks in GC, and despite the unambiguous identification, a single dominating representative cannot be shown. The main group is the mixture of 13-, 15-, 17- and 19-methylheptatriacontane. Trimethylalkanes with 17.3% follow and 13,17,21-trimethylpentatriacontane is the main individual component. Surface HC contain relative low amounts of iso- (3.6%) and anteiso- (2.9%) alkanes. The main representatives are 2-methyltriacontane and 3-methylhexacosane, respectively.

Fig. 2(B) represents the structural distribution in the internal HC. Straight-chain alkanes dominate (39.9%) with heptacosane as the main component (7.9%). Following are dimethyl- (21.1%), internally branched monomethyl- (18.5%) and trimethyl- (9.2%) alkanes. A single dimethyl- or monomethyl-component of significant quantity cannot be differentiated. The mixtures of 9,13- 11,15-, 13,17- and 15,19-dimethylpentatriacontanes (4.3%) and of 9-, 13-, 15- and 17-monomethyltritriacontanes (4.4%) dominate in the respective classes. 13,17,21-Trimethylpentatriacontane with 3.2% is the main trimethyl HC component. The quantities of iso- and anteiso-alkanes are 5.6 and 4.4%, respectively.

The composition of the total (surface plus internal) HC of insects put on willow leaves diet for 7 days and of insects starved for 3 days is shown in Fig. 2(C and D), respectively. The HC pattern of the two samples is similar with only small quantitative differences between the respective HC classes. Straight-chain alkanes (28.9 and 31.1%) are the most abundant compounds with nonacosane being the main component (6.3 and 9.0%). The internally branched dimethyl alkanes represent the second most abundant class (26.8 and 33.9%, respectively). The main group is a mixture of 11,15-, 11,19-, 13,17- and 15,19-dimethylheptatriacontanes (5.6 and 4.7%). The trimethyl alkanes follow with 19.2 and 20.2%. 13,17,21-Trimethylpentatriacontane (3.1%) is the main component in the fed and 5,15,23-trimethylpentatriacontane (4.1%) in the starved insects. The mixture of 13-, 15-, 17- and 19-methylheptatriacontane (4.3 and 4.5%) is the main group of internally branched monomethyl alkanes (18.6 and 17.7%). 2-Methyltriacontane (0.8%) and 3-methylhexacosane (0.9 and 1.0%) are the major components of the iso- (3.0 and 3.4%) and anteiso- (3.0 and 3.3%) alkanes.

The HC pattern of the excrement (Fig. 2(E)) is simple and is characterised by compounds with relatively short chain-length: up to 32 carbon atoms. Straight-chain alkanes are by far the most dominating type (86.9% of the total). Heptacosane (36.1%) and pentacosane (18.7%) are the major components. The excrements contain in addition only iso- and anteiso-alkanes in proportion 2:1. 2-Methyltetracosane (5.6%) and 3-methyloctacosane (1.1%) are the main terminally branched components. As in the insect samples alkenes are under 1%.

Finally, Fig. 2(F) shows the HC composition of the willow leaves. Straight chain alkanes (60.9%), iso- and anteiso-HC of practically the same quantity (13.5 and 13.9%, respectively), and relatively high amount of alkenes (11.6%) were detected in the leaves. Heptacosane (16.9%) is the main n-alkane; 2-methylhexacosane, 2,5%, and 3-methyloctacosane, 3.4% are the main terminally branched HC. The alkenes are of the same composition as those identified in the insect, with pentacosene (8.5%) being the main component.

Summarising the results presented in Table 2 and Fig. 2, it is evident that:

- Four odd-chain alkenes have been identified: pentacosene (25:1), nonacosene (29:1), hentriacontene (31:1) and tritriacontene (33:1). The 33:1 alkene has been detected only in surface HC (in traces) and in the willow leaves (0.4%). The other three alkenes have been found in all samples in different proportions, the highest being in the willow leaves. The position of the double bond remains unknown but according to the literature any position within the chain is possible [10].
- The total content of the iso- and the anteiso-HC (or terminally branched monomethyl alkanes according to Lockey [13]) in the insect does not exceed 10%. Interesting is that in all samples the ratio between the 2- and the 3-branched compounds is close to 1. The terminally branched alkanes found in the willow leaves differ by their significantly higher amount but not in composition and the ratio between the two structural types. The chain-lengths of terminally branched alkanes are relatively short

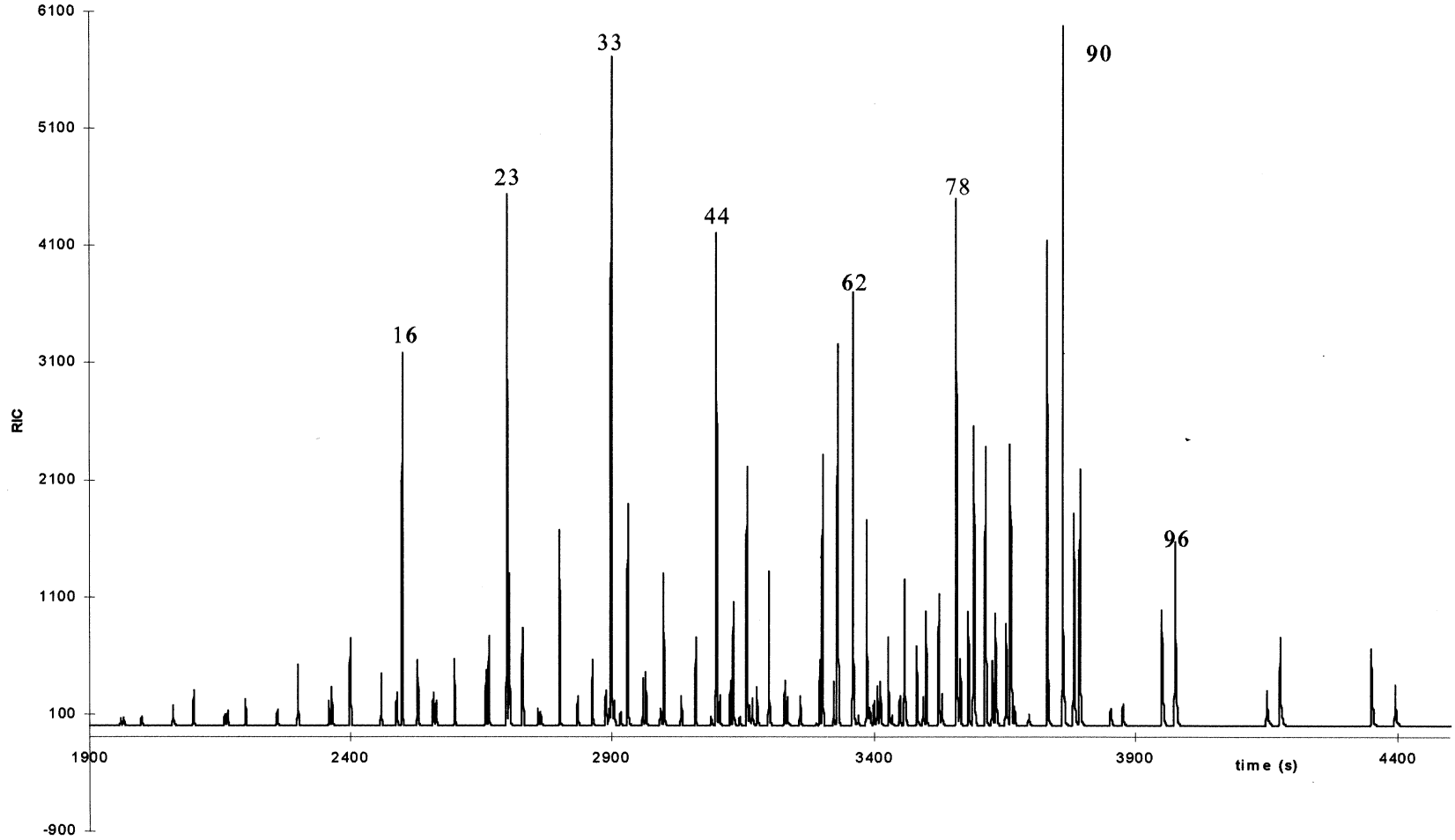

Fig. 1. GC–MS chromatogram of surface hydrocarbons of *C. vigintipunctata* (Scopoli) (Coleoptera: Chrysomelidae). 16-Pentacosane; 23-heptacosane, 33-nonacosane; 44-hentriacontane; 62-11,15 & 13,17 &11,17 & 15,19-Dimethyltritriacontane; 78-9,13 &11,15 & 13,17 & 15,19-Dimethylpentatriacontane; 90-11,15 & 11,19 & 13,17 & 15,19-Dimethylheptatriacontane; 96-11,15,19-Trimethylnonatriacontane.

Table 2
Hydrocarbon composition and content in *C. Vigintipunctata* (Scopoli)

Hydrocarbon	ECL[a]	Surface HC	Internal HC	Fed Insects	Starved Insects	Excrement	Willow leaves
Nonadecane	1900	0.1	0.1	0	0	0.2	0.1
2-Methylnonadecane	1960	0.1	0.2	0	0	0	0.1
3-Methylnonadecane	1965	0.1	0.1	0	0	0	0.2
Eicosane	2000	0.1	0.2	0.1	0.1	0	0.1
2-Methyleicosane	2060	0.2	0.2	0.1	0.1	0	1.0
Heneicosane	2100	0.3	0.5	0.1	0.1	0.5	1.1
2-Methylheneicosane	2160	0.1	0.2	0	0	0	0.5
3-Methylheneicosane	2165	0.1	0.3	0.1	0.1	0	0.4
Docosane	2200	0.2	0.4	0.1	0.1	1.1	1.0
2-Methyldocosane	2260	0.1	0.3	0.2	0.1	0	0.4
Tricosane	2300	0.5	0.8	0.3	0.3	6.2	1.1
2-Methyltricosane	2360	0.2	0.5	0.2	0.2	0.5	0.9
3-Methyltricosane	2365	0.3	0.6	0.4	0.4	0.6	1.6
Tetracosane	2400	0.8	1.2	0.7	0.6	5.2	1.9
2-Methyltetracosane	2460	0.4	0.8	0.3	0.2	5.6	2.5
Pentacosene	2490	0.3	0.5	0.1	0.1	0.3	8.5
Pentacosane	2500	3.2	5.7	3.4	3.8	18.7	10.7
11 & 13-Methylpentacosane	2530	0.6	0.8	0.3	0.2	0	0
2-Methylpentacosane	2560	0.3	0.5	0.2	0.2	0.8	0.9
3-Methylpentacosane	2565	0.2	0.4	0.2	0.2	0.6	1.3
Hexacosane	2600	0.6	1.0	0.4	0.5	0.5	1.1
2-Methylhexacosane	2660	0.5	0.8	0.3	0.4	0.6	2.5
3-Methylhexacosane	2665	0.8	1.2	0.9	1.0	0.9	3.2
Heptacosane	2700	4.5	7.9	5.0	5.4	36.0	17.0
2,6-Dimethylhexacosane	2704	1.3	1.9	1.1	1.1	0	0
9 & 11 & 13-Methylheptacosane	2730	0.8	1.2	1.0	1.1	0	0
2-Methylheptacosane	2765	0.2	0.2	0.1	0.1	0.3	1.0
3-Methylheptacosane	2765	0.1	0.2	0.1	0	0.1	0.9
Octacosane	2800	1.7	1.6	2.3	1.8	1.4	3.0
10-Methyloctacosane	2836	0.3	0.3	0.1	0.1	0	0
3-Methyloctacosane	2864	0.6	0.6	0.3	0.4	1.1	3.4
Nonacosene	2890	0.3	0.3	0.1	0.1	0.5	1.6
2,10 & 2,18-Dimethyloctacosane	2899	0.1	0.2	0.1	0.1	0	0
Nonacosane	2900	5.7	6.0	6.3	9.0	14.8	12.3
2,6-Dimethyloctacosane	2905	0.2	0.4	0.4	0.3	0	0
2,10,18-Trimethyloctacosane	2918	0.1	0.2	0.1	0.1	0	0
9 &11 & 13 & 15-Methyloctacosane	2931	1.9	2.0	1.6	1.2	0	0
2-Methylnonacosane	2960	0.4	0.5	0.4	0.5	0.1	0.6
3-Methylnonacosane	2965	0.5	0.7	0.5	0.7	0.3	0.8
11,19-Dimethyloctacosane	2995	0.1	0.3	0.1	0.2	0	0
Triacontane	3000	1.3	2.6	1.5	1.3	0.9	1.6
12-Methyltriacontane	3033	0.3	0.5	0.5	0.4	0	0
2-Methyltriacontane	3060	0.8	1.1	0.8	0.8	0.1	1.4
Hentriacontene	3090	0.1	0.1	0.1	0.1	0.2	1.2
Hentriacontane	3100	4.2	5.9	4.0	3.5	0.8	6.1
2,6-Dimethyltriacontane	3105	0.3	0.4	0.3	0.3	0	0
13 & 15-Methylhentriacontane	3127	0.4	0.8	0.6	0.5	0	0
9 & 11 & 13 & 15-Methylhentriacontane	3130	1.0	1.8	0.7	0.6	0	0
5 & 7-Methylhentriacontane	3145	0. 1	0.2	0.2	0.1	0	0
9,13 & 11,15 & 13,17-Dimethylhentriacontane	3158	2.2	2.0	1.4	1.6	0	0
2-Methylhentriacontane	3160	0.2	0.3	0.3	0.5	0.1	1.3
3-Methylhentriacontane	3165	0.2	0.4	0.4	0.6	0.3	2.0
5,15-Dimethylhentriacontane	3178	0.3	0.4	0.3	0.3	0	0
Dotriacontane	3200	1.3	2.6	1.5	1.2	0.3	2.2
11 & 13 & 14 & 16-Methyldotriacontane	3230	0.4	0.5	0.3	0.3	0	0
12-Methyldotriacontane	3233	0.2	0.2	0.3	0.3	0	0
2-Methyldotriacontane	3260	0.3	0.2	0.3	0.3	0	0.5

Table 2 (Continued)

Hydrocarbon	ECL[a]	Surface HC	Internal HC	Fed Insects	Starved Insects	Excrement	Willow leaves
Tritriacontene	3290	.0.1	0	0	0	0	0.4
2,8 & 2,10-Dimethyldotriacontane (possible 2,22 and 2,24)	3297	0.6	0.9	0.8	0.8	0	0
Tritriacontane	3300	2.3	2.5	1.7	2.0	0.5	1.1
2,10,16-Trimethyldotriacontane	3324	0.4	0.6	0.4	0.4	0	0
9 & 11 & 13 & 15 & 17-Methyl-tritriacontane	3330	3.2	4.4	3.5	2.8	0	0
11,15 & 13,17 & 11,17 & 15,19-Dimethyltritriacontane	3360	3.7	2.4	2.9	2.0	0	0
9,13 & 7,11-Dimethyltritriacontane	3370	0.1	0.1	0.1	0.1	0	0
5,23-Dimethyltritriacontane	3386	1.7	2.0	1.4	1.6	0	0
13,17,19 & 13,17,21-Trimethyl-tritriacontane	3390	0.2	0.2	0.3	0.2	0	0
Tetratriacontane	3400	0.2	0.4	0.2	0.2	0	0.2
3,23-Dimethyltritriacontane	3407	0.3	0.5	0.4	0.5	0	0
5,9,23 & 5,17,23-Trimethyl-tritriacontane	3409	0.4	0.5	0.6	0.5	0	0
10 & 11 & 12-Methyl-tetratriacontane	3430	0.7	1.1	0.9	1.2	0	0
Methyltetratriacontene	3431	0.1	0.2	0.1	0.1	0	0
10,18 & 12,18-Dimethyltetratriacontane	3449	0.3	0.3	0.3	0.3	0	0
13,17 & 12,16 & 11,15 & 10,14-Dimethyltetratriacontane	3458	1.2	1.3	1.4	1.4	0	0
10,14,18 & 11,15,19-Trimethyl-tetratriacontane	3482	0.7	0.8	0.9	0.9	0	0
2,10 & 2,12 & 2,14-Dimethyl-tetratriacontane	3494	0.2	0.3	0.2	0.2	0	0
Pentatriacontane	3500	1.0	0.7	1.4	1.3	0	0.1
2,10,16 & 2,16,24-Trimethyl-tetratriacontane	3524	1.1	1.1	1.4	1.4	0	0
11 & 13 & 15 & 17-Methyl-pentatriacontane	3530	2.8	3.1	2.2	2.2	0	0
9,13 & 11,15 & 13,17 & 15,19-Dimethylpentatriacontane	3560	4.5	4.3	4.6	3.5	0	0
11,19 & 11,23-Dimethyl-pentatriacontane	3561	0.6	0.7	0.8	0.7	0	0
11,15,19 & 9,13,17-Trimethyl-pentatriacontane	3580	1.0	0.4	1.6	1.3	0	0
13,17,21-Trimethylpentatriacontane	3590	2.5	3.2	3.1	2.9	0	0
5,15,23-Trimethylpentatriacontane	3612	2.4	1.5	1.9	4.0	0	0
11 & 12-Methylhexatriacontane	3626	0.6	0.6	0.8	0.8	0	0
13 & 14-Methylhexatriacontane	3630	1.0	0.5	1.2	1.3	0	0
12,16-Dimethylhexatriacontane	3652	0.9	0.5	1.0	1.0	0	0
Dimethylhexatriacontane	3660	2.4	1.6	1.2	1.4	0	0
12,16,20 & 11,15,19-Trimethyl-hexatriacontane	3677	1.7	0.1	2.4	2.1	0	0
2,12-Dimethylhexatriacontane	3695	0.1	0	0.1	0.1	0	0
13 & 15 & 17 & 19-Methyl-heptatriacontane	3730	4.1	0.8	4.3	4.5	0	0
11,15 & 11,19 & 13, 17 & 15,19-Dimethylheptatriacontane	3760	6.0	0.5	5.6	4.7	0	0
11,15,19 & 9,13,17-Trimethyl-heptatriacontane	3781	1.8	0.2	1.2	1.4	0	0
13,17,21-Trimethylheptatriacontane	3790	2.2	0.2	2.5	2.5	0	0
12,16-Dimethyloctatriacontane	3852	0.1	0	0.2	0.2	0	0
11,15,19-Trimethyloctatriacontane	3875	0.2	0	0.3	0.1	0	0
13,17 & 11,15 & 11,19-Dimethyl-nontatriacontane	3949	1.0	0.1	1.1	0.8	0	0
11,15,19-Trimethylnonatriacontane	3974	1.6	0.1	1.4	1.3	0	0
13,17-Dimethylhentetracontane	4149	0.3	0	0.3	0.3	0	0
11,15,19-Trimethylhentetracontane	4174	0.8	0	0.8	0.8	0	0
13,17-Dimethyltriatetracontane	4349	0.7	0	0.7	0.7	0	0
11,15,19-Trimethytriatetracontane	4394	0.3	0	0.3	0.3	0	0

[a] ECL, Equivalent chain length.

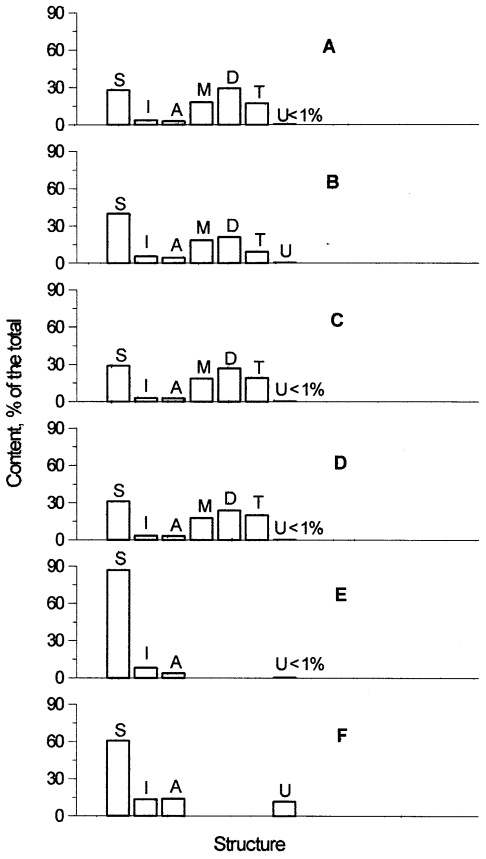

Fig. 2. The structural pattern of surface (A), internal (B), fed insects (C), starved insects (D), excrement (E) and willow leaves (F) hydrocarbons. S, straight-chain-; I, iso-; A, anteiso-; M, monomethyl-; D, dimethyl-; T, trimethyl-; U, unsaturated hydrocarbons.

when compared to those of the other branched alkanes: up to 32 carbon atoms. The same has been reported for other insects [17].

- The internally branched monomethyl HC form a homologue series of compounds with chain-lengths from 25 to 37 carbon atoms and branching at either the 5-, 7-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17- or 19-carbon atom. Most of the compounds give mixed peaks in GC. A C_{26} homologue was not detected, presumably because of its very low proportion.
- Dimethyl alkanes of two types have been found. The first type is represented by C-C*-Cx-C*-Cy and C-C-C*-Cx-C*-Cy structures (the * sign indicates the branching). The number of the separating methylene groups, x, varies in the interval 3–19. Thus, for example 2,18-dimethyloctacosane has 15, while 3,23-dimethyltritriacontane has 19 separating methylene groups. HC with a distance between the branching points longer than 13 methylene groups [11] have not been reported for Coleoptera beetles to the best of our knowledge. The chain lengths of these HC vary from 26 to 36 carbon atoms. The second type represents the genuine internally branched dimethylalkanes, the Cx-C*-Cy-C*-Cz structures [17]. The methyl groups are attached to either odd or even numbered carbon atom and are separated by an odd number of methylene groups, mostly by three. In addition, compounds were detected with 5, 7, 9 and 11 (which is not unusual [17]) but also with 17 separating methylene groups as in 5,17-dimethyltritriacontane. Internally branched dimethyl HC with chain-lengths from C_{31} up to C_{41} are detected.
- Trimethyl alkanes (Cw-C*-Cx-C*-Cy-C*-Cz) also comprise compounds with odd and even chain-lengths and branching at odd and even carbon atoms. HC with three separating methylene groups are most abundant but compounds with 5, 7, and even with the unusual 13 intervening groups are found. Most of the alkanes with more than three intervening methylene groups have an even-chain carbon skeleton with branching at even numbered carbon atom, and presumably derived from the terminally branched species, as 2,10,16- and 2,16,24-trimethyltetratriacontane, for example. Chain lengths vary in the interval from 31 to 43 carbon atoms.

4. Discussion

The results presented here give a general picture of the hydrocarbon composition of the adult *C. vigintipunctata* insects reared in laboratory conditions and fed with their usual diet.

The choice of ethanol to extract the surface HC was unusual and evidently, as compromising, as is the choice of non-polar solvent for the purpose. Our point was based on a previous experience with examination of plants where it has been found that polar solvents are able to extract lipophylic compounds from a material that contains also a high amount of polar compounds. In this case, the assumption was that opposite to the conventional procedure, co-extraction of internal HC will be substantially minimised. The data presented in Table 2 confirm, in our opinion, that ethanol ensures sufficiently full isolation of surface hydrocarbons, including long-chain (C_{43}) trimethyl-branched components, which were found in the ethanol washing but not in the internal HC (cf. the respective columns in Table 2). The efficiency of this extraction is confirmed also by the experiments with fed and starved insects when the total HC were also thoroughly extracted (Section 2). No differences in HC composition and in structural pattern but only in quantities, have been observed.

The HC pattern of all insect samples are similar. The HC in the adult *C. vigintipunctata* insects comprise normal-chain-, terminally branched monomethyl-, internally branched monomethyl-, dimethyl- (with either terminal or internal branching of the first methyl

group) and trimethyl alkanes. The alkanes are about 99% of the total hydrocarbons, while the alkene content is less than 1%. Compounds with odd chains dominate (approx. 75% of the total). The excrement and the willow leaves have much simpler HC pattern: n-alkanes, terminally branched alkanes and alkenes.

The surface HC of *C. vigintipunctata* (Scopoli) are characterised by the presence of longer chain components with higher level of branching when compared to the internal HC. Branched-chain compounds with chain-lengths from C_{41} to C_{43} were detected in the surface HC (Table 2).

Several dimethyl- and trimethyl-branched HC with unusual large distance between the branching points have been detected. We believe that these were unambiguously identified by their resolution in capillary gas chromatography and the specific fragmentation in the electron-impact mass-spectrometry (Figs. 3 and 4). Thus, for example, in the mass-spectra of 5,23-dimethyltritriacontane the abundance and intensity of M^+ at m/z 492, M-CH_3 at m/z 377 and the pairs of ions at m/z 351/350 and 168, and m/z 435/234 and 84 reveal a dimethylalkane of 35 carbon atoms and branching at carbon 5 and carbon 23.

We assume that the HC composition of *C. vigintipunctata* (Scopoli) reveals that the 'normal' elongation/decarboxylation pathway prevails in the synthesis of surface and internal HC. In accordance with this pathway, dominating are odd-chain HC, with branching at odd numbered carbon atoms and with three methylene groups separating the branch points in the dimethyl- and trimethyl-branched compounds [13]. On

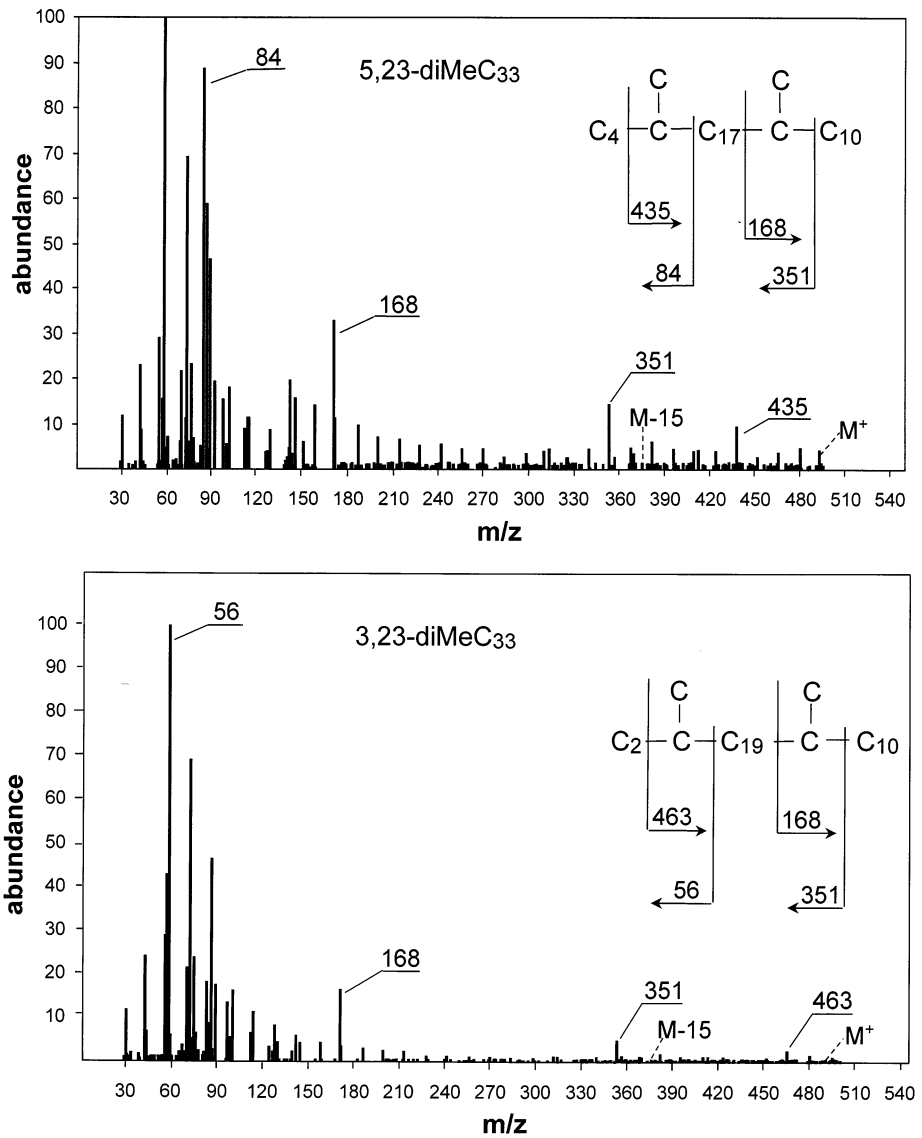

Fig. 3. Electron-impact mass-spectra scans and fragmentation of some unusual dimethylalkanes identification: 3,23-diMeC33, 3,23-dimethyltritriacontane, M^+ at m/z 492, M-15 at m/z 377; 5,23-diMeC33, 5,23-dimethyltriacontane, M^+ at m/z 492, M-15 at m/z 377.

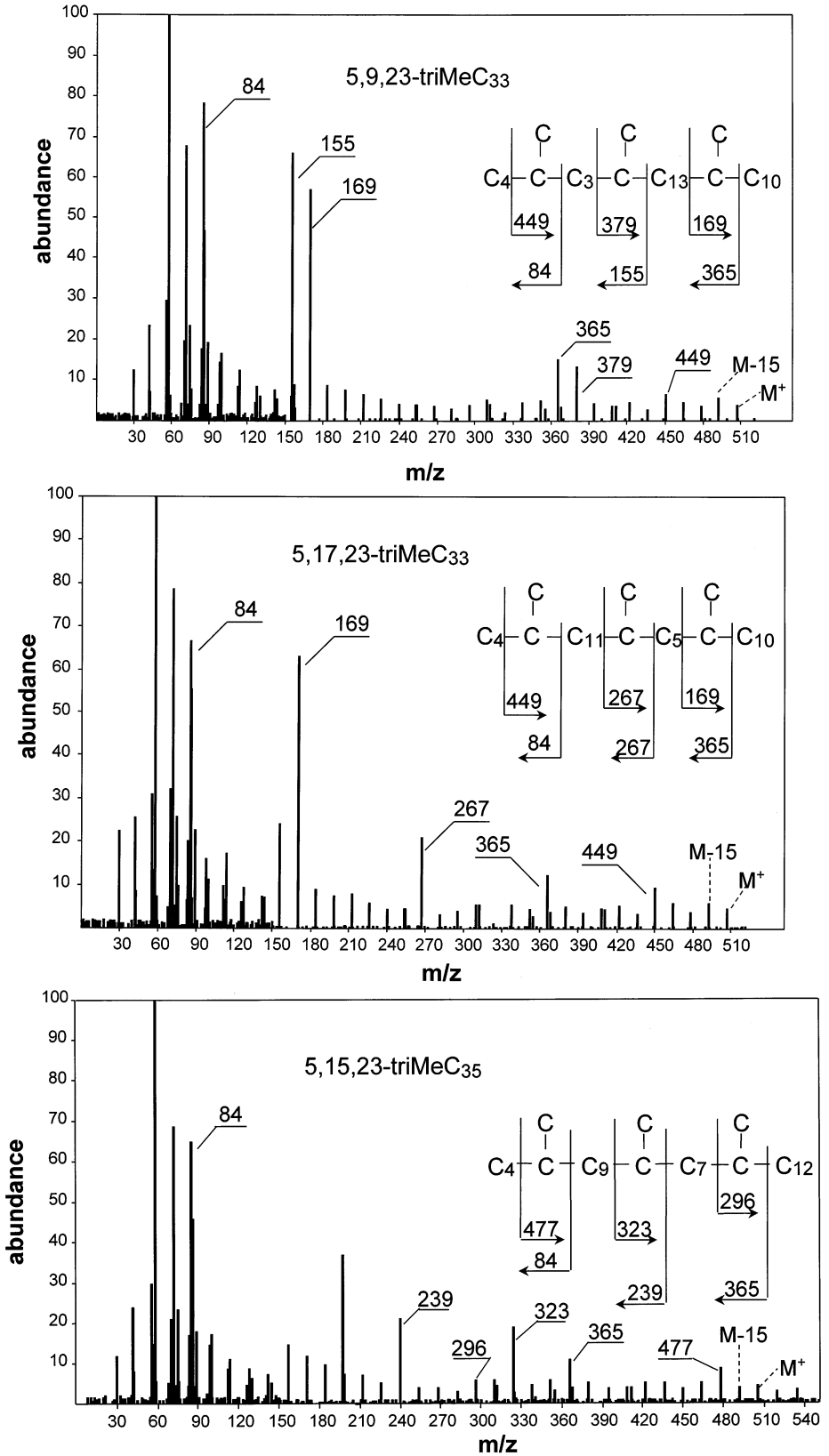

Fig. 4. Electron-impact mass-spectra scans and fragmentation of some unusual trimethylalkanes 5,9,23-triMeC33, 5,9,23-trimethyltritriacontane, M^+ at m/z 506, M-15 at m/z 491; 5,17.23-triMeC33, 5,17,23-trimethyltritriacontane, M^+ at m/z 506, M-15 at m/z 491; 5,15,23-triMeC35, 5,15,23-trimethylpentatriacontane, M^+ at m/z 506, M-15 at m/z 491.

the other hand, the relatively high amount (23.4% of the total) of even-chain normal and branched hydrocarbons, as well as the existence of di- and trimethyl branched compounds with more than three methylene groups between the branch points indicates that a modified pathway might be also in use [13,14].

It is obvious from the results that the HC composition of the insect is not effected by the diet, at least for the duration of the feeding and starving stages chosen in the present work. The hydrocarbon composition of both fed and starved insects is very close and the small quantitative differences cannot be considered to be of any significance. The incorporation of dietary hydrocarbons has been assumed in some studies [4] but the assumption is contradictory to the accepted mechanism of hydrocarbon synthesis in insects via an elongation/decarboxylation pathway [13]. No effect of the diet has been observed in the study of cuticular HC in the insects of Diabrotica (Coleoptera: Chrysomelidae) sibling species [6].

Another confirmation that the diet does not effect the HC composition of the insects is the general similarity between the HC pattern and composition between the excrement of the fed insects and those of the willow leaves. To the best of the authors' knowledge lipids and HC in insect excrement have not been examined so far. It has been assumed that due to regulatory mechanisms in the insect organism undesired compounds or excess of compounds should be discarded with the excrement. Evidently, that exactly happens with the n-alkanes and with some of the terminally branched alkanes. These compounds are the main components in both the leaves and the excrement and this eventually indicates that dietary HC of these types are predominately eliminated with the excrement. Unexplained at this stage is the remarkable quantitative difference between the alkene content in the willow leaves and in the rest of the samples. Equivalent amounts of alkenes are not found either in the insects or in the excrement, while the composition is the same in all samples. A possible explanation is that alkenes are degraded by intestinal bacteria of insects or by the insects own metabolism. Also, it was not possible to compare the HC composition of the willow leaves from the region with reported data. It has been shown that the HC composition of *Salix* sp. leaves is strongly effected not only by the species but also by the geographical region and climate changes [24].

The presence of methyl-branched alkanes with unusual number of separating methylene groups might be of interest for the chemotaxonomic characterisation of *C. vigintipunctata* (Scopoli).

Acknowledgements

This work was supported in part by the Bulgarian National Fund for Scientific Research.

References

[1] Blomquist GJ. Hydrocarbon and hydrocarbon-derived sex pheromones in insects: biochemistry and endocrine regulation. In: Stanley-Samuelson DW, Nelson DR, editors. Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln, NE: University of Nebraska Press, 1993:272–317.

[2] Craveiro D, Bao SN. Localization of carbohydrates in spermatids of three chrysomelid beetles. Biocell 1995;19:195–202.

[3] Dillwith JW, Neese PA, Brigham DL. Lipid biochemistry in aphids. In: Stanley-Samuelson DW, Nelson DR, editors. Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln, NE: University of Nebraska Press, 1993:389–434.

[4] Dubis E, Malinski E, Dubis A, Szafranek J, Nawrot J, Poplawski J, Wrobel J. Sex-dependent composition of cuticular hydrocarbons of the Colorado beetle *Leptinotarsa decemlineata* Say. Comp Biochem Physiol 1987;87A:839–43.

[5] Dubis E, Poplawski J, Wrobel JT, Kusmierz J, Malinski E, Szafranek J. Analysis of exoskeleton triglycerides in *Leptinotarsa decemlineata* Say. Lipids 1986;21:434–9.

[6] Golden KL, Meinke LJ, Stanley-Samuelson DW. Cuticular hydrocarbons discrimination of Diabrotica (Coleoptera: Chrysomelidae) sibling species. Ann Entomol Soc Am 1992;85:561–70.

[7] Gross J, Hilker M. Chemoecological studies of the exocrine glandular larval secretions of two chrysomelid species (Coleoptera): *Phaedon cochleariae and Chrysomela lapponica*. Chemoecology 1994;5/6:185–189.

[8] Gruev B, Tomov V. Fauna Bulgarica. Coleoptera: Chrysomelidae. Part II. Sofia: Academic Scientarium Bulgaricae, 1986:71.

[9] Hilker M, Schulz S. Composition of larval secretion of *Chrysomela lapponica* (Coleoptera: Chrysomelidae). J Chem Ecol 1994;20:1075–93.

[10] Howard RW. Cuticular hydrocarbons and chemical communication. In: Stanley-Samuelson DW, Nelson DR, editors. Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln, NE: University of Nebraska Press, 1993:179–226.

[11] Jackson LL. Cuticular hydrocarbons of the milk-weed bug *Oncopeltus fasciatus* by age and sex. Insect Biochem 1983;13:19–25.

[12] Jacob J, Hanssen H-P. Distribution and variability of cuticular hydrocarbons within the Coleoptera. Biochem Syst Ecol 1986;14:207–10.

[13] Lockey KH. Lipids of the insect cuticule: origin, composition and function. Comp Biochem Physiol 1988;89B:595–645.

[14] Lockey KH, Oraha VS. Cuticular lipids of adult *Locusta migratoria migratoriodes* (R and S), *Schistocerca gregaria* (Forskal) (Acrididae) and other orthopteran species—II. Hydrocarbons. Comp Biochem Physiol 1990;95B:721–44.

[15] Mitchell BK. Adult leaf beetles as models for exploring the chemical basis of host-plant recognition. J Insect Physiol 1988;34:213–25.

[16] Mittler TE, Dadd RH, editors. Metabolic Aspects of Lipid Nutrition in Insects. Boulder, CO: Westview Press, 1983.

[17] Nelson DR. Methyl-branched lipids in insects. In: Stanley-Samuelson DW, Nelson DR, editors. Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln, NE: University of Nebraska Press, 1993:227–70.

[18] Oldham NJ, Veith M, Boland W, Dettner K. Iridoid monoterpene biosynthesis in insects. Evidence for a de novo pathway in the defensive glands of *Phaedon amoricae* (Chrysomelidae) leaf beetle larvae. Naturwissenschaften 1996;83:470–3.

[19] Pasteels J M, Dobler S, Rowell-Rahier M, Ehmke A, Hartman T. Distribution of antogenous and host-derived chemical defences in Orina leaf beetles (Coleoptera: Chrysomelidae). J Chem Ecol 1995;21:1163–79.

[20] Pasteels JM, Daloze D, Rowell-Rahier M. Chemical defence in chrysomelid egs and neonate larvae. Physiol Entomol 1986;11:29–37.

[21] Pasteels JM, Rowell-Rahier M, Braekman JC, Daloze D, Duffey S. Evolution of exocrine chemical defense in leaf beetles (Coleoptera: Chrysomelidae). Experentia 1989;45:295–300.

[22] Pasteels JM, Rowell-Rahier M, Raupp MJ. Plant derived defense in chrysomelid beetles. In: Barbosa P, Letourneau D, editors. Novel Aspects of Insect-Plant Interactions. Chap. 7. New York: Wiley, 1988:235–70.

[23] Pasteels MJ, Rowell-Rahier M, Braekman JC, Daloze D. Chemical defences in leaf beetles and their larvae: the ecological, evolutionary and taxonomic significance. Biochem Systematics Ecol 1984;12:395–406.

[24] Rosenqvist H, Laakso S. Fatty acid and alkanes in leaves of frost-tolerant and frost-susceptible willows. Phytochemistry 1991;30:2161–4.

[25] Soetens P, Pasteels JM, Daloze D. A simple method for in vivo testing of glandular enzymatic activity on potential precursors of larval defensive compounds in Phratora species (Coleoptera: Chrysomelinae). Experientia 1993;49:1024–6.

[26] Stanley-Samuelson DW, Nelson DR. Preface. In: Stanley-Samuelson DW, Nelson DR, editors. Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln, NE: University of Nebraska Press, 1993:XI–XIII.

[27] Stanley-Samuelson DW, Nelson DR, editors. Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln, NE: University of Nebraska Press, 1993.