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Stage-specific surface chemicals of *Plodia interpunctella*: 2-acyl-1,3-cyclohexanediones from larval mandibular glands serve as cuticular lipids

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Abstract

Cuticular lipid compositions of all life stages of the stored product moth *Plodia interpunctella* have been determined. Eggs and adults of *P. interpunctella* have cuticular lipids consisting solely of hydrocarbons. The composition of eggs and adult females is qualitatively nearly identical with ca. 86 hydrocarbons (11 *n*-alkanes, 39 monomethyl alkanes, 19 dimethyl alkanes, 11 trimethyl alkanes and 6 monoenes) except females lack the 2-methyl alkanes found in eggs. Adult males have a hydrocarbon composition qualitatively nearly identical to females with the exception that they lack the monoenes. Larval and pupal cuticular lipids are dominated by a mixture of ca. 20 previously described 2-acyl-1,3-cyclohexanediones, with only minute amounts of *n*-alkanes on the larvae and pupae. The 2-acyl-1,3-cyclohexanediones are continuously secreted onto their silk webbing and food particles by the paired mandibular glands found in all larvae. Extracts from dissected mandibular glands have a qualitatively identical composition to larval cuticular extracts. The pupal stage (which does not have mandibular glands) is enclosed in a silk cocoon also coated with 2-acyl-1,3-cyclohexanediones laid down while the wandering stage larvae spin the cocoon. The 2-acyl-1,3-cyclohexanediones have physical properties which closely mimic those of cuticular hydrocarbons, including melting point and boiling point range and hydrophobicity. This is the first report of an insect with a life stage that does not use conventional cuticular lipids for conservation of water.
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Keywords: 2-Acyl-1,3-cyclohexanediones; Chemical ecology; Cuticular lipids; Desiccation resistance; Lepidoptera; Mandibular glands; Pyralidae; Waterproofing

1. Introduction

Numerous studies have been conducted on the chemistry and biology of cuticular lipids of insects (Howard, 1993; Lockey, 1988; Lockey, 1991; Nelson and Blomquist, 1995), but surprisingly little attention has been paid to members of the extremely species-rich order Lepidoptera (Coudron and Nelson, 1978, 1981; De Renobales and Blomquist, 1983; Espelie and Brown, 1990; Guo and Blomquist, 1991; Carlson and Milstrey, 1991; Nelson and Buckner, 1995; Buckner et al., 1996; Jurenka and Subchev, 2000; Howard, 2001; Arsene et al., 2002). Cuticular lipids of the family Pyralidae have only been surveyed twice (Hebanowska et al., 1990; Richmond and Page, 1995). Extensive chemical ecology studies have been conducted on larvae of the pyralid subfamily Phycitinae which are pests of stored products because of the semichemically active 2-acyl-1,3-cyclohexanediones secreted almost continuously from their paired mandibular glands (Corbet, 1971, 1973a,b; Mudd and Corbet, 1973; Mossadegh, 1978, 1980; Mudd, 1981; Kuwahara et al., 1983; Nemoto et al., 1987a,b; Strand et al., 1989; Phillips and Strand, 1994). As part of a larger program on the chemical ecology of pests of stored products, we have characterized the surface chemicals found on the cuticle of all life stages of the moth *Plodia interpunctella* (Lepidoptera: Pyralidae: Phycitinae). Surprisingly, the larval and pupal stages of this insect were found to have almost no conventional cuticular lipids such as hydrocarbons (Howard, 1993), alcohols, aldehydes, wax esters or fatty acids (Buckner, 1993). Instead their cuticular chemistry consists almost exclusively of the acylcyclohexanediones. We discuss this

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anomaly and suggest that the acylcyclohexanediones have physical properties which allow them to serve as a cuticular waterproofing layer. In addition, we discuss the normal hydrocarbon composition of the egg and adult stages.

2. Materials and methods

2.1. Insects

P. interpunctella was collected from shelled corn in El Paso, IL, in November 1998 and reared continuously in our laboratory on a diet of cracked wheat, wheat shorts, wheat germ, Brewer's yeast, honey, glycerin and water (McGaughy and Beeman, 1988). Cultures are maintained at 27 °C and 50–55% relative humidity in a 12-h photophase. Eggs were obtained by placing mated *Plodia* adults into a 570-ml jar containing fluted filter paper. Eggs laid overnight were collected by sifting them through a 0.42-mm mesh screen lid (=#40 sieve). First instar larvae were obtained from isolated eggs that were allowed to hatch. Third and fifth instar larvae were removed from the cultures at appropriate intervals. Pupae were obtained by dissection from cocoons located near the surface of the diet and newly emerged adults were obtained from pupae isolated into 13 × 100-mm glass tubes.

2.2. Dissection procedures and sample preparation

Mandibular glands were dissected by immersing chilled 5th instar larvae in cold saline (0.9% NaCl) containing 0.05% Triton-X100. With two pairs of forceps, the head capsule was gently torn away from the prothoracic region and pulled anteriorly, yielding the head capsule with attached mandibular glands and silk glands (Fig. 1). For extraction, the mandibular glands were pinched off at their mandibular attachment, rinsed 2 × in H_2O, blotted onto tissue paper while still on the tips of the forceps, and placed on a 3 × 5-mm piece of Whatman #1 filter paper pre-extracted with hexane. Filters with glands were placed in a 1-ml crimp-top vial, immersed in 0.1 ml hexane, a teflon-lined crimp top secured, and vials held at − 80 °C until analyzed.

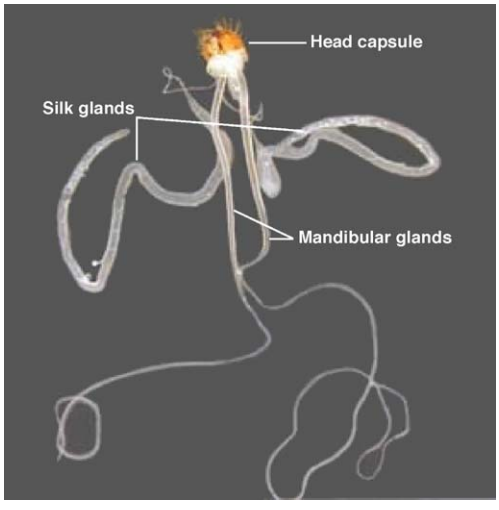

Fig. 1. Paired mandibular glands dissected from a 5th instar *P. interpunctella* larva.

Individual mandibular gland analyses and 5th instar larval cuticle analyses were conducted by solid phase microextraction (SPME) using a 7-μm polydimethylsiloxane bonded phase fiber in a Supelco SPME holder. Larvae were chilled briefly, grasped with forceps, and specific body regions rubbed onto the exposed fiber. The head/prothoracic region, 1st proleg segment and terminal (9th and 10th) abdominal segments were separately analyzed. Absorbed lipids were analyzed by gas chromatography–mass spectrometry (GC–MS) using the same parameters as listed below, with the exception that the fiber was desorbed for 2 min at 280 °C with the septum purge closed before beginning the temperature program.

Quantitative determination of the amount of surface lipids on 5th instar larvae was obtained by freezing 100 larvae at − 80 °C for 30 min, warming the larvae to room temperature, weighing 10 groups of 10 larvae separately in tared 5-ml beakers, extracting the larvae with two 1-ml portions of hexane for ca. 1 min each, combining the extracts, evaporating the solvent in the tared beaker, and determining residue weight.

Hemolymph was collected from 25 wandering stage 5th instar larvae by surface sterilizing them with ethanol, chilling in an ice bath, cutting the front proleg, gently squeezing the insect and aspirating the exuded drop of hemolymph into a 10-μl pipet. Approximately 50 μl hemolymph so obtained was diluted to 100 μl with saline, shaken with 100 μl of hexane and the hexane layer removed. A second extraction with another 100 μl hexane was pooled with the first aliquot and the combined extracts concentrated to dryness in a tapered mini-vial, re-dissolved in 3 μl hexane, and analyzed by GC–MS using the parameters below.

2.3. Chemical analyses

Cuticular lipids were extracted by immersing the insects in three successive 0.25-ml portions of hexane for 1 min each time. The combined portions from each sample were concentrated under a stream of N_2, and the crude extract was examined by gas chromatography–mass spectrometry. Hydrocarbons were isolated by chromatography on a 3-cm "mini-column" of BioSil A (Bio-Rad Laboratories, Hercules, CA) (Howard et al., 1978).

Electron impact mass spectral analyses were conducted by using a Hewlett-Packard 5790A gas chromatograph (Hewlett-Packard, San Fernando, CA) containing a DB-5 bonded phase capillary column (15 m long, 0.25 mm inside diameter) (J&W Scientific, Folsom, CA) connected to a Hewlett-Packard 5970 mass selective detector and a Hewlett-Packard 9133 data system. Ultrapure helium was the carrier gas, with a column head pressure of 0.75 kg/cm^2. Mass spectra were obtained at 70 eV. Analyses were

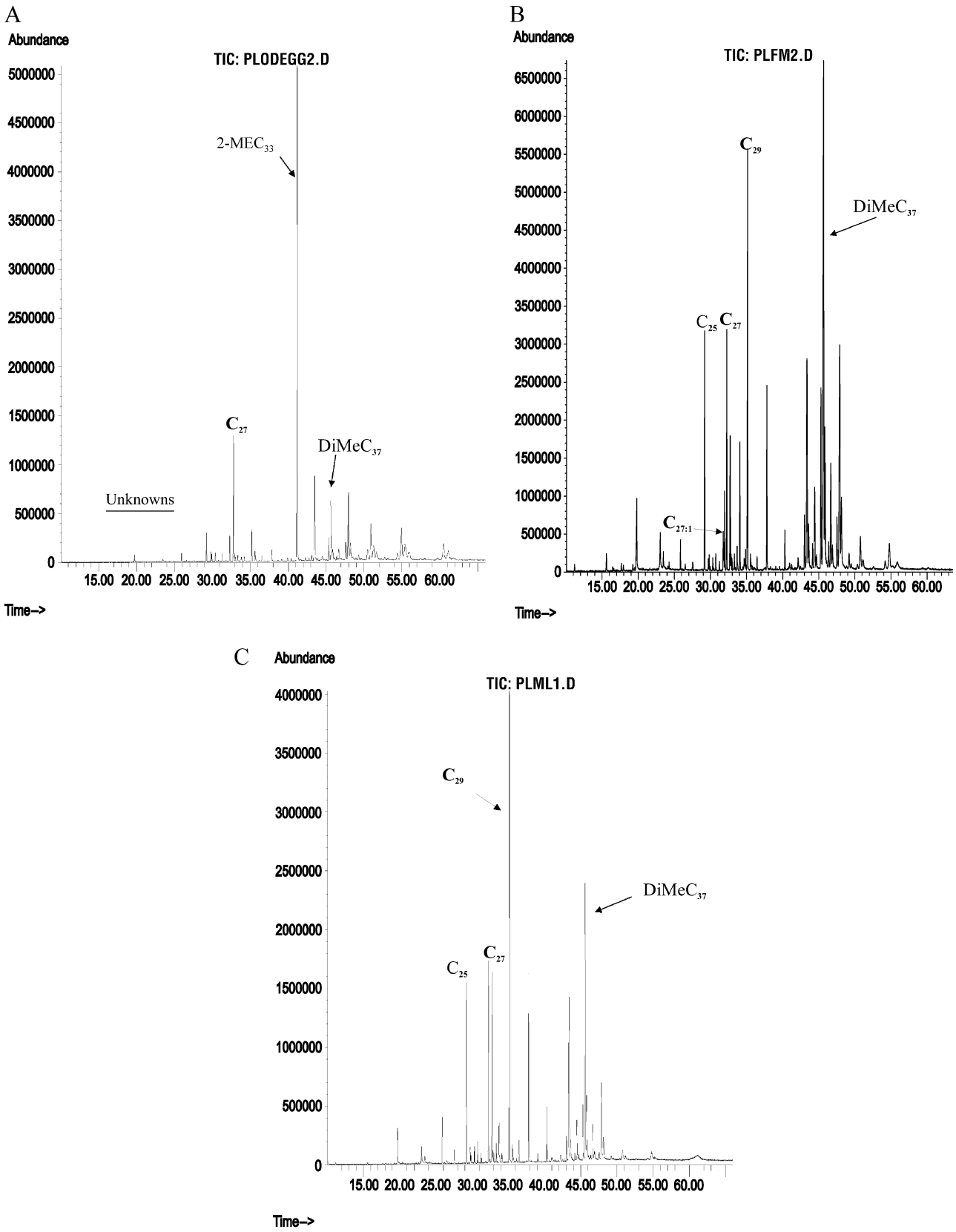

Fig. 2. (A) Reconstructed TIC of cuticular lipids of *P. interpunctella* eggs; (B) reconstructed TIC of cuticular lipids of an adult female *P. interpunctella*; (C) reconstructed TIC of cuticular lipids of an adult male *P. interpunctella*.

Table 1
Cuticular hydrocarbons of *P. interpunctella*

Hydrocarbon	CN	ECL	Diagnostic EI-MS ions *m/z*[a]	Present in life stage						
				Egg	la1[b]	la3[c]	la5[d]	Pu[e]	♀[f]	♂[g]
C_{23}	23	23.00	324	Y	N	N	N	Y	Y	Y
C_{24}	24	24.00	338	Y	N	N	N	N	Y	Y
C_{25}	25	25.00	352	Y	N	Y	N	Y	Y	Y
11-, 13-MeC_{25}	26	25.30	169, 225; 197; 351	Y	N	N	N	N	Y	Y
7-MeC_{25}	26	25.35	113, 281; 351	Y	N	N	N	N	Y	Y
5-MeC_{25}	26	25.40	85, 309; 351	Y	N	N	N	N	Y	Y
3-MeC_{25}	26	25.70	337; 351	Y	N	N	N	N	Y	Y
C_{26}	26	26.00	366	Y	N	N	N	N	Y	Y
12-, 13-MeC_{26}	27	26.30	183, 225; 197, 210; 365	Y	N	N	N	N	Y	Y
Z-11-$C_{27:1}$	27	26.69	378 [201, 271, 472]	Y	N	N	N	N	Y	N
Z-9-$C_{27:1}$	27	26.76	378 [173, 299, 472]	Y	N	N	N	N	Y	N
Z-7-$C_{27:1}$	27	26.82	378 [145, 327, 472]	Y	N	N	N	N	Y	N
C_{27}	27	27.00	380	Y	Y	Y	N	Y	Y	Y
11-, 13-MeC_{27}	28	27.30	169, 253; 197, 225; 379	Y	N	N	N	N	Y	Y
7-MeC_{27}	28	27.35	113, 309; 379	Y	N	N	N	N	Y	Y
5-MeC_{27}	28	27.40	85, 337; 379	Y	N	N	N	N	Y	Y
3-MeC_{27}	28	27.70	365; 379	Y	N	N	N	N	Y	Y
C_{28}	28	28.00	394	Y	N	N	N	N	Y	Y
3, 15-DiMeC_{27}	29	28.05	379, 197, 239; 393	Y	N	N	N	N	Y	Y
13-, 14-MeC_{28}	29	28.30	197, 239; 210-, 225; 393	Y	N	N	N	N	Y	Y
Z-11-$C_{29:1}$	29	28.69	406 [201, 299, 500]	Y	N	N	N	N	Y	N
Z-9-$C_{29:1}$	29	28.76	406 [173, 327, 500]	Y	N	N	N	N	Y	N
Z-7-$C_{29:1}$	29	28.82	406 [145, 355, 500]	Y	N	N	N	N	Y	N
C_{29}	29	29.00	408	Y	Y	Y	Y	Y	Y	Y
11-, 13-MeC_{29}	30	29.30	169, 281; 197, 253; 407	Y	N	N	N	N	Y	Y
7-MeC_{29}	30	29.35	113, 337; 407	Y	N	N	N	N	Y	Y
5-MeC_{29}	30	29.40	85, 365; 407	Y	N	N	N	N	Y	Y
3-MeC_{29}	30	29.70	393; 407	Y	N	N	N	N	Y	Y
C_{30}	30	30.00	422	Y	N	N	N	N	Y	Y
C_{31}	31	31.00	436	Y	Y	N	N	Y	Y	Y
C_{32}	32	32.00	450	Y	N	N	N	N	Y	Y
C_{33}	33	33.00	464	Y	N	N	N	Y	Y	Y
2-MeC33	34	33.59	435; 463	Y	N	N	N	N	N	N
12, 16-DiMeC_{34}	36	34.48	183, 351, 253, 281	Y	N	N	N	N	Y	Y
13-, 15-, 17-MeC_{35}	36	35.3	197, 337; 225, 309; 253, 281	Y	N	N	N	N	Y	Y
13, 17-DiMeC_{35}	37	35.53	197, 351, 267, 281; 505	Y	N	N	N	N	Y	Y
11, 15-DiMeC_{35}	37	35.53	169, 379, 239, 309; 505	N	N	N	N	N	Y	Y
2-MeC_{35}	36	35.59	463; 491	Y	N	N	N	N	N	N
13, 17, 21-TriMeC_{35}	38	36.05	197, 365, 267, 295, 225, 337	N	N	N	N	N	Y	Y
11, 15, 19-TriMeC_{35}	38	36.05	169, 393, 239, 323, 253, 309	N	N	N	N	N	Y	Y
14-, 15-, 16-MeC_{36}	37	36.32	211, 337; 225, 323; 239, 309	Y	N	N	N	N	Y	Y
12, 16-DiMeC_{36}	38	36.48	183, 379, 253, 309; 519	Y	N	N	N	N	Y	Y
14, 18-DiMeC_{36}	38	36.48	211, 351, 281; 519	Y	N	N	N	N	Y	Y
12, 16, 20-TriMeC_{36}	39	37.05	183, 393, 253, 323	N	N	N	N	N	Y	Y
13-, 15-, 17-, 19-MeC_{37}	38	37.32	197, 365; 225, 337; 253, 309; 281; 519	Y	N	N	N	N	Y	Y
15, 19-DiMeC_{37}	39	37.53	225, 351, 281, 295; 533	Y	N	N	N	N	Y	Y
13, 17-DiMeC_{37}	39	37.53	197, 379, 267, 309; 533	Y	N	N	N	N	Y	Y
11, 15-DiMeC_{37}	39	37.53	169, 407, 239, 337; 533	Y	N	N	N	N	Y	Y
13, 17, 21-TriMeC_{37}	40	38.05	197, 393, 267, 323, 253, 337	Y	N	N	N	N	Y	Y
11, 15, 19-TriMeC_{37}	40	38.05	169, 421, 239, 351, 281, 309	Y	N	N	N	N	Y	Y
14-, 16-MeC_{38}	39	38.32	211, 365; 239, 337	Y	N	N	N	N	Y	Y
14, 18-DiMeC_{38}	40	38.48	211, 379, 281, 309	Y	N	N	N	N	Y	Y
12, 16-DiMeC_{38}	40	38.48	183, 407, 253, 337	Y	N	N	N	N	Y	Y
14, 18, 22-TriMeC_{38}	41	39.05	211, 393, 281, 323, 253, 351	Y	N	N	N	N	Y	Y
12, 16, 20-TriMeC_{38}	41	39.05	183, 421, 253, 351, 281, 323	Y	N	N	N	N	Y	Y
13-, 15-MeC_{39}	40	39.32	197, 393; 225, 365	Y	N	N	N	N	Y	Y
13, 17-DiMeC_{39}	41	39.53	197, 407, 267, 337; 561	Y	N	N	N	N	Y	Y
11, 15-DiMeC_{39}	41	39.53	169, 435, 239, 365; 561	Y	N	N	N	N	Y	Y
13, 17, 21-TriMeC_{39}	42	40.05	197, 421, 267, 351, 281, 337	Y	N	N	N	N	Y	Y
11, 15, 19-TriMeC_{39}	42	40.05	169, 449, 239, 379, 309	Y	N	N	N	N	Y	Y
12, 16-DiMeC_{40}	42	40.48	183, 435, 253, 365	Y	N	N	N	N	Y	Y

Table 1 (*continued*)

Hydrocarbon	CN	ECL	Diagnostic EI-MS ions *m/z*[a]	Present in life stage						
				Egg	la1[b]	la3[c]	la5[d]	Pu[e]	♀[f]	♂[g]
15-MeC$_{41}$	42	41.32	225, 393	Y	N	N	N	N	Y	Y
13, 17-DiMeC$_{41}$	43	41.53	197, 435, 267, 365	Y	N	N	N	N	Y	Y
11, 15-DiMeC$_{41}$	43	41.53	169, 463, 239, 393	Y	N	N	N	N	Y	Y
13, 17, 21-TriMeC$_{41}$	44	42.05	197, 449, 267, 379, 309, 337	Y	N	N	N	N	Y	Y
13-, 15-MeC$_{43}$	44	43.32	197, 449; 225, 421	Y	N	N	N	N	Y	Y
13, 17-DiMeC$_{43}$	45	43.53	197, 463, 267, 393	Y	N	N	N	N	Y	Y
11, 15-DiMeC$_{43}$	45	43.53	169, 491, 239, 421	Y	N	N	N	N	Y	Y
13-MeC$_{45}$	46	45.32	197, 477	Y	N	N	N	N	N	Y
13, 17-DiMeC$_{45}$	47	45.53	197, 491, 267, 421	Y	N	N	N	N	Y	Y

CN, carbon number; ECL, equivalent chain length. Y, present; N, not detected.

[a] Ions in brackets are from the dithiomethyl ether derivatives.

[b] 1st instar larvae.

[c] 3rd instar larvae.

[d] 5th instar larvae.

[e] Pupa.

[f] Adult female.

[g] Adult male.

conducted using temperature programming, with an initial temperature of 100 °C, a final temperature of 320 °C, a program rate of 5 °C/min, and a 20-min final hold period. The splitless injector was set at 275 °C and the GC–MS interface was at 280 °C. Retention times of each component and equivalent chain length values (ECL) were obtained by comparison with known *n*-alkane standards (Howard et al., 1978). Individual components in the total ion scanning mode were identified from their characteristic EI-MS fragmentation patterns (Jackson and Blomquist, 1976; Nelson, 1978; Mudd, 1981; Nemoto et al., 1987a,b) in conjunction with equivalent chain length values. Double-bond locations in alkenes were obtained by preparing dithiomethyl ethers and examining their electron impact mass spectra (Francis and Veland, 1981). Stereochemistry of the parent alkenes was established by comparison to ECL values of known alkenes from previously identified cuticular hydrocarbon samples. Each isomer is nearly base-line separated from other isomers of the same carbon number and the ECL values are highly reproducible.

3. Results

3.1. Morphology

All stages of pyralid larvae have well-developed silk and mandibular glands (Fig. 1; Mossadegh, 1978). Both pairs of

Table 2
Composition of 2-acyl-1,3-cyclohexanediones present on the cuticle of various life stages of *P. interpunctella*

Component	CN	ECL	Diagnostic EI-MS ions m/z	Egg	la1[a]	la3[b]	la5[c]	Pu[d]	♀[e]	♂[f]
16:1 CHD	22	27.09	139, 154, 167, 348 (M^+)	N	Y	Y	Y	Y	N	N
16:0 CHD	22	27.20	139, 154, 167, 332 (M-18), 350 (M^+)	N	Y	Y	Y	Y	N	N
16:1 hydroxy CHD[i]	22	27.84	137, 168, 183, 346 (M-18), 364 (M^+)	N	Y	Y	Y	Y	N	N
16:2 hydroxy CHD	22	27.93	137, 168, 183, 344 (M-18), 362 (M^+)	N	Y	Y	Y	Y	N	N
16:1 hydroxy CHD	22	27.98	137, 168, 183, 346 (M-18), 364 (M^+)	N	Y	Y	Y	Y	N	N
16:0 hydroxy CHD	22	28.06	140, 170, 183, 348 (M-18), 366 (M^+)	N	Y	Y	Y	Y	N	N
18:2 CHD	24	28.97	139, 154, 167, 356 (M-18)	N	Y	Y	Y	Y	N	N
18:1 CHD	24	29.05	139, 154, 167, 358 (M-18)	N	Y	Y	Y	Y	N	N
18:1 CHD	24	29.22	139, 154, 167, 358 (M-18)	N	Y	Y	Y	Y	N	N
18:0 CHD	24	29.29	139, 154, 167, 360 (M-18), 378 (M^+)	N	Y	Y	Y	Y	N	N
18:1 hydroxy CHD	24	29.80	137, 168, 183, 374 (M-18), 392 (M^+)	N	Y	Y	Y	Y	N	N
18:2 hydroxy CHD	24	30.59	137, 168, 183, 372 (M-18), 390 (M^+)	N	Y	Y	Y	Y	N	N
20:1 CHD	26	31.17	139, 154, 167, 404 (M^+)	N	Y	Y	Y	Y	N	N

CN, carbon number; ECL, equivalent chain length. Y, present; N, not detected.

Nomenclature for 2-acyl-1,3-cyclohexanediones: CHD = 1,3-cyclohexanedione; 16:1 indicates a 2-acyl moiety of 16 carbons with one double bond; Hydroxy CHD = 4-hydroxy-1,3-cyclohexanedione. See Fig. 3 for structures.

[a] 1st instar larvae.

[b] 3rd instar larvae.

[c] 5th instar larvae.

[d] Pupa.

[e] Adult female.

[f] Adult male.

glands can be removed intact by careful dissection of the larval head from the thoracic region. The distinct paired mandibular glands usually remain attached to the head capsule and have a diameter of ca. 0.2 mm where they empty into the buccal cavity. The glands gradually narrow to a diameter of ca. 0.04 mm in the longer terminal regions that loop deep within the hemocoel.

3.2. Stage-specific cuticular chemistry

The surface lipids of eggs and adults of *P. interpunctella* consist solely of cuticular hydrocarbons such as are found in the vast majority of insects (Fig. 2A–C; Howard, 1993; Nelson and Blomquist, 1995). These comprise *n*-alkanes (C_{23}–C_{33}), monomethyl alkanes (2-, 3-, 5-, 7-,11-, 12-, 13-, 14-, 15-, 16-, 17-methyl), dimethyl alkanes (3, *X*- and internally branched *X,Y*-dimethyl with three methylene units between branches), trimethyl alkanes, all methyl groups internally located with three methylene units between each methyl branch point, and in adult females only, a series of monoenes with the double bond at Δ^{11}-, Δ^{9}- and Δ^{7} (Table 1). The cuticular hydrocarbons of eggs are qualitatively nearly identical to those of adult females with the exception that 2-methyl alkanes only occur in eggs (Table 1). 2-Methyl C_{33}, by far the largest egg component, constituted about 36% of the total ion count. However, distinctive quantitative differences among individual hydrocarbon components occur for the eggs and adult females (Figs. 2A vs. 2B).

Gender-specific differences were found for the adults, where females contained substantial quantities of Z-11-, Z-9-, and Z-7-$C_{27:1}$ and -$C_{29:1}$ monenes that were not present in males. Otherwise, the two sexes had qualitatively identical profiles (Fig. 2B,C). Based on total ion count in three replicate samples of males and females, the percentage composition (mean ± S.E.M.) of major hydrocarbons in adult females was C_{25} (4.4 ± 0.1%), C_{27} (4.7 ± 0.3%), MeC_{27} (3.3 ± 0.7%), C_{29} (10.7 ± 1.0%),a C_{31} (3.8 ± 0.3%), $DiMeC_{35}$ (8.2 ± 0.2%), $DiMeC_{37}$ (19.2 ± 0.3%) and $DiMeC_{39}$ (8.0 ± 0.7%). Similarly, the percentage composition of major hydrocarbons in adult males was C_{25} (3.3 ± 1.0%), C_{27} (3.4 ± 1.3%), MeC_{27} (3.2 ± 1.5%), C_{29} (9.3 ± 3.5%), C_{31} (3.7 ± 1.3%), $DiMeC_{35}$ (9.4 ± 0.6%), $DiMeC_{37}$ (19.7 ± 2.0%) and $DiMeC_{39}$ (7.8 ± 1.6%).

The larval and pupal stages also contained cuticular hydrocarbons, but in very small quantities (<5% of the total extract), and only *n*-alkanes were present (Table 1). The dominant chemicals extracted from the larvae and pupae were 2-acyl-1,3-cyclohexanediones (Table 2, Figs. 3 and 4A–D). They consist of homologous series of 2-acyl-1,3-cyclohexanediones with or without a hydroxy at C-4 of the cyclohexane ring, and with or without one or more double bonds in the 2-acyl side chain. We have not determined the location of double bonds or their stereochemistry for the unsaturated 2-acyl compounds. These chemicals are produced and stored in the paired mandibular glands (Mossadegh, 1978), and the contents of the glands

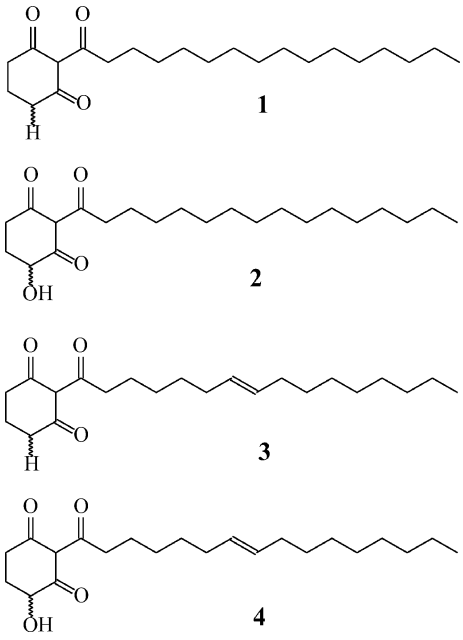

Fig. 3. Representative structures of 2-acyl-1,3-cyclohexanediones (**1** and **3**) and 2-acyl-4-hydroxy-1,3-cyclohexanediones (**2** and **4**) previously described from *P. interpunctella*. Depicted double bond stereochemistry in 2-acyl side chain is arbitrary.

(Fig. 4B) are qualitatively identical to the chemicals recovered from the larval and pupal cuticle (Fig. 4A,C,D). SPME analysis of the head, thorax and abdomen of 5th instar larvae indicated that the same mixtures of 2-acyl-1,3-cyclohexanediones were found on each body region (Fig. 5A–C). The isomeric mixture of pupal 2-acyl-1,3-cyclohexanediones contains more hydroxy isomers than found on the larval stages. Additional components eluting before the 2-acyl-1,3-cyclohexanediones have not been identified, but are found both in the glands and on the cuticle. Preliminary mass spectral analysis suggests that these early eluting components are possibly aldehydes and unusual unsaturated esters. Gravimetric analysis indicated that wandering stage 5th instar larvae have ca. 6.3 μg of mandibular gland chemicals on their cuticle which corresponds to ca. 0.05% of fresh body mass.

Analysis by GC–MS of the hexane soluble lipids in *P. interpunctella* larval hemolymph revealed only fatty acids, sterols and a low abundance of *n*-alkanes (C_{27}–C_{31}, with C_{29} as the major component). No 2-acyl-1,3-cyclohexanediones were recovered, indicating that the hydrocarbons observed were not cuticular contaminants.

4. Discussion

4.1. Cuticular hydrocarbons

Although many studies have been conducted on the cuticular hydrocarbons of insects (Howard, 1993; Lockey, 1988, 1991; Nelson and Blomquist, 1995), we have found

only two previous studies of the cuticular hydrocarbons of moths in the family Pyralidae: the adults of four species of pine coneworms (*Dioryctria* spp.; Richmond and Page, 1995) and larvae of the stored product pest *Ephestia* (*Anagasta*) *kuehniella* (Hebanowska et al., 1990). These species are members of the subfamily Phycitinae, as is *P. interpunctella*. The coneworms were not characterized by sex or age, and the *E. kuehniella* larvae were soaked for 2 weeks in methylene chloride before chemical analysis, thus mitigating any claim to have examined only "cuticular hydrocarbons." Among the adults of the four sibling coneworm species, 12 *n*-alkanes, 28 monomethyl alkanes, 18 dimethyl alkanes, 6 trimethyl alkanes and 2 alkenes were wholly or partially identified (Richmond and Page, 1995) and enough differences among species were observed to allow taxonomic separation. Some components found on the cuticle of the coneworm adults are also found on *Plodia* adults, but numerous differences also occur, especially among the dimethyl alkanes. In the coneworms, 11 methylene units occur between methyl branches, but in the dimethyl alkanes of *Plodia* only three methylene units occur between the methyl branches. We identified 86 hydrocarbons in *P. interpunctella*: 11 *n*-alkanes, 39 monomethyl alkanes, 19 dimethyl alkanes, 11 trimethyl alkanes and 6 monoenes. The adults contained 84 of these hydrocarbons, with 2-MeC_{33} and 2-MeC_{35} only occurring in the egg stage. Likewise, the 6 monoenes ($C_{27:1}$ and $C_{29:1}$) only occurred in the females and their eggs, suggesting that at least for the adult females, these alkenes may be involved in sexual recognition or as contact pheromones. The functional sig-

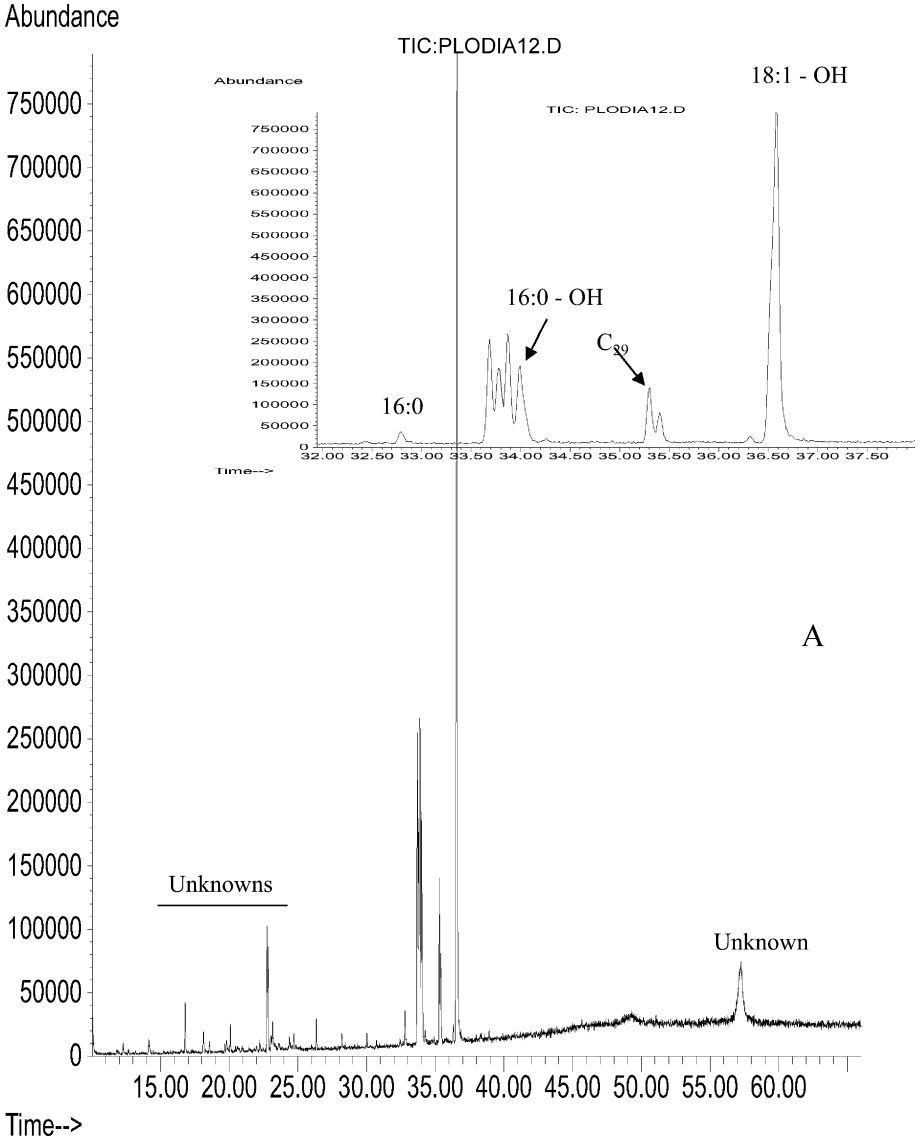

Fig. 4. (A) Reconstructed TIC of cuticular lipids from a 5th instar larval *P. interpunctella*; (B) reconstructed TIC of the hexane extract of dissected mandibular glands from a 5th instar larval *P. interpunctella*; (C) reconstructed TIC of the cuticular lipids from a female pupa of *P. interpunctella*; (D) reconstructed TIC of the cuticular lipids from a male pupa of *P. interpunctella*. Inset boxes are expansions of the chromatographic regions containing the 2-acyl- and 2-acyl-4-hydroxy-1,3-cyclohexanediones. See Table 2 for explanations of abbreviations used.

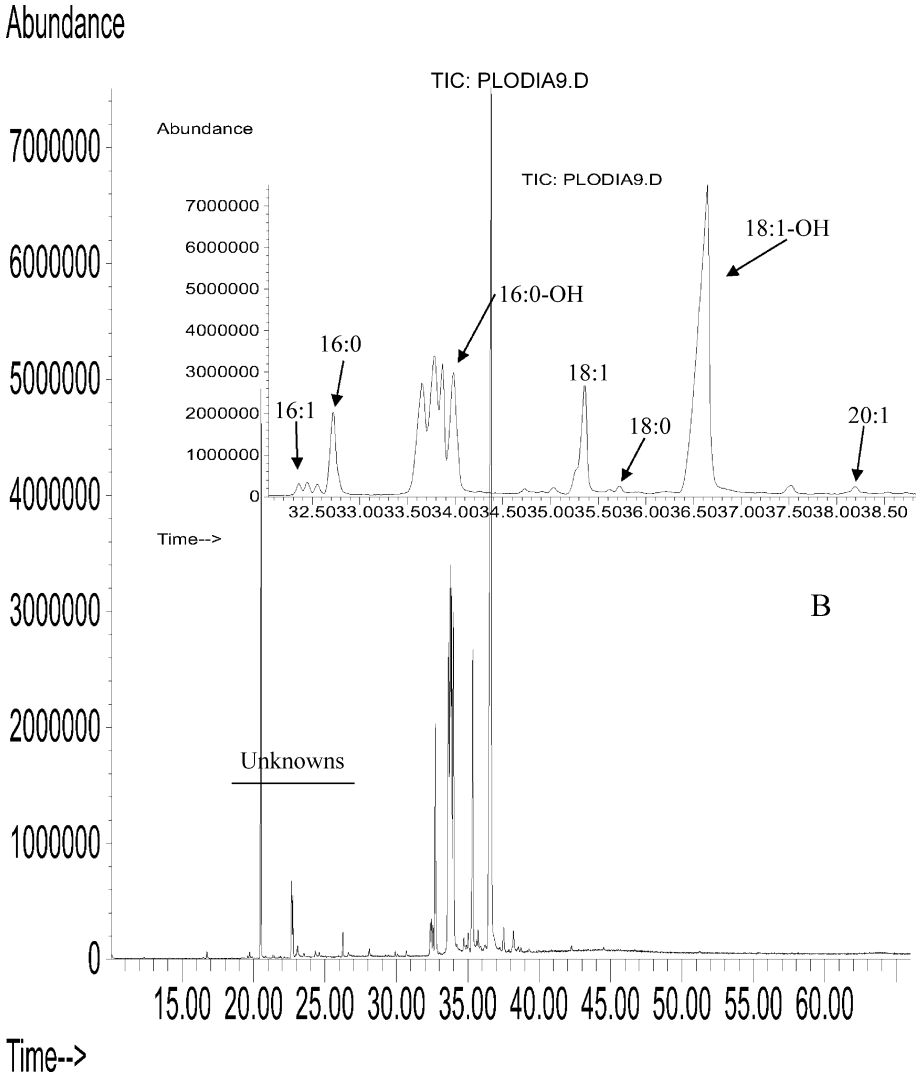

Fig. 4 (*continued*).

nificance of the 2-methyl alkanes on the eggs is unknown. Hydrocarbons were the only lipid components found on the eggs and adults of *P. interpunctella*.

The larval stages had only minor amounts of cuticular hydrocarbons, consisting solely of *n*-alkanes, and by the 5th instar the only hydrocarbon present on the cuticle was *n*-nonacosane. Likewise, the pupae had only minor amounts of cuticular hydrocarbons, again all *n*-alkanes. The low abundance of these hydrocarbons would argue against their providing an effective cuticular lipid layer. The question arises as to whether the near absence of hydrocarbons on the cuticle is a function of essentially no biosynthesis during the larval and pupal stages, or whether it is a case of active biosynthesis, but lack of transport to the cuticle from lipophorin bound hydrocarbons in the hemolymph (Van der Horst et al., 1993). Our analysis of larval hemolymph, where we found very low quantities of *n*-alkanes, clearly indicates that it is the former (low biosynthesis) rather than a lack of transport. We note that none of the acyl cyclohexanediones occurred in the hemolymph.

In the only other report of hydrocarbons from a pyralid larvae (*E. kuehniella*), the authors (Hebanowska et al., 1990) reported very low amounts of *n*-alkanes, monomethyl alkanes, and three dimethyl alkanes. Although the authors described these compounds as ‘‘cuticular hydrocarbons’’, it is likely that the samples were contaminated with internal hydrocarbons since the samples were soaked in solvent for 2 weeks before analysis. No mention is made of the 2-acyl-1,3-cyclohexanediones known from this species (Corbet, 1973b; Mudd, 1981), but their sample preparation involved a silica gel chromatography which would have removed them from their hydrocarbon sample. We have conducted preliminary examinations of the larvae of *E. kuehniella* using both short extraction times with hexane and SPME analysis, and have found abundant 2-acyl-1,3-cyclohexane-

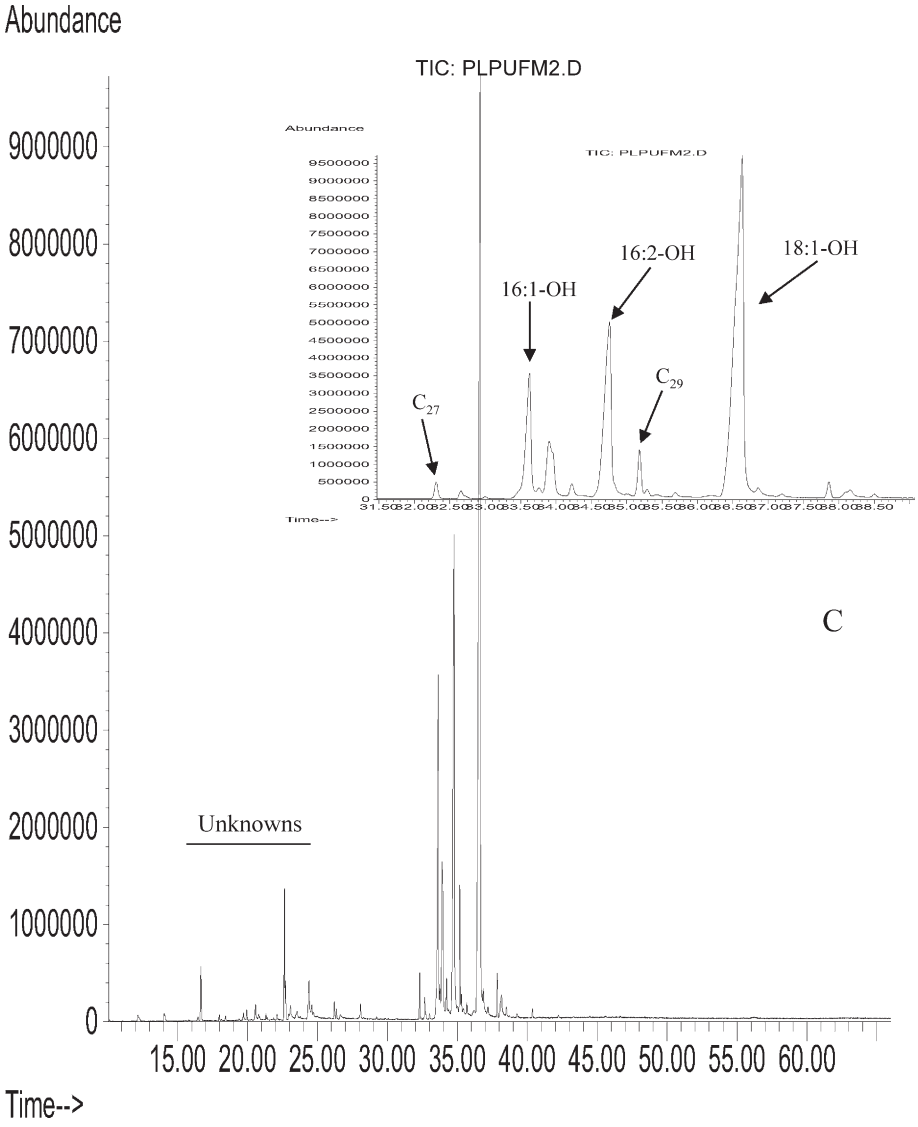

Fig. 4 (*continued*).

diones but no evidence for cuticular hydrocarbons (R.W. Howard, J.E. Baker, unpublished data).

4.2. 2-Acyl-1,3-cyclohexanediones

The foraging behavior of larvae of *P. interpunctella* has been shown to involve the secretion of mandibular chemicals onto silk fibers (which serve to connect food particles, to provide feeding tunnels, moulting shelters and cocoons) as discrete droplets and onto the substrate as a surface film (Mossadegh, 1978). Indeed, the mouthparts of all larval instars are constantly bathed in these secretions, and over the course of five larval instars, they deposit on the silk strands from 3959 to 6412 individual droplets, as well as an unknown (but likely substantial) amount on the substratum while crawling. Based on the scale in Fig. 4 in Mossadegh (1978), the ellipsoidal shape of the droplets, and the number of droplets that he counted (Table 1 in Mossadegh, 1978) during each larval instar including cocoon formation, we estimate that during larval development, 3.83 μl of oil is secreted onto the silk. Assuming a specific gravity of ca. 0.85, each larva would produce about 3.25 mg of oil during its lifetime. However, more oil than this is undoubtedly secreted because of the amount of oil that continually spreads over the larval surface as well as the oil deposited onto the food substrate that was not measured by Mossadegh (1978). When first deposited, the oily, irregular viscous droplets are opaque, but within 3 h they become ovoid and transparent and remarkably stable, surviving at room temperature for several months as well as heating at 90 °C for 24 h (Mossadegh, 1978).

The chemical identity by mass spectrometry, infrared spectrometry, NMR spectrometry and total synthesis of these mandibular gland secretions as 2-acyl-1,3-cyclohexanediones and 2-acyl-4-hydroxy-1,3-cyclohexanediones was first described by Mudd (1981) from *E. kuehniella*.

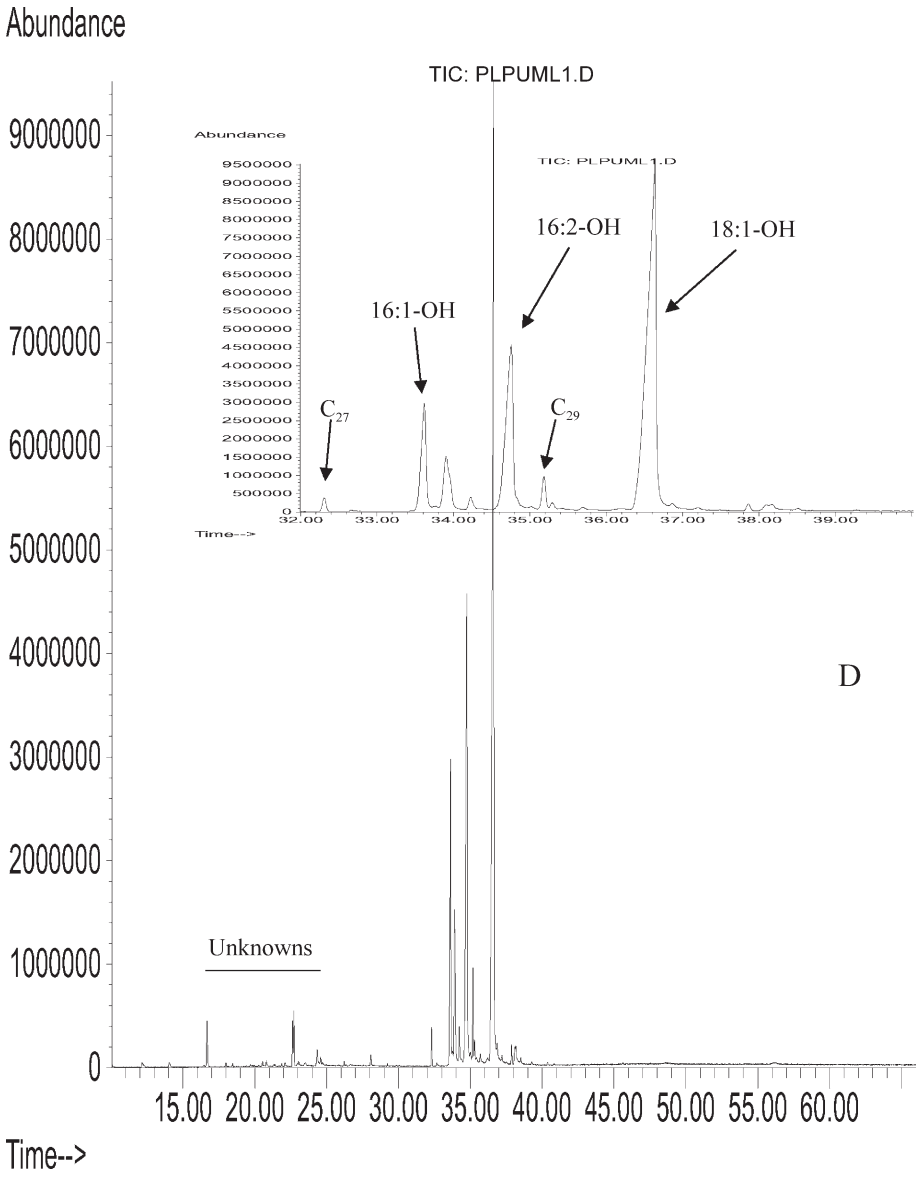

Fig. 4 (*continued*).

Similar mandibular gland secretions were characterized by mass spectrometry and total synthesis from three other pyralids including *P. interpunctella* (Nemoto et al., 1987a,b). The species-specific compositions of the mixtures produced by the mandibular glands of the four species examined differ only in the length and degree of unsaturation of the 2-acyl side chain for each component. We have found more isomers than reported by these early studies because we used capillary gas chromatography columns, whereas they were restricted to the packed columns available at that time.

Clearly, the biochemical investment required for these species to produce such copious quantities of relatively complex chemicals strongly suggests that they serve important functions in maximizing the fitness of these organisms. Indeed, several studies have shown that these compounds are involved in such behavioral functions as prevention of overcrowding by regulation of total numbers via dispersion (Corbet, 1971; Mossadegh, 1980), partitioning of food (Mossadegh, 1980), and regulation of egg density by ovipositing females (Corbet, 1973b; Phillips and Strand, 1994). At least two parasitoids have evolved to use these secretions as kairomonal cues to locate the hosts: *Venturia canescens* (Hymenoptera: Ichneumonidae; Corbet, 1971; Mudd and Corbet, 1973; Mossadegh, 1980) and *Bracon hebetor* (Hymenoptera: Braconidae; Strand et al., 1989).

We propose that an additional physiological function is being served by these mandibular gland secretions: that of a cuticular lipid layer to provide water proofing (Gibbs, 1998, 2002). Our conclusion is based on four major considerations: (1) the 2-acyl-1,3-cyclohexanediones are of a molec-

ular size that approximates many of the common cuticular hydrocarbons known from insects (ECL values of ca. 27 to 31); (2) they occur as homologous series, as do hydrocarbons; (3) the 2-acyl-1,3-cyclohexanediones with saturated 2-acyl side chains (Nemoto et al., 1987b) have melting points similar to that of cuticular hydrocarbons (38° to 57 °C) and the 2-acyl-1,3-cyclohexanediones with unsaturated 2-acyl side chains are high-boiling oils (Mudd, 1981); (4) they are amphiphilic, with the conjugated trione system likely binding via hydrogen bonds with cuticular proteins and chitin, while the strongly hydrophobic hydrocarbon side chains readily repel water (similar to the properties of other polar cuticular lipids) (Buckner, 1993). The stability of these 2-acyl-1,3-cyclohexanediones to environmental parameters such as temperature is noted above (Mossadegh, 1978). That these chemicals are found on all body segments is not surprising considering that the insects are constantly foraging over webs and substrate that have been coated with these secretions. In addition, we have for the first time partially characterized 2-acyl-1,3-cyclohexanediones from the pupal stage. Interestingly, the major pupal components are 2-acyl-4-hydroxy-1,3-cyclohexanediones and tend to have more unsaturation than the larval compounds. Since these compounds are all high-boiling, hydrophobic oils (Mudd, 1981), it is likely that the shift to the hydroxy compounds is a response to the need for desiccation resistance in the non-feeding pupal stage, as well as providing a deterrence to predators (Corbet, 1973a). It appears that the relative proportions of 2-acyl-1,3-cyclohexanediones and 2-acyl-4-hydroxy-1,3-cyclohexanediones vary markedly between the

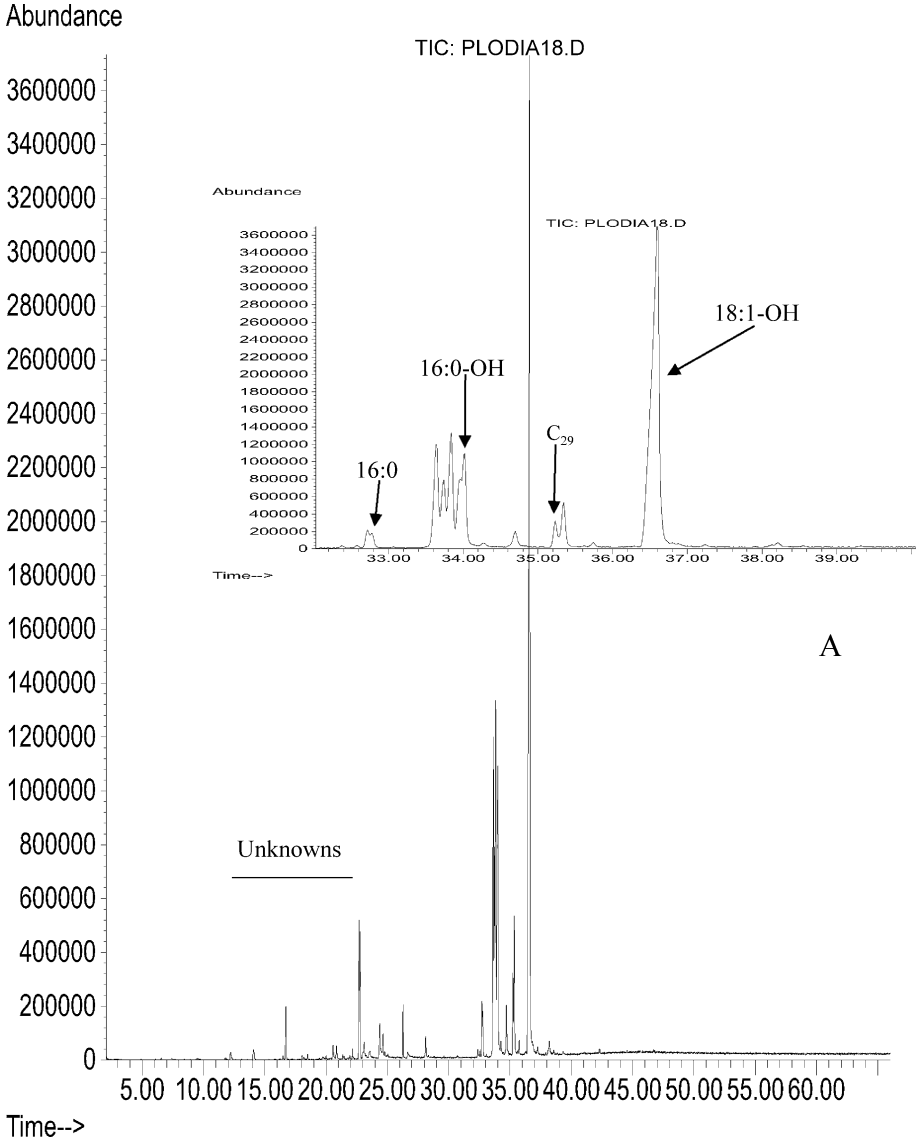

Fig. 5. (A) Reconstructed TIC of a SPME analysis of the head capsule and prothoracic segment cuticular lipids from a 5th instar larva of *P. interpunctella*; (B) reconstructed TIC of a SPME analysis of the cuticular lipids from the 1st proleg thoracic segment of a 5th instar larva of P. *interpunctella*; (C) reconstructed TIC of a SPME analysis of the cuticular lipids on the 9th and 10th abdominal segments of a 5th instar larva of *P. interpunctella*. Inset boxes are expansions of the chromatographic regions containing the 2-acyl- and 2-acyl-4-hydroxy-1,3-cyclohexanediones. See Table 2 for explanations of abbreviations used.

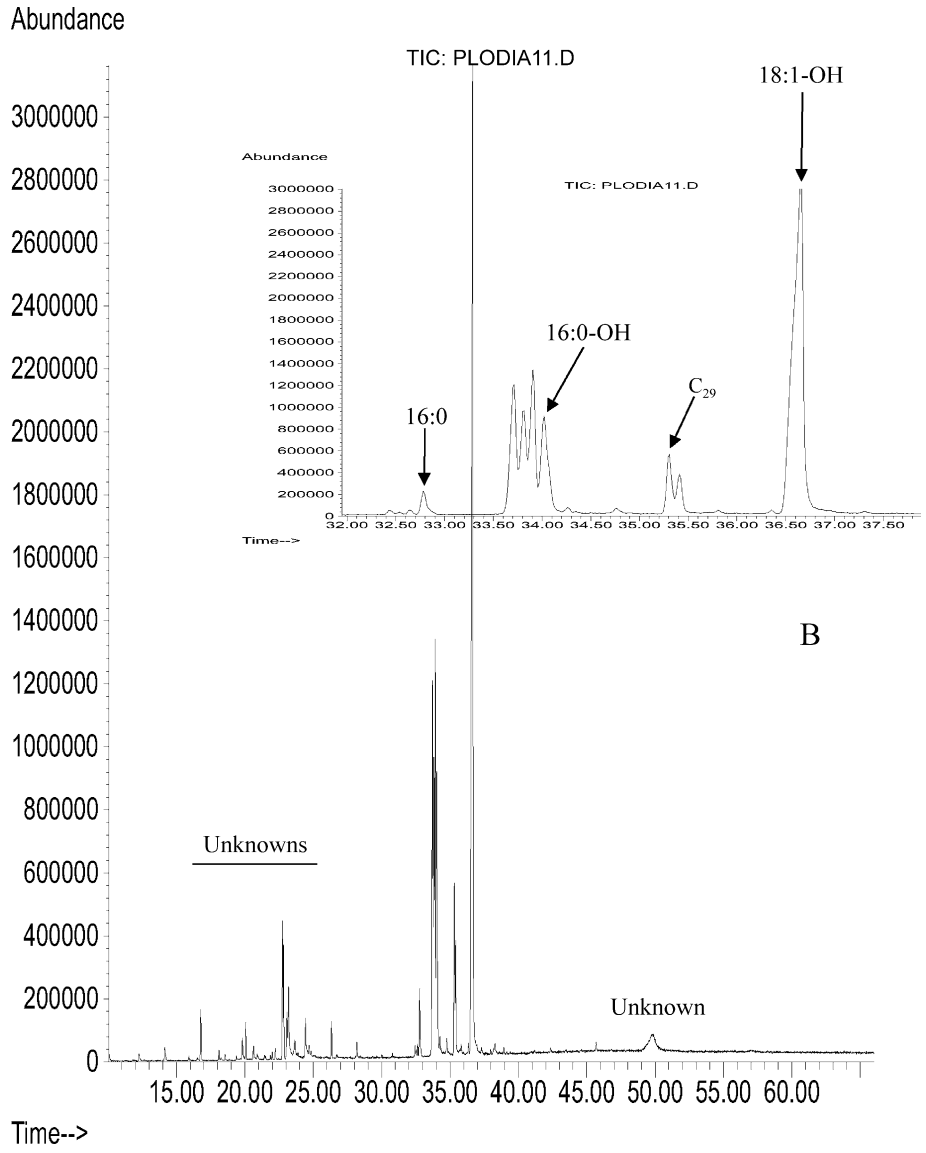

Fig. 5 (*continued*).

actively feeding larval stages and the wandering prepupal, non-feeding last larval stage. As noted in Table 2, all larval stages possess both the non-hydroxy and hydroxy compounds, but the 2-acyl-4-hydroxy-1,3-cyclohexanediones do not become major components of the mandibular gland secretions until the wandering phase begins. The pupae do not have mandibular glands, but in the course of spinning the silk cocoon, the wandering phase larvae completely coat the silk with the 2-acyl-4-hydroxy-1,3-cylohexanediones, and the pupae makes considerable movement during the process of converting to an adult, resulting in a mechanical transfer of the secretions to the pupal cuticle (Mossadegh, 1978, 1980).

It would appear that *P. interpunctella* has evolved a parsimonious approach to the problem of water retention and water proofing in its various life stages (Gibbs, 1998, 2002). Although other insects are known to use different lipid compositions in different life stages as a means of responding to different ecological niches (Howard, 1993; Nelson and Blomquist, 1995), we are not aware of any other example where cuticular hydrocarbons are virtually eliminated from the insect lipid profile. *P. interpunctella* (and likely other stored product pyralids known to have 2-acyl-1,3-cyclohexanediones) invests enormous amounts of biochemical energy resources to produce the mandibular gland secretions which play such a dominant role in their foraging and population ecology (Mossadegh, 1978). In as much as these secretions have physical and chemical properties which allow them to effectively function as cuticular lipids, it is not surprising that these insects effectively put hydro-

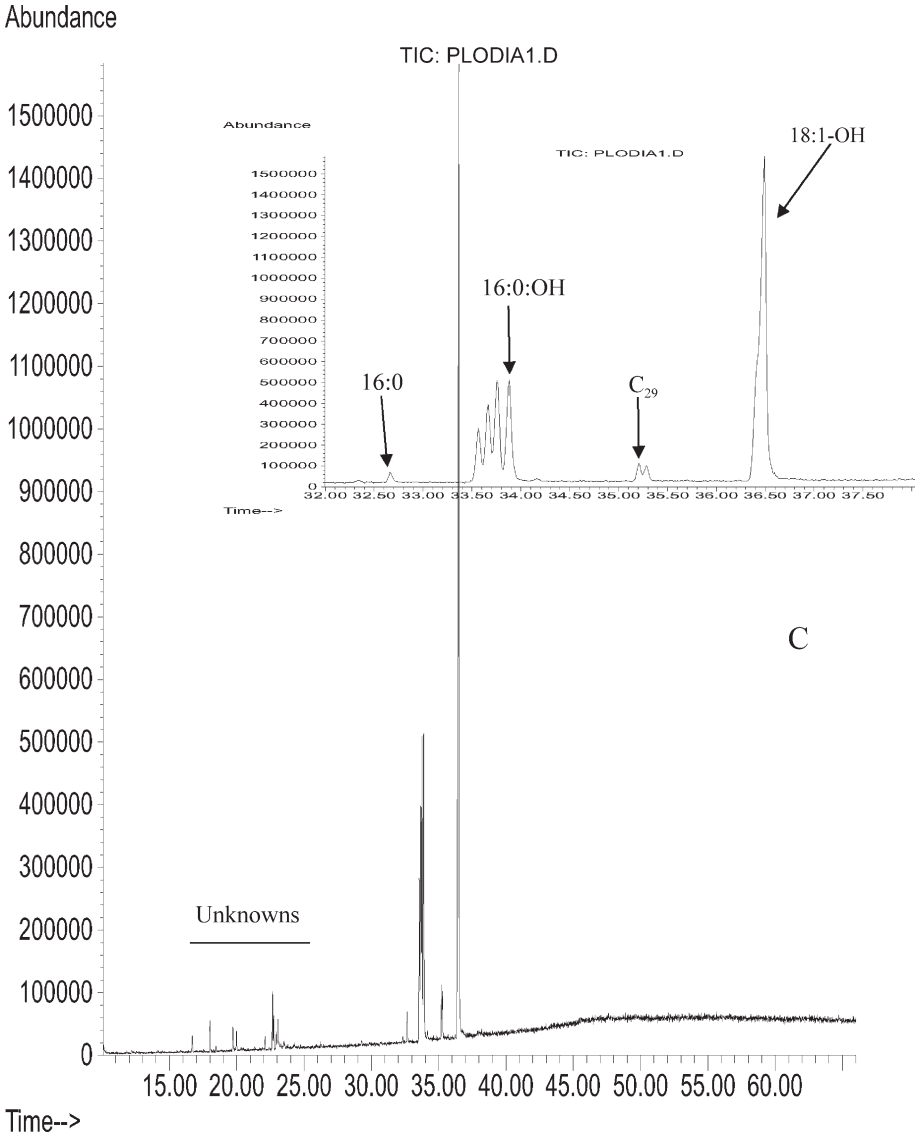

Fig. 5 (*continued*).

carbon production and use on hold until a life stage is reached where the 2-acyl-1,3-cyclohexanediones are no longer present. The biochemical pathways by which these two approaches to water conservation are regulated would seem to offer a very interesting problem that deserves further attention.

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