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Acetate esters of saturated and unsaturated alcohols (C_{12}–C_{20}) are major components in Dufour glands of *Bracon cephi* and *Bracon lissogaster* (Hymenoptera: Braconidae), parasitoids of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae)

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Abstract

Chemistry of Dufour glands associated with the venom complex in *Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck (Hymenoptera; Braconidae), two parasitoids of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), was examined by solid phase micro-extraction (SPME) and gas chromatography/mass spectrometry. Homologous series of five chemical classes were detected in individual glands from each species. Major classes included: (1) acetate esters of saturated and unsaturated primary alcohols with parent chain lengths from C_{12} to C_{20}. Hexadecanyl acetate, octadecanyl acetate, and octadecenyl acetate were major components in *B. cephi*. The composition of the acetate series in *B. lissogaster* was

Abbreviations: 13-MeC29, 13-methylnonacosane.

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similar except that the octadecanyl acetate was only a minor component. (2) A homologous series of monoenes from $C_{23:1}$ to $C_{35:1}$ were detected in both species, with $C_{29:1}$, $C_{31:1}$ and $C_{33:1}$ being the major components. Dienes from $C_{31:2}$ to $C_{35:2}$ and trienes ($C_{33:3}$–$C_{35:3}$) were also detected in both species. (3) A homologous series of *n*-alkanes from C_{19} to C_{31} was detected in both species. *n*-Tricosane was the major component. Minor components in both species included homologous series of both mono- and dimethyl branched alkanes. The Dufour gland hydrocarbon components in both *B. cephi* and *B. lissogaster* have some similarities to the composition of cuticular hydrocarbons of their host *C. cinctus*, a species with a complex pheromonal signaling system.
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Keywords: Braconidae; Parasitoid; Dufour gland; Semiochemicals; Acetate esters; Hydrocarbons; Alkenes

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The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) is a major insect pest of wheat grown in the Great Plains, including Montana and North Dakota. Larval feeding within the stem reduces phloem tissue and decreases seed quality and yield. Another problem caused by large infestations of this species is the severe lodging that occurs because of stem girdling by mature larvae. The biology and population dynamics of this sawfly have been described (Ainslie, 1920; Holmes, 1982) and progress has been made in understanding the complex chemical ecology of this species (Bartelt et al., 2002; Cossé et al., 2002). Chemical control of *C. cinctus* is generally not cost effective because of the prolonged flight period of up to 6 weeks, when the short-lived adults emerge and oviposit (Weiss and Morrill, 1992). Insecticides are ineffective against the immature stages that are protected within the stems. Agronomic practices, such as planting resistant cultivars (varieties with stems containing solid pith) and swathing before harvest, can reduce the impact of this pest (Weiss and Morrill, 1992). However, in addition to these cultural controls, endemic, host-specific, congeneric parasitoids can also suppress sawfly populations. *Bracon cephi* (Gahan) (Hymenoptera: Braconidae) and *Bracon lissogaster* Muesebeck are two parasitoids associated with infestations of *C. cinctus* (Morrill, 1997; Runyon et al., 2002). The biology of these two braconids has been studied (Nelson and Farstad, 1953; Somsen and Luginbill, 1956) but little information is available on their semiochemical and possible chemically-mediated searching behavior and/or host location/parasitization by these two species.

Dufour glands in female Hymenoptera contain a broad array of semiochemicals that mediate a wide range of behaviors (Ali and Morgan, 1990). Among hymenopterous parasitoids Dufour gland secretions can deter oviposition (Vinson and Guillot, 1972; Guillot et al., 1974), elicit courtship behavior (Syvertsen et al., 1995), mediate oviposition responses (Mudd et al., 1982; Marris et al., 1996), and can elicit egg-mass leaving behavior in an egg parasitoid (Rosi et al., 2001). To provide an initial basis for understanding the chemical ecology of the wheat stem sawfly parasitoids, we have removed the Dufour glands from *B. cephi* and *B. lissogaster*,

and analyzed chemical components of individual glands by gas chromatography/mass spectrometry.

1. Materials and methods

1.1. Insects

Wheat stems containing wheat stem sawflies parasitized by *B. cephi* and *B. lissogaster* were collected from stubble in a wheat field near Conrad, MT, in the fall of 2002 and held at 4 °C. The stems were shipped to Manhattan, KS, in April 2003, and maintained at 22 °C and 45–55% RH. Adult parasitoids began emerging after 2 weeks (emergence was hastened by slightly misting the straw with tap water). *B. cephi* and *B. lissogaster* were separated according to venation characteristics of the forewings (Runyon et al., 2001) and held in vials with a source of diluted honey for 2–3 days prior to dissection and analysis.

1.2. Dissection of venom apparatus

Procedures for dissection of the venom apparatus in *B. cephi* and *B. lissogaster* were similar to those described by Howard et al. (2003) and Howard and Baker (2003a). Adult females were chilled on ice and immersed in cold 0.9% NaCl containing 0.05% TritonX-100. The thorax was held with one pair of fine forceps and the ovipositor grasped with a second pair of forceps. The ovipositor was gently torn from the abdomen. If done carefully, the ovipositor along with the venom apparatus, including venom reservoir, venom gland and associated duct, and Dufour gland, are removed intact. By separating the sheaths and stylets that make up the ovipositor, the gland complex can be exposed and removed. Gland size was measured with an ocular micrometer at 25×. Glands were photographed through a stereomicroscope equipped with a digital camera.

1.3. Chemical analyses

The elongated, sac-like Dufour glands were pinched off at their base, rinsed 2× in distilled H_2O, and the excess H_2O blotted while the gland was held with forceps. A solid phase micro-extraction (SPME) fiber insert (7 μm polydimethylsiloxane bonded phase, Supelco) in a Supelco SPME holder was positioned under a stereomicroscope and the whole gland macerated onto the tip region of the fiber.[2]

Samples were immediately analyzed on a Hewlett-Packard 5790A gas chromatograph equipped with a DB-5 bonded phase capillary column (15 m long, 0.25 mm i.d.) that was connected to a Hewlett-Packard 5970 mass selective detector and

[2] Mention of a commercial or proprietary product does not constitute an endorsement or a recommendation by USDA for its use.

a Hewlett-Packard 9133 data system. Chromatographic parameters were identical to those of Howard et al. (2003). The fiber was desorbed for 2 min at 280 °C before beginning the temperature program. Individual components detected in the total ion-scanning mode were identified by their characteristic fragmentation pattern, retention time, and equivalent chain length (ECL) relative to known standards. Mean percentage composition of each major peak (based on total ion current) was determined from replicate glands dissected from each species.

2. Results

2.1. Morphology of venom gland complex

The general morphology of the venom gland complex, including the venom reservoir, venom gland filaments, duct, and Dufour gland was similar in *B. cephi* and *B. lissogaster* (Fig. 1). The elongated, translucent, pouch-shaped Dufour glands ranged from 0.5 to 0.9 mm in length in both species. Mean length ± SD was 0.8 ± 0.2 mm for *B. cephi* ($n = 4$), and 0.7 ± 0.1 mm for *B. lissogaster* ($n = 3$). The pear-shaped venom reservoirs are about 0.2–0.3 mm in length and are connected to the ovipositor via a glandular duct approximately 0.6 mm in length. A chitinized

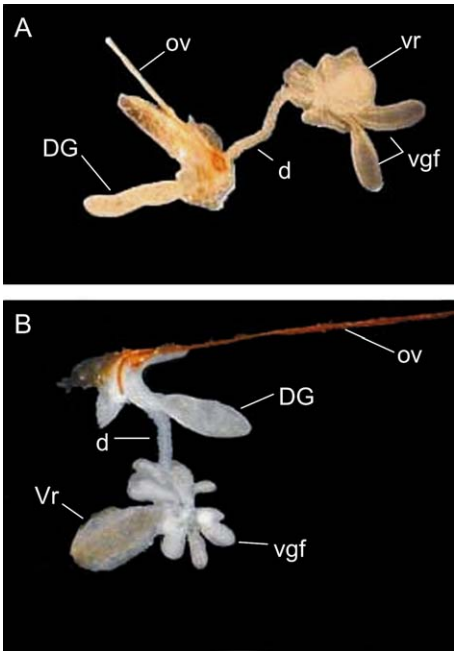

Fig. 1. Venom gland complex dissected from newly emerged *B. cephi* (A) and *B. lissogaster* (B). ov, ovipositor; d, venom gland duct; DG, Dufour gland; vr, venom gland reservoir; vgf, venom gland filaments.

spiral band lines the interior region of the reservoir (Bender, 1943). The venom gland filaments cluster about the base of the venom reservoir in both species. There are differences in the morphologies of the gland filaments between the 2 species (compare Fig. 1A and B). In *B. lissogaster*, about 16 gland filaments are closely appressed to the base of the venom reservoir. These filaments are similar in size and are approximately 0.2–0.28 mm in length. In *B. cephi*, two of the gland filaments are significantly larger (ca. 0.5 mm in length) than the remaining filaments which are similar in size to those of *B. lissogaster*.

2.2. Chemical components of Dufour glands

A complex of chemical components including acetate esters of primary alcohols, unsaturated hydrocarbons, and normal and branched alkanes from the Dufour glands of both species was resolved on the capillary GC column (Fig. 2). For *B. cephi*, about 92 peaks were detected with baseline resolution and 83 had fragment intensities that were sufficient for tentative identification. For *B. lissogaster*, 105 peaks were detected and 71 were similarly identified. Although many components were unsaturated, the location and stereochemistry of double bond locations could not usually be characterized because of the SPME sampling method (the destructive analysis of a small one-time sample), and because insufficient parasitoids were available to conduct the necessary derivatization reactions. For the acetate esters, comparison of retention times and mass spectra of known standards allowed tentative assignment of the double bond location and stereochemistry for some of the components.

2.2.1. Acetate esters

Homologous series of acetate esters of medium chain length (C_{12}–C_{20}) saturated and unsaturated *n*-alcohols were detected in both *B. cephi* and *B. lissogaste*r (Table 1). About 16 acetate components were identified in each species and they represented 29.4% and 23.5% of the total ion count in *B. cephi* and *B. lissogaster*, respectively. Major components in *B. cephi* were hexadecanyl acetate and a peak containing a mixture of octadecenyl acetates. Other major components in *B. cephi* were octadecanyl acetate, hexadecenyl acetate, and tetradecanyl acetate. The parent chain length of the acetates in *B. lissogaster* ranged from C_{12} to C_{22} and also consisted of mono- and di-unsaturated alcohols. Octadecanyl acetate, a major component in *B. cephi*, was only a trace component in *B. lissogaster*. Otherwise, the qualitative composition of acetates detected by SPME in both species was similar.

2.2.2. Unsaturated hydrocarbons

About 23 unsaturated hydrocarbons were resolved by GC in both *B. cephi* and *B. lissogaster*. These components made up 35.5% and 36.8% of the total ion count in these 2 species, respectively. Homologous series of alkenes from $C_{23:1}$ to $C_{35:1}$, alkadienes from $C_{29:2}$ to $C_{35:2}$, and alkatrienes from $C_{33:3}$ to $C_{35:3}$ were detected (Table 2). $C_{29:1}$, $C_{31:1}$, and $C_{33:1}$ were the major components in both *B. cephi* and

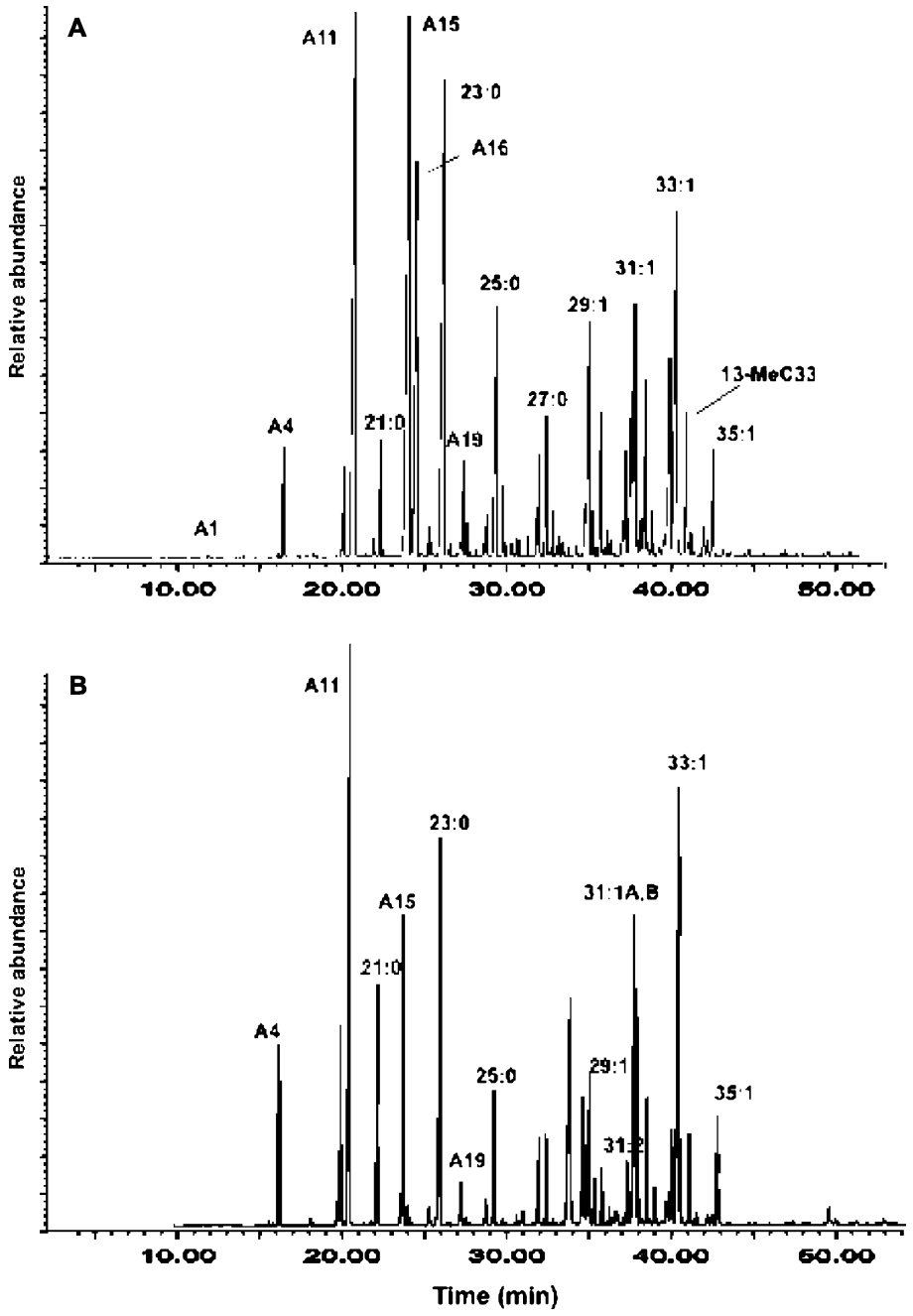

Fig. 2. Total ion chromatogram of the Dufour gland contents of *B. cephi* (A) and *B. lissogaster* (B). Peaks labeled A1, A2, etc., are acetates (see Table 1). Peaks marked with numbers xx:x are hydrocarbons (see Tables 2–5). Note that for clarity not all peaks were labeled.

Table 1
Acetate esters detected in single Dufour glands from the wheat stem sawfly parasitoids *Bracon cephi* and *Bracon lissogaster*

Dufour gland component[a]	Peak number[b]	% of TIC[c]		Diagnostic EI-MS ion fragments[d]
		B. cephi	*B. lissogaster*	
Dodecanyl acetate	A1	Tr	Tr	61, 168*
Z,Z-11,14-tetradecandienyl acetate	A2	ND	Tr	61, 192*
Z-9-tetradecenyl acetate	A3	Tr	Tr	61, 192 (M-62)
Tetradecanyl acetate	A4	0.80 ± 0.07	1.9 ± 1.8	61, 196*
Pentadecanyl acetate	A5	Tr	Tr	61, 210*
Z,Z-11,14-hexdecadienyl acetate	A6	ND	Tr	61, 220*
Z,Z-9,12-hexadecadienyl acetate	A7	Tr	ND	61, 220*
Z-11-hexadecenyl acetate	A8	ND	0.85 ± 0.02	61, 222*
Z-9-hexadecenyl acetate	A9	0.20 ± 0.09	2.9 ± 0.15	61, 222*
Z-7-hexadecenyl acetate	A10	0.60 ± 0.15	Tr	61, 222*
Hexadecanyl acetate	A11	10.4 ± 1.5	12.8 ± 0.63	61, 224*
Z-9-heptadecenyl acetate	A12	ND	Tr	61, 236*
Heptadecanyl acetate	A13	0.1 ± 0.1	ND	61, 238*
Z,Z-9,12-octadecadienyl acetate	A14	Tr	0.24 ± 0.04	61, 248*
Octadecenyl acetate	A15	10.6 ± 1.6	4.0 ± 0.68	61, 250*
Octadecanyl acetate	A16	5.6 ± 1.0	0.10 ± 0.02	61, 252*
Nonadecenyl acetate	A17	Tr	ND	61, 264*
Z,Z-11,14-eicosadienyl acetate	A18	Tr	0.10 ± 0.02	61, 276*
Z,Z,Z-11,14,17-eicosatrienyl acetate	A19	1.1 ± 0.1	0.5 ± 0.08	61, 274*, 334 (M+)
Eicosanyl acetate	A20	0.02 ± 0.02	ND	61, 280*
Docosanyl acetate	A21	ND	0.1 ± 0.01	61, 294*

[a] In order of elution from capillary column. Compounds with assigned double bond location and stereochemistry were identified by comparison to retention times and mass spectra of standards.
[b] Peak number as designated in Fig. 2.
[c] Percentage of total ion count of all Dufour gland components. Mean (S.E.) of 3 replicate glands. Tr = trace amount (area count not integrated). ND, not detected.
[d] The *m*/*z* 61 ion is a protonated acetic acid and is a diagnostic ion fragment for alkyl acetates. Asterisk indicates the (M-60) fragment.

B. lissogaster. The alkadienes and alkatrienes were present at lower levels in both species.

2.2.3. *n*-Alkanes

Dufour glands in both *B. cephi* and *B. lissogaster* contained homologous series of *n*-alkanes with parent chain length from C_{19} to C_{31} (Table 3). *n*-Tricosane was the major *n*-alkane in both species. The *n*-alkanes made up 23.8% and 19.6% of the total ion count in *B. cephi* and *B. lissogaster*, respectively.

2.2.4. Monomethyl branched alkanes

Monomethyl branched alkanes with parent chain lengths from C_{22} to C_{35} were detected in *B. cephi* (Table 4). About 30 positional isomers were detected and they represented 8.3% of the total ion count. The major components were 13-Me-C_{31}, 15-Me-C_{31}, and 13-Me-C_{29} although the concentration of these alkanes was much

Table 2
Unsaturated hydrocarbons detected in single Dufour glands from the wheat stem sawfly parasitoids *Bracon cephi* and *Bracon lissogaster*

Dufour gland component[a]	% of TIC[b]		Diagnostic EI-MS ion fragments (M+)
	B. cephi	*B. lissogaster*	
C23:1 (2 isomers)	0.1 ± 0.02	0.4 ± 0.03	322
C25:1 (2 isomers)	0.4 ± 0.04	0.5 ± 0.02	350
C27:1 (3 isomers)	1.9 ± 0.20	1.53 ± 0.1	378
C29:1 (2 isomers)	6.2 ± 0.9	3.0 ± 0.15	406
C29:2	0.01 ± 0.01	ND	404
C30:1	Tr	ND	420
C31:2	1.5 ± 0.06	1.4 ± 0.08	432
C31:1 (2 isomers)	9.2 ± 1.1	10.7 ± 0.3	434
C32:2	0.1 ± 0.01	ND	432
C33:3	0.1 ± 0.03	0.6 ± 0.06	458
C33:2	4.2 ± 0.07	1.1 ± 0.5	460
C33:1	9.3 ± 0.34	14.7 ± 0.8	462
C34:1	0.2 ± 0.0	0.2 ± 0.01	476
C35:3	0.3 ± 0.06	0.2 ± 0.02	486
C35:2 (2 isomers)	0.1 ± 0.02	0.3 ± 0.02	488
C35:1	1.9 ± 0.2	2.2 ± 0.13	490

[a] In order of elution from capillary column.
[b] Percentage of total ion count. Mean (S.E.) of 3 replicate glands. Tr = trace amount (area count not integrated). ND, not detected.

lower than those of the acetates, the unsaturated hydrocarbons, and the *n*-alkanes. 2- (or 4-)-Me-C_{22} and 2- (or 4-)-Me-C_{24} were detected in Dufour glands of *B. cephi* but were not detected in *B. lissogaster*. Otherwise, the composition of monomethyl alkanes in *B. lissogaster* was similar to that of *B. cephi* with 13-Me-C_{31}, 15-Me-C_{31},

Table 3
n-Alkanes detected in single Dufour glands from the wheat stem sawfly parasitoids *Bracon cephi* and *Bracon lissogaster*

Dufour gland component[a]	% of TIC[b]		Diagnostic EI-MS ion fragments (M+)
	B. cephi	*B. lissogaster*	
C19	Tr	0.1 ± 0.02	268
C21	1.3 ± 0.1	3.7 ± 0.4	296
C22	0.3 ± 0.06	0.3 ± 0.01	310
C23	13.8 ± 0.8	9.9 ± 1.0	324
C24	0.3 ± 0.1	0.1 ± 0.02	338
C25	4.6 ± 0.5	2.4 ± 0.4	352
C26	0.1 ± 0.01	0.1 ± 0.02	366
C27	2.2 ± 0.3	1.7 ± 0.3	380
C28	0.5 ± 0.3	ND	394
C29	0.5 ± 0.1	0.8 ± 0.1	408
C31	0.2 ± 0.03	0.5 ± 0.1	436

[a] In order of elution from capillary column.
[b] Percentage of total ion count. Mean (S.E.) of 3 replicate glands. Tr = trace amount (area count not integrated). ND, not detected.

Table 4
Monomethyl branched alkanes detected in single Dufour glands from the wheat stem sawfly parasitoids *Bracon cephi* and *Bracon lissogaster*

Dufour gland component[a]	% of TIC[b]		Diagnostic EI-MS ion fragments
	B. cephi	*B. lissogaster*	
2-MeC22	0.2 ± 0.03	ND	309, 281
11-MeC23	0.1 ± 0.1	Tr	169, 197
7-MeC23	ND	Tr	113, 253
3-MeC23	Tr	Tr	309
11-, 12-MeC24	0.03 ± 0.01	Tr	169, 211; 183, 197
2- or 4-MeC24	0.08 ± 0.06	ND	337, 309
11-, 13-MeC25	0.62 ± 0.06	0.07 ± 0.01	169, 225; 197
9-MeC25	ND	Tr	141, 253
5-MeC25	0.08 ± 0.02	Tr	85, 309
3-MeC25	0.1 ± 0.00	ND	337
13-MeC26	0.07 ± 0.04	ND	197, 211
11-, 13-MeC27	0.37 ± 0.08	Tr	169, 197, 225, 253
5-MeC27	Tr	Tr	85, 337
3-MeC27	0.15 ± 0.02	ND	365
12-, 13-, 14-MeC28	0.06 ± 0.01	ND	183, 253; 197, 239; 211, 225
13-MeC29	1.61 ± 0.20	0.75 ± 0.05	197, 253, 407 (M-15)
5-MeC29	0.02 ± 0.01	ND	85, 365
12-, 14-, 16-MeC30	0.47 ± 0.10	0.18 ± 0.02	183, 309; 211, 281; 197, 253
13-, 15-MeC31	2.48 ± 0.22	2.38 ± 0.4	197, 281; 225, 253, 435 (M-15)
12-, 14-MeC32	ND	Tr	183, 309; 211, 281
13-MeC33	1.82 ± 0.06	1.48 ± 0.10	197, 309, 463 (M-15)
12-, 14-MeC34	Tr	Tr	183, 337; 211, 309
13-, 15-MeC35	0.02 ± 0.02	0.02 ± 0.03	197, 337; 225, 309

[a] In order of elution from capillary column.
[b] Percentage of total ion count. Mean (S.E.) of 3 replicate glands. Tr = trace amount (area count not integrated). ND, not detected.

and 13-Me-C_{29} being the major components. About 20 positional isomers were detected which represented 4.9% of the total ion count.

2.2.5. Dimethyl branched alkanes

Dimethyl branched alkanes with parent chain lengths from C_{25} to C_{41} (*B. cephi*) and from C_{29} to C_{41} (*B. lissogaster*) were detected by SPME but were very minor components of the Dufour glands in both species (Table 5). Two series of dimethyl alkanes were present: a homologous series of 5,17-dimethyl alkanes and a series of internally-branched alkanes with 3 methylene units between methyl branches. Dimethyl alkanes represented 0.79% and 0.16% of the total ion count in *B. cephi* and *B. lissogaster*, respectively.

3. Discussion

Venom gland filaments that cluster at the base of the venom reservoir in *B. cephi* and *B. lissogaster* are similar to those described by Bender (1943) for another

Table 5
Dimethyl branched alkanes detected in single Dufour glands from the wheat stem sawfly parasitoids *Bracon cephi* and *Bracon lissogaster*

Dufour gland component[a]	% of TIC[b]		Diagnostic EI-MS ion fragments
	B. cephi	*B. lissogaster*	
9,13-DiMe-C25	Tr	ND	141, 267, 211, 197
5,17-DiMe-C25	0.14 ± 0.00	ND	85, 323, 141, 267
11,15-DiMe-C27	0.17 ± 0.01	ND	169, 267, 197, 239
5,17-DiMe-C27	0.02 ± 0.01	ND	85, 351, 169, 267
13,17-DiMe-C29	0.02 ± 0.02	ND	197, 267
5,17-DiMe-C29	0.15 ± 0.03	0.07 ± 0.04	85, 379, 197, 267
13,17-DiMe-C31	Tr	Tr	197, 225, 267, 295
5,17-DiMe-C31	0.11 ± 0.01	ND	85, 407, 225, 267
13,17-DiMe-C33	0.15 ± 0.01	Tr	197, 323, 253, 267
5,17-DiMe-C33	Tr	ND	85, 435, 253, 267
13,17-DiMe-C41	0.03 ± 0.02	0.09 ± 0.05	197, 267, 365, 435

[a] In order of elution from capillary column.
[b] Percentage of total ion count. Mean (S.E.) of 3 replicate glands. Tr = trace amount (area count not integrated). ND, not detected.

braconid, *Habrobracon hebetor* (Say). Although *B. cephi* and *B. lissogaster* are quite similar in size and external morphology, the difference in venom gland filament morphology may be a useful internal marker for species separation in newly emerged adults. The ectodermally-derived Dufour glands are found in all aculeate Hymenoptera and are generally simple tube- or pear-shaped structures (Jervis and Kidd, 1996; Quicke, 1997), and this is the case for the glands found in these sawfly parasitoids. The elongated pouch-shaped Dufour glands of *B. cephi* and *B. lissogaster* are also similar in structure to the Dufour gland present in *H. hebetor* except that they are 2× to 3× larger. In contrast, the Dufour glands of two pteromalid parasitoids, *Anisopteromalus calandrae* Howard and *Pteromalus cerealellae* (Ashmead) are similarly elongated but are quite tubular in shape (Howard and Baker, 2003a).

Bender (1943) hypothesized that the function of the Dufour gland in *H. hebetor* [= *H. juglandis*] (termed lubricating gland in his paper) was to aid in passage of the egg through the valvulae that make up the ovipositor. A similar lubricating function for Dufour gland secretion was also postulated by Robertson (1968) and Copland and King (1971). Nevertheless, Howard et al. (2003) found no evidence for diterpenoids on the egg surface of *H. hebetor* despite the high amount of diterpenoids present in its Dufour gland secretion. Rather, lipids from the egg surface of *H. hebetor* contained numerous hydrocarbons and wax esters that were similar in composition to hemolymph lipids in this braconid. Although a lubricating function for the Dufour gland secretion cannot be ruled out for *H. hebetor* as well as for *B. cephi* and *B. lissogaster*, the data for *H. hebetor* suggest that the gland contents in these braconids may have a physiological and/or semiochemical role.

Hydrocarbons are common constituents of Dufour glands and in many species the hydrocarbon composition in the glands is very similar to that found on the

cuticle. Although some qualitative and quantitative differences exist and unique compounds are found in each species, the hydrocarbon composition of the Dufour glands of the bethylids *Cephalonomia tarsalis*, *Cephalonomia watersoni*, and the pteromalids *A. calandrae*, and *P. cerealellae* are nearly identical to the hydrocarbon composition of the adult cuticle (Howard and Baker, 2003a). In contrast, in the braconid parasitoids we have examined, the glands have a major chemical class that is not present on the adult cuticle. For example, diterpenes and homoditerpenes are major components in the Dufour gland of *H. hebetor* but are not present in the adult (Howard and Baker, 2003b). In addition, wax esters are major cuticular components of *H. hebetor*, and these components are not found in the Dufour gland. In *B. cephi* and *B. lissogaster*, acetates are major components in the Dufour glands but they were not detected on adult cuticle (Howard and Baker, unpublished data). However, similar to *H. hebetor*, the surface lipids of the cuticle of the *B. cephi* and *B. lissogaster* also contain significant levels of wax esters that are not found in their Dufour glands (Howard and Baker, unpublished data).

Acetate esters of C_{12}–C_{20} alcohols and $C_{12:1}$–$C_{20:1}$ alcohols that are found in the Dufour glands of *B. cephi* and *B. lissogaster* are biologically active in many insect species, with activities ranging from repellency to attraction. C_{12} acetates are present in the volatile emissions from the Dufour glands of Formacine ants (Bergström and Löfqvist, 1968) and acetates of C_{12}–C_{20} and $C_{12:1}$–$C_{20:1}$ alcohols are very common constituents of lepidopteran pheromones (http://www.nysaes.cornell.edu/pheronet/cpds.html). Acetate esters of saturated and unsaturated C_{16}–C_{20} alcohols are primary components of a defensive secretion that forms at the tip of the abdomen in thrips (*Suocerathrips linguis*) (Tschuch et al., 2001). The secretion is thought to repel ants and other predators. Similarly, a defensive secretion from ventral glands of larvae of the larch sawfly, *Pristiphora erichsonii* (Hartig) (Hymenoptera, Tenthredinidae) contains tetradecanyl acetate, hexadecanyl acetate, and octadecanyl acetate (Jonsson et al., 1988). The C_{18} acetate was a major component of the defensive secretion which was shown to repel predaceous ants in laboratory studies.

Clearly, the complex chemistry of the Dufour glands of *B. cephi* and *B. lissogaster* provide an array of potential semiochemicals that may mediate both parasitoid:parasitoid interactions as well as parasitoid:predator and parasitoid:host interactions. Nevertheless, in the absence of behavioral studies we cannot speculate on specific behaviors that could be elicited by the gland secretions in these parasitic wasps. Interestingly, the hydrocarbons found in Dufour glands of *B. cephi* and *B. lissogaster* have some striking similarities to the composition of cuticular lipids extracted from adults of their host, *C. cinctus* (Bartelt et al., 2002). Both parasitoids and host have *n*-tricosane as the predominant *n*-alkane and have a series of unsaturated hydrocarbons from about C_{23} to C_{31}. In addition, both parasitoids and host have acetate esters although they differ in that *C. cinctus* has a series of alkene-1,ω-diacetates with parent chain lengths of C_{22}, C_{24}, and C_{26}, whereas the parasitoids have monoacetate esters of both saturated and unsaturated primary alcohols from C_{12} to C_{20}. Bartelt et al. (2002) provide evidence that both the alkenes and diesters from the cuticle of *C. cinctus* undergo passive oxidation to aldehydes which are found in volatiles trapped from adults and which are behaviorally active in both

laboratory and field bioassays (Cossé et al., 2002). Whether the alkenes and acetates in the oily Dufour gland secretions of *B. cephi* and *B. lissogaster* undergo similar chemical changes subsequent to their deposition remains to be determined.

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References

Ainslie, C.N., 1920. The western grass stem sawfly. United States Department of Agriculture Bulletin 841. US Government Printing Office, Washington, DC.

Ali, F.A., Morgan, E.D., 1990. Chemical communication in insect communities: a guide to insect pheromones with special emphasis on social insects. Biol. Rev. 65, 227–247.

Bartelt, R.J., Cossé, A.A., Petroski, R.J., Weaver, D.K., 2002. Cuticular hydrocarbons and novel alkenediol diacetates from wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. J. Chem. Ecol. 28, 385–405.

Bender, J.C., 1943. Anatomy and histology of the female reproductive organs of *Habrobracon juglandis* (Ashmead) (Hym., Braconidae). Ann. Entomol. Soc. Am. 36, 537–545.

Bergström, G., Löfqvist, J., 1968. Odour similarities between the slave-keeping ants *Formica sanguinea* and *Polyergus rufescens* and their slaves *Formica fusca* and *Formica rufibarbis*. J. Insect Physiol. 14, 995–1011.

Copland, M.J.W., King, P.E., 1971. The structure and possible function of the reproductive system in some Eulophidae and Tetracampidae. Entomologist 104, 4–28.

Cossé, A.A., Bartelt, R.J., Weaver, D.K., Zilkowski, B.W., 2002. Pheromone components of the wheat stem sawfly: identification, electrophysiology, and field bioassay. J. Chem. Ecol. 28, 407–423.

Guillot, F.S., Joiner, R.L., Vinson, S.B., 1974. Host discrimination: isolation of hydrocarbons from Dufour's gland of a braconid parasitoid. Ann. Entomol. Soc. Am. 67, 720–721.

Holmes, N.D., 1982. Population dynamics of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae) in wheat. Can. Entomol. 114, 775–788.

Howard, R.W., Baker, J.E., Morgan, E.D., 2003. Novel diterpenoids and hydrocarbons in the Dufour gland of the ectoparasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae). Arch. Insect Biochem. Physiol. 54, 95–109.

Howard, R.W., Baker, J.E., 2003a. Morphology and chemistsry of Dufour glands in four ectoparasitoids: *Cephalonomia tarsalis*, *C. waterstoni* (Hymenoptera: Bethylidae), *Anisopteromalus calandrae*, and *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). Comp. Biochem. Physiol. (B) 135, 153–167.

Howard, R.W., Baker, J.E., 2003b. Cuticular hydrocarbons and wax esters of the ectoparasitoid *Habrobracon hebetor*: ontogenetic, reproductive and nutritional effects. Arch. Insect Biochem. Physiol. 53, 1–18.

Jervis, M., Kidd, N., 1996. Insect Natural Enemies: Practical Approaches to Their Study and Evaluation. Chapman and Hall, London.

Jonsson, S., Bergström, G., Lanne, B.S., Stensdotter, U., 1988. Defensive odor emission from larvae of two sawfly species, *Pristiphora erichsonni* and *P. wesmaeli*. J. Chem. Ecol. 14, 713–721.

Marris, G.C., Hubbard, S.F., Scrimgeour, C., 1996. The perception of genetic similarity by the solitary parthenogenetic parasitoid *Venturia canescens*, and its effects on the occurrence of superparasitism. Entomol. Exp. Applicata 78, 167–174.

Mudd, A., Fisher, R.C., Smith, M.C., 1982. Volatile hydrocarbons in the Dufour's gland of the parasite *Nemeritis canescens* (Grav.) (Hymenoptera: Ichneumonidae). J. Chem. Ecol. 8, 1035–1042.

Morrill, W.L., 1997. The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), and associated parasitoids in the northern Great Plains of North America. Trends Entomol. 1, 171–174.

Nelson, W.A., Farstad, C.W., 1953. Biology of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae), an important parasite of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae), in western Canada. Can. Entomol. 85, 103–107.

Quicke, D.L.J., 1997. Parasitic Wasps. Chapman and Hall, London.

Robertson, P.L., 1968. A morphological and functional study of the venom apparatus in representatives of some major groups of Hymenoptera. Aust. J. Zool. 16, 133–166.

Rosi, M.C., Isidoro, N., Colazza, S., Bin, F., 2001. Source of the host marking pheromone in the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae). J. Insect Physiol. 47, 989–995.

Runyon, J.B., Hurley, R.L., Morrill, W.L., Weaver, D.K., 2001. Distinguishing adults of *Bracon cephi* and *Bracon lissogaster* (Hymenoptera: Braconidae), parasitoids of the wheat stem sawfly (Hymenoptera: Cephidae). Can. Entomol. 133, 215–217.

Runyon, J.B., Morrill, W.L., Weaver, D.K., Miller, P.R., 2002. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat fields bordering tilled and untilled fallow in Montana. J. Econ. Entomol. 95, 1130–1134.

Somsen, H.W., Luginbill Jr., P., 1956. *Bracon lissogaster* Mues., a parasite of the wheat stem sawfly. U.S. Department of Agriculture Technical Bulletin 1153 (Washington, DC).

Syvertsen, T.C., Jackson, L.L., Blomquist, G.J., Vinson, S.B., 1995. Alkadienes mediating courtship in the parasitoid *Cardiochiles nigriceps* (Hymenoptera: Braconidae). J. Chem. Ecol. 21, 1971–1989.

Tschuch, G., Lindemann, P., Moritz, G., 2001. Chemical defence in thrips. In: Marullo, R., Mound, L. (Eds.), Proceedings of the Seventh International Symposium. Thysanoptera, Reggio Calabria, Italy. Available on cd at: http://laurence.mound@csiro.au.

Vinson, S.B., Guillot, F.S., 1972. Host marking: source of a substance that results in host discrimination in insect parasitoids. Entomophaga 17, 241–245.

Weiss, M.J., Morrill, W.L., 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. Am. Entomol. 38, 241–245.