Chemoecology 11:43–47 (2001)
0937–7409/01/010043–05 $1.50 + 0.20

CHEMOECOLOGY

Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae)

J.-L. Hemptinne[1], G. Lognay[2], M. Doumbia[2] and A. F. G. Dixon[3]

[1]Ecole nationale de Formation agronomique, Jeune Equipe 000271JE1 "Laboratoire d'Agroécologie", BP 87, F-31326 Castanet-Tolosan, France
[2]Faculté universitaire des Sciences agronomiques, Passage des Déportés, 2, B-5030 Gembloux, Belgium
[3]School of Biological Sciences, University of East Anglia, Norwich NR4 7 TJ, UK

Summary. Aphidophagous ladybirds are reluctant to oviposit in patches of prey where conspecific larvae are present. This is adaptive as larval cannibalism is a major threat to egg survival. Ladybirds avoid laying eggs in such patches by responding to a species specific oviposition deterring pheromone present in the tracks of larvae. This study revealed that the oviposition deterring pheromone consists of a mixture of alkanes of which n-pentacosane is the major component (15.1%). These alkanes are likely to spread easily on the hydrophobic cuticle of plants and so leave a large signal. In addition, they are not quickly oxidized and therefore provide a long lasting signal. The latter was confirmed by the observation that 10 day old tracks still deterred oviposition.

Key words. Alkanes – ladybird beetles – oviposition deterring pheromone – persistence – semiochemicals

Introduction

The larvae of some species of ladybird beetles feed exclusively on aphids. Females of these aphidophagous ladybirds tend to lay their eggs over a short period of time early in the development of a colony of aphids, the "egg window" (Hemptinne *et al.* 1992; Dixon 1997). As a consequence their numerical reproductive response is only weakly, if correlated at all, with the abundance of prey. This reproductive strategy is adaptive as prey abundance fluctuates dramatically over a 5 to 6 week period and only ladybird larvae born early in the development of a patch of prey are likely to mature before the aphids disappear.

In selecting suitable patches of prey ovipositing females do not respond to cues associated with the age structure of the aphid colony, as has been reported for hoverflies, or the phenology of the host-plant (Kan 1988a,b; Hemptinne *et al.* 2000a). They refrain from ovipositing in patches where there are few aphids (Dixon 1959) and conspecific larvae are present (Hemptinne *et al.* 1992). For the latter females do not need to encounter larvae but respond to an oviposition deterring pheromone present in the tracks of the larvae. Oviposition is severely inhibited when mature females are placed in Petri dishes lined with filter paper on which larvae had previously crawled. The deterrent effect is dependent on the number but not on the age of the larvae. A chloroform extract of the tracks also deters oviposition. That is, the oviposition deterrent is a chloroform soluble chemical present in the tracks of larvae. However, these extracts may have been contaminated by faeces produced by the larvae (Doumbia *et al.* 1998).

In this paper, up to 78% of the molecules making up the oviposition deterring pheromone are identified and the persistence of this pheromone in the tracks of larvae of the two spot ladybird is determined.

Correspondence to: J.-L. Hemptinne, e-mail: jean-louis.hemptinne@educagri.fr

Material and methods

Ladybird culture

Two-spot ladybirds, *Adalia bipunctata* (L.), were reared at 15 ± 1°C and a photoperiod of 16 h light and 8 h darkness. Groups of approximately twenty males and twenty females were kept in 5 litre plastic boxes, which also contained a piece of corrugated filter paper to increase the surface area. Every other day the ladybirds were fed an excess of pea aphids, *Acyrthosiphon pisum* (Harris). A section of stem of broad bean, *Vicia faba* L., was provided as a source of humidity and of food for the aphids. Once a week the ladybirds were transferred to clean containers to stimulate egg laying.

The eggs laid on the filter paper were collected every day, and were used either to maintain the stock culture or produce larvae for the experiments. They were incubated in 175 cm^3 plastic boxes kept under the same laboratory conditions as the adults. To reduce the risk of cannibalism the number of larvae per box never exceeded 15.

Chemical nature and biological activity of the extract

Extraction of larval tracks. Late third instar larvae of *A. bipunctata* were isolated in 5 cm diameter Petri dishes and after moulting to the fourth instar, were fed an excess of pea aphids for 24 h before being moved to clean Petri dishes and left without food for another 24 h. Each larva was then carefully placed in a glass tube (1 cm diameter;

6 cm long) where it remained without food for 24 h. Each tube was sealed with a cotton plug. Larvae treated in this way did not produce faeces. They did not contaminate the tube by reflex bleeding as is the case when larvae interact with one another or are roughly handled. As the larvae were hungry they spent most of the time walking. After 24 h the larva was removed and the tube washed twice with 1 ml of chloroform for 5 min.

The chloroform extracts of the tracks of 25 larvae were pooled and treated as a replicate, passed through a silanized glass wool filter and concentrated at 35°C under reduced pressure. These extracts were then dissolved in 500 µl of chloroform. There were 15 replicates.

GC-MS identification of the chemicals. The GC-MS (Gas Chromatography–Mass spectrometry) was performed on a Hewlett-Packard HP5989 Mass spectrometer coupled to a HP5890 Serie II gas chromatograph equipped with a HP-5 (cross-linked 5% phenylmethylpolysiloxane) column (28 m × 0.25 mm I.D.; film thichness 0.25 µm). The operating conditions were: cold on-column injection, carrier gas: helium at 1.3 ml min^{-1}; temperature programme: from 50°C to 140°C at 20°C min^{-1} then from 140°C to 290°C at 5°C min^{-1} with a final hold of 20 min at 290°C. The mass spectra were recorded in the electron impact mode at 70 eV (Source temperature: 200°C, transfer line at 275°C; scanned mass range: 25 to 500 amu).

The peaks detected on the chromatograms were identified on the basis of retention data and fragmentation patterns. The identification of the straight-chain alkanes and squalene were confirmed by co-injection of pure reference samples and comparison of spectra with those recorded in of the NBS 75K.L and WILEY 138.L computer MS libraries. The structure of internally branched (methyl-branched) alkanes was determined from the incidence of C_nH_{2n+1} and C_nH_{2n} fragments after α-cleavage at the branch position(s) (McCarthy *et al.* 1968). For the 3-methylalkanes, both the M-29 (loss of an ethyl group) and the M-57 (loss of a secondary butyl group) ions were the "diagnostic" fragments. For the 2-methylalkanes, M-43 and M-15 were the diagnostic ions, and correspond to the loss of an isopropyl- and a methyl-group, respectively.

Measurement of track constituents. The track constituents were measured by Gas Liquid chromatography (GLC) on a Carlo Erba Mega 5160 gas chromatograph fitted with a cold on-column injector and a FID detector maintained at 290°C. A fused silica OPTIMA 1 (30 m × 0.32 mm I.D.; film thickness 0.30 µm) column from Macherey-Nagel was used for the analysis. The carrier gas was Helium at 70 KPa and the temperature programme was similar to that used for the GC-MS analyses. For the identification of the peaks, their retention times relative to that of n-tricosane and an homologous series of C_{19} to C_{29} n-alkanes similarly treated, were used. Fifteen samples, each consisting of the chloroform extract of 25 tracks, were subjected to GLC in order to determine the proportion of the different constituents. For the GLC quantification, 50 µl of a chloroform solution of n-nonadecane (1.23 $mg \cdot ml^{-1}$) was added as an internal standard (the response factors were fixed to 1 for each product detected).

Biological activity of the extract. A chloroform extract of larval tracks was obtained as outlined above. A quantity of extract equivalent to that from the tracks of 25 fourth instar larvae of *A. bipunctata* was applied to a 9-cm diameter filter paper and the chloroform evaporated off in a fume cupboard for 20 min, after which the filter paper was placed in a 9-cm diameter Petri dish. A gravid female and an excess of pea aphid were added to the dish at 08.00 h and after 3 h the number of eggs laid was recorded. This was repeated 20 times. There were also 20 replicates of the control the filter paper of which was just treated with 1 ml of pure chloroform. The female ladybirds were reared in isolation, and were between 15 and 25 days old at the beginning of the experiment. They had been recently mated. Five days before each test, the number of eggs laid by each females was counted daily. Only those producing at least one clutch per day were selected for the experiments. All the experiments were done at 21°C and a light intensity of 2000 lux. The numbers of eggs laid were compared by a Student's *t* test performed with Systat 9 (Zar 1996)

Persistence of the larval tracks

Ten fourth instar larvae of *A. bipunctata* were supplied with an excess of a mixture of different instars of the pea aphid in a 9 cm diameter Petri dish, the base of which was covered with filter paper. After 24 h the larvae and all aphid material were carefully removed. A gravid female of *A. bipunctata* was then placed in the Petri dish at 08.00 h and supplied with an excess of pea aphids. The number of eggs the female laid was recorded 3 h later. This, the fresh track treatment, was replicated 20 times.

The above procedure was followed, but in this case, after the removal of the larva and aphid material, 20 Petri dishes were placed open for 2 days in a large plastic box measuring 36 × 24 × 14 cm. The two smaller vertical faces had two 3 cm diameter holes covered with metallic mesh (diameter of threads: 0.18 mm; mesh size: 0.3 × 0.3 mm). The Petri dishes were so protected from contamination by dust or wandering aphids. The relative volume of the box to the Petri dishes is compatible with ventilation and evaporation of volatile compounds. The same procedure was successively repeated for groups of 20 Petri dishes than were placed open for 5, 7 or 10 days. Then, a female was placed in each dish at 08.00 h and the influence of these 'aged' larval tracks on its egg production recorded after 3 h. In the control the filter paper in the Petri dishes was not contaminated by tracks of larvae, but otherwise similarly treated.

All the experiments were done at 21°C and a light intensity of 2000 lux. In order to reduce variability the females were standardised as outlined above.

The average number of eggs laid in each treatment were compared by an ANOVA and a least significant difference test performed with Systat 9® (Zar 1996).

Results

Chemical nature of the tracks

There are at least forty different components, mostly alkanes, in the tracks of larvae of *A. bipunctata* (Table 1; Appendix 1 and Fig. 1). The elution order of the C_{23} to C_{27} molecules was: n-alkanes < 15- to 2-methyl alkanes < 3-methyl alkanes. For the C_{29} to C_{31} molecules, the following order was observed: n-alkanes < 15- to 7-methyl alkanes < dimethyl alkanes. This sequence is similar to that obtained by Liepert & Dettner (1996) for the cuticular hydrocarbons from an aphid parasitoid.

The main compounds were methyl-branched (C_{23} to C_{33}) and straight chain hydrocarbons (C_{20} to C_{31}), respectively, representing $32.5 \pm 4.5\%$ and $29.9 \pm 4.2\%$ of the extract. Among the methyl alkanes, the internally branched molecules consisting of complex mixtures of 15-, 13-, 11-, 9-, 7- and 5-isomers, were dominant ($22.9 \pm 3.0\%$), and the 2- and 3-monomethyl derivatives were present in lower proportions ($6.8 \pm 1.9\%$ and $2.8 \pm 0.6\%$ respectively). The 2-, 3- and 5-methyl alkanes were separated, the 9- and 7 isomers were partially

Table 1 The compounds that made up 1% or more of the chloroform extract of the tracks of larvae of *Adalia bipunctata*

Compounds	Mean[1]	Compounds	Mean[1]
nC_{21}	3.2	nC_{29}	1.7
nC_{23}	3.3	Me-C_{26}	1.4
Me-C_{23}	3.9	Me-C_{29}	4.7
Me-C_{24}	4.3	di-Me-C_{29}	3.9
nC_{25}	15.1	nC_{31}	3.7
Me-C_{25}	7.7	Me-C_{31}	6.9
nC_{27}	1.8	Me-C_{33}	4.8
Me-C_{27}	1.6	di-Me-C_{33}	2.9
		Squalene	4.4

[1] Expressed as percentage of the total weight of detected molecules (mean of 15 analyses)

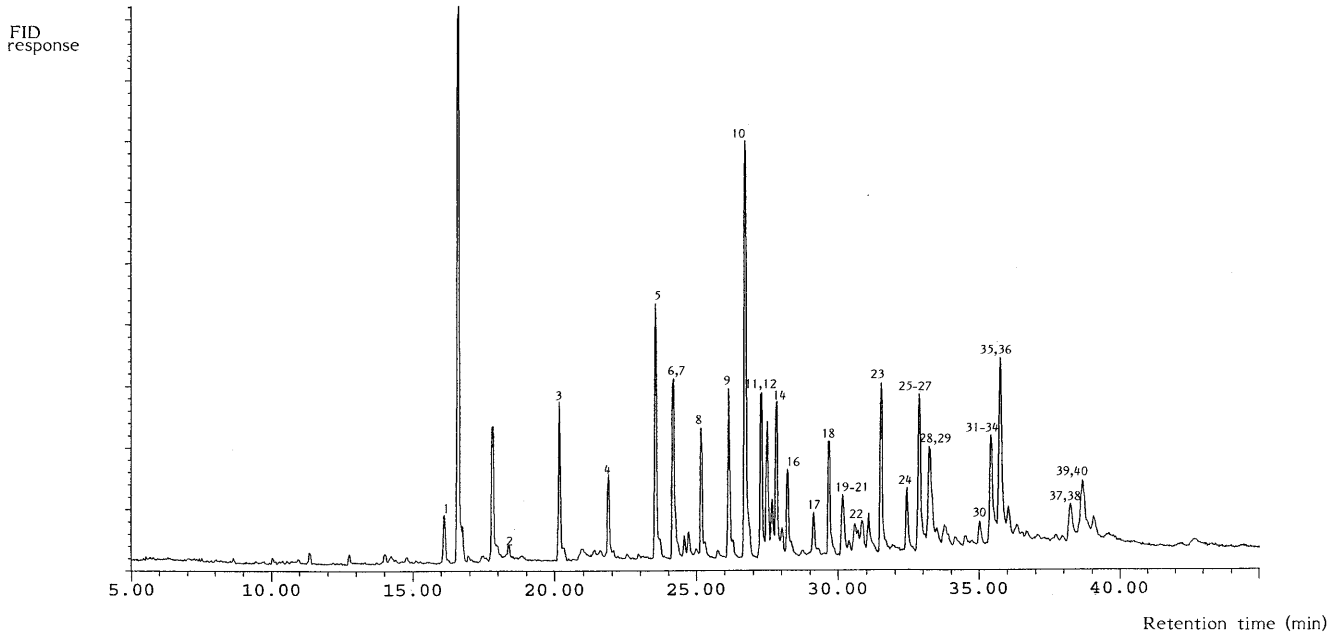

Fig. 1 Chromatogram of an extract of 25 larval tracks of *Adalia bipunctata*
Legend: **1** = bis(2-methylpropyl)phtalate; **2** = nC_{20}; **3** = nC_{21}; **4** = nC_{22}; **5** = nC_{23}; **6** = 9-Me-C_{23}; **7** = 7-Me-C_{23}; **8** = nC_{24}; **9** = 2-Me-C_{24}; **10** = nC_{25}; **11** = 9-Me-C_{25}; **12** = 7-Me-C_{25}; **13** (not labelled) = 2-Me-C_{25}; **14** = 3-Me-C_{25}; **15** (not labelled) = diisooctyl phtalate; **16** = nC_{26}; **17** = 2-Me-C_{26}; **18** = nC_{27}; **19** = 11-Me-C_{27}; **20** = 9-Me-C_{27}; **21** = 7-Me-C_{27}; **22** = 5-Me-C_{27}; **23** = squalene; **24** = nC_{29}; **25** = 13-Me-C_{29}; **26** = 11-Me-C_{29}; **27** = 9-Me-C_{29}; **28** = 11,15 diMe-C_{29}; **29** = 13,17 diMe-C_{29}; **30** = nC_{31}; **31** = 15-Me-C_{31}; **32** = 13-Me-C_{31}; **33** = 11-Me-C_{31}; **34** = 9-Me-C_{31}; **35** = 11,15 diMe-C_{31}; **36** = 13,17 diMe-C_{31}; **37** = 13-Me-C_{33}; **38** = 11-Me-C_{33}; **39** = 11,15 diMe-C_{33}; **40** = 13,17 diMe-C_{33}. The major peak eluting after peak 1 is the internal standard, *n*-nonadecane

separated and the 11-, 13- and 15-homologous series co-eluted because chromatographic resolution decreases as a function of the branch position. The quantity of the compounds that were not or only partially separated was determined collectively. Six dimethyl-alkanes ($7.7 \pm 1.7\%$), with odd numbered carbon backbones (C_{29} to C_{33}) and exhibiting isoprenoid spacings of the methyl groups (i.e 11,15- and 13,17-), were also detected as isomeric mixtures. A single unsaturated molecule, squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) accounted for $4.4 \pm 2.1\%$ of the secretion. This compound is present in the oviposition deterring pheromone of *Ceutorhynchus assimilis* Payk (Mudd *et al.* 1997). In addition to these hydrocarbons, two minor peaks corresponding to phtalate platicizer contaminants were also detected in all samples.

The principal constituent of the tracks of larvae was n-pentacosane accounting for $15.1 \pm 4.1\%$ of the extract. An average of 226 ± 19 μg of extract was obtained from the tracks of twenty-five individuals (mean of five replicates), which corresponds to 8 to 10 μg per larva.

Biological activity of the extract

The chloroform extract of the tracks of larvae had a very marked deterrent effect on oviposition by *A. bipunctata*. The activity of the extract used in this study is greater than that used in the previous study (Doumbia *et al.* 1998), which may have been contaminated with larval faeces (Table 2: $t = 3.279$; 38 d.f., $P = 0.002$). Therefore, the oviposition deterring pheromone is a chloroform soluble chemical present in the tracks of larvae.

Persistence of the oviposition deterring pheromone

The fresh tracks of ten fourth instar larvae significantly reduced the average number of eggs laid by two-spot females (Table 3; $F = 66.30$; 1 and 190 d.f.; $P < 0.001$). In addition, the deterrent effect of the tracks persisted for up to 10 days (Table 3).

Discussion

The tracks left by larvae of the two-spot ladybird have a marked deterrent effect on oviposition of conspecific females. Chloroform extracts of these tracks mainly

Table 2 The average number of eggs laid in 3 h by females of *Adalia bipunctata* in Petri dishes that contained filter paper treated with a chloroform extract of conspecific larval tracks that may have been contaminated with larval faeces, an extract which was not contaminated by faeces and in control Petri dishes

	Replicates	Average number of eggs (standard error)
Uncontaminated extract	20	3.9 (1.7)
Control	20	11.6 (1.6)
Contaminated extract[1]	30	7.5 (1.9)
Control[1]	30	15.5 (1.6)

[1] Doumbia *et al.* 1998

Table 3 The average numbers of eggs laid in 3 h by females of *Adalia bipunctata* kept in Petri dishes lined with clean filter paper (control), filter paper contaminated with fresh, or 2, 5, 7 or 10 day old conspecific larval tracks

	Number of eggs[1]
Control	9.0 (0.6) a
Treatments:	
fresh tracks	0.1 (1.3) b
2 days old tracks	2.6 (1.3) b
5 days old tracks	2.4 (1.3) b
7 days old tracks	2.6 (1.3) b
10 days old tracks	1.6 (1.3) b

[1] Figures followed by the same letter did not differ significantly ($F = 0.32$; 4 and 190 d.f.; $P > 0.05$)

consist of a mixture of alkanes in which methyl-branched (C_{23} to C_{33}) and straight chain (C_{20} to C_{31}) hydrocarbons are dominant. These molecules are similar to those found on the elytra of the adults and on the surface of the eggs of the same species (Hemptinne *et al*. 1998, 2000). Alkanes are common in nature. Those that cover the surface of plants are known to stimulate oviposition in their specialist insect herbivores and deter oviposition in some generalists (Eigenbrode & Espelie 1995; Udayagiri & Mason 1997). Herbivorous insects are also known to produce oviposition deterring pheromones that deter other individuals of the same species from ovipositing on a plant already being attacked. In the case of the cabbage seed weevil, *C. assimilis*, the oviposition deterring pheromone is also mainly made up of alkanes (Mudd *et al*. 1997). Most of the reports on the chemical identification of oviposition deterring pheromones relate to insect herbivores (*e.g*., Schoonhoven 1990; Blaakmeer *et al*. 1994; Gabel & Thiery 1996) and few to parasitoids (*e.g*., Millar & Hare 1993). This is the first time that the chemical nature of the oviposition deterring pheromone of an insect predator has been investigated.

The fact that the tracks of larvae contain alkanes is interesting for two reasons. Firstly, these molecules spread easily on the hydrophobic cuticle of plants and so leave a large signal. Secondly, saturated hydrocarbons are not quickly oxidized and are therefore stable, which was confirmed by the fact that tracks up to 10 days old deterred oviposition. How do ladybirds distinguish the oviposition deterring pheromone from the waxy background of plant cuticles? Although similar to each other, it seems that plant cuticular waxes contain fewer or no methyl-branched compounds and no squalene (Lognay, personal communication). In view of the fact that many insects are able to perceive the presence of very low concentrations of specific substances, it is not unreasonable to suggest that ladybirds can detect the presence of the oviposition deterring pheromone

By responding to the mixture of alkanes present in the tracks of their larvae adults avoid ovipositing in patches where larvae are already present. This is adaptive as it reduces the risk of the eggs being eaten by conspecific larvae. The fact that the alkanes are hydrophobic and stable facilitates this signalling. For the larvae these alkanes possibly have a locomotory function. A mixture of alkanes, similar to the oviposition deterring pheromone in its composition, is secreted by the hairs on the under surface of ladybird tarsi. These alkanes enable ladybirds to adhere to smooth surfaces (Kosaki & Yamaoka 1996). Interestingly, similar alkanes on the elytra of adults facilitate species recognition in mating (Hemptinne *et al*. 1998) and on the surface of the eggs signal the potential danger of attacking and eating the eggs (Hemptinne *et al*. 2000b). That is, these alkanes present three different context specific signals, which if confirmed would be an example of semiochemical parsimony (Blum 1996).

Acknowledgements

We are greatly indebted to The Royal Society for the award of a Joint Project Grant to A.F.G. D. and J-L. H. that greatly facilitated this study.

References

Blaakmeer A, Stork A, Vanveldhuizen A, Vanbeek TA, Degroot A, Vanloon JJA, Schoonhoven LM (1994) Isolation, identification and synthesis of miriamides, new hostmarkers from eggs of *Pieris brassicae*. Journal of Natural Products 57:90–99

Blum MS (1996) Semiochemical parsimony in the Arthropoda. Annual Review of Entomology 41:353–374

Dixon AFG (1959) An experimental study of the searching behaviour of the predatory coccinellid beetle *Adalia decempunctata* (L.). Journal of Animal Ecology 28:259–281

Dixon AFG (1997) Patch quality and fitness in predatory ladybirds. *in* Dettner *et al*. (eds) Vertical Food Web Interactions, Ecological Studies 130:205–223

Doumbia M, Hemptinne J-L, Dixon AFG (1998) Assessment of patch quality by ladybirds: role of larval tracks. Oecologia 113:197–202

Eigenbrode SD, Espelie KE (1995) Effect of plant epicuticular lipids on insect herbivores. Annual Review of Entomology 40:171–194

Gabel B, Thiery D (1996) Oviposition response of *Lobesia botrana* females to long-chain free fatty acids and esters from its eggs. Journal of chemical Ecology 22:161–171

Hemptinne J-L, Dixon AFG, Goffin J (1992) Attack strategy of ladybird beetles (Coccinellidae): factors shaping their numerical response. Oecologia 90:238–245

Hemptinne J-L, Lognay G, Dixon AFG (1998) Mate recognition in the two-spot ladybird beetle, *Adalia bipunctata*: role of chemical and behavioural cues. Journal of Insect Physiology 44:1163–1171

Hemptinne J-L, Doumbia M, Dixon AFG (2000a) Assessment of patch quality by ladybirds: role of aphids and plant phenology. Journal of Insect Behaviour 13:353–359

Hemptinne J-L, Lognay G, Gauthier C, Dixon AFG (2000b) Role of surface alkanes in egg cannibalism and intraguild predation in ladybirds (Coleoptera: Coccinellidae). Chemoecology 10:in press

Kan E (1988a) Assessment of aphid colonies by hoverflies. I. Maple aphids and *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae). Journal of Ethology 6:39–48

Kan E (1988b) Assessment of aphid colonies by hoverflies. II. *Betasyrphus serarius* (Wiedemann), *Metasyrphus frequens* Matsumara and *Syrphus vitripennis* (Meigen) (Diptera: Syrphidae). Journal of Ethology 6:135–142

Kosaki A, Yamaoka R (1996) Chemical composition of footprints and cuticula lipids of three species of Lady Beetles. Japanese Journal of Applied Zoology and Entomology 40:47–53

Liepert C, Dettner K (1996) Role of hydrocarbons of aphid parasitoids in their relationship to aphid-attending ants. Journal of Chemical Ecology 22:695–707

McCarthy ED, Han J, Calvin M (1968) Hydrogen atom transfer in mass spectrometric fragmentation patterns of saturated aliphatic hydrocarbons. Annals of Chemistry 40:1475–1480

Millar JG, Hare JD (1993) Identification and synthesis of a kairomone inducing oviposition by parasitoids *Aphytis melinus* from California red scale covers. Journal of Chemical Ecology 19:1721–1736

Mudd A, Ferguson AW, Blight MM, Williams IH, Scubla P, Solinas M, Clark SJ (1997) Extraction, isolation and composition of oviposition-deterring secretion of cabbage seed weevil *Ceutorhynchus assimilis*. Journal of Chemical Ecology 23:2227–2240

Schoonhoven LM (1990) Host-marking pheromones in Lepidoptera, with special reference to 2 Pieris spp. Journal of Chemical Ecology 16:3043–3052

Udayagiri S, Mason CE (1997) Epicuticular wax chemicals in Zea mays influence oviposition in *Ostrinia nubialis*. Journal of Chemical Ecology 23:1675–1687

Zar JH (1996) Biostatistical Analysis. Prentice Hall International Editions, Upper Saddle River, N.J., 662 pp

Received 22 May 2000; accepted 28 November 2000.

Appendix 1: The compounds present in the chloroform extract of the tracks of larvae of *Adalia bipunctata*

Identified compounds	CN[1]	RRT[2]	Diagnostic ions (m/z)		Others	Percentage[3] Mean ± SD
			M^+	$[M–CH_3]^+$		
bis(2-methylpropyl) phtalate	–	0.713	278		223-149-104	2.8(0.8)
nC_{20}	20	0.779	282			0.2(0.1)
nC_{21}	21	0.866	296			3.2(1.0)
nC_{22}	22	0.928	310			0.3(0.2)
n-C_{23}	23	1.000	324			3.3(0.9)
9-Me-C_{23}	24	1.028	338	323	140/141–224/225	Σ X-Me-C23 =
7-Me-C_{23}	24	1.030	338	323	112/113–252/253	3.9(1.1)
nC_{24}	24	1.069	338			0.3(0.1)
2-Me-C_{24}	25	1.112	352	337	309	4.3(0.9)
nC_{25}	25	1.136	352			15.1(4.1)
9-Me-C_{25}	26	1.159		351	140/141–252/253	Σ X-Me-C_{25} =
7-Me-C_{25}	26	1.159		351	112/113–280/281	3.8(0.9)
2-Me-C_{25}	26	1.177	366	351	323	1.1(0.1)
3-Me-C_{25}	26	1.183	366	351	337	2.8(0.6)
diisooctyl phtalate	–	1.193	390		279-167-149-113	0.5(0.1)
nC_{26}	26	1.200	366			0.3(0.1)
2-Me-C_{26}	27	1.240	380	365	337	1.4(0.2)
nC_{27}	27	1.262	380			1.8(0.4)
11-Me-C_{27}	27	1.278		379	168/169–252/253	Σ X-Me-C_{27} =
9-Me-C_{27}	27	1.278		379	140/141–280/281	1.0(0.3)
7-Me-C_{27}	27	1.278		379	112/113–308/309	
5-Me-C_{27}	27	1.282			84/85–337/338	0.7(0.5)
Squalene	30	1.327	410		149-137-81-69	4.4(2.1)
nC_{29}	29	1.384	408			1.7(0.3)
13-Me-C_{29}	30	1.404		407	196/197–252/253	
11-Me-C_{29}	30	1.404		407	168/169–280/281	Σ X-Me-C_{29} =
9-Me-C_{29}	30	1.404		407	140/141–308/309	4.7(0.6)
11,15 diMe-C_{29}	31	1.424		421	168/169–238/239–224/225–294/295	Σ diMe-C_{29} =
13,17 diMe-C_{29}	31	1.497	434		97,83,69,55	3.9(0.9)
nC_{31}	31	1.558	426			3.7(0.5)
15-Me-C_{31}	32	1.585		435	224/225–252/253	
13-Me-C_{31}	32	1.585				Σ X-Me-C_{31} =
11-Me-C_{31}	32	1.585		435	168/169–308/309	6.9(1.1)
9-Me-C_{31}	32	1.585		435	140/141–336/337	
11,15 diMe-C_{31}	33	1.608		449	168/169–322/323–252/253–238/239	Σ diMe-C_{31} =
13,17 di Me-C_{31}	33	1.608		449	196/197–224/225–266/267–294/295	0.8(0.2)
13-Me-C_{33}	34	1.779		463	196/197–308/309	Σ X-Me-C_{33} =
11-Me-C_{33}	34	1.779		463	168/169–336/337	1.9(0.7)
11,15 diMe-C_{33}	35	1.815			168/169–350/351–252/253–280/281	Σ diMe-C_{33} =
13,17 diMe-C_{33}	35	1.815			196/197–322/323–252/253–266/267	2.9(0.7)

[1] CN = total carbon number

[2] Relative retention time (nC_{23} as reference)

[3] Average percentage of the total weight of the detected molecules (mean +/− of 15 analyses)

In 5 of the 15 analyses, dibutylphtalate (RRT = 0.734) has been detected