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Assessment of patch quality by ladybirds: relative response to conspecific and heterospecific larval tracks a consequence of habitat similarity?

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Summary. Aphid colonies can reach high levels of abundance but last for short periods of time. The larvae of aphidophagous ladybirds (Coleoptera: Coccinellidae) that feed on these colonies might therefore suffer from starvation, which favours the occurrence of cannibalism and intraguild predation. Thus, the assessment of patch quality becomes crucial and it has been shown that female ladybirds refrain from laying eggs in the presence of an oviposition deterring semiochemical deposited by their larvae.

Adalia bipunctata (L.)*, Adalia decempunctata* (L.) and *Coccinella septempunctata* L. are 3 sympatric species of ladybirds, which can co-occur in aphid colonies. As a consequence, their eggs and larvae are under threat, not only from cannibalism but also intraguild predation. Females should, therefore, also use the tracks deposited by heterospecific larvae to assess the quality of aphid colonies as oviposition sites. The expectation is that: 1- the strength of the reaction to each other's larval tracks should be correlated with percentage habitat overlap and that 2- the reaction to conspecific larval tracks should be stronger than to heterospecific tracks. In order to test these hypotheses, females' oviposition behaviour was analysed and a chemical analysis of the tracks of their larvae undertaken.

The results show that oviposition behaviour is not related to habitat overlap. Both species of *Adalia* react to tracks of their own larvae and those of *C. septempunctata*, but *A. decempunctata* reacted more strongly than *A. bipunctata*. *C. septempunctata* reacted very slightly to its own tracks but not to those of either species of *Adalia*.

The larval tracks are mainly composed of alkanes. Those of the two species of *Adalia* are qualitatively 100% similar and 60% so when the quantitative results are compared. They are, however, only 24-29 % similar to those of *Coccinella septempunctata*.

Key words. Cannibalism – intraguild predation – patch quality – oviposition-deterring semiochemicals – alkanes – Coleoptera: Coccinellidae

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Introduction

Aphid colonies last for relatively short periods of time and rapidly change in numbers, often reaching high levels of abundance for only very short periods (Dixon, 1998). Associated with large aggregations of prey are usually large numbers of insect predators belonging to several taxa (Rosenheim *et al.*, 1995). Larvae of aphidophagous ladybirds are exposed to three main causes of mortality: starvation when aphids are scarce, cannibalism and intraguild predation (IGP).

Cannibalism is commonly observed under laboratory conditions (e.g. Agarwala & Dixon, 1991, Agarwala *et al.,* 1998) and in the field (e.g. Mills, 1982, Osawa, 1993). As for IGP between aphidophagous ladybirds, many laboratory experiments indicate it occurs. Rigorous field studies on its incidence and significance, however, are rare (Dixon, 2000).

In order to maximise fitness, ladybird females should assess the quality of patches in terms of their potential to sustain the development of their larvae, and lay eggs or refrain from doing so accordingly. Ladybirds start laying eggs in a colony when first instar aphids are abundant enough for their vulnerable first instar larvae to catch sufficient prey to sustain their development (Dixon, 1959). They stop ovipositing when they detect tracks deposited by conspecific larvae (Dixon, 2000). This has been shown for *Adalia bipunctata* (Doumbia *et al.,* 1998), *Coccinella septempunctata* (Doumbia *et al.,* 1998, Růžička, 1997, 2001)*, Harmonia axyridis* Pallas (Yasuda *et al.*, 2000), *Cycloneda limbifer* Casey and *Ceratomegilla undecimnotata* (Schneider) (Růžička, 2001, 2003), *Semiadalia undecimnotata* (Schneider) (Růžička, 2001) and *Aphidecta obliterata* (L.) (Oliver *et al.,* 2006). In the case of *A. bipunctata,* the oviposition deterring semiochemical is present in the larval tracks and is mainly a mixture of alkanes (Hemptinne *et al.,* 2001). Although adult ladybirds also deposit chemical tracks (e.g. Kosaki & Yamaoka, 1996, Nakashima *et al.*, 2004), these do not seem to have oviposition-deterring properties (Doumbia *et al.*, 1998).

In addition, the expectation is that a female of one species should also refrain from laying eggs in the presence of tracks deposited by larvae of other species. There are a

Table 1. Habitat overlap between three ladybird species calculated using their abundance in natural habitats (after Honek, 1985).

Species	Habitat overlap (%)
Adalia bipunctata × *Adalia decempunctata*	14.9
Adalia bipunctata × *Coccinella septempunctata*	16.5
Coccinella septempuncata × *Adalia decempuncata*	6.0

few studies on the oviposition-deterring effect of heterospecific larval tracks on aphidophagous ladybirds (Doumbia *et al.*, 1998, Yasuda *et al.,* 2000, Růžička, 2001, 2003, Oliver *et al.*, 2006) and of the larval tracks of ladybirds on other aphidophagous predators (Růžička, 1997, 2001, Oliver *et al.,* 2006). However, for ladybirds there appears to be no pattern in the results: females of different species react differently to heterospecific larval tracks. The evolutionary mechanisms associated with this are still under discussion; several explanations based on geographical distribution, habitat similarity or low risk of predation have been suggested (e.g. Růžička, 2001, Oliver *et al.,* 2006, Yasuda *et al*., 2000).

This paper presents the results of an analysis of the oviposition behaviour of females of 3 sympatric species – *A. bipunctata, A. decempunctata* and *C. septempunctata* - in the presence of the larval tracks of their own and other species.

The preferred habitat and prey of ladybird species is very poorly documented (Dixon, 2000). However, data collected by Hodek & Honek (1996) depict *A. bipunctata* and *C. septempunctata* as more generalist predators (41 essential aphid prey for the former and 25 for the later) than *A. decempunctata* (14 aphid prey). In terms of habitat preference during the reproductive period, *A. bipunctata* occurs mainly on shrubs and less frequently on trees and herbaceous plants. *C. septempunctata* is more restricted to herbaceous plants. Finally, *A. decempunctata* is a tree-dwelling species that sometimes occurs on shrubs. Therefore, the habitats of these three species overlap to some extent (table 1)**.**

As these species are likely to encounter each other in the field, it is suggested that females should react more strongly to the larval tracks of those species they are most likely to meet.

The objective of this paper is to test the above hypothesis, check whether ovipositing females of *A. decempunctata* and *C. septempunctata* react to conspecific larval tracks and provide qualitative and quantitative information on the chemical nature of the larval tracks of 3 species of ladybird beetles.

Material and methods

Ladybird culture

The *A. bipunctata* and *A. decempunctata* used in this study came from stock cultures maintained in the laboratory. These consisted of adults reared at 20 ± 1°C, LD 16:8, in 5-litre plastic boxes, which contained a piece of corrugated filter paper on which the females laid eggs. Three times a week the ladybirds were fed an excess of pea aphids, *Acyrthosiphon pisum* Harris. Two stems of broad bean, *Vicia faba* L. (variety "Primabel"), were added to each box to improve the survival of the aphids. Adults of *C. septempunctata* were collected in the field and reared in the same conditions.

Ladybirds used in the experiments:

a) Larvae

Eggs were taken from the stock cultures and incubated in 175 cm^3 plastic boxes kept under the same conditions as the stock cultures. After hatching larvae were fed 3 times a week with an excess of pea aphids. Second instar larvae were isolated in 5 mm Petri dishes, fed 3 times a week and checked daily for moulting. Freshly emerged L4 larvae (1 to 48 hours old) were used to produce tracks (see below).

b) Adults

Freshly emerged adults of *A. bipunctata* and *A. decempunctata* were isolated within 24 hours of emerging from pupae. When their cuticles had hardened, they were sexed and couples, consisting of a male and a female, were isolated in 90 mm Petri dishes containing a piece of corrugated filter paper. They were kept at 20 °C ± 1° C, LD 16:8. Each day, the couples were transferred to clean Petri dishes and fed an excess of pea aphids and the eggs they laid were counted. Ladybirds selected for the experiments were between 15 and 25 days old and had laid daily one egg batch over the previous 5 days. In order to standardize hunger, females were deprived of food 16 hours overnight before the beginning of the experiments.

Adults of *C. septempunctata*, used in the experiments came from the field. As this species is monovoltine and each generation undergoes diapause, it is difficult to continuously rear it in the laboratory. The adults were sexed and couples, consisting of a male and a female, were isolated in 90 mm Petri dishes containing a piece of corrugated paper and kept at 20 °C ± 1 °C, LD 16:8. These couples were then treated and selected for the experiments in the same way as those of *A. bipunctata* and *A. decempunctata.*

Experiment 1: *Do females of A. decempunctata and C. septempunctata refrain from ovipositing in the presence of conspecific larval tracks?*

Experiment 2: *Do females of A. bipunctata, A. decempunctata and C. septempunctata refrain from ovipositing in the presence of heterospecific larval tracks?*

At the beginning of both these experiments couples were isolated in 90 mm Petri dishes with about 50 aphids of mixed instars. These couples were randomly subjected to the following treatments:

(1) a filter paper contaminated with tracks of larvae
- of their own species in experiment 1
- of each of the other two species in experiment 2

(2) a clean filter paper as a control, in both experiments.

Depending on the treatment, the number of replicates of the reciprocal experiments varied between 25 and 32.

1, 2, 3, 6, 9 and 24 hours after the beginning of the experiment it was noted whether the females had laid eggs and how many. The proportions of females that laid eggs in the different treatments were compared by χ^2 tests. *These tests were applicable since the average expected values were always bigger than 6.0 (Zar, 1996).*

Larval tracks were produced by placing a 90 mm diameter Whatman® filter paper in the lids of 90 mm diameter Petri dishes in which there were five 1-48 hour old fourth instar larvae of a particular species provided with an excess of pea aphids. After 24 hours at 20 ± 1 °C, LD 16:8, the larvae and aphids were removed and the filter paper brushed to remove faeces, surviving aphids and aphid remains.

Table 2. The number of females of *A. bipunctata* (A2) that laid eggs on clean filter paper (controls) and filter paper contaminated with the tracks of larvae of *A. decempunctata* (A10) or C. *septempunctata* (C7), 1,2,3,6,9 and 24 hours after the beginning of the experiment. (*n* = number of replicates; df = degrees of freedom; * = Significant differences at 0.05; ** = S. diff. at 0.01).

Treatment	n	1h	2h	3h	6h	9h	24h
Control	57	4	9	10	29	41	54
A10	30	0	0	0	8	17	26
C7	28	0	0	1	11	19	25
χ^2 tests							
Global (2 d. f.)		4.217 NS	9.935 **	8.531 *	4.829 NS	2.083 NS	1.802 NS
Control versus pooled tracks (A10+C7) (1 d. f.)		4.217 *	9.935 **	8.317 **	3.881 *	1.263 NS	1.677 NS
Among tracks (1 d. f.)		(1)	(1)	1.090 NS	1.047 NS	0.770 NS	0.094 NS

(1) : Tests cannot be calculated because no female laid eggs after 1 and 2h.

Chemical nature of the larval tracks

a) Production and extraction of larval tracks

Freshly emerged L4 larvae (1-24 hours old) were isolated in 5 mm Petri dishes, and deprived of food for 24 h. Each larva was then carefully introduced into a glass tube (12 mm diameter; 75 mm long). These tubes were sealed with a cotton plug and kept at 20 °C ± 1°C, LD 16:8. As the larvae were hungry, they spent most of the time walking. After 24 hours the larvae were removed and the tubes were stored at – 18 °C. For each species, three batches of 30 tubes were produced. Tubes with no larvae were handled similarly and used as a control.

To extract the larval tracks, the 30 tubes of each batch were successively washed with 1 mL of hexane (Hexane Merck, HPLC grade for liquid chromatography). They were washed a second time with 1 ml of hexane for maximum extraction. Finally, the 2 mL of extract for each batch of tubes was transferred to a small vial and evaporated off under a current of nitrogen. The residue was re-dissolved in 40 µL of hexane for GC-MS analysis. The control tubes were similarly treated. The fasting period imposed on the larvae prior to the experiment and the careful introduction of these larvae into the tubes reduced the likelihood of the tracks being contaminated by faeces or reflex bleeding.

b) Chemical analyses

The analyses were performed on a Finnigan Trace 2000 chromatograph directly coupled to a mass spectrometer quadrupole detector. The whole system was controlled by Xcalibur dada system, 1.2 version. The temperature source was set at 200 °C, the interface at 250 °C and the injection port at 280 °C. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The apolar capillary column was a Restek RTX-5MS (5% diphenyl and 95% dimethylpolysiloxane), 30 m × 0.25 mm, 0.25 µm film thickness. The oven was programmed from 50 to 140 °C at 20 °C /min and from 140 to 300 °C at 3 °C/min. The analysis was carried out by electron impact (EI: 70 eV) with the mass spectra scanned from m/z 60 to m/z 450.

Preliminary experiments using 1µL of the extract (*A. bipunctata*, *A. decempunctata* and *C. septempunctata*) were run to determine the best temperature programme for separation of alkanes. Then, 20 µL of two internal standards nC_{20} and nC_{30}, respectively at 0.02 and 0.1 mg/mL, were added to 20 µL of the larval tract extract. This mixture was then evaporated off under a current of nitrogen and the residue was re-dissolved in 10 µL of hexane, 2 µL of which were analysed using GC-MS.

Identification of substances is based upon mass spectrometry (Xcalibur dada system, 1.2 version library) and comparison with published results (Hemptinne *et al.*, 2001).

After identification, the quantity of each compound present in the mixture was determined. Times of retention and area of the peaks were compared with those of our two internal standards (C_{20} and C_{30}). A standard alkane mixture Supelco from C_{12} to C_{60} (0.01 % w/w each component) was used as a retention time and quantitative standard. Each peak was adjusted by applying a correction factor derived from the chromatogram of a mixture of linear alkanes Supelco. In this way the concentration and quantity of each product in the mixture produced by 30 larvae of ladybirds was determined.

The chemical compositions of the larval tracks of the three species were compared by means of a similarity index – percentage of similarity (Krebs, 1989).

Results

Females of *A. bipunctata* refrained from egg laying for up to 6 hours in the presence of tracks deposited by *A. decempunctata* and *C. septempunctata* (table 2). The females responded similarly to the tracks of these two species (table 2).

For females of *A. decempunctata*, the oviposition-deterrent effect of conspecific and heterospecific larval tracks was detectable for up to 9 hours. They did not differ in their response to the three kinds of tracks. Moreover, based on the level of significance of the χ^2 tests, females of *A. decempunctata* responded more strongly to the tracks than *A. bipunctata* females (table 3).

Females of *C. septempunctata* only responded to conspecific larval tracks after 6 hours. They were not inhibited by tracks deposited by larvae of the two species of *Adalia,* to which they responded similarly (table 4).

Chemical nature of the larval tracks

The hexane extracts of the 3 species consisted mainly of alkanes (90 %) (Figure 1).

Larval tracks of *A. bipunctata* and *A. decempunctata* each contain thirty-eight hydrocarbons: ten linear alkanes (from nC_{21} to nC_{29} and nC_{31}), twenty-two monomethylalkanes and six dimethylalkanes. There are thirty-four hydrocarbons in the larval tracks of *C. septempunctata*: seven linear alkanes (from nC_{23} to nC_{29} and nC_{31}), twenty-three monomethylalkanes, three dimethylalkanes and one unidentified alkane. The quantitative analysis of the tracks of each

Table 3. The number of females of *A. decempunctata* that laid eggs on clean filter paper (controls) and filter paper contaminated with the tracks of conspecific larvae (A10) or larvae of *A. bipunctata* (A2) or *C. septempunctata* (C7), 1,2,3,6,9 and 24 hours after the beginning of the experiment (N = number of replicates; df = degrees of freedom; * = Significant differences at 0.05; ** = S. diff. at 0.01; *** = S. diff. at 0.001).

Treatment	n	1h	2h	3h	6h	9h	24h
Control	85	13	18	25	51	69	85
A10	25	1	1	1	5	18	25
A2	31	0	1	5	13	20	29
C7	30	0	2	4	9	17	29
χ^2 tests							
Global (3 d. f.)		11.734 **	10.588 *	9.638 *	16.820 ***	7.979 *	6.367 NS
Control versus pooled tracks (A10+A2+C7) (1 d. f.)		11.35 7 ***	10.41 4 **	8.305 **	14.10 0 ***	6.362 *	3.018 NS
Adalia versus *Coccinella* (A10+A2 versus C7) (1 d. f.)		0.542 NS	0.422 NS	0.130 NS	0.042 NS	1.061 NS	0.003 NS
Between *Adalia* sp. (A10 versus A2) (1 d. f.)		1.263 NS	0.024 NS	2.138 NS	3.053 NS	0.355 NS	1.673 NS

Table 4. The number of females of *C. septempunctata* that laid eggs on clean filter paper (controls) and filter paper contaminated with the tracks of conspecific larvae (*C7*) or larvae of *A. bipunctata* (A2) or *A. decempunctata* (A10), 1,2,3,6,9 and 24 hours after the beginning of the experiment. (N = number of replicates; df = degrees of freedom; * = Significant differences at 0.05).

Treatment	n	1h	2h	3h	6h	9h	24h
Control	92	1	7	12	31	44	73
C7	31	0	1	2	5	12	20
A2	30	0	2	5	9	13	22
A10	30	1	3	6	15	18	25
χ^2 tests							
Global (3 d. f.)		2.067 NS	1.132 NS	2.648 NS	8.077 *	3.053 NS	3.838 NS
Control versus pooled tracks (A10+A2+C7) (1 d. f.)					0.069 NS		
Adalia versus *Coccinella* (A10+A2 versus C7) (1 d. f.)					5.364 *		
Between *Adalia* sp. (A10 versus A2) (1 d. f.)					2.500 NS		

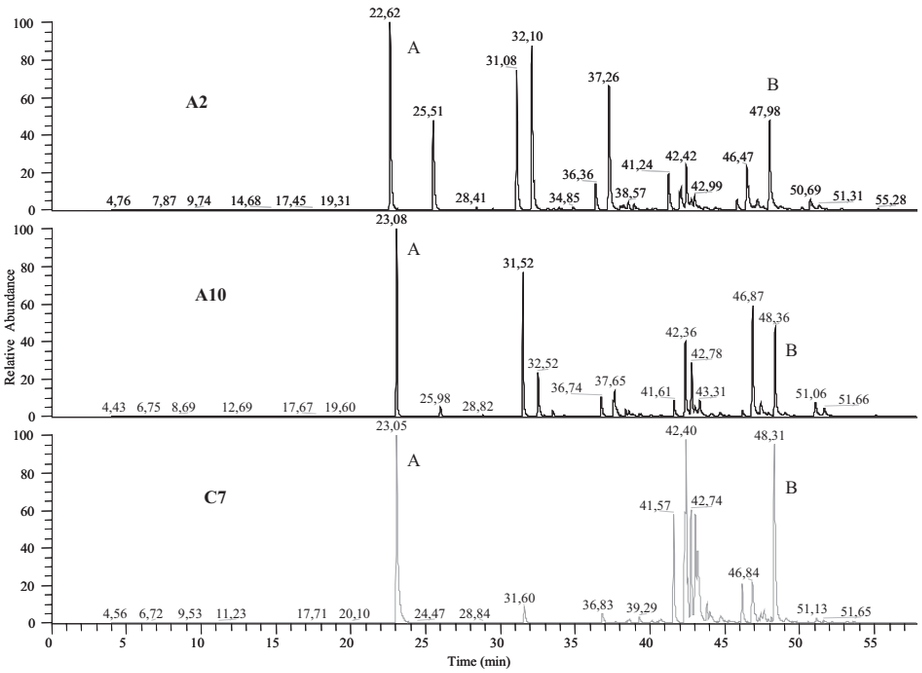

Fig. 1 Chromatograms of the larval tracks of *A. bipunctata* (A2), *A. decempunctata* (A10) and *C. septempunctata* (C7) obtained using a CG-MS, after selected ion monitoring (SIM, Xcalibur dada system) at m/z = 85. The labeled peaks are: A = Eicosane; B = Triacontane

Table 5. Comparison of the relative quantity (%) of compounds in the larval tracks of *A. bipunctata* (A2), *A. decempunctata* (A10) and *C. septempunctata* (C7). 0 = absent.

	Relative quantity (%)		
Compounds	**A2**	**A10**	**C7**
nC21	5.73	0.66	0
9MeC21	0.07	0.03	0
nC22	0.15	0.09	0
nC23	10.78	9.42	0.69
9MeC23	13.83	3.47	0
7MeC23	0.1	0.03	0
nC24	2.33	0.58	0
2MeC24	0.2	0.04	0
nC25	3.58	2.72	0.62
9MeC25	16.96	4.97	0.04
7MeC25	0.43	0.77	0.14
3MeC25	0.56	1	0
2MeC25	0.01	0.52	0
nC26	1.01	0.31	0.38
9MeC26	0	0	0.07
5MeC26	0	0	0.15
8MeC26	0	0	0.08
2MeC26	0.76	0.28	0
nC27	10.64	4.31	11.90
11MeC27	8.39	18.36	>0.00
9MeC27	11.97	11.29	28.70
7MeC27	2.61	4.73	>0.00
5MeC27	0.57	1.09	10.26
3MeC27	0	0	11.99
2MeC27	0	0	10.29
nC28	0.74	1.4	1.79
12MeC28	0	0	1.66
8MeC28	0	0	1.26
nC29	0.63	0.39	5.71
13MeC29	2.97	6.40	0
11MeC29	0.65	0.77	8.67
9MeC29	0.09	0.14	0
5MeC29	0	0	0.85
11,15diMeC29	0.17	1.78	1.74
13,17diMeC29	0.11	0.2	0
7,12diMeC29	0	0	0.11
Unidentified	0	0	0.19
11MeC30	0	0	0.44
12MeC30	0	0	0.6
nC31	0.27	0.27	0.51
15MeC31	0.75	9.07	0.72
13MeC31	0.09	4.7	0.17
11MeC31	0.05	0.16	0.05
9MeC31	0.03	0.49	0.05
11,15diMeC31	0.22	1.25	0
13,17diMeC31	0.41	0.91	0.06
13MeC33	0.3	2.08	0.06
11MeC33	0.37	1.09	0.06
11,15diMeC33	1.06	3.03	0
13,17diMeC33	0.44	1.22	0
Total mass (µm/30 larvae)	7.82	10.73	14.58

species revealed an average of 7.82, 10.73 and 14.58 µg of extract, respectively, in the tracks of thirty larvae of *A. bipunctata*, *A. decempunctata* and *C. septempunctata* (table 5).

The larval tracks of the two species of *Adalia* are qualitatively similar and are composed of 38 alkanes. *C. septempunctata* larval tracks are composed of 34 alkanes, of which only 22 are present in the tracks of the two species of *Adalia*. The quantitative analysis indicates that the most similar tracks are those of the two *Adalia* species, which are 59.9% similar, followed by *A. bipunctata* and *C. septempunctata,* which are 28.6 % similar and, finally, *A. decempunctata* and *C. septempunctata,* which are 24.2% similar.

Discussion and Conclusion

A. bipunctata females can recognise conspecific larval tracks and refrain from ovipositing when they are present

Annexes

Table a. Identification and concentration of compounds present in the hexane extract of the tracks of larvae of *A. bipunctata*. C = concentration in mg/ml based on a µl of solution; Q = quantity of each component in the track material collected from thirty larvae (µg).

Retention time (min)	Compounds	Total carbon number	m/z	[m/z - CH3]	other m/z	other m/z	C	Q
22,62	nC20	20	282				0.0200	0.8
25,51	nC21	21	296				0.0112	0.4482
26,39	9MeC21	22			140-141	196-197	0.0001	0.0052
28,41	nC22	22	310				0.0003	0.0113
31,08	nC23	23	324				0.0211	0.8432
32,1	9MeC23	24	338	323	140-141	224-225	0.0270	1.0812
33,15	7MeC23	24	338	323	112-113	253-254	0.0002	0.0077
33,55	nC24	24	338				0.0045	0.1819
34,85	2MeC24	25	352	337	309		0.0004	0.0153
36,36	nC25	25	352				0.0070	0.2797
37,26	9MeC25	26		351	140-141	252-253	0.0332	1.3265
38,03	7MeC25	26		351	112-113	280-281	0.0008	0.0332
38,21	3MeC25	26	366	351	337		0.0011	0.0440
38,31	2MeC25	26	366	351	323		0.0000	0.0010
38,57	nC26	26	366				0.0020	0.0788
38,93	2MeC26	27	380	365	337		0.0015	0.0596
41,24	nC27	27	380				0.0208	0.8319
42,08	11MeC27	28		379	168-169	252-253	0.0164	0.6558
42,42	9MeC27	28		379	140-141	280-281	0.0234	0.9361
42,99	7MeC27	28		379	112-113	308-309	0.0051	0.2042
43,77	5MeC27	28			84-85	337-338	0.0011	0.0445
44,35	nC28	28	394				0.0014	0.0578
45,8	nC29	29	408				0.0012	0.0493
46,47	13MeC29	30		407	196-197	252-253	0.0058	0.2325
47,18	11MeC29	30		407	168-169	281-282	0.0013	0.0508
47,56	9MeC29	30		407	140-141	308-309	0.0002	0.0069
47,98	nC30			422			0.0105	4
48,71	11,15diMeC29	31		421	168-169/ 238-239	224-225/ 297-295	0.0003	0.0130
49,43	13,17diMeC29	31	434		97/83	69/55	0.0002	0.0086
50,15	nC31	31	436				0.0005	0.0208
50,69	15MeC31	32		435	224-225	252-253	0.0015	0.0586
51,31	13MeC31	32					0.0002	0.0072
52,32	11MeC31	32		435	168-169	308-309	0.0001	0.0040
52,8	9MeC31	32		435	140-141	336-337	0.0001	0.0024
51,08	11,15diMeC31	33		449	168-169/ 322-323	252-253/ 238-239	0.0004	0.0169
53,33	13,17diMeC31	33		449	196-197/ 224-225	266-267/ 294-295	0.0008	0.0318
54,49	13MeC33	34		463	196-197	308-309	0.0006	0.0236
54,52	11MeC33	34		463	168-169	336-337	0.0007	0.0288
54,98	11,15diMeC33	35			168-169/ 350-351	252-253/ 280-281	0.0021	0.0828
55,01	13,17diMeC33	35			196-197/ 322-323	252-253/ 266- 267	0.0009	0.0341

(Hemptinne *et al.,* 1992; Doumbia *et al.,* 1998). The present study indicates that another species of the same genus -. *decempunctata* – responds similarly and confirms the results already obtained for *C. septempunctata* by Doumbia *et al.* (1998) and Růžička, (1997, 2001). In addition, the results indicate that the response of *A. decempunctata* to conspecific larval tracks is much stronger than that of *C. septempunctata.*

The significant response of *C. septempunctata* to conspecific larval tracks is recorded over a much longer period than for *A. decempunctata.* This might be because *C. septempunctata* prefers to oviposit late in the day or during the night (Omkar *et al.,* 2004).

That *A. decempunctata* responds to conspecific larval tracks adds another species to the growing list of aphidophagous ladybird beetles that assess the quality of aphid colonies as oviposition sites. All these species exploit aphid colonies, which are a time limited resource. Due to their ephemeral nature, the optimal strategy is for ladybirds to lay a few eggs at the beginning of the development of an aphid colony (Kindlmann & Dixon, 1993). As soon as they hatch, larvae deposit an oviposition-deterring pheromone each time they attach their anal disk to the substrate (Laubertie *et al.*, 2006). By responding to this signal/cue, females avoid colonies already being exploited by larvae and so reduce the chance of

Table b. Identification and concentration of compounds present in the hexane extract of the tracks of larvae of *A. decempunctata*. C = concentration in mg/ml based on a µl of solution; Q = quantity of each component in the track material collected from thirty larvae (µg).

Retention time (min)	Compounds	Total carbon number	m/z	[m/z - CH3]	other m/z	other m/z	C	Q
23,08	nC20	20	282				0.0200	0.8
25,98	nC21	21	296				0.0018	0.0706
26,39	9MeC21	22			140-141	196-197	0.0001	0.0036
28,82	nC22	22	310				0.0002	0.0092
31,52	nC23	23	324				0.0253	1.0111
32,52	9MeC23	24	338	323	140-141	224-225	0.0093	0.3728
32,97	7MeC23	24	338	323	112-113	253-254	0.0001	0.0037
33,5	nC24	24	338				0.0015	0.0618
35,18	2MeC24	25	352	337	309		0.0001	0.0043
36,74	nC25	25	352				0.0073	0.2918
37,65	9MeC25	26		351	140-141	252-253	0.0133	0.5332
38,38	7MeC25	26		351	112-113	280-281	0.0021	0.0826
38,58	3MeC25	26	366	351	337		0.0027	0.1070
38,82	2MeC25	26	366	351	323		0.0014	0.0559
39,28	nC26	26	366				0.0008	0.0328
40,01	2MeC26	27	380	365	337		0.0007	0.0296
41,61	nC27	27	380				0.0116	0.4624
42,36	11MeC27	28		379	168-169	252-253	0.0493	1.9705
42,78	9MeC27	28		379	140-141	280-281	0.0303	1.2124
43,31	7MeC27	28		379	112-113	308-309	0.0127	0.5074
44,08	5MeC27	28			84-85	337-338	0.0029	0.1167
44,67	nC28	28	394				0.0038	0.1501
46,16	nC29	29	408				0.0010	0.0418
46,87	13MeC29	30		407	196-197	252-253	0.0172	0.6872
47,81	11MeC29	30		407	168-169	281-282	0.0021	0.0830
47,9	9MeC29	30		407	140-141	308-309	0.0004	0.0151
48,36	nC30			422			0.1	4
48,99	11,15diMeC29	31		421	168-169/ 238-239	224-225/ 297-295	0.0048	0.1909
50,36	13,17diMeC29	31	434		97/83	69/55	0.0005	0.0212
50,87	nC31	31	436				0.0007	0.0287
51,06	15 MeC31	32		435	224-225	252-253	0.0243	0.9736
51,66	13 MeC31	32					0.0126	0.5040
52,11	11 MeC31	32		435	168-169	308-309	0.0004	0.0173
53,14	9 MeC31	32		435	140-141	336-337	0.0013	0.0528
53,35	11,15diMeC31	33		449	168-169/ 322-323	252-253/ 238-239	0.0034	0.1343
53,66	13,17diMeC31	33		449	196-197/ 224-225	266-267/ 294-295	0.0024	0.0974
54,49	13 MeC33	34		463	196-197	308-309	0.0056	0.2235
54,52	11 MeC33	34		463	168-169	336-337	0.0029	0.1173
54,98	11,15diMeC33	35			168-169/ 350-351	252-253/ 280-281	0.0081	0.3251
55,01	13,17diMeC33	35			196-197/ 322-323	252-253/ 266-267	0.0033	0.1314

cannibalism, which is common in many coccinellid species (Mills, 1982, Osawa, 1989).

Predatory ladybirds, in common with many phytophagous and parasitic insects, rely on cues associated with the presence of eggs and larvae, and avoid depositing eggs on resources that are being exploited (see Nufio & Papaj, 2001 for a review of this subject).

Large aggregations of aphids are likely to attract several species of predators, with several species of ladybird exploiting the same colonies of aphids in the same habitat. For example, although the typical habitat of *A. bipunctata* is mainly shrubs, that of *A. decempunctata* trees and that of *C. septempunctata* herbaceous plants, these three species overlap. That is, in addition to cannibalism, interspecific predation by larvae may threaten a female's fitness. Again, this poses a problem for females looking for a safe place to lay their eggs and it is likely they have also developed strategies to assess whether patches of prey are already being exploited by heterospecific larvae. In addition it seems logical that the strength of the response should reflect the degree of habitat overlap.

The results presented here show that heterospecific larval tracks inhibit oviposition by *A. bipunctata* and *A. decempunctata,* but that *C. septempunctata* is insensitive to tracks deposited by larvae of both *Adalia* species. Based on the significance of the chi squared tests, *A. decempunctata* seems to react most strongly to heterospecific tracks, followed by *A. bipunctata* and then *C. septempunctata.* Thus, the evolution of oviposition behaviour does not seem to be connected to habitat overlap, although further studies of species with greater habitat overlap are needed. In addition, intraguild predation

Table c. Identification and concentration of compounds present in the hexane extract of the tracks of larvae of *C. septempunctata*. C = concentration in mg/ml based on a µl of solution; Q = quantity of each component in the track material collected from thirty larvae (µg).

Retention time (min)	Compounds	Total carbon number	m/z	[m/z - CH3]	other m/z	other m/z	C	Q
22,62	nC20	20	282				0.0200	0.8
31,55	nC23	23	324				0.0025	0.100
36,74	nC25	25	352				0.0023	0.090
37,62	9MeC25	26		351	140-141	252-253	0.0002	0.007
38,45	7MeC25	26		351	112-113	280-281	0.0005	0.020
39,18	nC26	26	366				0.0014	0.055
40,02	9MeC26	27	380	365	140-141	266-267	0.0003	0.010
40,37	5MeC26	27		365	84-85	324-325	0.0005	0.021
40,65	8MeC26	27		365	126-127	280-281	0.0003	0.012
41,46	nC27	27	380				0.0434	1.735
42,21	11MeC27	28		379	168-169	252-253	0.0000	>0.000
42,28	9MeC27	28		379	140-141	280-281	0.1046	4.184
42,42	7MeC27	28		379	112-113	308-309	0.0000	>0.000
42,63	5MeC27	28			84-85	337-338	0.0374	1.495
42,92	3MeC27			337/379			0.0437	1.749
43,09	2MeC27			337/379			0.0375	1.501
43,71	nC28	28	394				0.0065	0.261
43,88	12MeC28	29		393	182-183	239-240	0.0060	0.242
44,62	8MeC28	29		393	126-126		0.0046	0.183
46,07	nC29	29	408				0.0208	0.833
46,76	11MeC29	30		407	168-169	281-282	0.0316	1.264
47,22	5MeC29	30		407	84-85		0.0031	0.124
47,35	11,15diMeC29	31	436		168-169/ 238-239	224-225/ 294-295	0.0063	0.2530
47,61	7,12 diMeC29	31		407	112-113/ 239-240	182-183/ 350-351	0.0004	0.0158
47,9	Unidentified compound						0.0007	0.0276
48,31	nC30		422				0.1000	4
48,44	11MeC30	31		421	168-169	294-295	0.0016	0.0641
49,02	12MeC30	31		421	182-183	239-240	0.0022	0.0879
50,49	nC31	31	436				0.0019	0.0750
51,07	15MeC31	32		435	224-225	252-253	0.0026	0.1050
51,65	13MeC31	32		435	196-197	280-281	0.0006	0.0249
52,11	11MeC31	32		435	168-169	308-309	0.0002	0.0078
53,2	9MeC31	32		435	140-141	336-337	0.0002	0.0078
53,65	13,17diMeC31	33		449	19-197/ 266-267	224-225/ 294-295	0.0002	0.0091
55,14	13MeC33	34		463	196-197	308-309	0.0002	0.0091
55,58	11MeC33	34		463	168-169	366-337	0.0002	0.0091

does not appear to be a uniform threat for the three species studied and the similarities and differences in the chemical nature of larval tracks might reflect more the phylogeny of these species.

Although there might be a phylogenetic basis for the similarity in the ODP and oviposition behaviour of *A. bipunctata* and *A. decempunctata* (indicated by the similarities in the chemical composition of the larval tracks), the following might also be important. First, aphid colonies are exploited by a sequence of aphidophagous insect predators. In spring syrphids tend to attack aphid colonies earlier and their larvae are present in colonies in autumn later than those of ladybirds, which is related to differences in the lower developmental thresholds of these two groups of predators (Dixon *et al*., 2005). Small species of ladybirds tend to attack aphid colonies before large species (Agarwala & Bandhanroy, 1999, Agarwala & Yasuda, 2001, Smith, 1966), which is thought to be determined by geometrical and physiological constraints associated with body size (Dixon, 2006). The two *Adalia* species are similar in size and *A. bipunctata* attacks aphid colonies before the relatively bigger *C. septempunctata* (Smith, 1966). Thus, it is possible that *C. septempunctata* experiences greater difficulties finding unexploited aphid colonies in which to lay its eggs than the *Adalia* species. Cannibalism or IGP might therefore be less costly in terms of fitness than not laying eggs at all.

Secondly, *C. septempunctata* mainly exploits aphids living on herbaceous plants and readily falls off plants when disturbed, which is not hazardous as it can quickly locate and climb back on to aphid infested plants. The habitat of the two spot and ten spot ladybirds is mainly shrubs and trees, and dropping off is far more risky. Thus it is not surprising that the larvae of both these species have a well developed adhesive disk at the end of their abdomen, which they attach to the substrate to prevent themselves falling off. *C. septempunctata*'s strategy of dropping off is possibly an effective defence against intra or interspecific predators. This is supported by the results presented by Sato *et al.*

(2005), who show that in the presence of larvae of the ladybird *Harmonia axyridis* Pallas, 44.3 % of the larvae of *C. septempunctata* but less then 2% of those of *A. bipunctata* dropped from the plant and 95 % of the *A. bipunctata* larvae fell victim to intraguild predation by *H. axyridis* vs. only 54.5 % of the *C. septempunctata.*

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