THE ROLE OF SEMIOCHEMICALS IN THE AVOIDANCE OF THE SEVEN-SPOT LADYBIRD, *Coccinella septempunctata* BY THE APHID PARASITOID, *Aphidius ervi*

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Abstract – The role of semiochemicals in mediating intraguild interactions between the seven-spot ladybird, *Coccinella septempunctata*, and the aphid parasitoid, *Aphidius ervi* was investigated. Female parasitoids significantly avoided leaves visited by *C. septempunctata* adults and larvae during the previous 24 hr. Ethanol extracts of *C.septempunctata* adults and larvae also induced avoidance responses by *A. ervi*. Two of the hydrocarbons identified by gas chromatography (GC) and coupled GC-mass spectrometry (GC-MS), n-tricosane ($C_{23}H_{48}$) and n-pentacosane ($C_{25}H_{52}$), when tested individually at levels found in the adult extract, significantly induced avoidance by *A. ervi*. Further investigation of the larvae extract, and footprint chemicals deposited by adults in glass Petri dishes, confirmed the presence of the hydrocarbons. Parasitism rates of the pea aphid, *Acyrthosiphon pisum*, on broad bean plants, *Vicia faba*, which had been sprayed with a mixture of the chemicals, were significantly lower than those on control plants. The effect, however, was no longer evident if parasitoid foraging was delayed by 24 hr after the plants were treated. The ecological significance of intraguild avoidance behavior and implications for possible use of the semiochemicals involved in future biological control programs are discussed.

Key Words – Intraguild predation, predator avoidance, trail, oviposition

decision, biological control.

INTRODUCTION

Trophic interactions between organisms sharing the same resource, specifically those interactions involving intraguild predation (IGP), have been well documented in several recent studies (Rosenheim et al., 1993; Colfer and Rosenheim, 1995; Rosenheim et al., 1998; Ferguson and Stiling, 1996; Raymond et al., 2000; Synder and Ives 2001). These reports suggest that IGP changes the extent to which top-down forces by predator guilds affect herbivore populations. Although less is known about the avoidance of intraguild predation, it has been documented both in predator–predator (Doumbia et al., 1998) and predator-parasitoid (Taylor et al., 1998; Nakashima and Senoo, 2003) interactions. In intraguild predator avoidance, the species at risk, i.e. intraguild prey, tends to avoid patches or microhabitats where the aggressors, i.e. intraguild predators, are already or potentially present. Such avoidance behavior may be categorized into two types, avoidance by the potential victims themselves, or avoidance by the parents of victims (e.g. oviposition avoidance).

There are several reports of intraguild avoidance behavior in aphidophagous predators. For example, chrysopid and coccinellid females were deterred from ovipositing when exposed to areas where heterospecifics were present (Růžička, 1998; Růžička, 2001; Agarwala et al, 2003). However, very few studies of intraguild predator avoidance have been conducted on predator–parasitoid interactions. In this system, parasitoids are usually the intraguild prey because parasitized hosts are potentially consumed by predators (Wheeler et al, 1968; Hoelmer et al., 1994; Wells et al., 2001). It

has been demonstrated that the aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Braconidae) avoids places where the intraguild predator, the seven-spot ladybird, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) is present or was present recently (Taylor et al., 1998). Such avoidance behavior was rationalized by the detection of chemical trails left by the predator. No studies, however, have identified the semiochemicals mediating this interaction.

The pea aphid, *Acyrthosiphon pisum* is attacked by a large guild of arthropod natural enemies (Wheeler, 1974, 1977), in which *A. ervi* and *C. septempunctata* are usually the dominant species (Ekbom, 1994; Takahashi, 1996; Senoo et al., 2001). The seasonal distribution of *A. ervi* overlaps with that of foraging *C. septempunctata* ladybirds on alfalfa (Takahashi, 1996), and parasitized aphids can be consumed by the ladybirds (Nakashima, unpublished data). Using *A. pisum*, *A. ervi* and *C. septempunctata* as the model prey-predator-parasitoid system, the chemical characteristics of trails left by foraging ladybirds on plant surfaces, and effects of these chemical compounds on parasitoid behavior were investigated in a series of laboratory experiments. The objectives of our study were to: (1) identify the semiochemicals involved in this intraguild predator avoidance, (2) determine the effective period over which the avoidance behavior functions, and (3) determine the effects of specific compounds on avoidance behavior and parasitism rates of aphids. The overall objective was to assess the role of semiochemicals involved in intraguild predator avoidance, and to consider their potential use in the enhancement of parasitoids in biological control programs.

MATERIALS AND METHODS

Insects. Aphidius ervi were obtained from a laboratory colony that had been initiated with mummies of pea aphids, *A. pisum*, collected from pea fields in Hertfordshire and Bedfordshire, UK during spring 2001. Overwintered adult seven-spot ladybirds, *C. septempunctata*, were collected from evergreen shrubs at Rothamsted Research during March and April, 2002. Both parasitoids and ladybirds were kept at 20˚C and a 16L:8D photoperiod, and maintained with *A. pisum*, reared on broad bean plants, *Vicia faba* L. (Fabaceae) (var. Sutton), until further use. Parasitoids were removed from the colony as final instar larvae or pupae at the mummy stage, and were kept in Petri dishes (9 cm diameter, 1.5 cm height) containing cotton wool with honey solution as adult food until emergence. Two days after the first adult emergence was observed, females were individually confined in small Petri dishes (5.0 cm diameter, 1.5 cm height) with approximately 50 *A. pisum* and cotton with honey solution. All females used in bioassays were 4-5 days old.

Ladybird chemicals. Coccinella septempunctata adults (500, mixed sex) and fourth-instar larvae (150) were cooled with liquid nitrogen and extracted with freshly distilled ethanol (400 ml and 50 ml respectively) for 24 hr at 25^{o}C. The extracts and washings were recovered by filtration (gravity), and diluted to a volume of 500 ml and 150 ml respectively, *i.e.* 1 ladybird equivalent/ml ethanol. A portion of the adult extract (100 ml) was subjected to distillation under high vacuum (0.04 torr) for 24 hr at 25^{o}C, as

described by Pickett and Griffiths (1980), to produce a distillate (100 ml) containing volatile components, and a residue, containing components with little or no volatility, that was re-dissolved in ethanol (100 ml). The crude extract, along with the vacuum distillate and residue fractions, were kept at 4^{o}C until use in the bioassays.

Components with little or no volatility in the adult extract were separated by repeating the vacuum distillation on another portion of the extract (100 ml), and subjecting the residue to liquid chromatography over Florisil (60-100 mesh, Aldrich Chemical Company, Gillingham, UK) using distilled hexane, diethyl ether and ethanol as eluants (150 ml each). The fractions were evaporated to dryness and re-dissolved in ethanol (100 ml) prior to use in bioassays. For GC/GC-MS analysis, a portion of the hexane eluant that had been re-dissolved in ethanol was retained (50 ml) and evaporated to dryness to yield a waxy solid (10.00 mg), from which a solution in hexane (1 mg/ml) was prepared. The larvae extract (50 ml) was also separated into hexane, diethyl ether and ethanol eluting fractions by Florisil column chromatography. For GC/GC-MS analysis, a portion of the hexane eluant (25 ml) was retained and evaporated to dryness to yield a waxy solid (3.12 mg), from which a solution in hexane (1 mg/ml) was prepared. Compounds in the hexane solutions were quantified on the basis of percentage of the total integrated peak area. Male and female adult footprint extracts were collected by placing ladybirds in clean Petri dishes (9 cm x 1.5 cm) for 18 hr at 20^{o}C, then removing the ladybirds and washing the dishes with distilled hexane (10 ml per dish). The extracts were evaporated to 10 µl under a gentle stream of high purity nitrogen and stored in microvials at -20^{o}C until further use.

Gas chromatography (GC). The hexane eluants arising from liquid chromatography of the adult and larval ladybird extracts, and the adult footprint extracts, prepared as described above, were analyzed on a Hewlett-Packard 5880A gas chromatograph equipped with a split-splitless injector, a flame-ionization detector (FID), and a 10 m x 0.53 mm i.d. HP-1 bonded-phase fused-silica capillary column. The oven temperature was maintained at 40^{o}C for 1 min, then programmed at 10^{o}C/min to 150^{o}C, held at this temperature for 0.1 min, then programmed at 10^{o}C/min to 250^{o}C. The carrier gas was hydrogen.

Coupled GC-Mass Spectrometry (GC-MS). A capillary GC column (50 m x 0.32 mm i.d. HP-1) fitted with a cool on-column injector was directly coupled to a mass spectrometer (VG Autospec, Fisons instruments, UK). Ionization was by electron impact at 70eV, 250^{o}C. The oven temperature was maintained at 30^{o}C for 5 min, and then programmed at 5^{o}C/min to 250^{o}C. The carrier gas was helium. Tentative identifications by GC-MS were confirmed by peak enhancement on GC with authentic samples obtained from commercial sources (Pickett, 1990).

Chemicals. n-Tricosane ($C_{23}H_{48}$), n-pentacosane ($C_{25}H_{52}$) and n-heptacosane ($C_{27}H_{56}$) (all 99% purity) were purchased from the Aldrich Chemical Company (Gillingham, UK). For behavioral studies, individual and mixed solutions of these chemicals were prepared in distilled ethanol at the concentrations at which they were found in the ladybird extracts. All solvents used were distilled prior to use.

Application of chemical compounds on plants. Ladybird extracts and pure authentic compounds in ethanol were applied on broad bean seedlings (6-8 leaf stage, 1 plant per pot) in plastic plant pots (8.0 cm in height and 8.0 cm in diameter) for use in behavioral bioassays. The solutions described above were applied to the plants using a rotary atomizer mounted on a multi speed track. The atomizer operated at 4500 rpm and produced a drop size of approximately 110 μm VMD. A small peristaltic pump was fitted to the atomizer to provide stable control of flow rate. Applications were made at a velocity of 0.4 ms^{-1} at a height of 25 cm above the plants, providing an application rate of 1.04 ml/m^2. Control leaves were prepared in a similar manner using distilled ethanol only.

Leaf square experiments. The effects of *C. septempunctata* trails and extracts on *A. ervi* responses were investigated using a dual-choice bioassay. Treatment leaves containing trails were prepared by inserting a leaf from a bean plant (6-8 leaf stage) into a plastic container *via* a slit, releasing a ladybird adult or larva into the container, allowing it to walk upon the leaf for 24 hr, and then removing it from the plant. Control leaves were left untouched for a similar length of time. Treated and control leaves containing extracts/pure compounds or solvents respectively were prepared using a sprayer as described above. Leaves were used for experiments either immediately (0 hr) or 24 hr after exposure ended. Treated and control leaf squares (1.5 cm x 1.5 cm) were taken from the plants and placed 0.5 cm apart in a Petri dish (5 cm diameter), into which a single *A. ervi* female was then released. After allowing the parasitoid to settle (1 min), the time spent on each leaf square was measured for a period of 10 min. Each experiment was repeated twenty times.

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Effects of ladybird chemicals on aphid parasitism. Treated plants were sprayed with a mixed solution of n-tricosane and n-pentacosane in ethanol at the concentrations found in the ladybird extracts, and control plants were sprayed with distilled ethanol. Fifty mixed age *A. pisum* were released onto single treated and control bean plants, placed individually in cylindrical cages (30 cm diameter x 30 cm height). A single *A. ervi* female was released into each cage and removed after 18 hr. Parasitism rates of *A. pisum* were estimated by rearing the aphids on the tested plants in the laboratory, each plant being kept in a cylindrical tube (10 cm diameter x 24.5 cm height) for 10-14 days in a growth room (20˚C, photoperiod of 16L:8D) to allow mummy formation. Rates of parasitism were estimated by dividing the number of mummified aphids by the initial number of released aphids. Experiments were initiated both 0 hr and 24 hr after spraying. Each experiment was repeated twenty times.

Statistical analysis. The duration of visits by *A. ervi* to treated and control leaf squares were analyzed using Wilcoxon's signed rank tests. Rates of parasitism were also compared by Wilcoxon's signed rank tests. Additive effects of chemical compounds on the degree of avoidance were analyzed by a two way ANOVA with application of each chemical compound as main effects. The data were transformed to logarithms to stabilize the variance before this analysis.

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RESULTS

In dual choice leaf square bioassays conducted immediately after leaf exposure to *C. septempunctata* adults and larvae, the total residence times of *A. ervi* females on treated leaf squares were significantly shorter than on control leaves. This effect was not observed when leaves were tested 24 hr after exposure to the ladybird (Figure 1a). Similar results were also obtained for leaf squares sprayed with crude ethanol extracts of *C. septempunctata* adults and larvae (Figure 1b). However, when the crude adult extract was separated by vacuum distillation into a distillate containing volatile components and a residue containing components with little or no volatility, *A.ervi* responses varied. The residence time on leaf squares treated with the residue was significantly shorter than on control squares, and statistically similar to that for the crude extract, whereas the distillate showed little biological activity (Figure 2). Further separation of the residue by liquid chromatography over Florisil using high purity hexane, diethyl ether and ethanol as eluants yielded three fractions comprising compounds of increasing polarity. Of these, the hexane-eluted fraction retained biological activity, suggesting that non-polar chemicals were responsible for the parasitoid avoidance behavior. The diethyl ether and ethanol-eluted fractions showed little or no activity (Figure 3).

To identify the chemicals responsible for parasitoid avoidance, the biologically active hexane-eluted fraction was analysed by high resolution GC and coupled GC-MS. The sample comprised almost entirely of aliphatic hydrocarbons (Table 1; Figure 4a). Components identified included n-tricosane, n-pentacosane and n-heptacosane, which were identified by comparison with published MS spectra (NIST, 1990) and peak enhancement on GC using authentic samples. Other major components were identified

tentatively as branched hydrocarbons from their characteristic fragmentation patterns and by comparison with MS data from similar studies elsewhere (Kosaki and Yamaoka, 1996; Hemptinne et al, 2001). Similar arrays of straight-chained and branched hydrocarbons were found in the hexane-eluted fraction obtained from extracted *C. septempunctata* larvae, and from footprint extracts collected in glass Petri dishes in which *C. septempunctata* male and female adults had been allowed to walk (Figure 4b, c and d respectively). Solutions of the straight-chained hydrocarbons in ethanol, at the levels found in the adult ladybird extract, were prepared for bioassays. Leaf squares sprayed with n-tricosane and n-pentacosane were significantly avoided by *A. ervi*, but n-heptacosane did not induce avoidance behavior (Figure 5). A two way ANOVA showed that both n-tricosane and n-pentacosane significantly induced avoidance behavior (for n-tricosane, F=12.38, 1 and 76 d. f., $P < 0.01$; for n-pentacosane, F=8.00, 1 and 76 d.f., P<0.01), and reduced residence time on treated leaf squares (Figure 6). The interaction between these two chemicals was not significant (n-tricosane x n-pentacosane, F=0.064, 1 and 76 d.f., P=0.80), indicating that their effects on parasitoid avoidance responses were statistically similar. A mixture of these two hydrocarbons significantly reduced the level of parasitism on treated broad bean plants, compared with untreated controls, although the effect disappeared within 24 hr (Figure 7).

DISCUSSION

To our knowledge, this study is the first identification of semiochemicals that mediate intraguild predator avoidance. The aphid parasitoid, *A. ervi*, avoided low-volatile compounds in chemical trails deposited on leaf surfaces by both adults and larvae of *C. septempunctata* ladybirds. However, components present in the trails retained biological activity during relatively short periods, and parasitoids did not avoid areas treated 24 hours previously. From the active fraction isolated by liquid chromatography, n-tricosane and n-pentacosane were identified as the main active stimuli, inducing intraguild predator avoidance by this parasitoid. These two hydrocarbons negatively affected parasitism rates of pea aphids on broad bean plants, but the duration of activity was also less than 24 hr.

n-Tricosane and n-pentacosane have been reported previously as components of trails of seven-spot ladybirds that occur in Japan (Kosaki and Yamaoka, 1996). In the same study, it was shown that the hydrocarbons in trails were identical with cuticular surface chemicals, and large amounts of these chemicals were also secreted from the tarsi. These findings, along with our results, indicate that *A.ervi* avoids n-tricosane and n-pentacosane in the 'footprints' of seven-spot ladybirds, and these two compounds additively affect avoidance responses. Mixtures of hydrocarbons, of which n-pentacosane is the major component, are also present in larval trails of the two-spot ladybird, *Adalia bipunctata*, and it has been shown that the mixture functions as an oviposition deterring pheromone for *A. bipunctata* (Hemptinne et al., 2001). Our findings suggest that *A.ervi* may also use hydrocarbons to avoid trails of *A. bipunctata*.

The results in this study are consistent with previously published work, in that

ladybird trails have a relatively short period of activity (<24 hr) (Nakashima and Senoo, 2003). Even though n-tricosane and n-pentacosane are relatively stable chemicals, their activities disappear quickly. This may be due to absorption or dilution of the hydrocarbons into the plant cuticular lipid layer, or even evaporation/sublimation from the leaf surface over time, as even higher molecular weight hydrocarbons are known to possess vapour pressures at ambient temperatures.

The use of semiochemicals in predator avoidance can be advantageous to parasitoids, because chemicals deposited by *C. septempunctata* give parasitoids both spatial and temporal information on predator presence. Information from predator trails is a reliable indicator of areas where intraguild predators are located, and this spatial information is vital for parasitoids in reducing the risk of predation, especially from aphidophaous ladybirds, as larval ladybirds are likely to stay and complete development within an aphid patch (Dixon, 2000). Additionally, temporal information may also be important for parasitoids. Contrary to larval ladybirds, adults of *C. septempunctata* are highly mobile, and it has been estimated that the average residence time of adult ladybirds in an aphid patch lasts for several hours (van der Werf et al, 2000). Thus, the limited activity (<24 hr) of ladybird trails is advantageous to parasitoids, because they are allowed to forage in patches no longer occupied by adult ladybirds. However, larval ladybirds would continuously renew the chemical signals whilst continuing to forage in a single aphid-infested patch.

Coccinella septempunctata displays aposematic warning coloration and is highly toxic, although its own parasitoid, *Dinocampus coccinellae*, uses the free base, toxic

alkaloid precoccinelline as a kairomone (Al Abassi et al., 2001). There could therefore be an element of toxin avoidance in the case of *A. ervi*, using the hydrocarbons as an extension of the aposematic cues deployed by *C. septempunctata*. However, such a general effect of these particular compounds is unlikely as 2-isopropyl-3-methoxypyrazine is known to provide this signal as well as having a role as an aggregation pheromone for this particular ladybird (Al Abassi et al., 1998).

Intraguild predator avoidance would reduce the likelihood of IGP, and this has been suggested in predator-predator (Schellhorn and Andow 1999; Pallini et al., 1998) and predator-parasitoid (Taylor et al., 1998; Raymond et al., 2000) interactions. In the *A. ervi–C. septempunctata* system, n-tricosane and n-pentacosane function as kairomones for *A. ervi* because these chemicals help them to reduce predation risks to their progeny. The avoidance responses of parasitoids mediated by kairomones can reduce foraging opportunities in patches that contain intraguild predators, and thus rates of parasitism may decrease in these aphid patches. Thus, intraguild predator avoidance may be a factor affecting parasitoid population dynamics, and may affect aphid population suppression. The commercially available chemicals, n-tricosane and n-pentacosane, which are the active compounds in ladybird trails, may be useful for the enhancement of parasitoid roles in biological control programs by helping to concentrate them in target areas through reduction of unproductive foraging in non-target areas. Although it is possible to develop materials and formulations that will extend the short effective period of such compounds, initial field evaluation of the effects of the semiochemicals on parasitism rates is required to assess fully their impact and potential.

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TABLE 1. COMPOUNDS IDENTIFIED FROM COUPLED GC-MS ANALYSIS OF SEVEN-SPOT LADYBIRD, *Coccinella septempunctata*, EXTRACTS

Peak Number[a]	Compound	Percentage (%)[b]
1	n-Tricosane	9.5
2	n-Pentacosane	4.9
3	7,11-Dimethylpentacosane	3.1
4	n-Heptacosane	4.2
5	13-Methylheptacosane	
6	9-Methylheptacosane	27.1
7	7-Methylheptacosane	
8	9,13-Dimethylheptacosane	
9	7,11-Dimethylheptacosane	26.8
10	7,11,15-Trimethylheptacosane	10.0
	Other (unidentified)	14.4

[a] Peak numbers correspond to labeled peaks in GC-MS traces in figures 4a, b, c and d.

[b] Percentage based on peak area calculated by integration on GC of adult body extract.

Figure legends

FIG. 1. Effect of seven-spot ladybird, *Coccinella septempunctata* trails and extracts on residence times of *Aphidius ervi* females on broad bean leaf squares tested immediately or 24 hr after treatment (a) *Coccinella septempunctata* adult and larval trails (b) ethanol extracts of whole adult and larval *C. septempunctata.* Data are expressed as differences from residence times on control leaf squares in choice bioassays (control time – treatment time). Vertical lines with plots indicate ±1 SE. Asterisks indicate significant difference from control (Wilcoxon's signed rank test, $P<0.05$).

FIG. 2. Effect of vacuum distillation fractions of adult *Coccinella septempunctata* ethanol extract on residence times of *Aphidius ervi* females on broad bean leaf squares. Data are expressed as differences from residence times on control leaf squares (a, b), differences from residences times on leaf squares treated with the total extract (c, d) and difference of residence time on leaf squares treated with the distillation residue from that on leaves treated with the vacuum distillate (e) in choice bioassays. Vertical lines with plots indicate ±1 SE. Asterisks indicate significant difference (Wilcoxon's signed rank test, $P<0.05$).

FIG. 3. Effect of hexane, diethyl ether and ethanol-eluted fractions, prepared by liquid chromatography through Florisil, of adult *Coccinella septempunctata* vacuum distillation residue on residence times of *Aphidius ervi* females on broad bean leaf squares. Data are expressed as differences from residence times on control leaf squares in choice bioassays.

Vertical lines with plots indicate ± 1 SE. Asterisks indicate significant difference (Wilcoxon's signed rank test, $P<0.05$).

FIG. 4. Coupled gas chromatography-mass spectrometry (GC-MS) analysis of seven-spot ladybird, *Coccinella septempunctata* extracts a) adult whole body extract, hexane-eluted fraction b) larvae whole body extract, hexane-eluted fraction c) adult male footprint extract d) adult female footprint extract. Peak numbers correlate to identifications listed in table. X = dioctyl phthalate.

FIG. 5. Effect of n-tricosane ($C_{23}H_{48}$), n-pentacosane ($C_{25}H_{52}$) and n-heptacosane ($C_{27}H_{56}$) on residence times of *Aphidius ervi* on broad bean leaf squares. Data expressed as differences from residence times on control leaf squares in choice bioassays. Vertical lines with plots indicate ±1 SE. Asterisks indicate significant difference (Wilcoxon's signed rank test, $P<0.05$).

FIG. 6. Differences in residence times of *Aphidius ervi* females on a leaf disc treated with n-tricosane ($C_{23}H_{48}$), n-pentacosane ($C_{25}H_{52}$), a n-tricosane/n-pentacosane mixture (treatment) and ethanol (control) in choice situations. Vertical lines indicate ± 1 SE (two-way ANOVA).

FIG. 7. Differences in percentages of parasitism by *Aphidius ervi* on broad bean seedlings sprayed with a n-tricosane/n-pentacosane mixture in ethanol or with ethanol only (control),

immediately and 24 hr after release of a parasitoid female in an experimental arena with both types of seedlings. Vertical lines indicate ±1 SE (Wilcoxon's signed rank test, $P<0.01$).

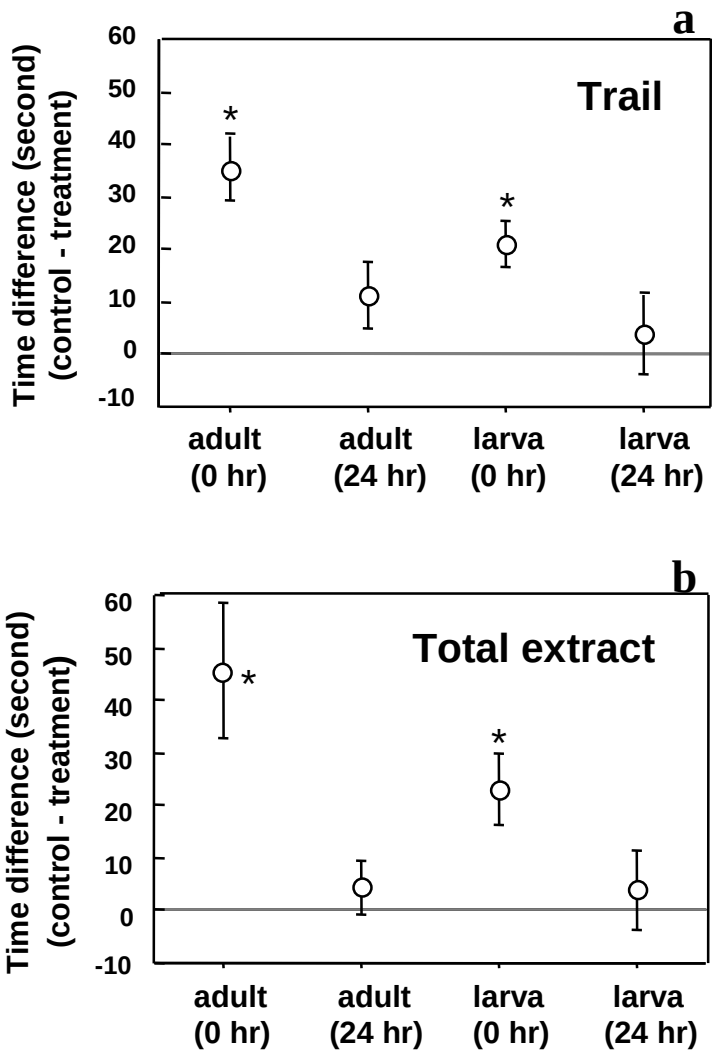
a
Trail
Time difference (second)
(control - treatment)
60
50
40
30
20
10
0
-10
*
*
adult
(0 hr)
adult
(24 hr)
larva
(0 hr)
larva
(24 hr)
b
Total extract
Time difference (second)
(control - treatment)
60
50
40
30
20
10
0
-10
*
*
adult
(0 hr)
adult
(24 hr)
larva
(0 hr)
larva
(24 hr)

Fig. 1

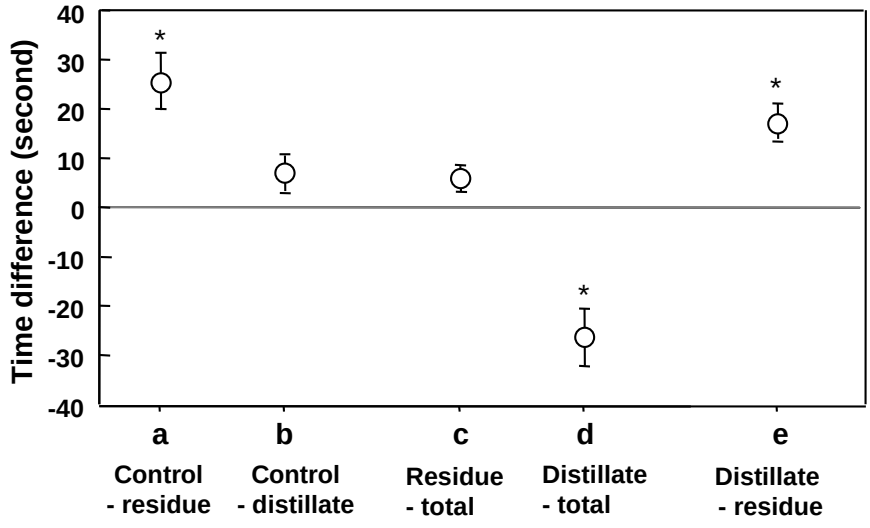
Time difference (second)
40
30
20
10
0
-10
-20
-30
-40
*
*
*
a
b
c
d
e
Control
- residue
Control
- distillate
Residue
- total
Distillate
- total
Distillate
- residue

Fig. 2

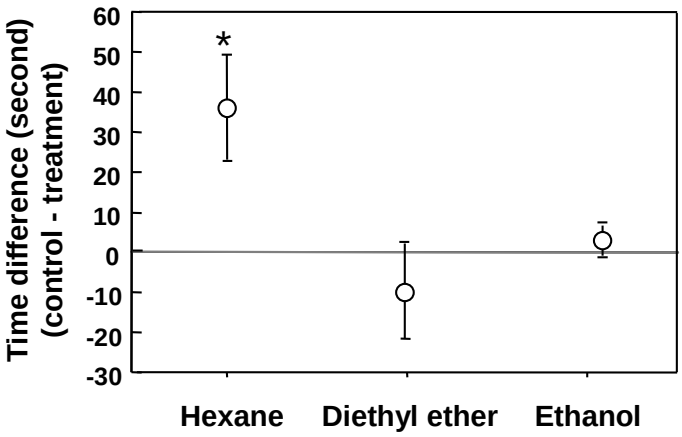
Time difference (second)
(control - treatment)
60
50
40
30
20
10
0
-10
-20
-30
*
Hexane
Diethyl ether
Ethanol

Fig. 3

a

b

c

d

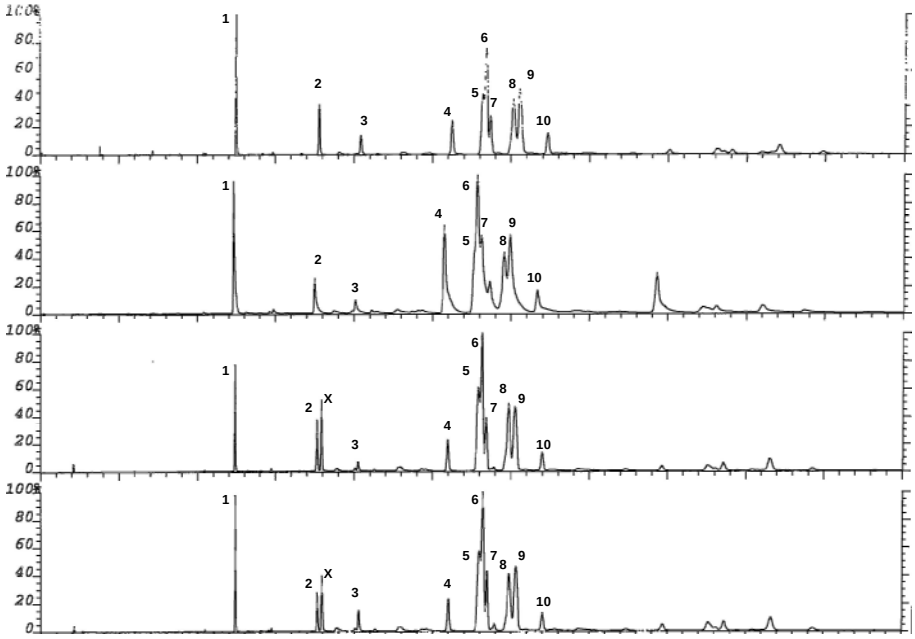

Fig. 4

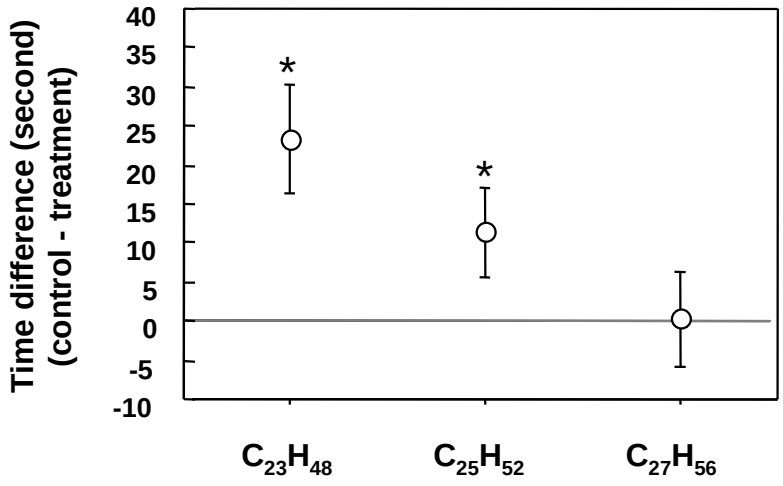
Time difference (second)
(control - treatment)
40
35
30
25
20
15
10
5
0
-5
-10
*
*
C23H48
C25H52
C27H56

Fig. 5

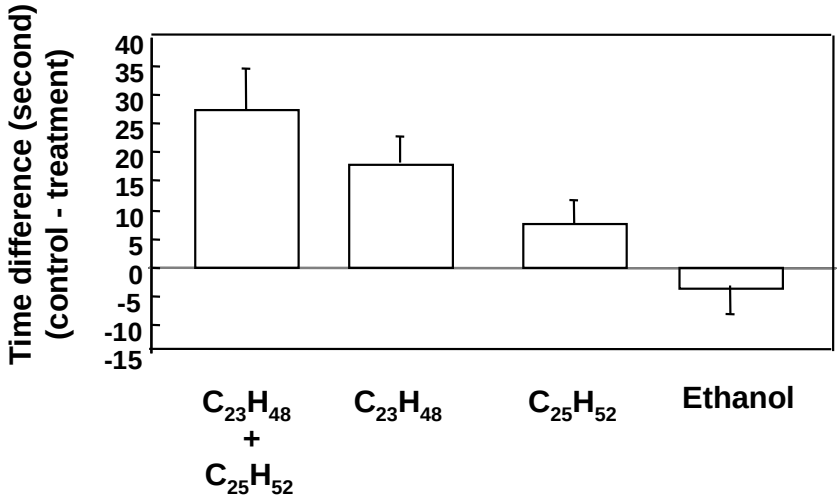
Time difference (second)
(control - treatment)
40
35
30
25
20
15
10
5
0
-5
-10
-15
C23H48 + C25H52
C23H48
C25H52
Ethanol

Fig. 6

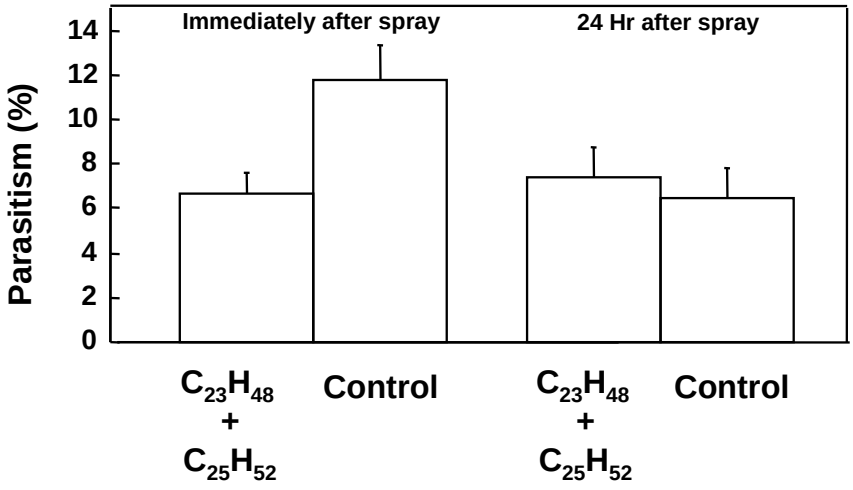
Immediately after spray
24 Hr after spray
Parasitism (%)
14
12
10
8
6
4
2
0
C23H48 + C25H52
Control
C23H48 + C25H52
Control

Fig. 7