RESEARCH ARTICLE

Locomotory behaviour of the seven-spotted ladybird, *Coccinella septempunctata*, in response to five commonly used insecticides

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Abstract

Coccinella septempunctata is known to actively avoid substrates treated with an insecticide containing the organophosphate dimethoate. This study examines the responses of *C. septempunctata* to a range of different insecticide products from three chemical classes, carbamates, organophosphates and pyrethroids. Five formulated product insecticides were compared with the active ingredients (AIs) at two different application rates and using two different spray patterns (conferring choice or no-choice designs) in a randomised block design. Coccinellids' responses differed between insecticide classes. Pyrethoid treatments significantly reduced locomotion. Organophosphates effected mixed locomotory responses, as found in previous studies. Carbamate treatments effected very few changes in locomotory activity. Similar results were found at both application rates tested and under different test designs. The results of the AI tests indicated that different components of the products were responsible for the different reactions, with the AIs being responsible for some responses but the carriers being responsible for others. Results are discussed in relation to the insecticides' modes of action and to their potential to increase the efficiency of integrated aphid control.

Introduction

Behavioural responses to the organophosphate insecticide dimethoate have been reported for *Coccinella septempunctata* (Singh *et al.*, 2001, 2004). Dimethoate is an acetylcholinesterase inhibitor present in a number of widely used insecticide formulations for the control of aphids (LIAISON; Central Science Laboratory, 2004). It is only one of several organophosphates used for aphid control, and organophosphates are only one of several chemical classes of insecticide used for this purpose (Garthwaite *et al.*, 2003). As potential biocontrol agents for aphids, coccinellids such as *C. septempunctata* are therefore likely to experience a diversity of insecticides, with varying modes of action.

Natural enemies of aphids have been found to exhibit different responses to different insecticides. The organophosphate, dimethoate, produces no response to treated prey in carabids (Mauchline *et al.*, 2004) but significant effects on predatory efficiency in coccinellids (Singh *et al.*, 2004). Different organophosphates and pyrethroids have been shown to lead to significantly different responses even within a single species of neuropteran (Hodge & Longley, 2000).

The variable responses of insects to different insecticides (Hodge & Longley, 2000; Mauchline *et al.*, 2004; Singh *et al.*, 2004; also see review by Desneux *et al.*, 2007) make it imperative to understand coccinellids' behavioural reactions and their physiological origins to groups of insecticidal crop protection chemicals. This extends to

different products using active ingredients (AIs) from the same chemical class. Furthermore, as consumer safety concerns led to a review of all anticholinesterases in the UK, Danadim, the organophosphorus product investigated in previous behavioural studies using *C. septempunctata* (Singh *et al.*, 2001, 2004) is currently in the final stages of a phased revocation from the approved list in the UK (Pesticides Safety Directorate, 2005). Other organophosphorus products need to be examined to determine whether the behavioural responses previously reported remain relevant to current crop protection methods in the UK.

This study reports on investigations of locomotory behaviour in *C. septempunctata* exposed to both full-rate and half-rate applications of five insecticides and their active ingredients. The study aimed to determine whether changes in locomotory behaviour, such as those seen in *C. septempunctata* exposed to organophosphates, are also effected by other classes of insecticide currently being used in agriculture in the UK. Where locomotory differences existed, we aimed to quantify these and to determine, using a randomised block design, which components and application rates of the different insecticides might be most effective in instigating these.

Materials and methods

Insects

Cultures of the pea aphid, *Acyrthosiphon pisum* Harris, were maintained on dwarf broad beans, *Vicia faba* Linnaeus (cv. Bunyards Exhibition) following husbandry protocols of Morgan *et al.* (2001). The culture environment was maintained at 13–27°C, with a photoperiod of 18L:6D, with natural daylight being supplemented by 400 W Holophane® lamps (Milton Keynes, UK) where necessary.

Coccinella septempunctata were reared in controlled environment (CE) cabinets using the protocol established by Majerus *et al.* (1989). CE cabinets were maintained at 16–24°C and 48–68% relative humidity with a photoperiod of 16L:8D depending on reproductive rate requirements (Dixon, 2000). Plastic containers (16 × 8 × 5 cm) were used as culture boxes. Twice weekly, excess aphids were provided to adult *C. septempunctata* in clean culture boxes lined with paper. Cultured *A. pisum* were provided as food. Up to 10 adult *C. septempunctata* were transferred to each prepared box using a fine sable brush ensuring that at least one of each sex was included in new boxes.

Wild caught individuals from the York and Edinburgh areas were routinely added to the culture to maintain genetic diversity. These individuals were kept in isolation for 2 weeks prior to their inclusion into the general culture

to avoid the introduction of parasitoids or fungal disease (Majerus *et al.*, 1989).

All *C. septempunctata* used in experiments were 1–5 weeks old (postadult eclosion) and F1–F4 generation.

Insecticides

Insecticides were chosen on the basis of masses used, areas sprayed and crop versatility in Britain (Whitehead, 1998; Garthwaite & Thomas, 1999; Stoddart *et al.*, 2001; Garthwaite *et al.*, 2003). The following insecticides were used in the study: the carbamate pirimicarb, the organophosphates dimethoate and chlorpyrifos, and the pyrethroids cypermethrin and λ-cyhalothrin. Samples of product insecticides (PPs) were obtained from leading manufacturers, and samples of the AIs were obtained from laboratory suppliers. The test PPs were Aphox™ (pirimicarb; Syngenta, Cambridge, UK), Hallmark with Zeon Technology™ (λ-cyhalothrin; Syngenta), Toppel 10™ (cypermethrin; United Phosphorus, Warrington, UK) and Dursban 75WG™ (chlorpyrifos; Dow Agro-Science, Hertfordshire, UK). These 4 chemicals, in addition to BASF Dimethoate 40™ (dimethoate; BASF, Nottingham, UK) as a toxic standard and High Performance Liquid Chromatography (HPLC) standard water as a non-toxic control, constituted the 6 treatments used in behavioural assays. The names of the products are simplified henceforth to pirimicarb PP, dimethoate PP, chlorpyrifos PP, λ-cyhalothrin PP and cypermethrin PP.

A median application rate was established for aphid treatment on a range of crops for each product selected using the LIAISON on-line database (Central Science Laboratory, 2004). Each of these was termed the 'full' rate (Table 1).

Behavioural tests

Treatments were applied by a calibrated bench-top computer-controlled sprayer (Burkard Scientific, Uxbridge,

Table 1 The 'full' application rates for the insecticide products used were the recommended maximum rates for the listed crops (Central Science Laboratory, 2004)

Insecticide Product	'Full' Application Rate	Relevant Crops
Aphox	0.28 kg ha^{-1}	Beans, blackcurrant, oats and wheat
Dimethoate	1.00 L ha^{-1}	Broccoli, garlic and cauliflower
Dursban	1.00 kg ha^{-1}	Beans, blackcurrant and cereals
Hallmark	0.075 L ha^{-1}	Beans
Toppel	0.025 L ha^{-1}	Asparagus, beans, broccoli, potatoes and winter wheat

Middx, UK) in a balanced, randomised block design, encompassing PPs or AIs, full or half application rates and choice or no-choice test designs in 4 experimental blocks (Table 2). A full factorial design was not possible because of time constraints. Therefore, the 4 designs that would provide the greatest detail and allow the fullest comparisons were carried out (Table 2). For the 'no-choice' test, the entire dish was sprayed. For the 'choice' tests, half of the dish was sprayed. The treated dishes were then left in a fume cupboard to dry for 1 h.

Behavioural experiments were carried out in a CE cabinet at a mean of 21°C (± 0.02°C) with a 16L:8D photoperiod and a mean of 55% (± 0.6%) RH. Any effect of environmental variability was controlled for by ensuring that, for any given replicate, all six treatments were tested simultaneously. Coccinellids were sexed, following the methods of Randall *et al.* (1992), and acclimated for a minimum of 12 h prior to experiments. All experiments were started 3–5 h after the onset of photophase, when coccinellids are most active (Nakamuta, 1985).

Behavioural recordings used Ethovision® behavioural analysis software (Noldus, the Netherlands) focussing a video camera on the experimental arena and feeding it directly into a computer. The lids of up to 12 inverted 90-mm glass Petri dishes were fitted into the field of view. Each dish was defined to the software as an arena, allowing 12 independent observations to be recorded simultaneously. In choice experiments, straight lines superimposed onto the arena definitions described two zones. The orientations of the dishes in the experimental field were varied to eliminate phototactic bias from the experiments.

A single coccinellid was placed in the centre of each arena and allowed to acclimate for 15 min in their experimental arenas before data collection. Twelve coccinellids were tracked simultaneously and their locations recorded every second for 3 h. After this time, individuals were maintained within the experimental CE cabinet, with an excess of food, for 24 h. Postexposure mortality was then assessed by gentle prodding to induce movement (Singh *et al.*, 2004).

Analysis

For each of the independent variables, data were obtained for times during which the coccinellids were 'moving'.

Table 2 The four experimental blocks used in the study

Application Rate	Chemicals Used	Test Design
Full	Commercial Products (PP)	Choice
Full	Active Ingredients (AI)	Choice
Half	Commercial Products (PP)	Choice
Half	Commercial Products (PP)	No choice

Moving was defined as travelling at speeds ≥ 5 mm s^{-1} for distances ≥ 10 mm. Movement was considered synonymous with 'walking' and 'activity' because movement was very rarely by flight after the acclimation period. Locomotory variables analysed were the frequency of walking events (periods in which movement was sustained for ≥ 2 s, as defined by the conditions for movement), the mean duration of activity periods, the total duration of activity, the turning frequency (where turning was defined as rotating through $\geq 60°$ in successive movement vectors), the mean walking speed during active periods, the mean distance travelled during activity periods and the total distance travelled. Where the relative degree of linear movement, which might indicate an avoidance behaviour, could not be determined from these variables, turn frequency was divided by the total distance travelled to obtain a measure of overall 'tortuosity' (degree per centimetre) – how tortuous (or linear) the overall path shape was (*sensu* Biesinger & Haefner, 2005).

Locomotor variables were transformed using $\log_{10}$ $(n + 1)$. These variables and the untransformed survival data were analysed using factorial ANOVA. Independent variables included as factors in the analysis were experiment block, insecticide treatment, sex, time period (first, second or third hour) and zone of arena (treated or untreated). Pairwise multiple comparisons were tested using least significant differences (LSD) test for low numbers of comparisons, such as when examining within-block variation from controls or the more conservative Bonferroni test for large numbers of comparisons, such as when examining between-block variation. Although sex was included in the analysis throughout, few differences were found between the two sexes and they are therefore mentioned seldomly. Twenty coccinellids were used for each treatment, 10 of each sex. A total of 480 coccinellids were used in the four experimental blocks.

Results

The full-rate formulated product (PP) choice test

There were trends for all aspects of the locomotor behaviour recorded for *C. septempunctata* to decrease with time on all surfaces (Fig. 1). Superimposed on this trend, both of the pyrethroid insecticides (λ-cyhalothrin PP and cypermethrin PP) and both of the organophosphate insecticides (dimethoate PP and chlorpyrifos PP) suppressed locomotion in several of the parameters (Fig. 1). However, despite significant decreases in the turning frequencies in three treatment groups, there were no significant changes in tortuosity ($F_{10,359} = 0.3$, $P = 0.97$).
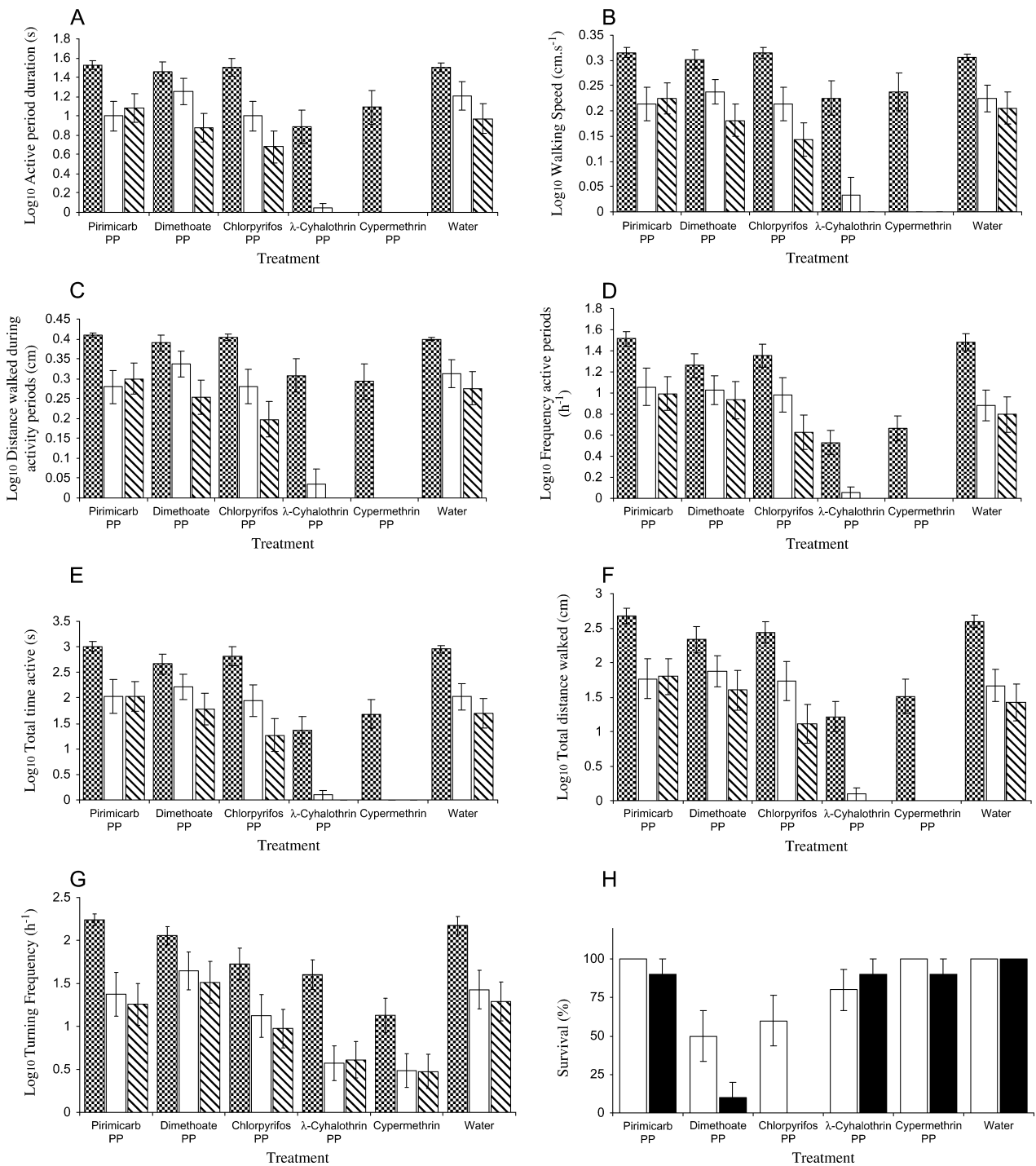
Figure 1 Results of the full-rate product choice tests. (A–G) Movement variables against treatment and time period: (A) mean duration of activity periods ($F_{10,719} = 11.3$, $P < 0.001$); (B) mean walking speeds ($F_{10,719} = 10.4$, $P < 0.001$); (C) mean distance walked during activity periods ($F_{10,719} = 12.7$, $P < 0.001$); (D) frequency of activity periods ($F_{10,719} = 6.6$, $P < 0.001$); (E) total duration of activity ($F_{10,719} = 9.7$, $P < 0.001$); (F) total distance walked ($F_{10,719} = 9.3$, $P < 0.001$) and (G) turning frequency ($F_{10,719} = 4.9$, $P < 0.001$). Shaded bars, hour 1; open, hour 2 and striped, hour 3. (H) Coccinellid survival 24 h following exposure against treatment and sex ($F_{10,719} = 22.3$, $P < 0.001$); open bars, female; filled, male. Error bars, standard error of the mean.

Additionally, the two organophosphate treatments reduced coccinellid survival, with males being more affected than females (Fig. 1H; LSD test, $P < 0.05$). Pirimicarb PP did not significantly affect either coccinellid behaviour or mortality (Fig. 1). The effects of the two pyrethroids were greater than those of the organophosphates, and they were similar in nature (more so than those of the two organophosphates). Finally,

C. septempunctata demonstrated no preference to move more in either the treated or untreated zones of arenas ($F_{5,719} < 2.1$, NS).

The half-rate formulated product choice test

With the exception of one insecticide treatment (pirimicarb PP; Table 3), there was a general decrease in activity

Table 3 Movement variables from half-rate formulated product choice test for each insecticide treatment in each hour. Factorial ANOVA results are presented, and multiple comparisons (least significant differences test) results are presented alongside the back-transformed mean values[a]

Variable Units (ANOVA Statistics)	Treatment	Hour 1		Hour 2		Hour 3	
		Mean Value	Multiple Comparisons	Mean Value	Multiple Comparisons	Mean Value	Multiple Comparisons
Mean duration of walking events (s)	Pirimicarb PP	18.1	b_1	14.1	a_1	14.8	c_1
($F_{10,359} = 4.493$, $P < 0.001$)	Dimethoate PP	34.9	a_1	16.0	a_1	6.0	a_2
	Chlorpyrifos PP	35.2	a_1	10.7	a_2	6.2	a_2
	λ-cyhalothrin PP	11.9	b_1	0.0	b_2	0.0	b_2
	Cypermethrin PP	22.7	ab_1	0.0	b_2	0.0	b_2
	Water	50.3	a_1	8.2	a_2	5.5	a_2
Mean walking speed (cm s^{-1})	Pirimicarb PP	0.8	d_1	0.7	d_1	0.8	f_1
($F_{10,359} = 7.045$, $P < 0.001$)	Dimethoate PP	1.0	d_1	0.7	d_2	0.5	d_2
	Chlorpyrifos PP	1.0	d_1	0.6	d_2	0.5	d_2
	λ-cyhalothrin PP	0.9	d_1	0.0	e_2	0.0	e_2
	Cypermethrin PP	0.9	d_1	0.0	e_2	0.0	e_2
	Water	1.1	d_1	0.5	d_2	0.4	d_2
Mean distance walked during individual	Pirimicarb PP	1.2	g_1	1.1	g_1	1.3	i_1
walking events ($F_{10,359} = 7.750$, $P < 0.001$)	Dimethoate PP	1.5	g_1	1.1	g_2	0.8	g_2
	Chlorpyrifos PP	1.5	g_1	0.9	g_2	0.7	g_2
	λ-cyhalothrin PP	1.2	g_1	0.0	h_2	0.0	h_2
	Cypermethrin PP	1.4	g_1	0.0	h_2	0.0	h_2
	Water	1.6	g_1	0.8	g_2	0.6	g_2
Frequency of walking events	Pirimicarb PP	19.8	j_1	11.9	l_1	11.8	l_1
($F_{10,359} = 1.260$, $P = 0.252$)	Dimethoate PP	19.4	j_1	5.5	j_2	2.9	j_2
	Chlorpyrifos PP	21.9	j_1	5.8	j_2	2.7	j_2
	λ-cyhalothrin PP	3.8	k_1	0.0	k_2	0.0	k_2
	Cypermethrin PP	4.3	k_1	0.0	k_2	0.0	k_2
	Water	19.5	j_1	5.1	j_2	3.8	j_2
Total duration of activity(ies)	Pirimicarb PP	369.7	m_1	179.1	o_1	181.2	o_1
($F_{10,359} = 2.664$, $P = 0.004$)	Dimethoate PP	648.8	m_1	93.6	m_2	23.1	m_2
	Chlorpyrifos PP	744.7	m_1	71.4	m_2	21.7	m_2
	λ-cyhalothrin PP	50.3	n_1	0.0	n_2	0.0	n_2
	Cypermethrin PP	91.6	n_1	0.0	n_2	0.0	n_2
	Water	953.0	m_1	50.1	m_2	28.7	m_2
Total distance walked	Pirimicarb PP	178.0	p_1	90.3	p_1	100.3	r_1
($F_{10,359} = 2.710$, $P = 0.003$)	Dimethoate PP	242.0	p_1	37.7	p_2	11.7	p_2
	Chlorpyrifos PP	245.5	p_1	32.0	p_2	11.0	p_2
	λ-cyhalothrin PP	41.1	q_1	0.0	q_2	0.0	q_2
	Cypermethrin PP	35.4	q_1	0.0	q_2	0.0	q_2
	Water	307.1	p_1	26.1	p_2	17.6	p_2
Turning frequency	Pirimicarb PP	109.0	s_1	55.4	u_1	48.3	u_1
($F_{10,359} = 2.268$, $P = 0.014$)	Dimethoate PP	59.3	s_1	22.3	s, u_1	16.0	$s_{1,2}$
	Chlorpyrifos PP	70.0	s_1	15.1	s_2	8.7	s_2
	λ-cyhalothrin PP	18.9	t_1	0.0	t_2	0.0	t_2
	Cypermethrin PP	16.5	t_1	0.0	t_2	0.0	t_2
	Water	135.9	s_1	13.9	s_2	6.9	s_2

[a]Values sharing a letter were not significantly different within hours, between treatments (comparing within columns). Within a variable, mean values sharing a subscript number were not significantly different within a treatment, between hours (comparing within rows).

with time for all locomotory parameters. These patterns mirrored those in the full-rate test ($F_{6,386} < 1.4$, NS). Reductions in locomotion were greatest in response to the pyrethroid-based insecticides (Table 3).

Relative to the control group, the locomotor activity of coccinellids in the pirimicarb PP treatment group increased significantly in all the variables tested (Table 3). Within the half-rate product choice test, females walked more frequently ($F_{5,119} = 3.6$, $P = 0.004$) and covered greater distances ($F_{5,119} = 2.4$, $P = 0.04$) than males in the pirimicarb PP treatment. These differences (Table 3) between the treatments within the half-rate product choice test did not, however, represent significant departures from the activity patterns of the full-rate test for either sex ($F_{8,599} < 2.9$, NS) or time ($F_{4,199} < 5.6$, NS).

Coccinella septempunctata again demonstrated no preference to move more in either the treated or untreated zones of arenas in the half-rate formulated product choice test ($F_{5,119} < 0.7$, NS).

In summary, although coccinellids appeared to respond to reduced rates of pirimicarb PP by increasing their movement with respect to a number of variables, these locomotor increases were not statistically significant departures from the responses to full-rate applications. Overall, no significant differences were found between the responses of *C. septempunctata* to full- and half-rate insecticide applications for any of the five insecticides tested.

The full-rate active ingredient choice test

Within the AI choice test, the interaction of insecticide treatment and time period was statistically significant in four of the seven movement variables examined (Table 4). Upon examination (*post hoc* LSD test), only the λ-cyhalothrin AI treatment decreased locomotion in *C. septempunctata* (Fig. 2; LSD test, $P < 0.05$), indeed dimethoate AI even led to an increase in locomotion (Fig. 2; LSD test, $P < 0.05$). However, when the 3 h were considered together, there were no significant differences between treatment groups.

Table 4 Significance levels of interactions involving treatment and time period for each movement variable tested in the full-rate AI choice test

Variable	$F_{10,359}$	P
Mean duration of activity period(s)	1.33	0.213
Mean walking speed (cm s^{-1})	1.81	0.057
Mean distance walked during activity periods (cm)	2.10	0.024
Frequency of activity periods	2.22	0.017
Total duration of activity(ies)	2.29	0.013
Total distance walked (cm)	2.42	0.009
Turning frequency	1.33	0.214

Male *C. septempunctata* were found to travel significantly further during individual walking events on dimethoate AI-treated substrates than they did in the control group ($F_{5,359} = 3.1$, $P = 0.01$). Additionally, female coccinellids exposed to λ-cyhalothrin AI were found to walk significantly shorter distances during activity periods than coccinellids from any other insecticide treatment group ($F_{5,359} = 3.0$, $P = 0.01$).

Comparison of the AI choice test with the PP choice test showed significant differences between the two experimental blocks in all the locomotory variables examined (Fig. 3). Differences were attributable to both the sex of the coccinellids ($F_{10,1499} \geq 2.2$, $P < 0.05$) and the time period of the experiments ($F_{20,1499} \geq 9.1$, $P < 0.001$). In the majority of cases (19 of 28), these differences arose in 2 and 3 h, suggesting a cumulative effect over time.

In four of the seven locomotor variables, the mean values over the 3 h calculated for pirimicarb AI, dimethoate AI and chlorpyrifos AI did not differ significantly from those of their respective PPs (e.g. Fig. 3), but the mean values for both pyrethroid AIs were significantly higher than those of their respective PPs. The other three locomotor variables also highlighted differences in responses between PPs and AIs, although a pattern was less obvious (Fig. 3). Only dimethoate AI significantly reduced post-treatment survival in the AI experiment block ($F_{4,199} = 7.8$, $P < 0.001$; Fig. 3H). In the chlorpyrifos treatment group, coccinellid survival was significantly lower in the PP test than in the AI test. The survival of coccinellids in the other AI treatment groups was not significantly different to 100%.

The half-rate formulated product no-choice test

Comparing the results of this no-choice test to those of the half-rate product choice test, factorial ANOVA indicated that there were significant differences attributable to the experimental blocks ($F_{4,201} > 2.5$, $P < 0.05$). However, *post hoc* multiple comparisons procedures revealed that in all cases, differences were those (reported above) from within the choice test not between the two test designs.

Discussion

Previous studies have determined that the presence of insecticides on treated surfaces causes modifications in the behaviour of *C. septempunctata* that reduce exposure levels (Wiles & Jepson, 1994; Singh *et al.*, 2001). Organophosphates (specifically dimethoate) were previously the only group of insecticides to which detailed locomotory responses in *C. septempunctata* had been studied under laboratory conditions (Singh, 2001). This study aimed to build on earlier work by considering locomotory
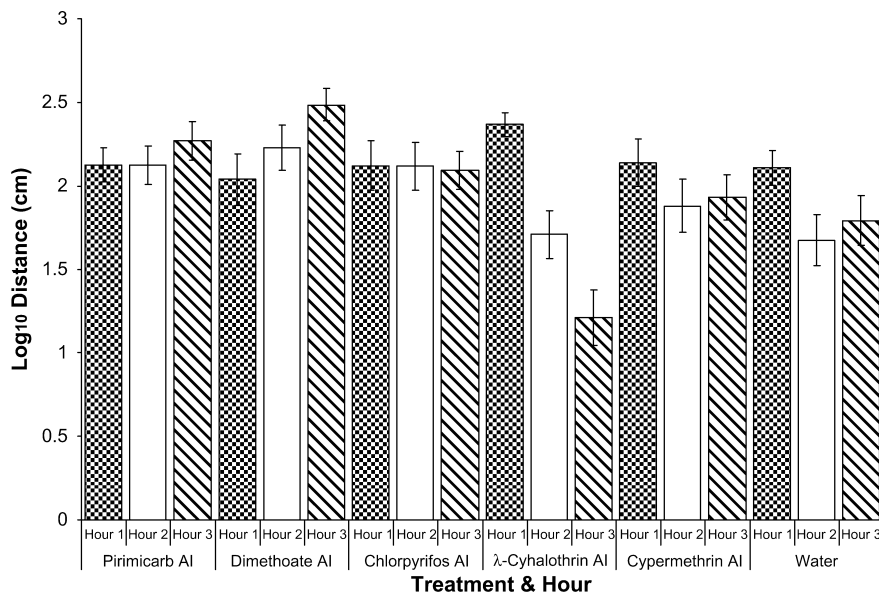
Figure 2 Example of within-block results from full-rate active ingredient choice test: total distance walked by coccinellids against treatment and time. Shaded bars, hour 1; open, hour 2 and striped, hour 3. Statistics are provided in text and Table 4. Error bars, standard error of the mean.

behaviour using a wider range of parameters and insecticides. This is particularly important as responses of other beneficial insects have been demonstrated to vary considerably according to both insecticide product and dose (see review by Desneux *et al.*, 2007).

Postexposure mortality

Mortality has traditionally been used to assess insecticide efficacy but is increasingly becoming recognised as only one of a suite of effects insecticides have (Desneux *et al.*, 2007). In this study, postexposure survival rates were high in ladybirds exposed to pyrethroid- and carbamate-treated surfaces and low after organophosphate exposure (Fig. 1D). The increased mortality rates seen on organophosphates in this instance may be attributable, in part, to the differences in the concentrations of insecticides presented according to the recommended application rates (Table 1). Further study might elucidate the degree to which this is the case, but for the purposes of this study, we felt that the biological significance of using concentrations relevant to crop protection and thus similar to those encountered by ladybirds in the field was paramount.

Differential susceptibilities to organophosphates, carbamates and pyrethroids might also be explained by their respective modes of action on the central and peripheral nervous systems (Sogorb & Vilanova, 2002). Interestingly, males were found to succumb to the toxic effects of the organophosphates more readily than females

(Fig. 1H). This is most likely to be attributed to differential rates of neurotoxin uptake because of physical differences between the sexes, such as in mass or differences in tarsal morphology (Thornham *et al.*, 2007). Sex-related differences in locomotion were also identified occasionally and although attributing biological significance to these differences is difficult, sexual selectivity by insecticides may warrant further investigation if there is a risk of skewing sex ratios in wild populations.

Behavioural modifications in response to formulated products

In all formulated product treatment groups, there was a general decrease in locomotion with time at both full-rate and half-rate applications (Fig. 1 and Table 3). However, as the control group also showed a similar decrease in locomotor behaviour over time, the actions of the insecticides cannot be considered to be the sole factor involved. The decrease in mean walking speed seen in the control group over time (Fig. 1B) suggests that reduced locomotion is unlikely to be attributable to hunger, as previous work on ladybirds has shown that starvation leads either to increased or maintained walking speeds (Carter & Dixon, 1982; Biesinger & Haefner, 2005). Gravid *C. septempunctata* females are known to avoid depositing eggs where conspecific footprint chemicals are present (Oliver *et al.*, 2006) and larvae avoid conspecific chemical secretions when foraging to increase search efficiency (Marks, 1977; Nakashima *et al.*,
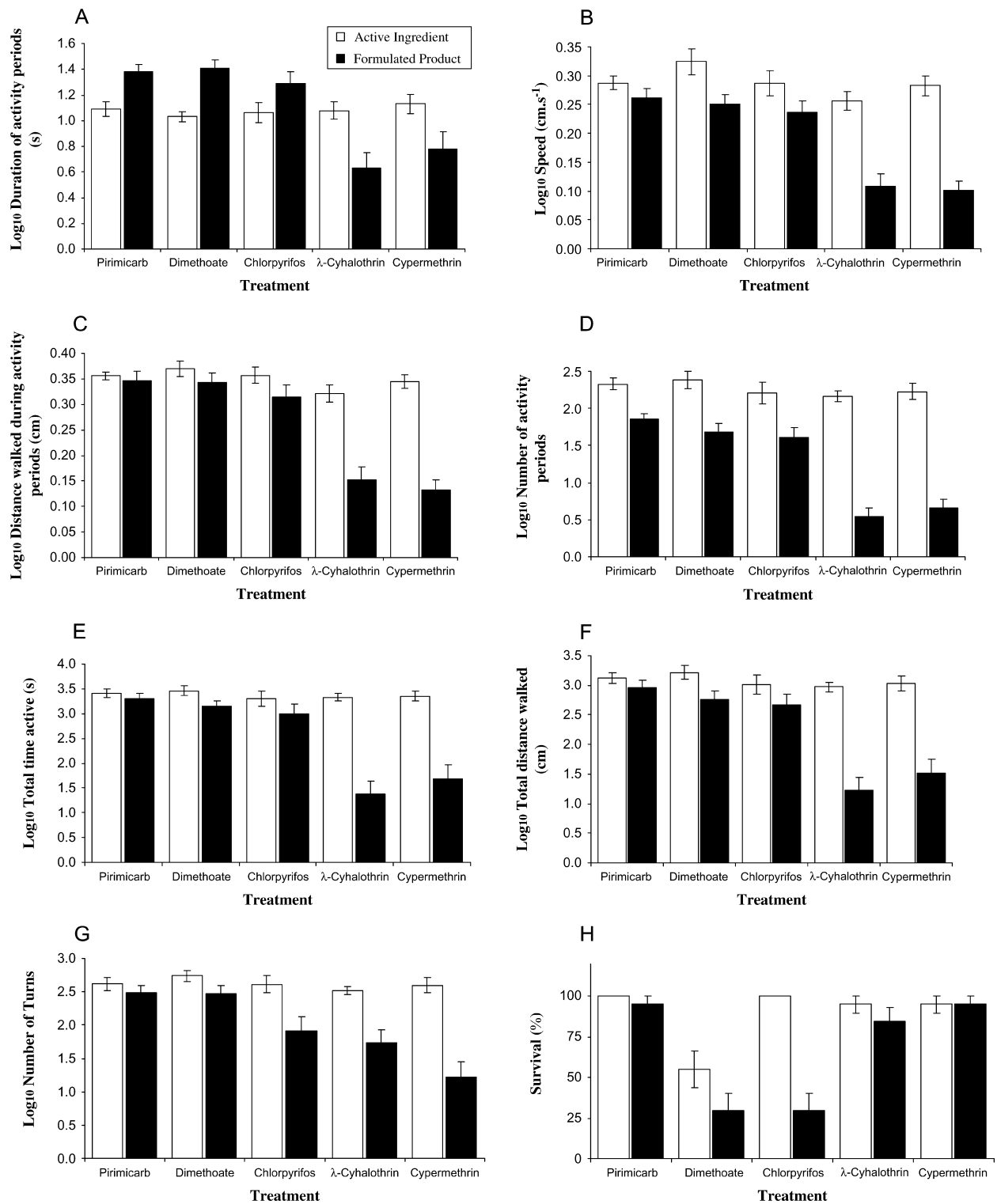
Figure 3 Results of the AI choice test (open bars) compared with those of the full-rate PP choice test (filled bars). (A–G) Movement variables against treatments: A, mean duration of activity periods ($F_{4,199}$ = 10.8, P < 0.001); (B) mean walking speed ($F_{4,199}$ = 6.5, P < 0.001); (C) mean distance walked during activity periods ($F_{4,199}$ = 12.8, P < 0.001); (D) number activity periods ($F_{4,199}$ = 12.6, P < 0.001); (E) total duration of activity ($F_{4,199}$ = 12.6, P < 0.001); (F) total distances walked ($F_{4,199}$ = 11.0, P < 0.001) and (G) number of turns ($F_{4,199}$ = 5.7, P < 0.001). (H) Survival after 24 h ($F_{4,199}$ = 7.8, P < 0.001). Error bars, standard errors of the means.

2002), and it may be that the decrease in locomotor behaviour over time is, in part at least, a response to increased levels of footprint chemicals present as coccinellids explore the arena.

Pyrethroid PPs significantly reduced movement in the first hour of the experiment, when accumulation of footprint chemicals was likely to be minimal and caused almost complete cessation of movement by the end of the 3 h observation period (Fig. 1). The survival rates of *C. septempunctata* indicate that this was not evidence that prolonged exposure to this class of insecticides decreased locomotion through progressive neurological poisoning. Instead, decreases in locomotor activity may have reduced coccinellids' exposure to pyrethroid insecticides and consequently increased postexposure survival. However, this is not supported by the results of the AI test, in which locomotion was not significantly reduced but survival remained high. Further work is necessary to determine whether similar behavioural responses equate to decreased mortality in the field as they do in laboratory conditions, and thus, whether they are likely to provide a selection pressure for the evolution of an avoidance response.

Decreased tortuosity has previously been used as an indicator of an avoidance response to insecticides (Singh *et al.*, 2001). While significant reductions in turning frequency seen in response to both pyrethroid treatments and to chlorpyrifos PP (Fig. 1G) might suggest an increase in straight-line locomotion, there were no significant differences in tortuosity, either between treatments or between any of the interacting independent variables measured. The observed decreases in turning in these experiments are not likely, therefore, to constitute an avoidance response to these chemicals by *C. septempunctata*.

In both formulated product pyrethroid treatments and in chlorpyrifos PP, similar reductions in locomotory behaviour were observed at both half-rate and full-rate applications. This might indicate that the behavioural modifications seen in response to both products are independent of concentration and that the levels present at both application rates were sufficient to stimulate a presence/absence response. Equally, it is possible that the detection mechanism (whether sensory based or toxicological) is concentration dependent but that both application rates tested are above saturation level, resulting in 'extreme' reactions. Further work at lower concentrations is required to determine the relative effects of the dose rate on ladybird behaviour.

Responses to active ingredients

The coccinellids' responses to the AIs of the insecticide products revealed that the beetles were responding to different components of the insecticides' formulation in different products.

Pyrethroid PP treated substrates caused the coccinellids to significantly reduce their locomotor activity compared with the controls. As previously mentioned, it is possible that the decreases in locomotor activity decreased the coccinellids' exposure to the pyrethroid insecticides and the subsequent rate at which the chemicals took effect on their nervous systems. However, no such similar behavioural results were observed in the AI tests but similarly low levels of mortality were observed. It therefore seems more likely that pyrethroids were simply less toxic to the coccinellids than the organophosphates. Moreover, the behavioural responses to the products must have been to formulation chemicals rather than the AIs.

The coccinellids' responses to the AIs of the insecticide products revealed that the beetles were responding to different components of the insecticides' formulation. Evidence from the pyrethroids and further work examining the carrier formulation of the dimethoate PP product reinforce this conclusion (Thornham, 2005), confirming that different chemicals in the formulated product stimulated different behavioural responses from *C. septempunctata*. Additionally, the difference in susceptibility to chlorpyrifos AI and PP (Fig. 3H) highlights how effective the carrier formulation can be, not just in terms of influencing behaviour but in altering insecticide uptake and subsequent mortality.

Of particular interest is the observation that for the pyrethroids, large locomotor changes were observed in response to the products, λ-cyhalothrin PP and cypermethrin PP (Fig. 1) but not the AI treatments (Fig. 3). This provides strong evidence that the stimuli for the behavioural alterations observed in the pyrethroid product groups were components of the carrier formulation rather than the AIs. This situation is slightly different from that of the organophosphates, where both the carrier and the AIs were concluded to stimulate responses. Although coccinellids' responses to the carrier formulations of organophosphate insecticides must be concluded to override their responses to the AIs, the situation for pyrethroids would make the outcome of any attempt to manipulate insect behaviour by altering the makeup of an insecticide a lot easier to predict.

Attempting to categorise behavioural responses as results of repellency – occurring through the normal sensory pathways – or of irritancy, an adverse neurotoxic event (Haynes, 1988; Desneux *et al.*, 2007), has proved difficult. No changes in tortuosity between treatment groups or different movement patterns between the zones of choice arenas were found, indicating that repellency was not a contributing factor. However, neither can we conclude that the insecticides in question were irritant

because in many cases, responses were to non-neurotoxic carrier components of the PPs. Instead, a suite of loco-motory responses was stimulated by a wide variety of chemicals of differing toxicities. Individual responses could be variously categorised as arrestment, excitant or stimulant. However, much more work is needed to tease apart all the responses to the different components of the insecticides used here as no one term could be applied to any of the PPs studied.

Relevance to integrated pest management

The consequences of the results of these experiments to integrated pest management (IPM) strategists and to insecticide manufacturers who wish to promote IPM are significant. A decrease in locomotor activity in response to pyrethroid-based insecticides has the potential to maintain a population of coccinellids in an area of sprayed crop, without resulting in high rates of mortality. Such a situation would allow for a rapid recovery of the coccinellids' biocontrol potential because with limited movement, coccinellids would resume feeding in the crop rather than moving to escape the insecticide. The only food available would therefore be the prey that the insecticide had failed to destroy (Singh *et al.*, 2004). This effect would act to limit the initiation and subsequent growth of an aphid colony in the crop at that time when such predatory activity by coccinellids has been shown to be most effective for biocontrol (Kehrli & Wyss, 2001). Furthermore, by removing remaining aphids in a sprayed crop, the risk of pest resurgence, recognised as one of the greatest problems facing pest control (Kogan, 1998), would be reduced.

That the pyrethroids' effects on locomotor behaviour were present at both the application rates tested also has implications for IPM. Lower dose rates could be used to stimulate the same behavioural modifications seen at higher rates, while providing the control of pests coupled with the survival benefits to non-target invertebrates that other studies have reported (Poeling, 1987; Wiles & Jepson, 1995).

Additionally, both the pyrethroids (λ-cyhalothrin PP and cypermethrin PP) and organophosphates (dimethoate PP and chlorpyrifos PP) tested here present great opportunities to develop insecticides that manipulate beneficial arthropods' behaviour to make them more efficacious in IPM. By demonstrating that both classes of insecticide already contain carrier formulation chemicals that limit coccinellid movement, the effects discussed above could be achieved in either. In the case of the organophosphates, although the mortality data collected here suggest that efficacy might be instead increased by formulating insecticides that stimulate avoidance behaviour rather than arrestment. In this way, greater numbers of coccinellids may

survive outside treated areas (*sensu* Singh *et al.*, 2004), and their numerical response to subsequent aphid populations would be greater.

Conclusions

This study into the locomotor responses of *C. septempunctata* to five widely used commercial insecticides has provided clear evidence that behavioural responses in *C. septempunctata* are not limited to one or two insecticides (Wiles and Jepson, 1994; Singh *et al.*, 2001). Instead, behavioural responses differ according to the presence of different insecticides. In most cases, the responses were also observed at reduced dose rates, suggesting they would also be prevalent in the field. Furthermore, a significant proportion of the responses to insecticides were to components of the carrier formulation rather than the AIs. This was particularly the case for the organophosphates and the pyrethroids. The potential to affect behaviour of insects through the manipulation of accessory formulation chemicals of an insecticide opens up new avenues of research that must be explored if we are to use IPM to its greatest potential.

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