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Behaviour of the adult seven spot ladybird, *Coccinella septempunctata* (Coleoptera: Coccinellidae), in response to dimethoate residue on bean plants in the laboratory

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Abstract

The sub-lethal effects of dimethoate residue on the distribution and locomotor activity of adults of *Coccinella septempunctata* Linnaeus on *Vicia faba* plants were investigated in the laboratory. The presence of dimethoate residues on the upper half of the plant caused *C. septempunctata* to spend significantly less time on the plants overall. When given a choice of treated and untreated surfaces, *C. septempunctata* spent proportionately more time on the untreated areas of the plant. The locomotor activity of *C. septempunctata* was also altered following encounter with dimethoate residues resulting in a significant increase in the proportion of time spent walking and a reduction in the proportion of time spent resting. The proportion of time spent on plant parts was also affected, with a reduction in the time spent on the apex and an increase in the proportion of time spent on the stem, indicating a possible avoidance response. The proportion of time spent on the adaxial leaf surface was significantly increased following dimethoate treatment. The implications for integrated pest management are considered.

Introduction

Coccinellids are important predators of many aphid species (Frazer, 1988), but attempts to incorporate them into integrated pest management (IPM) strategies have resulted in varied success (Oakley *et al.*, 1996). The variable response may, in part, be due to exposure to insecticide residues on plant surfaces, both through mortality, or sub-lethal effects of pesticides resulting in altered foraging and searching patterns and decreased effectiveness of coccinellids as aphid predators.

Coccinellids locate their prey by initially searching their habitat extensively, but switch to intensive searching following an encounter with prey or honeydew (Banks, 1957; Nakamuta, 1982, 1983, 1985). They are also known to be negatively geotactic and positively phototactic and hence are often found on the apex of plants (Dixon, 1959; Majerus & Kearns, 1989) where pesticide residues are thought to be greatest (e.g. Cilgi & Jepson, 1992). These behaviours may result in variable exposure of natural predators such as coccinellids to insecticide residues.

Although lethal effects of insecticides on non-target organisms including natural enemies of pests have been widely studied (e.g. Coats *et al.*, 1979; Vickerman *et al.*, 1987; Poehling, 1988; Tripathi *et al.*, 1988; Zoebelein, 1988; Unal & Jepson, 1991; Wiles & Jepson, 1992), little information exists on the sub-lethal effects that contact with insecticide residues may have (Jepson, 1989; Jepson *et al.*, 1990; Wiles & Jepson, 1994). Such effects may include changes to fecundity, longevity, egg viability (Moriarty, 1969; Wiles & Jepson, 1994; Ruberson *et al.*, 1998), altered foraging patterns, disrupted sexual communication and host recognition (Elzen, 1989).

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It has been suggested that reduced dose rates (Poehling, 1987, 1988; Unal & Jepson, 1991; Oakley *et al*., 1996) and the careful selection of chemicals (Alford *et al*., 1995; Head *et al*., 2000) can be used to encourage natural predators and parasitoids and alleviate the severity of pesticide side-effects following spraying. Poehling (1987) found that following exposure to reduced dose rates of the aphicides pirimicarb and fenvalerate in a winter wheat crop, surviving aphid populations had no detrimental effects on yield. The surviving aphids provided an important food source for surviving predators, such as coccinellids, and as hosts for parasitoids that would otherwise not be available following full rate application.

If coccinellids are to be encouraged and incorporated into IPM systems more widely, it is important to understand the sub-lethal effects that insecticides may have on the behaviour of these beetles. This study investigated the sub-lethal effects of residues of the organophosphate insecticide dimethoate on behaviour patterns of adult *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) foraging on *Vicia faba* L. (Fabaceae).

Materials and methods

Experimental insects and plants

Aphid free *V. faba* plants (cv. Bunyard's Exhibition) were grown in 8 cm pots and John Innes No. 3 potting compost, in a glasshouse at 21°C ± 7°C and a 16:8 light:dark regime supplemented by 400 W daylight bulbs in Holophane® lamps when necessary. As plant architecture plays an important role in the searching behaviour of coccinellids (Carter *et al*., 1984; Grevstad & Klepetka, 1992; Clark & Messina, 1998; Eigenbrode *et al*., 1996) plants with two sets of developed and one set of undeveloped leaf branches (approximately 10 days old and 15 ± 5 cm high) were used in all experiments. A new plant was used for each replicate in the experiment.

Coccinella septempunctata were laboratory reared following the method of Majerus *et al.* (1989), using the pea aphid, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), as food. The cultures were initiated using wild beetles collected in the York area. Adult coccinellids used in experiments were all 10–14 weeks old, F1–F5 generation, starved and acclimated to experimental conditions for 24 h prior to the experiment. Equal numbers of males and females were used for each experiment. All experiments were carried out in a controlled environment chamber with a temperature of 20°C ± 2°C, 65% rh and 16:8 light:dark regime. The experimental period commenced 5–6 h, and terminated 11–12 h after the start of the light period, as coccinellids were observed to be active during this period.

Experimental procedure

Bean plants were divided into five zones for insecticide treatment and event recording; apex (growing shoot), stem (the main central stem and branches leading to petioles), adaxial and abaxial leaf surface and margin of the leaf. The upper half of the plant was defined as the apex plus one set of undeveloped leaves and one set of developed leaves. The lower half of the plant included the base, first set of developed leaves and the length of the stem until the second set of leaves was encountered.

Dimethoate (Danadim® Dimethoate 400 g/l EC, Cheminova, Denmark) diluted to the recommended field rate for aphids (840 ml in 400 litres) was used in all experiments. Five experimental treatments were applied, each using *V. faba* plants: (i) full plant painted with dimethoate (full); (ii) upper half of plant painted with lower half unpainted (upper); (iii) lower half of plant painted with upper half unpainted (lower), (iv) whole plant sprayed with hand held boom (boom); and (v) control plants unpainted. Dimethoate was applied in treatment 4 using a hand held 1 m boom lunch box sprayer (Coleman Engineering Ltd, Essex, UK) fitted with flat fan nozzles at 2 bar pressure, to mimic field sprayer deposition. In treatments, two to four plants were painted with insecticide using a camel-hair brush, applying an even coating of dimethoate to all surfaces. During this procedure unpainted sections of plant were covered in a polythene bag and tied with a rubber band at the stem to prevent any contamination with pesticide. The insecticide was allowed to dry in a fume cupboard for 60–90 min prior to introduction of coccinellids. Treatments (i) and (ii) above were replicated 30 times each, following this the replicate number was reduced to 20 due to low availability of coccinellids.

Adults were introduced onto the soil using a camel-hair brush. Event recording was initiated when the coccinellid first climbed onto the plant and terminated once the beetle walked, dropped or flew from the plant. Time spent walking (intensive and extensive searching behaviour), resting (movement arrested often entailing the abdomen and elytra being dropped to fully contact the plant surface) and grooming (cleaning of antennae, palps, legs and other body parts) were recorded using Observer® software (Version 2.0, Noldus Information Technology, Wageningen, Netherlands). Overall time spent on the upper and lower halves of the plant was also recorded.

Statistical analyses

Total length of time *C. septempunctata* spent on plants following treatments were transformed ($\log_{10}$) and subjected to one-way analysis of variance (ANOVA) and Fisher's pairwise comparison. Data for duration on upper and lower halves of the plant were transformed using arcsine for percentages and subjected to one-way ANOVA and Fisher's pairwise comparison for least significant differences (LSD). Time spent grooming and resting were expressed as a logged ($\log_{10}$) ratio of walking and analysed using multivariate analysis of variance (MANOVA) using Wilk's test. The data were divided into time spent on different plant parts (apex, stem, abaxial, adaxial and margin) before being expressed as a logged ratio of apex and the resulting data compared using MANOVA using Wilk's test. Further analyses to determine individual differences were by one-way ANOVA between treatments using arcsine transformation followed by Fisher's pairwise comparisons. Method of departure from the plants was compared using a chi-square test.

Results

Throughout the study no significant differences were found between male and female coccinellids in any aspect of behaviour ($P > 0.05$).

Time on plant

Average time spent on bean plants by adult *C. septempunctata* varied between treatments (fig. 1). Individuals on control and lower treatments spent significantly more time (25–35 min) on *V. faba* ($F_{4,119}$ = 6.58, P < 0.05) than full, upper and boom treated plants (10–15 min). There was no significant difference between control and lower, or between full, upper and boom treatments (LSD: P > 0.05).

Time on upper and lower half of the plant

In all cases, *C. septempunctata* spent a substantially greater proportion of time on the upper half of the plant than the lower half of the plant, but proportions differed between treatments ($F_{4,119}$ = 3.23, P < 0.05; fig. 2). Where the upper half of the plant was treated, *C. septempunctata* spent proportionately more time on the lower half of the plant than in the controls. Overall, individuals on full, upper and boom treatments spent proportionately less time on the upper half of the plant than those individuals on either control or lower treated plants. There were no differences between responses to control and lower, or between full, upper and boom treatments (LSD: P > 0.05).

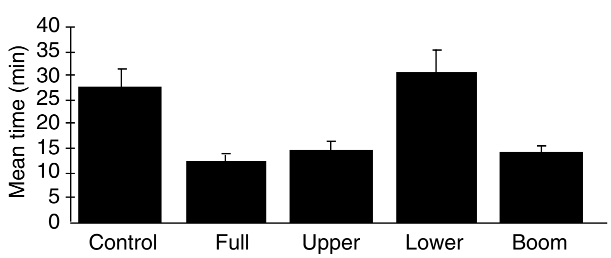

Fig. 1. Mean total time spent on *Vicia faba* plants by adult *Coccinella septempunctata* following four different dimethoate (336 g a.i. ha^{-1} in 400 litres of water) treatments. Whole plant painted with dimethoate (full), upper half of plant painted (upper), lower half of plant painted (lower), plant sprayed with hand held boom (boom). Bars represent standard error of the mean; control and full n = 30; upper, lower and boom n = 20.

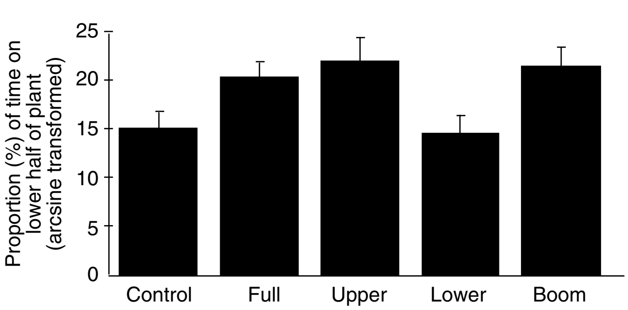

Fig. 2. Proportion of time spent on lower half of *Vicia faba* plant by adult *Coccinella septempunctata*. Whole plant painted with dimethoate (full), upper half of plant treated (upper), lower half of plant painted (lower), plant sprayed with insecticide using a hand held boom (boom).

Time spent walking, grooming and resting

The proportion of time spent walking, grooming and resting differed significantly between treatments ($F_{8,228}$ = 5.69, P < 0.01) (fig. 3). *Coccinella septempunctata* spent a greater proportion of time walking on treated plants than on controls ($F_{4,119}$ = 9.52, P < 0.01). Similarly, a greater proportion of time was spent walking by individuals exposed to upper compared with lower treated plants (LSD P < 0.05).

Total time spent resting was significantly different among treatments ($F_{4,119}$ = 12.39, P < 0.01), with significant reductions recorded on all dimethoate treated plants. Individuals exposed to plants in which the lower half of the plant had been treated spent a greater proportion of time resting than upper and boom treatments. Overall, individuals on control plants spent approximately equal proportions of time walking and resting whereas individuals on treated plants spent proportionately more of their time walking than grooming or resting (LSD P < 0.05). There were no significant differences among the five treatments for time spent grooming ($F_{4,119}$ = 1.24, P > 0.05).

Time in different plant zones

Significant differences among treatments were recorded in the proportion of time spent searching on the different parts of the plants ($F_{16,342}$ = 2.647, P < 0.01; fig. 4). The proportion of time spent on the apex was significantly different between the treatments ($F_{4,119}$ = 5.49, P < 0.01). In those treatments where dimethoate was applied to the apex (full, upper and boom), *C. septempunctata* spent less time on the apex (LSD P < 0.05). Time spent on the apex was significantly greater in controls than in any other treatments. There were no further differences found among treatments full, upper, lower and boom with the exception of full and lower, coccinellids on lower treated plants spent a greater proportion of time on apex than on full painted plants (LSD P < 0.05).

Proportion of time spent on the stem was significantly different among the treatments ($F_{4,119}$ = 3.80, P < 0.01). Coccinellids on treated plants spent more time on the stem than those on control plants (LSD P < 0.05).

Time spent on the abaxial surface of the leaf did not differ between treatments ($F_{4,119}$ = 0.96, P > 0.05). However, the proportion of time spent on adaxial surface was significantly different between treatments ($F_{4,119}$ = 2.47, P < 0.05). Coccinellids on full and upper treated plants were found to spend significantly more time on the adaxial leaf surface than those on control plants. No other differences were found. Time spent on the adaxial surface of the leaf was consistently greater than on the abaxial leaf surface for all dimethoate treated plants (LSD P < 0.05), with no difference found for control plant (LSD P > 0.05).

Overall, the proportion of time spent on the leaf margins was significantly different among treatments ($F_{4,119}$ = 3.39, P < 0.05). Coccinellids on full treated plants were found to spend significantly more time on the margins than those on upper or lower treated plants. Similarly, coccinellids on boom treated plants spent significantly more time on the margins than those on lower treated plants (LSD P < 0.05).

Method of departure

The presence or absence of dimethoate did not influence the method by which *C. septempunctata* adults departed from

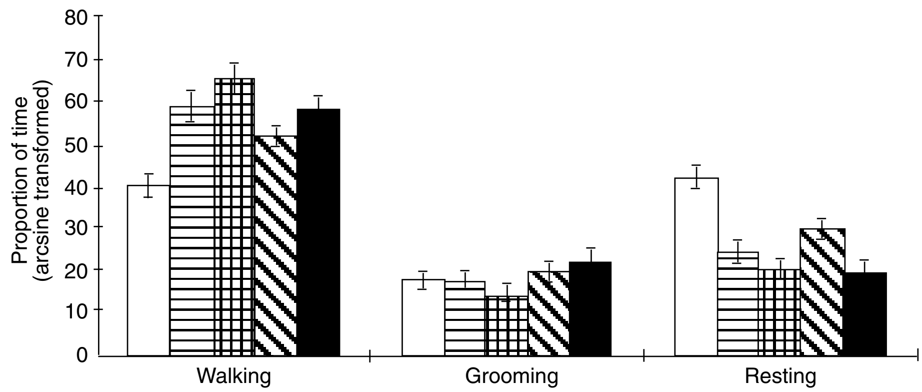

Fig. 3. Proportion of time spent walking, grooming and resting by adult *Coccinella septempunctata* on dimethoate treated *Vicia faba*. □, Control plants; ▤, whole plant painted with dimethoate (full); ▦, upper half of plant painted (upper); ▧, lower half of plant painted (lower); ■, plant sprayed with insecticide using a hand held boom (boom). Bars represent standard error of the mean; control and full n = 30; upper, lower and boom n = 20.

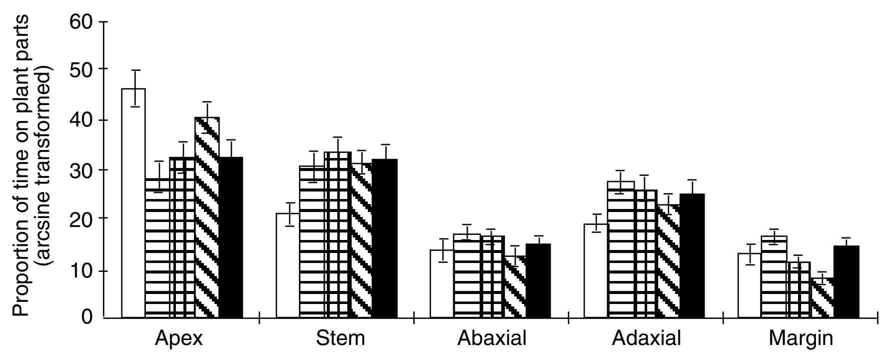

Fig. 4. Proportion of time spent on dimethoate treated *Vicia faba* plant parts by adult *Coccinella septempunctata*. □, Control plants; ▤, whole plant painted with dimethoate (full); ▦, upper half of plant painted (upper); ▧, lower half of plant painted (lower); ■, plant sprayed with insecticide using a hand held boom (boom). Bars represent standard error of the mean; control and full n = 30; upper, lower and boom n = 20.

the plants. The majority of individuals walked off the plant onto the soil with a few individuals either flying from or dropping from the plant (table 1). No significant differences were found among the five treatments ($\chi^2 = 3.17$; $P = > 0.05$; df = 8).

Discussion

Coccinellids are negatively geotactic and positively phototactic (Dixon, 1959; Majerus & Kearns, 1989), and on crops which have not received an insecticide treatment they often spend longer searching the apex of plants where dense aphid colonies are also most likely to occur. As spray deposition declines in lower regions of the canopy and on the adaxial surface of leaves as a consequence of sheltering by vegetation (Cilgi & Jepson, 1992; Kjaer & Jepson, 1995), it has been suggested that this might increase exposure to insecticidal residues. In this study, when the upper half of the plant was treated with an insecticide, coccinellids spent significantly shorter periods of time on the plant, coupled with a significant disruption to searching and resting behaviour. In particular, a reduction in the time spent resting and an increase in the proportion of time spent walking on treated plants was recorded. This conclusion is supported by field observations of coccinellid behaviour (Wiles & Jepson, 1994), and it has been suggested that such responses may further exacerbate exposure to insecticide residues (Jepson *et al.*, 1990).

Behavioural changes that reduce exposure of natural enemies to treated surfaces have been recorded in several studies. For example the aphid parasitoid, *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) exposed to Brussels sprouts and oilseed rape plants treated with the insecticide pirimicarb displayed reduced searching and walking behaviour on sprayed surfaces (Jui & Waage, 1990; Umoru *et al.*, 1996). Although coccinellids in the current study spent proportionately more time on the upper half of the plant than the lower half even after treatments were applied, the presence of insecticide affected the time spent on the different plant parts. This resulted in *C. septempunctata* spending proportionately less time on the apex of the plant compared with untreated controls and an increased proportion of time on the stem of the plant and adaxial surface of the leaves. Coupled with the increased proportion of time that was spent on the untreated areas, this suggests a possible avoidance response to the pesticide residues.

Table 1. Method of departure from *Vicia faba* plant by adult *Coccinella septempunctata*. Values represent proportion of adult *C. septempunctata* that flew, walked or dropped from the bean plants. Whole plant painted with dimethoate (full), upper half of plant painted (upper), lower half of plant painted (lower), plant sprayed with insecticide using a hand held boom (boom).

	Control	Full	Upper	Lower	Boom
	n = 30	n = 30	n = 20	n = 20	n = 20
Fly	16.7	23.3	20	25	20
Walk	76.7	63.3	70	65	60
Drop	6.7	13.3	10	10	20

In addition, these changes to the searching behaviour may reduce the impact of increased movement if pesticides are not deposited evenly throughout all canopy horizons as described above (Cilgi & Jepson, 1992; Kjaer & Jepson, 1995). Although a previous, less intensive, study suggested that *C. septempunctata* exposed to residues of deltamethrin were observed more often on the abaxial leaf surface (Wiles & Jepson, 1994), even the avoidance of the apex and increased time spent on the lower regions of plants are likely to reduce exposure. If the result of detailed observations in the current work, indicating an increase in the time spent on the stem and adaxial surface of the leaf are also reflected in behavioural changes occurring after spraying in the field, then a further reduction of the impact of insecticide residues may be expected.

Successful use of *C. septempunctata* as part of an integrated pest management strategy for the control of aphids relies on both survival, and maintenance of its ability to find sufficient prey to avoid it leaving the crop in search of food, after spray application. The results of this study suggest that in the field, post-treatment searching activity would be concentrated on parts of the bean plants where aphids were least likely to occur (i.e. increased time spent on the stem and reduced time on the apex). However, possible avoidance of treated surfaces will concentrate them in the lower crop horizon where more aphid survivors might be found. Aphid free plants were used throughout this study, and it is possible that the presence of aphids or honeydew on the plant may further alter the behaviour of coccinellids, as honeydew is known to provide an arrestant stimulus for coccinellid larvae and can increase the amount of time spent searching a particular patch (Carter & Dixon, 1984).

This study has shown that dimethoate residues can disrupt the normal behaviour pattern of aphidophagous coccinellids such as *C. septempunctata*. These sub-lethal effects may have important consequences for the post-treatment predatory efficiency of coccinellids, both those present in a crop and those entering crops from refuges following a spray application. Although it has been proposed that application of reduced dose rates limit the severity of the initial effects of pesticides (Poehling, 1987, 1988; Unal & Jepson, 1991; Oakley *et al*., 1996), this study has indicated that sub-lethal effects of insecticide residues may result in an immediate reduction in the efficiency with which coccinellids locate their prey. However, the behavioural responses may also reduce exposure to insecticides thus increasing both survival and the potential for aphid control in the future. Further work is required to establish specific application rates at which behavioural responses begin to occur, and the relative effects of increased survival and decreased searching efficiency on aphid population depression under field conditions. However, this study has highlighted the importance of improving our understanding of sub-lethal effects of insecticides on natural enemies to support the development of IPM strategies.

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