

Consumption rates and predatory activity of adult and fourth instar larvae of the seven spot ladybird, *Coccinella septempunctata* (L.), following contact with dimethoate residue and contaminated prey in laboratory arenas

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Abstract

The sub-lethal effects of dimethoate residues on the condition and predatory efficiency of adult and fourth instar larvae of *Coccinella septempunctata* were investigated in laboratory arenas. Overall, predatory efficiency of both adult and larval *C. septempunctata* was significantly reduced following encounter with dimethoate residues and treated prey. Prey-choice experiments revealed that adult coccinellids consumed significantly fewer treated than untreated aphids over the 5-h experimental period. Fourth instar larvae preferentially consumed untreated aphids when given the choice of full rate dimethoate treated aphids or untreated aphids. The implications for post-treatment coccinellid survival and integrated pest management are considered.

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1. Introduction

The seven spot ladybird *Coccinella septempunctata* (L.) is a member of the family Coccinellidae that includes nearly 5000 species, most of which are predaceous. The eggs of *C. septempunctata* are laid on the surface of leaves and hatch to give rise to a sequence of four larval instars before pupating. Both the adult and larval stages are known to be widely polyphagous, feeding on the Aphidoidea, Psylloidea, Coccoidea, and mites found on a variety of plants (Dolling, 1991).

The importance of coccinellids as aphid predators has long been recognised (Frazer, 1988) and several species have been utilised as biocontrol agents. As with other predatory species their ability to suppress the development of pest populations is affected by both the environment surrounding the crop and the crop

management techniques that are used, and may be enhanced when they form a component of an integrated pest management (IPM) strategy that includes both a range of other natural enemies and conventional chemical insecticides (Ba M'Hamed and Chemseddine, 2002; Bommarco and Fagan, 2002; Vichitbandha and Wise, 2002). To fully realise the potential of the group as components of integrated pest management strategies, the lethal and sub-lethal responses to insecticide applications need to be better understood.

Exposure of coccinellids to insecticide residues on plant surfaces and through the consumption of contaminated prey may lead to mortality, or sub-lethal effects resulting in altered foraging and searching patterns, and thus altered effectiveness of coccinellids as aphid predators (Singh et al., 2001). The prey capture efficiency of coccinellids is dependent on their searching behaviour. Following prey encounter, the search path becomes convoluted keeping the beetle in the vicinity of the site of the first prey encounter (Nakamuta, 1985). In the absence of further prey items, this activity is not normally

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prolonged for longer than 70 s. This switch to area-concentrated search is advantageous for coccinellids as their prey mostly live in clusters (Nakamuta, 1982).

Previous studies have indicated that sub-lethal effects of insecticide residues may result in an immediate disruption of predatory behaviour and a potential reduction in the efficiency with which coccinellids locate their prey (Singh et al., 2001). However, the behavioural responses may also reduce exposure to insecticides thus increasing both survival and the potential for future aphid control. Studies with dimethoate residues on plants resulted in *C. septempunctata* spending proportionately less time on the upper half and apex of the plant compared with untreated controls and an increased proportion of time on the stem of the plant (Singh et al., 2001). Coupled with the increased proportion of time that was spent on the untreated areas, this suggests a possible avoidance response to the pesticide residues. Avoidance of treated surfaces would concentrate coccinellids in the lower crop horizon where more aphid survivors might be found. However, a shortage of aphids in the lower crop may encourage coccinellids to climb back up the plant to contaminated areas where contaminated aphids may be found. As prey were not provided in these earlier studies, it is possible that the presence of aphids or honeydew 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 and Dixon, 1984).

Detailed behavioural studies are urgently required to further the knowledge of pesticide effects and to develop our understanding of foraging behaviour of predatory coccinellids. Such information will enable more effective incorporation of coccinellids in biological control schedules (Obrycki and Kring, 1998) and integrated pest management (IPM) strategies. This study assesses the effect of three concentrations of dimethoate residue on the predatory capacity of adult and fourth instar larvae of the seven spot ladybird, *C. septempunctata*.

2. Materials and methods

Adult and fourth instar larvae of *C. septempunctata* were reared following the method of Majerus et al. (1989). Cultures were initiated using wild beetles caught in the York area and maintained in a glasshouse at $21 \pm 7^\circ\text{C}$ and 16:8 (L:D) h photoperiod supplemented by 400 W daylight bulbs in holophane lamps when necessary. The pea aphid, *Acyrtosiphon pisum* Harris was provided as food. 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 adult males and females were used for each experiment. Lar-

val coccinellids were 2- to 3-day-old fourth instars. All experiments were carried out in a controlled environment chamber at a temperature of $20 \pm 2^\circ\text{C}$, 65% RH, and 16:8 (L:D) h photoperiod.

2.1. Prey-residue experiments

Dimethoate (Danadim Dimethoate 40, Cheminova, Denmark) diluted to 100, 50, 25 and 0% of the recommended field rate for aphids (336 g a.i./ha in 400 litres of water) was used for prey-residue experiments. Dimethoate was applied to arenas or aphids using a Potter Precision Laboratory Spray Tower (Burkard Scientific, Uxbridge, UK) fitted with a medium spray nozzle at 0.7 bar pressure. The arenas were 9 cm glass petri dishes lined with filter paper (Whatman No. 1). Untreated prey and arenas were sprayed with water as the control. The rim of each arena was painted with fluon (Whitford Plastics, Runcorn, UK) before the pesticide was allowed to dry in a fume cupboard for 60–90 min prior to introduction of coccinellids.

Dimethoate was used in three experimental treatments: (1) surface of the arenas treated combined with untreated (water-sprayed) prey; (2) surface of the arenas untreated (water-sprayed) with treated prey; and (3) surface of arena treated combined with treated prey. Adult coccinellids were provided with an excess of prey (20 adult, 15 third and fourth instar, and 15 first and second instar pea aphids) at 0 and 24 h, whereas fourth instar larvae were provided with 10 adult, 10 third and fourth instar, and 10 first and second instar pea aphids at these times. The condition of the coccinellids was recorded after 24 and 48 h when any remaining prey was also removed. Each individual coccinellid was induced to move by gently prodding with a dissecting needle and classed as dead (no movement), un-coordinated (stumbling or poorly coordinated movement) or healthy (normal, coordinated movement). The percentage of aphids eaten was also recorded. Only data on prey eaten by surviving (un-coordinated or healthy) coccinellids was analysed. There were 20 replicates of each treatment.

2.2. Prey-choice experiments

Choice arenas were constructed consisting of a 150 mm petri dish with a filter paper disc in the base. On top of the filter paper six 30 mm diameter prey-rings were arranged in a circle and equidistant from each other. The prey-rings were made of plastic, with walls that rose to a height of 10 mm from the floor of the petri dish. The sides of the petri dish were coated with fluon to prevent coccinellids climbing onto the sides and lid of the arena. These arenas were used in a series of choice and no-choice treatments.

In each treatment either insecticide treated or untreated aphid prey were placed in each of the prey-rings.

A single adult or larval *C. septempunctata* was then introduced into the arena and the percentage of treated and untreated aphids that were eaten recorded after 5 h.

Three choice and two no-choice treatments were applied. Choice treatments included: (A) each of three of the prey-rings in the arena containing two aphids both of which had been treated with the recommended full field rate of dimethoate, whilst the other three prey-rings each contained a pair of aphids treated with half field rate dimethoate; (B) three prey-rings each containing a pair of aphids treated with full field rate of the dimethoate and the other three prey-rings with a pair of aphids sprayed with water; and (C) three prey-rings each containing a pair of aphids treated with half field rate dimethoate and the other three prey-rings with a pair of aphids sprayed with water. No-choice treatments included: (A) each of the six prey-rings containing two aphids, both of which had been treated with the recommended full field rate of dimethoate only; (B) each prey-ring containing two aphids, both of which had been sprayed with water (control). Each treatment was replicated 20 times and a separate experiment was run for adult and larval *C. septempunctata*.

Spraying of the prey-items used in the experiments followed a standard procedure. Adult apterous pea aphids were placed in a deep freeze for 25 min to kill them. Each was then sprayed with either an insecticide dilution or water using a Potter Precision Laboratory Spray Tower.

2.3. Statistical analyses

The data describing coccinellid condition in the prey-residue experiments were analysed using χ^2 tests to examine the null hypothesis that the effects of the dimethoate dilutions on coccinellid condition were independent of the experimental treatments. As the data for aphids eaten had different sample sizes and appropriate transformations failed to normalise them, we were unable to use either a two-way ANOVA or the Scheirer–Ray–Hare extension to the Kruskal–Wallis test. These data were therefore analysed using a Kruskal–Wallis test on each factor separately to test the null hypotheses that neither dimethoate dilution nor experimental treatment had an effect on the number of aphids consumed.

Percentages of aphids eaten in choice experiments were transformed using the angular transformation and analysed using one-way ANOVA within each experimental treatment to test the null hypothesis that the coccinellids had no preference for treated or untreated aphids. Overall data on the total number of aphids eaten in the choice and no-choice experiments were transformed using the angular transformation for percentages and subjected to ANOVA followed by Fisher's pairwise comparison for least significant differences (LSD) to test the null hypotheses that the experimental

set ups and combinations of treatments did not affect the total number of aphids eaten.

3. Results

Throughout the study no significant differences were found between adult male and female coccinellids ($P > 0.05$).

3.1. Prey-residue experiments

The condition of adult and fourth instar larvae of *C. septempunctata* was affected by dimethoate treatments to varying degrees. The severity of dimethoate side effects on coccinellid condition can be ranked. The treatment combining a treated surface with treated prey resulted in the greatest proportion of dead and un-coordinated individuals at both 24 and 48 h, followed by treated surface combined with untreated prey, and untreated surface combined with treated prey. The greatest deleterious effects (number dead/un-coordinated) were in treatments at 100% of field rate with those at 25% field rate leaving the greatest number of coccinellids healthy (Table 1).

Survival and movement of adult coccinellids varied between dimethoate concentrations and the three experimental treatments at both 24 and 48 h assessments (Table 1A). The χ^2 tests for the dead and uncoordinated coccinellids showed that the effects of the dimethoate dilutions were independent of the three experimental treatments at both 24 and 48 h ($P > 0.05$). The effect of the dimethoate dilutions on the number of healthy coccinellids after 24 and 48 h was shown to be significantly affected by the three experimental treatments (24 h: $\chi^2 = 19.02$, $df = 6$, $P < 0.01$; 48 h: $\chi^2 = 11.45$, $df = 6$, $P < 0.01$). After the application of treatment 2, untreated surface combined with treated prey; markedly more healthy individuals were recorded than following the other experimental treatments at both 24 and 48 h.

The larval coccinellids were severely affected by dimethoate exposure resulting in high mortality in all treatments and at all concentrations (Table 1B). The data were not analysed further due to the high mortality.

The percentage of pea aphids eaten by adult coccinellids varied significantly at both 24 h ($H = 53.58$; $df = 2$; $P < 0.01$) and 48 h ($H = 44.56$; $df = 2$; $P < 0.01$) following the three experimental treatments regardless of dimethoate dilution. At 24 h surviving coccinellids presented with treated prey, ate fewer aphids than those offered untreated aphids (Fig. 1A). Similarly, at 48 h surviving adult coccinellids presented with treated prey ate less than those with untreated prey (Fig. 1B). The percentage of aphids eaten by adult coccinellids between dimethoate dilutions regardless of treatment of surface varied significantly at 48 h

Table 1

The condition of (A) adult and (B) larval *Coccinella septempunctata* following application of four dilutions of dimethoate (0, 25, 50, and 100% of field rate: 336 g a.i./ha in 400 litres of water) in three experimental treatments at 24 and 48 h following spray application

	% field rate dimethoate	24 h % dead	48 h % dead	24 h % un-coord	48 h % un-coord	24 h % healthy	48 h % healthy
(A)							
Treat 1	0	0	0	0	0	100	100
	25	15	35	40	65	45	0
	50	20	55	50	45	30	0
	100	30	65	60	35	10	0
Treat 2	0	0	0	0	0	100	100
	25	5	20	30	50	65	30
	50	10	25	30	45	60	30
	100	20	45	55	55	25	0
Treat 3	0	0	0	0	0	100	100
	25	25	60	70	40	5	0
	50	45	80	55	20	0	0
	100	65	85	35	15	0	0
(B)							
Treat 1	0	0	0	0	0	100	100
	25	50	100	50	0	0	0
	50	65	100	35	0	0	0
	100	75	100	25	0	0	0
Treat 2	0	0	0	0	0	100	100
	25	70	100	30	0	0	0
	50	100	100	0	0	0	0
	100	100	100	0	0	0	0
Treat 3	0	0	0	0	0	100	100
	25	100	100	0	0	0	0
	50	100	100	0	0	0	0
	100	100	100	0	0	0	0

Treat 1, treated surface combined with untreated prey; Treat 2, untreated surface combined with treated prey; and Treat 3, treated surface with treated prey. Dead, no movement; un-coord, stumbling or poorly coordinated movement; and healthy, normal, coordinated behaviour.

($H = 47.66$; $df = 3$; $P < 0.01$). The percentage of aphids eaten was greater in the 0% treatment, with no differences between 25, 50 and 100% treatments at 24 h (Fig. 1A). At 48 h the greatest percentage of aphids eaten was in the 0% treatment with fewer eaten in all other treatments (Fig. 1B).

The proportion of pea aphids eaten by larval coccinellids also varied significantly at 24 h following the three experimental treatments ($H = 58.53$; $df = 2$; $P < 0.01$). At 24 h individuals presented with treated prey, regardless of dilution, ate fewer aphids than those experiments with untreated aphids (Fig. 2). There were no data collected at 48 h due to the high percentage mortality.

The percentage of aphids eaten by larval coccinellids between dimethoate concentrations regardless of treatment of surface varied significantly at 24 h ($H = 51.37$; $df = 3$; $P < 0.01$). The percentage of aphids eaten was greater in the 0% treatment with differences between 25, 50, and 100% treatments resulting in larvae exposed to lower concentrations (25 and 50%) consuming proportionately more aphids than those treated at full rate (Fig. 2).

3.2. Prey-choice experiments

Overall, in the choice experiments involving adult coccinellids, there were no significant differences in the percentage of treated/untreated aphids eaten in each of the experimental treatments ($P > 0.05$). The overall percentage of aphids eaten was significantly different between experimental treatments ($F = 5.64$; $df = 4.95$; $P < 0.01$; Fig. 3). Adults in treatments containing dimethoate ate significantly fewer aphids than the control treatment (LSD $P < 0.05$). There were no differences between the dimethoate treatments.

Overall, the percentage of aphids eaten by larval coccinellids varied significantly with treatment. When given a choice of untreated aphids together with either full or half field rate treated aphids, larval coccinellids ate significantly more untreated prey (full rate versus untreated: $F = 4.29$; $df = 1.19$; $P < 0.05$, half rate versus untreated: $F = 5.21$; $df = 1.19$; $P < 0.01$; Fig. 4). No significant differences were found in the percentage of aphids eaten in the full rate versus half rate treatment ($P > 0.05$). The overall percentage of aphids eaten was found to be significantly different between experimental

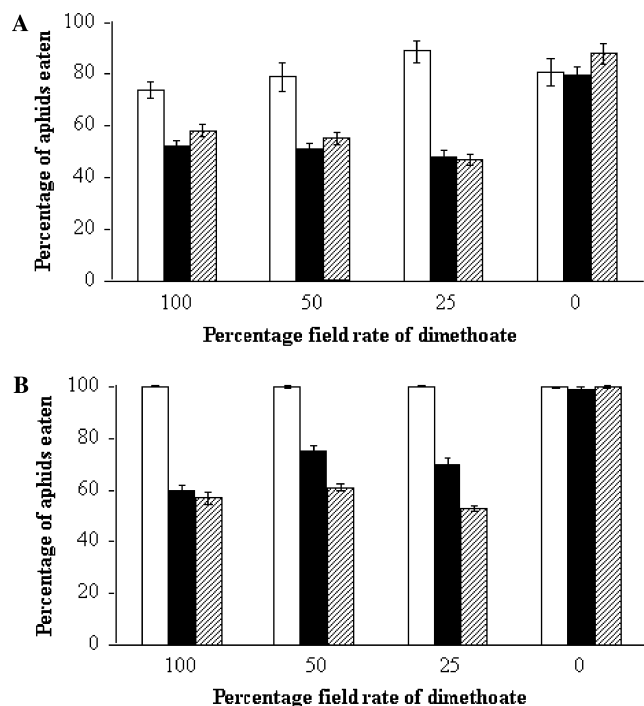


Fig. 1. Mean percentage of pea aphids eaten by adult *Coccinella septempunctata* following application of four dilutions of dimethoate (0, 25, 50, and 100% of field rate: 336 g a.i./ha in 400 litres of water) in three experimental treatments at (A) 24 and (B) 48 h following spray application. (□) Treated surface combined with untreated prey, (■) untreated surface combined with treated prey, and (▨) treated surface with treated prey. Bars represent standard error of the mean, $n = 20$.

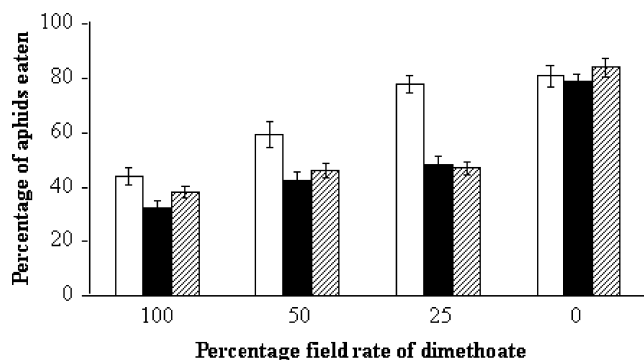


Fig. 2. Mean percentage of pea aphids eaten by fourth instar larvae of *Coccinella septempunctata* following application of four dilutions of dimethoate (0, 25, 50, and 100% of field rate: 336 g a.i./ha in 400 litres of water) in three experimental treatments at 24 h following spray application. (□) Treated surface combined with untreated prey, (■) untreated surface combined with treated prey, and (▨) treated surface with treated prey. Bars represent standard error of the mean, $n = 20$.

treatments ($F = 5.64$; $df = 4.95$; $P < 0.01$; Fig. 5). Larval beetles in treatments containing aphids sprayed with full field rate dimethoate ate significantly fewer aphids than the control, or half rate with untreated aphid experimental treatments (LSD $P < 0.05$). Overall, the greatest percentage of aphids eaten was in the untreated control treatment. There were no differences

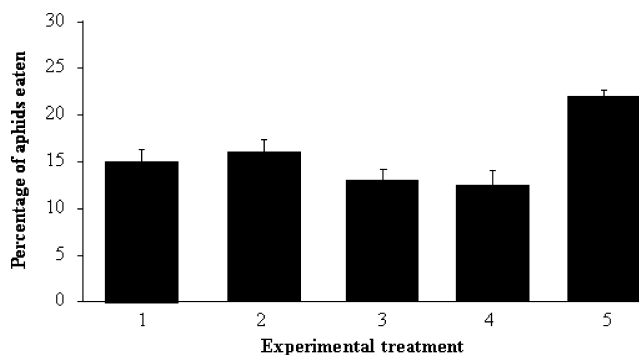


Fig. 3. Mean percentage of pea aphids eaten by adult *Coccinella septempunctata* following five experimental treatments at 5 h following spray application. 1, All aphids treated with full field rate dimethoate; 2, aphids treated with full field rate compared to half field rate dimethoate; 3, aphids untreated (water only) compared with full field rate dimethoate; 4, aphids untreated compared with half field rate dimethoate; and 5, all aphids untreated. Bars represent standard error of the mean; $n = 20$ for each experimental treatment.

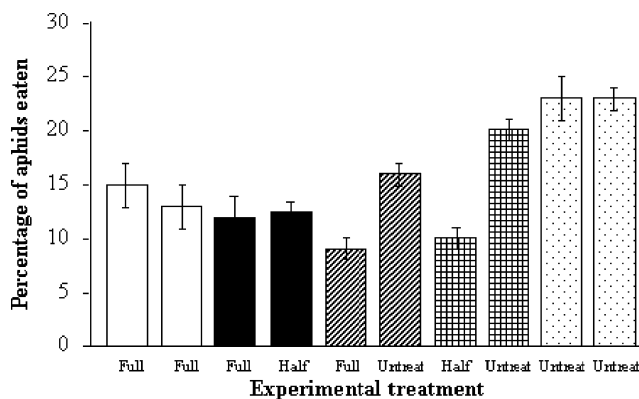


Fig. 4. Mean percentage of pea aphids eaten by fourth instar larvae of *Coccinella septempunctata* in experiments with five experimental choice treatments at 5 h following spray application. (□) All aphids treated with full field rate dimethoate; (■) aphids treated with full field rate compared to half field rate dimethoate; (▨) aphids untreated (water only) compared with full field rate dimethoate; (▩) aphids untreated compared with half field rate dimethoate; and (□) aphids untreated. Bars represent standard error of the mean; $n = 20$ for each experimental treatment.

between the percentage of aphids eaten that had received full or half rate dimethoate treatments ($P > 0.05$).

4. Discussion

The cumulative effects of two routes of insecticide exposure greatly increased the toxicity of dimethoate to *C. septempunctata* adults and larvae in this study. Present pesticide testing procedures are often designed to test one route of exposure of an organism, normally dried residue or direct spraying (Banken and Stark, 1998), whereas in the field three routes of exposure to an insecticide are possible; direct exposure to spray

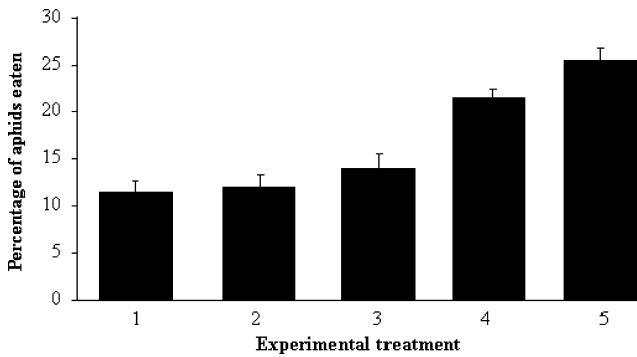


Fig. 5. Mean percentage of pea aphids eaten by fourth instar larvae of *Coccinella septempunctata* following five experimental treatments at 5 h following spray application. 1, All aphids treated with full field rate dimethoate; 2, aphids treated with full field rate compared to half field rate dimethoate; 3, aphids untreated (water only) compared with full field rate dimethoate; 4, aphids untreated compared with half field rate dimethoate; and 5, aphids untreated. Bars represent standard error of the mean; $n = 20$ for each experimental treatment.

droplets, contact with residue from contaminated surfaces, and consumption of contaminated prey (Longley and Stark, 1996). Although laboratory experiments can overestimate the effects of a pesticide, as the complex architecture of the plant affects the behaviour of the organism (Singh et al., 2001), this study has shown that dimethoate residues can reduce prey consumption rate of aphidophagous coccinellids such as *C. septempunctata* even in small arenas.

The higher sensitivity of larvae to dimethoate found in this study may have detrimental consequences for coccinellid populations and survival in crops following spraying. However, a previous study has shown that larvae on dimethoate treated bean plants avoided treated surfaces and concentrated their search on untreated areas of the plant (S.R. Singh, K.F.A. Walters, and G.R. Port, unpublished). Coupled with the avoidance of contaminated prey observed in this study, larvae may avoid mortality by concentrating their search in the lower levels of the crop canopy where untreated aphids may also exist.

The use of reduced concentrations of pesticides may benefit coccinellid survival and assist the depression of aphid populations below economic threshold levels (Mann et al., 1991; Oakley et al., 1996). Reduced rates of insecticide can potentially reduce initial toxic effects on populations of natural enemies by leaving a proportion of aphids and a high proportion of predators alive (Oakley et al., 1996). A reduction of dimethoate to a quarter and half of field rate in the current study still resulted in a detrimental effect on the condition of the coccinellids although it was less severe than a full rate treatment. However, there was a preference for untreated aphids, which in the field may be found surviving in the lower crop canopy following spray application where reduced pesticide residue levels may also be found (Kjaer and Jepson, 1995).

These sub-lethal effects may have important consequences for the post-treatment predatory efficiency of coccinellids, both those present in a crop during spray application and those entering crops following spraying. It has been proposed that application of reduced dose rates limit the severity of the initial effects of pesticides (Oakley et al., 1996; Poehling, 1987, 1988; Unal and Jepson, 1991). This study has indicated that sub-lethal effects of insecticide residues may result in increased mortality or knockdown and overall reduction in the efficiency with which coccinellids locate and consume their prey. However, other behavioural and foraging responses may also reduce exposure to insecticides (Singh et al., 2001) thus partially mitigating these effects on 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 foraging efficiency on aphid population depression under field conditions where the presence of patchily distributed prey may further affect the predatory ability of coccinellids.

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