

Toxicity of pesticides to *Halmus chalybeus* (Coleoptera: Coccinellidae) and the effect of three fungicides on their densities in a citrus orchard

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Abstract The effect of three fungicides in five programmes on densities of *Halmus chalybeus* (steelblue ladybird) was tested in a citrus orchard. Programmes of five and nine applications of cupric hydroxide or chlorothalonil reduced densities by c. 35% and 70% respectively, compared with unsprayed plots. One application of copper sulphate and lime (Bordeaux mixture) reduced ladybird density by 81%, similar to the two nine-application programmes. These reductions in numbers were probably due to a repellent effect since the fungicides had a relatively low toxicity. Ten fungicides, nine insecticides, and a miticide were screened for toxicity to adult *H. chalybeus* by a dip method. Copper-based fungicides were more toxic than non-copper fungicides at 3 times label rates, but not at label rates. The insecticides taufluvinate, permethrin/pirimphos-methyl, diazinon, and potassium salt were highly toxic at label rates. Two mineral oils, chlorpyrifos, and the miticide azocyclotin were moderately toxic, whereas buprofezin and one mineral oil were not toxic. *H. chalybeus* is an important predator of wax scales and the implications of these pesticide effects for biological control of these pests are discussed.

Keywords *Halmus chalybeus*; Coccinellidae; fungicides; insecticides; toxicity; biological control; citrus

INTRODUCTION

Halmus chalybeus (Boisduval) (steelblue ladybird) (Coleoptera: Coccinellidae) is a generalist predator whose prey includes armoured scales, wax scales, and mites (Valentine 1967; Beattie & Gellatley 1983; Drea & Gordon 1990; Flynn 1995). *H. chalybeus* was imported to New Zealand from Australia over a century ago as a predator of black scale (*Saissetia oleae*) (Dumbleton 1936). It is now the most common ladybird on citrus orchards in Northland, New Zealand (Lo 2000), where both larvae and adults are important predators of wax scales (Hemiptera: Coccidae) (Lo & Chapman 2001).

Wax scales excrete honeydew, which acts as a food source for sooty mould fungi (e.g., *Capnodium* spp.). These fungi cover plants with a black layer that reduces photosynthesis and fruit yield, as well as disfiguring fruit (Brun 1986). Two species of wax scale are pests of citrus in New Zealand. *Ceroplastes sinensis* Del Guercio (Chinese wax scale) is more widespread, but *C. destructor* (Newstead) (soft wax scale) is more prone to outbreaks (Lo et al. 1996). In Northland, *C. destructor* occurred at higher densities on a greater proportion of citrus orchards where organophosphate insecticides had been used compared with other orchards (Lo et al. 1996). It was thought that these outbreaks were caused by the destruction of natural enemies, which is a common side effect of insecticide applications (Croft 1990; DeBach & Rosen 1991).

Citrus in New Zealand is susceptible to four main fungal diseases: verrucosis (*Elsinoe fawcetti* Bitanc. & Jenk.); botrytis (*Botrytis cinerea* Pers.); melanose (*Diaporthe citri* Wolf); and alternaria (*Alternaria citri* Ellis & Pierce) (Sale 1990). These diseases may require spraying at c. 3-week intervals from spring to autumn. Protectant fungicides, such as copper oxychloride and cupric hydroxide, have been the traditional means of disease control. The main pests are thrips and scale insects, which are generally controlled by spraying with organophosphate insecticides, principally diazinon, acephate, and chlorpyrifos; and mineral oils.

The development of an integrated pest and disease management programme for citrus in New Zealand requires information about the impact of pesticides on both pests and beneficial insects. This study investigated the disruptive effect of three fungicides on densities of *H. chalybeus* in a citrus orchard. In a laboratory study, the toxicity of 10 fungicides, nine insecticides, and one miticide was also tested on adult *H. chalybeus*.

METHODS

Field applications of fungicides

The trial was conducted on a commercial block of tangelos (*Citrus reticulata* Blanco × *C. paradisi* Macf.) near Whangarei, between October 1990 and May 1991. The 12-year-old trees were 4 m high, 3 m apart, with 5 m between rows. Along rows there were no gaps between trees so the foliage formed a hedge. Plots comprised three or four adjacent trees with no unsprayed buffer between plots. The five fungicide treatments and an unsprayed control were assigned to plots using a randomised block design with four replicates.

Cupric hydroxide at 45 g a.i./100 litres or chlorothalonil at 150 g a.i./100 litres, were applied at moderate (five sprays) and high (nine sprays) frequencies (Fig. 1). Sprays were applied as follows (dates for the five-spray programme are asterisked): 29 October*, 16 November, 29 November*, 23 December*, 31 January, 21 February, 7 March, 22 March*, and 1 May*. Copper sulphate plus hydrated lime (Bordeaux mixture) at 600 and 800 g a.i./100 litres was applied once on 21 February. Fungicides were applied with a hand gun and hydraulic sprayer operating at 1200 kPa. Each tree received c. 4 litres of spray (12–16 litres/plot), which was equivalent to 2700 litres/ha. No other sprays were applied to the trees during the experiment.

The research on ladybirds took advantage of a fungicide efficacy trial against melanose and alternaria (Olson et al. 1992). Ideally, monitoring of ladybirds would have started before the first fungicide applications, but it did not begin until 20 February. This was 2 months after the third spray in the five-spray programmes, and 3 weeks after the fifth spray in the nine-spray programmes. Numbers of live ladybirds (larvae, pupae, adults) were assessed at weekly intervals until 25 March and then every 2 weeks until numbers on the unsprayed plots declined in late May. Each plot (a continuous "hedge" of 3–4 trees) was visually scanned for 3 min, 1.5 min on

each side of the row. Areas within half a tree of neighbouring plots were not assessed. Counts were conducted between 1100 and 1500 h.

The results of the cupric hydroxide and chlorothalonil treatments were analysed in three periods by 2-way ANOVA. The periods corresponded to intervals between the fungicide applications on 22 March and 1 May as follows: Period 1, 20 February–18 March; Period 2, 25 March–22 April; Period 3, 6–23 May. The result of the copper sulphate/lime treatment was analysed separately, over all three periods combined. Ladybird densities were square root transformed before analysis. The number of larvae in untreated and sprayed plots was compared by *t*-test.

Toxicity of fungicides and insecticides

Each chemical (Table 1) was tested at 0.1×, 1×, and 3× label rates, except for the 0.1× rate of buprofezin, and the 3× rate for pyrethroid and organophosphate insecticides. Water was used as the control. Adult ladybirds were stuck on their backs onto glass microscope slides using double-sided adhesive tape. They were immersed for 10 s in agitated solutions. Excess fluid was blotted off and the slides were allowed to dry before being placed in Petri dishes at 21 ± 1°C under natural daylight.

Preliminary results showed that mortality increased in some treatments up to 72 h after dipping. Subsequent changes in mortality were ≤ 5%, so this interval was used to assess all treatments. After 72 h ladybirds were removed from the slides and assessed as alive or dead; those classified as dead were motionless when prodded or were clearly affected and unable to walk. "Affected" ladybirds did not recover.

The experiment was conducted on five occasions between 25 February and 19 March 1993. Each treatment was tested once, using two slides with 10 ladybirds per slide. A fresh control with 20 ladybirds was used on each of the five occasions. Diazinon and chlorpyrifos were re-tested on 19 March at label rates with a further 30 ladybirds each. Ladybirds were collected from orchards and held for up to 5 days in a refrigerator before testing. They were acclimatised for 24 h at 21 ± 1°C before being dipped.

Data were analysed by comparing the mortality caused by different groups of chemicals using independent sample *t*-tests. Mortality among the three concentrations was compared by ANOVA in the copper and non-copper fungicide treatments. Mortality caused by the two organophosphates was compared using a Chi-square test with Yates' correction.

RESULTS

Field applications of fungicides

All five fungicide programmes reduced ladybird populations and trees sprayed 9 times had fewer ladybirds than those sprayed 5 times (Fig. 1; Table 2). A total of 735 *H. chalybeus* were recorded on

control trees and in comparison, the two five-spray programmes reduced ladybird densities overall by 31–41% (Fig. 1A). During Period 1, there was a 35% reduction in ladybird densities on cupric hydroxide treated plots compared with untreated plots ($P < 0.05$). This was 8.5–12 weeks after the third fungicide application. In contrast, ladybird densities on

Table 1 Pesticides used in toxicity experiments on *Halmus chalybeus* with rates of product and grams of active ingredient (g a.i.).

Group	Chemical	Trade name	Label rate (/100 litre)	
			Product	g a.i.
Fungicides				
Copper	cupric hydroxide	Kocide 101WP	200 g	100
	cupric hydroxide	Champ 18F	300 ml	70
	copper oxychloride	Copper oxychloride 50WP	300 g	150
	copper sulphate and lime	Bordeaux WP	600 g and 800 g	600 and 800
	copper sulphate pentahydrate	Phyton 27	600 ml	150
Non-copper	iprodione	Rovral 250F	200 ml	50
	triforine	Saprol 19EC	100 ml	20
	chlorothalonil	Bravo 500F	300 ml	150
	mancozeb	Dithane M-45 80WP	200 g	160
	benomyl	Benlate DF	25 g	12.5
Insecticides				
Mineral oil	mineral oil	Sunspray	1000 ml	1%
	mineral oil	DC Tron	1000 ml	1%
	mineral oil	Ultrafine	1000 ml	1%
Pyrethroid	taufluvallinate	Mavrik SC	20 ml	4.8
Pyrethroid/ organophosphate	permethrin	Attack EC	100 ml	2.5 and 47.5
Organophosphate	pirimiphos-methyl and diazinon	Diazinon 50WP	100 g	25
	chlorpyrifos	Lorsban 50W	75 g	37.5
Potassium salts	C12–C18 fatty acids	Defender 25SC	2000 ml	500
Insect growth regulator	buprofezin	Applaud 25W	100 g	12.5
Organotin	azocyclotin	Peropal 25WP	75 g	19

Table 2 Percentage reduction in number of ladybirds (*Halmus chalybeus*) in each fungicide programme compared with untreated trees and results of ANOVA tests. (NS, not significant.)

Programme	% reduction				Comparison (P value)	
	Period 1	Period 2	Period 3	All periods	With untreated	Between programmes
Five sprays						
(1) cupric hydroxide	35	46	80	41	<0.01	Prog. 1 versus 2
(2) chlorothalonil	15	53	70	31	<0.01	NS
Nine sprays						
(3) cupric hydroxide	64	74	85	68	<0.001	Prog. 3 versus 4 versus 5
(4) chlorothalonil	69	83	90	75	<0.001	NS
One spray						
(5) copper sulphate and hydrated lime	81	82	75	81	<0.001	Prog. 1 and 2 versus 3 and 4 <0.001

A. 5-spray programmes of cupric hydroxide & chlorothalonil

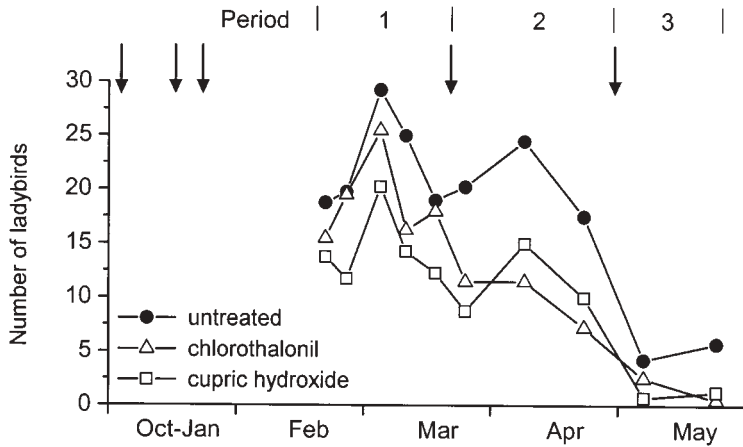
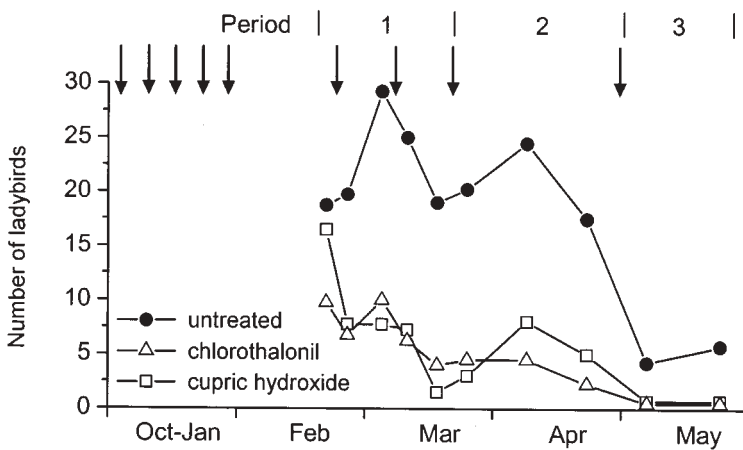


Fig. 1 Effect of five fungicide programmes on densities of *Halmus chalybeus* (mean number/plot per 3-min search) in a citrus orchard from 20 February to 23 May 1991. Arrows indicate spray applications for each programme (see text for dates).

B. 9-spray programmes of cupric hydroxide & chlorothalonil



C. 1-spray programme of copper sulphate & hydrated lime

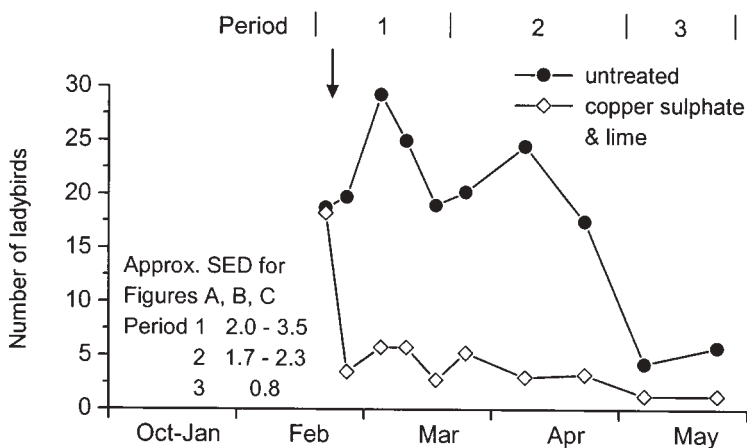
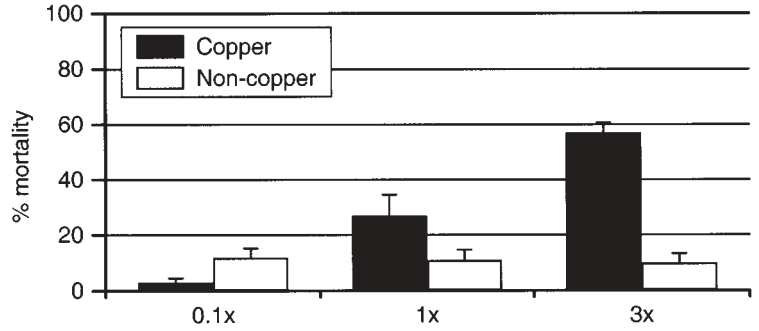
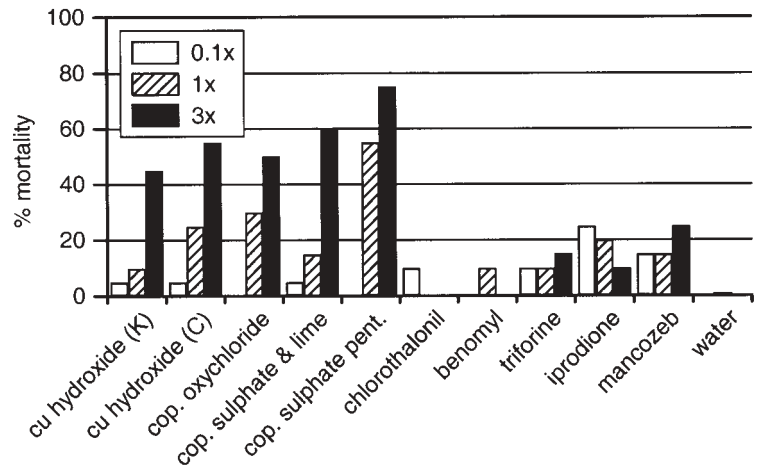


Fig. 2 Mortality (A, mean + SEM) of adult *Halmus chalybeus* 72 h after being dipped in pesticides at 0.1x, 1x, and 3x label rates. Full treatment names are given in Table 1. (*, not tested.)

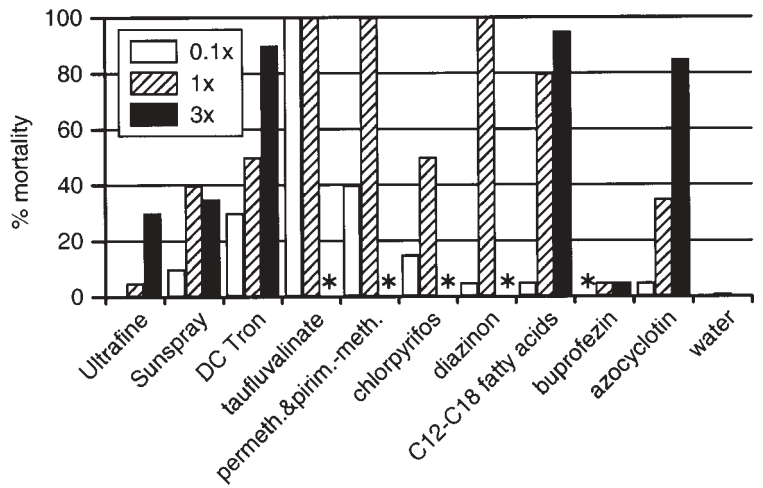
A. Copper and non-copper fungicides



B. Fungicides



C. Insecticides



chlorothalonil-treated plots were similar to those on untreated plots. After the fourth spray on 22 March (Periods 2 and 3), both fungicide treatments had ladybird densities that were similar ($P > 0.05$) and approximately half ($P < 0.01$) those on untreated plots.

The nine-spray programmes reduced ladybird densities by 68–75% overall (Table 2; Fig. 1B). There was no difference in numbers of ladybirds between these two programmes in all three periods. For both cupric hydroxide and chlorothalonil, trees sprayed 9 times had fewer ladybirds than those sprayed 5 times. The single application of copper sulphate and hydrated lime caused a similar reduction in ladybird densities as the two nine-spray programmes (Table 2; Fig. 1C). On 20 February, the number of ladybirds per plot averaged 18.8 in control trees, and 18.3 in the Bordeaux plots before the Bordeaux was applied. After the Bordeaux application there were fewer than 6 ladybirds per plot and 3 months later there was still no sign of a recovery in numbers.

Larvae and pupae comprised 1% ($n = 29$) of the ladybirds observed. Four times more immature ladybirds ($P < 0.05$) were recorded on unsprayed trees (0.54/plot per 3 min) than on fungicide-treated trees (0.13/plot per 3 min).

Toxicity of fungicides and insecticides

Overall, the copper-based fungicides tested killed more ladybirds than non-copper fungicides ($t_{(0.05, 18)} = 2.59$, $P < 0.01$), particularly at $3\times$ label rates ($t_{(0.05, 8)} = 6.71$, $P < 0.01$) (Fig. 2A,B). There was no difference between the two groups at $0.1\times$ label rate ($t_{(0.05, 8)} = 2.12$, $P > 0.05$) or label rate ($t_{(0.05, 8)} = 1.88$, $P > 0.05$). The toxicity of copper fungicides increased significantly ($F_{(0.05, 2, 12)} = 24.54$, $P < 0.001$) between $0.1\times$ and $3\times$ label rates. There was no such rate effect ($F_{(0.05, 2, 12)} = 0.06$, $P > 0.05$) for the non-copper fungicides, which all caused little mortality even at the $3\times$ rate.

There were large differences in toxicity amongst the insecticides (Fig. 2C). The insect growth regulator, buprofezin, and Ultrafine oil were the least toxic at label rates. The other two mineral oils, the miticide azocyclotin, and the organophosphate chlorpyrifos, were moderately toxic at label rates. Highly toxic insecticides included both pyrethroids, the other organophosphate and the fatty acid soap. Taufluvinate killed all ladybirds even at one-tenth label rate. Both organophosphates caused a similar low mortality at $0.1\times$ label rates ($\chi^2 = 0.3$, d.f. = 1, $P > 0.05$), but diazinon was more toxic than

chlorpyrifos at label rates ($\chi^2 = 10.8$, d.f. = 1, $P < 0.01$). This difference was confirmed by the repeated test when dipping in diazinon resulted in 100% mortality compared with 17% for chlorpyrifos ($\chi^2 = 39.5$, d.f. = 1, $P < 0.001$).

DISCUSSION

The undesirable side effects of insecticides on natural enemies have been well documented (e.g., Croft 1990; DeBach & Rosen 1991), but similar effects of fungicides are less well known. The field trial showed that applications of three fungicides reduced densities of *H. chalybeus*. In both the five-spray and nine-spray programmes the reduction in numbers of ladybirds increased between Periods 1 and 2 following fungicide sprays. The effect of Bordeaux mixture was even more dramatic. These effects were probably due largely to repellency, since the fungicides were unlikely to kill many adults. Other studies have demonstrated disruptive effects of fungicides on *H. chalybeus*. Lo & Blank (1992) found that predation of *C. destructor* was reduced on fungicide-treated leaves. Hely et al. (1982) reported an outbreak of *C. destructor* following spraying with Bordeaux mixture and believed the deposits repelled *H. chalybeus* thereby reducing predation.

Non-copper fungicides are likely to be less disruptive to *H. chalybeus* than copper fungicides. Chlorothalonil is therefore preferable to cupric hydroxide and Bordeaux mixture for an integrated pest and disease management programme. It gave better control than cupric hydroxide of alternaria and melanose on navel oranges, although the latter was more effective on tangelos (Olson et al. 1992). Importantly, chlorothalonil was less toxic than copper fungicides to *H. chalybeus* and had less effect on their feeding. In laboratory experiments, predation of *C. destructor* by *H. chalybeus* was drastically reduced by cupric hydroxide and Bordeaux mixture, less so by chlorothalonil (Lo & Blank 1992). Fortunately, given its severe effect on the activity of *H. chalybeus*, Bordeaux mixture has been superseded by newer copper fungicides such as cupric hydroxide. Unfortunately, the multiple applications of cupric hydroxide and chlorothalonil needed under New Zealand conditions are likely to be equally disruptive to ladybirds as a single Bordeaux spray.

The scarcity of ladybird larvae on sprayed trees suggests that the fungicides may have inhibited oviposition, either directly because adults avoided laying on sprayed leaves, or indirectly because fewer

adults were present. Another possible reason was that the fungicides increased mortality of ladybird larvae, as Olszak (1999) found for *Adalia bipunctata*. None of these possibilities were tested, but all have the effect of reducing numbers of larvae available to prey on scale insects.

The detrimental effects of fungicides, particularly copper-based ones, are likely to reduce the ability of *H. chalybeus* to control wax scales and other pests. This may have greater consequences for biological control of *C. destructor* than *C. sinensis*, and could help explain why *C. destructor* reached pest levels more often than *C. sinensis*. Tangelo fruit are most susceptible to fungal diseases (and hence require most frequent spraying) during the 3–5 months following fruit set in November (Olson et al. 1992). This period coincides with the presence of first- and second-instar *C. destructor* (Lo et al. 1996), which are the stages that are vulnerable to predation by *H. chalybeus* (Lo & Chapman 2001). The results of these experiments suggest that the use of fungicides, particularly copper-based ones, should be minimised from about November to February, where *C. destructor* is a pest. This will help reduce disruption to ladybird predation of the vulnerable scale stages. *C. sinensis* should be less susceptible to any negative effects of fungicides on *H. chalybeus* because its first-instars do not appear until February (Lo et al. 1996), when fruit are less susceptible to diseases.

Among insecticides, buprofezin is potentially a good candidate for an integrated pest management programme. It had the lowest direct toxicity of the insecticides in this trial and did not disrupt predation of *C. destructor* by adult *H. chalybeus* (Lo & Blank 1992). Furthermore it has good efficacy against scale insects and other Hemipteran pests, and like other insect growth regulators is relatively safe towards hymenopterous parasitoids. However, several studies have recorded longer-term negative effects on ladybirds. These include increased mortality of larvae or inhibition of pupation and egg hatch (Peleg 1983; Smith & Papacek 1990; Mendel et al. 1994). Further studies are needed on the longer-term effects of insect growth regulators on adult and larval *H. chalybeus*.

Mineral oils are used widely to control both armoured and wax scales on citrus and are considered to be relatively selective in favour of beneficial insects and mites (Beattie et al. 1989; Anon. 1991). Ultrafine caused the least mortality of ladybirds and being a more highly refined oil than the other two is less likely to be phytotoxic. It therefore appeared to be the best of the three oils tested for an integrated

programme. Mineral oils, however, can be potentially disruptive without being highly toxic. In the laboratory, Sunspray oil reduced predation by adult *H. chalybeus* of *C. destructor* by 67% over 24 h compared with unsprayed scales (Lo & Blank 1992).

Potassium salts are active against several groups of pests. Although Defender caused high mortality when *H. chalybeus* were immersed (this study), it was not toxic and did not interfere with predation when ladybirds fed on newly treated wax scales (Lo & Blank 1992). Dipping clearly gave the ladybirds a higher exposure to the chemical than they might receive in orchards. Soaps should still be able to be used safely in orchards with *H. chalybeus*.

Organophosphates and pyrethroids are well known for their toxicity to beneficial insects and mites. *H. chalybeus* was no exception and these insecticides are likely to be highly disruptive to biological control of scale insects. Chlorpyrifos may be less disruptive than diazinon because of its lower contact toxicity, but the persistence of these two products needs to be checked in orchards. Diazinon was highly toxic when *H. chalybeus* walked over freshly sprayed leaves and ingested treated scales (Lo & Blank 1992). The miticide, azocyclotin, is also likely to be disruptive in orchards as it interfered with predation by *H. chalybeus* (Lo & Blank 1992) and was moderately toxic. Olszak (1999) found azocyclotin was the most toxic of seven miticides tested on *A. bipunctata*.

The results of this study re-emphasise the need for an integrated approach to pest and disease management in citrus. Although fungicides should kill few *H. chalybeus* in orchards, they were by no means benign towards ladybird populations and potentially disruptive to their activities. Non-copper fungicides appear to be more compatible than copper-based fungicides for disease management programmes where *H. chalybeus*, and perhaps other ladybirds, are important predators. Further work is needed on the compatibility and longer-term effects of both fungicides and insecticides to *H. chalybeus* and other beneficial insects in New Zealand conditions.

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