Toxicity of pesticides to *Aphelinus mali*, the parasitoid of woolly apple aphid

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Abstract The parasitoid *Aphelinus mali* controls woolly apple aphid (WAA; *Eriosoma lanigerum*) in New Zealand apple orchards. The effects of seven pesticides on *A. mali* exposed to residues on filter paper were assessed in a laboratory bioassay immediately post-application. Spinosad at label rate was moderately to highly toxic, but other compounds and lower rates of spinosad had no detrimental effects on *A. mali*. Another bioassay incorporating field application and exposure to aged residues on leaves determined the toxicity of carbaryl, diazinon, indoxacarb and lime sulphur. Carbaryl had the greatest residual toxicity to *A. mali* on harvested leaves, causing 85% mortality 21 days after application, declining to 40% by 28 days. In contrast, diazinon initially caused high mortality but residues were not toxic 15 days after application, while indoxycarb and lime sulphur were not toxic. Minimising the use of pesticides toxic to *A. mali* should benefit the sustainable management of WAA in apple orchards.

Keywords woolly apple aphid, *Aphelinus mali*, off target toxicity, *Eriosoma lanigerum*, pesticides.

INTRODUCTION

Woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausemann) (Homoptera: Pemphigidae), is a prevalent pest within Hawke's Bay apple orchards and can cause significant damage to the trees (Bradley et al. 1997; Mols & Boers 1999) by sucking sap from new branches and shoots. WAA forms colonies that become covered in waxy filaments, which prevent effective chemical contact (Monteiro et al. 2004). If WAA build up to high numbers, they can be a serious pest of apples and this species is an increasing threat to the New Zealand apple industry in the absence of effective aphicides. *Aphelinus mali* (Hald.) (Hymenoptera: Encyrtidae) is a specific endoparasitoid of WAA

and is the New Zealand apple industry's primary control strategy for managing WAA. *Aphelinus mali* is an important biological control agent regulating WAA populations (Shaw & Walker 1996; Monteiro et al. 2004) and thus enabling Integrated Fruit Production (IFP). For an IFP programme to be effective, selective chemicals with the least toxicity to *A. mali* should be utilised in apple orchards (Bradley et al. 1997).

Serious WAA outbreaks that occurred in Hawke's Bay during the 2007–08 pipfruit season coincided with the introduction of a programme targeting negligible pesticide residues on harvested fruit. This new pest control programme is believed

to have initiated the observed disruption of biological control of WAA by *A. mali* in 2007–08. This study sought to conduct a laboratory bioassay using methods developed by Bradley et al. (1997) for the rapid screening of pesticides for direct toxicity to adult *A. mali*, as well as developing a more comprehensive bioassay to include field-aged residues of selected products.

MATERIALS AND METHODS

Filter paper bioassay

The bioassay procedure was carried out as described by Bradley et al. (1997) during April 2007. Briefly, it consisted of a filter paper disc placed into the lid of a 90-mm Petri dish with 0.5 ml of pesticide solution applied evenly over the surface of the paper and left to dry (20–25 min). Pesticides were tested that had not been available for testing in 1997 (Bradley et al. (1997); water was used for the control (Table 1). Approximately 30 adult *A. mali* were collected into a pottle, by use of aspirators, from a Hawke's Bay apple orchard. *Aphelinus mali* were taken from various parts of the apple trees and wind-fallen apples in the mornings and tested the same afternoons.

Each replicate constituted a Petri dish with about 30 *A. mali* and either six or twelve replicates

were tested per product and concentration. The specimens from the pottles were transferred into their respective treatments, and then quickly covered with the Petri dish base, which was sealed with cling film. The number of insects in each dish that had died as a result of "handling" was recorded immediately after this procedure and subtracted from the bioassay results. The remainder were held at 20°C for 16 h then examined to determine treatment mortalities. Insects were considered to be alive if any movement was observed.

Field-aged laboratory bioassay

Insecticides were applied to mature 'Pinkie' and 'Fuji' apple trees on 30 March 2009 and 19 April 2010 respectively (Day 0), at Plant & Food Research, Hawke's Bay. Treatments were sprayed at standard label rates and compared with an unsprayed control (Table 2). Each product was mixed into 3 litre solutions and applied until run-off by a motorised knapsack sprayer (Honda®) to the bottom tier of previously unsprayed trees. One tree was sprayed per treatment, and an untreated guard tree separated neighbouring treatments to minimise spray drift. Apple leaves of a similar size were collected on Day 0 immediately after the spray deposits had dried,

Table 1 Pesticide rates and formulations applied to filter paper prior to exposure of field-collected *Aphelinus mali* for 16 h.

Trade name	Active ingredient	Formulation	Active ingredient g/litre
Insecticides			
Success™	spinosad	120 g/litre suspension concentrate	0.048
Success™	spinosad	120 g/litre suspension concentrate	0.024
Success™	spinosad	120 g/litre suspension concentrate	0.012
Prodigy*	methoxyfenozide	240 g/litre suspension concentrate	0.09
Altacor®	chorantraniliprole	350 g/kg water dispersible granule	0.032
Calypso®	thiacloprid	480 g/litre suspension concentrate	0.144
Proclaim®	emamectin benzoate	50 g/kg water soluble granule	0.001
Fungicides			
Chorus®	cyprodinil	500 g/kg water dispersible granule	0.15
Flint®	trifloxystrobin	500 g/kg water dispersible granule	0.05

Table 2 Product rates applied to unsprayed apple trees at Plant & Food Research, Hawke's Bay, for *Aphelinus mali* bioassays during March 2009 and April 2010.

Product	Active ingredient and formulation	Active ingredient g/litre
Control	Unsprayed	
Avaunt®	300 g/kg indoxacarb, water dispersible granule	0.06
Gro-Chem Lime Sulphur	200 g/litre polysulphides of calcium, soluble concentrate	2
Sevin® Flo	500 g/litre carbaryl, suspension concentrate	0.6
Diazol®	500 g/litre diazinon, oil/water emulsion	0.5

and on additional days after spraying (Figure 1). The leaves from an untreated tree, well separated from treated trees, were used for the control. Leaf sampling from the treated trees was continued until the pesticide residue caused little toxicity. *Aphelinus mali* were collected as described for the filter paper bioassay. Approximately 30 *A. mali* adults were exposed to an apple leaf with residues within a Petri dish for 24 h at 20°C, after which the insects were assessed for mortality as above. Ten replicates (Petri dishes with leaves and about 30 *A. mali*) were used for each treatment on each day and compared with the control. In 2009 the carbaryl bioassay was abandoned after Day 15 through circumstances beyond our control and was therefore repeated in 2010. The 2009 carbaryl data have still been included to add support to the 2010 data.

Statistics

Results were corrected for mortality in the untreated controls using Abbott's correction formula (Abbott 1925). Minitab (Minitab® 15.1.0.0. ©Minitab 2006 Inc.) was used to calculate means and standard errors. Probit regression analysis was conducted on the field-aged residue data using a generalised linear model in Genstat and LT_{50} s predicted (Genstat® 12.2). Model adequacy checks were carried out by examining various plots (scatter, histograms and normal probability) of the residuals.

RESULTS

Filter paper bioassay

Of the products tested in this bioassay, spinosad

residues were the most toxic to adult *A. mali* (Table 3). At label rate (0.048 g ai/litre) spinosad was "moderately to highly toxic" to adults as categorised by the rating system developed by Croft (1982). Exposure of the parasitoids to half and a quarter of the label rate showed a rate response, whereby mortality increased with increasing insecticide concentration. All other products tested in this bioassay had little or no detrimental effect on *A. mali* adults. The mean mortality of the water controls was 11%.

Field-aged laboratory bioassay

Residues of both carbaryl and diazinon caused high mortality of adult *A. mali* on Day 0 (Figure 1). Carbaryl (2010 data) had the greatest persistent residual toxicity to *A. mali*, causing 85% mortality 21 days after application, which declined to about 40% by 28 days. In contrast, diazinon residues caused high toxicity on Day 3 and then declined sharply and any remaining residue caused little mortality 15 days after application. The relative difference in the toxicity of the two product residues over time is further illustrated by the very different predicted median lethal time (LT_{50}) values. The LT_{50} s for carbaryl and diazinon were 24.6 (21.6–29.0; 95% CI) and 9.09 days (7.67–10.7; 95% CI) respectively, for field decay of the insecticide residue that results in 50% mortality of *A. mali*. Data from the abandoned 2009 carbaryl treatment have been included to demonstrate consistency with the 2010 data (Figure 1). Rainfall following Day 0 in 2009 was minimal. The timing of significant rainfalls, over 2 mm per day, in 2010

Figure 1 Mean mortality (%; corrected using control mortalities) of *Aphelinus mali* after exposure to pesticide residues on an apple leaf at progressive intervals after field application of products to apple trees during March 2009 and April 2010. Error bars denote SEM.

Table 3 The corrected mean mortality (%; $\pm$ SEM) of *Aphelinus mali* specimens exposed to a range of pesticide residues on filter paper. Treatment means are from six replicates, unless otherwise stated.

Trade Names	Active ingredient (g/litre)	Corrected mortality (%)	Toxicity ¹
Insecticides			
spinosad	0.048	72 $\pm$ 7.6 ²	++++
spinosad	0.024	29 $\pm$ 8.3 ²	++
spinosad	0.012	12 $\pm$ 4.9 ²	++
methoxyfenozide	0.09	7.3 $\pm$ 2.7	+
chorantraniliprole	0.032	7.5 $\pm$ 2.8	+
thiacloprid	0.144	4.8 $\pm$ 1.1	+
emamectin benzoate	0.001	2.9 $\pm$ 1.4	+
Fungicides			
cyprodinil	0.15	6.8 $\pm$ 3.8	+
trifloxystrobin	0.05	0	+

¹Toxicity rating taken from Croft (1982) + (<10%) no or little toxicity; ++ (10-40%) low toxicity; +++ (50-70%) moderate toxicity; ++++ (75-90%) high toxicity; +++++ (99.99%) kill.

²Denotes 12 replicates.

are shown on Figure 1. Indoxacarb and lime sulphur were not toxic to *A. mali* at the rates applied in this bioassay (Figure 1). The mean control mortality was 6.6 and 11.3% respectively for 2009 and 2010.

DISCUSSION

The filter paper bioassay identified spinosad as a product that may potentially be disruptive to biological control of WAA. However, it is clearly not as toxic as some of the organophosphate insecticides (e.g. chlorpyrifos and diazinon) and carbaryl that Bradley et al. (1997) categorised as “highly toxic”. Furthermore, under IFP programmes, spinosad is mostly applied only once or twice close to harvest and therefore less likely to be disruptive to *A. mali*.

However, the degree to which a pesticide is disruptive to biocontrol within the orchard environment also depends upon additional factors, such as the use pattern of the product, how selective it is and how quickly residual toxicity is lost, all of which interact with the phenology of the biological control agent. For example, diazinon has historically been used to manage WAA outbreaks despite being highly toxic to adult *A. mali*. However, it loses its residual efficacy quickly and like other organophosphate insecticides it does not appear to harm internally-developing parasitoids and therefore does not disrupt biological control to the same extent (Cohen et al. 1996; Bradley et al. 1997). In contrast, carbaryl, used for fruit thinning, is very disruptive to WAA management because it is toxic to *A. mali* with long residual activity but not toxic to WAA.

It is important to note that neither the filter paper bioassay nor the more comprehensive field-aged laboratory bioassay is directly equivalent to the field application of these products to natural populations of *A. mali* in commercial apple orchards. However, these types of bioassays have successfully identified pesticides as toxic to *A. mali* that have later been shown to be disruptive to WAA control in an orchard setting (Cohen et al. 1996; Bradley et al. 1997). As discussed by

Bradley et al. (1997), the filter paper bioassay does not address any of the effects on the developing parasitoid within the aphid, potential effects on insect behaviour, or the rate of pesticide decay in the field. The field-aged laboratory bioassay has the advantage of determining the toxicity of a weathered pesticide residue and also uses a natural apple leaf rather than filter paper. Williams et al. (2003) has standardised this type of bioassay further by using a leaf disc, rather than whole leaves of a similar size as in our bioassay.

Carbaryl was expected to be highly toxic (~100% mortality) to *A. mali*. What was surprising about the present field-aged residue bioassay was that this product was so persistent and still able to affect *A. mali* after 28 days of field-weathering, which included two periods of moderate rainfall. Carbaryl is used early in the season for fruit thinning and, especially on varieties like ‘Braeburn’, may need to be applied twice to achieve the ideal crop load. This use pattern can be particularly disruptive to WAA biological control and has led to a strategy of applying diazinon at the same time as carbaryl to minimise WAA outbreaks later in the season (Shaw & Wallis 2009). The present study has demonstrated that diazinon residues decay relatively quickly, which helps explain why judicious use of this product mid-season can provide good effective control of WAA without depleting *A. mali* populations too much.

Lime sulphur is the most commonly used pesticide for disease control in organic apple production (Tate et al. 2000). Furthermore, sulphur compounds are known to be insecticidal and suppress insect populations. This study has demonstrated that lime sulphur residues on apple leaves do not appear to be toxic to *A. mali*. When integrated pest management programmes rely on both natural enemies and pesticides for pest control, there is a need to select pesticides cautiously to ensure minimal impacts on natural enemies (Rill et al. 2008). The present results suggest that a more sustainable strategy to control WAA and preserve other natural enemies would

be to use alternative crop thinners to carbaryl and minimise the use of products known to be disruptive to *A. mali* (Shaw et al. 2003). This is especially important for the Hawke's Bay region to avoid future WAA outbreaks such as occurred in 2007-08.

ACKNOWLEDGEMENTS

Funding from Pipfruit New Zealand Inc. is gratefully acknowledged. Tara Taylor, Tony White, Lyn Cole, Peter Lo, Keith Vincent, Mary Malone, Terrance Makea and Alan Coombs assisted with insect collection. We would also like to thank Apollo Pac for the use of two of their orchards for collecting *A. mali*. Duncan Hedderley provided valuable statistical advice.

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