THE TOXICITIES OF CHLORPYRIFOS AND FENITROTHION TO *MICROCTONUS AETHIOPOIDES* AND *SITONA DISCOIDEUS*

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SUMMARY

The effect of chlorpyrifos and fenitrothion on *Sitona discoideus* and its parasitoid *Microctonus aethiopoides* was evaluated by topical dosing and residue exposure experiments. At LD_{50}'s of 4.3 mg/g, both chemicals were highly toxic to the parasitoid adult but were less so to sitona. Preliminary tests indicated that *M. aethiopoides* pupae were much more tolerant of the insecticides. Field application of chlorpyrifos showed that residues were highly toxic to *M. aethiopoides* for 3 days after spraying, but by day 6 mortality had declined significantly.

INTRODUCTION

Sitona weevil *Sitona discoideus* was accidentally introduced into New Zealand in the early 1970's and thereafter spread rapidly throughout the country infesting lucerne stands and annual medicks. The adult weevil can cause widespread defoliation in lucerne stands particularly in the late spring when teneral adults emerge. However, in Canterbury's light soils, larvae cause the most significant yield losses (Goldson *et al* 1988).

Recent research has shown that sitona can be practically controlled by a single application of chlorpyrifos (0.2 - 0.4 kg/ha) or fenitrothion (0.6 kg/ha) insecticide in autumn once the post-aestivatory flights into lucerne are completed and before the onset of egg laying (Goldson 1984).

In an effort to achieve long term biological control of sitona weevil a parasitoid, *Microctonus aethiopoides*, which attracts the adult was introduced into New Zealand in 1982 (Stufkens *et al* 1987). Originally from the Mediterranean region, the parasitoid is now starting to show significant promise in suppressing some weevil populations (Goldson *et al* in prep). This is in spite of density-dependent effects that can off-set the impact of the parasitoid (Goldson *et al* 1988).

Until the effects of biological control are shown to be effective, chemical control may still be necessary in some lucerne stands (Goldson and Muscroft-Taylor 1988). For this reason an investigation into the relative toxicities of chlorpyrifos and fenitrothion insecticides to *M. aethiopoides* and *S. discoideus* adults was considered to be useful for assessing their possible detrimental effects on the parasitoid. The toxicities of field weathered residues of these insecticides to *M. aethiopoides* were also evaluated.

MATERIALS AND METHODS

Topical dosing

Topical application tests were carried out on sitona adults and on *M. aethiopoides* adults and pupae. Weevils were collected at fortnightly intervals in 1987-1988, from lucerne stands near Darfield, Canterbury. Weevils were held at laboratory temperature (20 °C) to permit the emergence of *M. aethiopoides* prepupae that dropped through a gauze floor of the holding cage. Pupation occurred under sections of filter paper provided below the cages.

Because of limited numbers, experiments on adult parasitoids had to be run over several weeks and the data pooled. For similar reasons, female and male *M. aethiopoides* were combined, as preliminary observations had indicated that there was no significant difference in susceptibility to insecticide (McNeill unpublished). Only parasitoids between 1-3 days old were used which, prior to dosing, were held at 15 °C in cages supplied with a honey-water solution.

For testing of *M. aethiopoides* pupae, cocoons were extracted from collecting dishes and placed on moist filter paper until required. Only those pupae which had developed eyespots were used for toxicity testing.

Prior to topical dosing, weevils were starved for 12-18 hours and held at 15 °C in 12:12 L:D photoperiod. No attempt was made to separate the sexes although exceptionally small specimens were discarded.

Test chemicals were restricted to those currently registered for use against *S. discoideus*; chlorpyrifos (Lorsban 40EC) and fenitrothion (Caterkill 600EC). Insecticide solutions were applied using a SGE microsyringe in an Arnold micro-applicator. The 250 µl syringe was calibrated to deliver a volume of 0.5 µl,. and 0.35 µl to the weevil and parasitoid cocoons respectively. A 100 µl microsyringe was calibrated to deliver a volume of 0.2 µl to *M. aethiopoides* adults.

Dosing was carried out in the laboratory at ambient temperatures ranging between 20-23 °C. Weevils and *M. aethiopoides* adults were immobilised by exposure to CO_2 for 30 seconds. To delay recovery the insects were placed in a glass petri dish, set over a bed of crushed ice. Weevils were manipulated with fine touch forceps, while a suction pencil proved to be most suitable for handling the adult parasitoids. A sprig of lucerne with the cut stem wrapped in moist cotton wool was added to each petri dish. *M. aethiopoides* cocoons were treated by positioning them on a glass plate and applying the insecticide droplet to the dorsal surface. After treatment, insects were placed in plastic petri dishes and held at 15 °C. Mortality of *S. discoideus* was recorded at 48 hours and of *M. aethiopoides* adults at 24 hours. Pupae were held until emergence was completed.

Dosages were converted to µg/g body weight and LD_{50} values obtained by subjecting the mortality data to log-probit analysis. The selectivity ratio proposed by Metcalf (1972) was used to compare the parasitoid adult/weevil response to each chemical.

Residue exposure

Chlorpyrifos was applied during autumn to early regrowth lucerne using a 6 metre boom spray unit, at the recommended rate of 0.3 kg/ha. Plots were 12 m x 12 m with the control being water only. There were seven replicates per treatment. Several strips were also treated with chlorpyrifos at 1.0 kg/ha.

Lucerne sprigs were randomly selected from within these plots at 1,3,6,10,15 and 22 days after application. Plant samples were taken to the laboratory and processed within 2 hours. Parasitoid adults, 1-4 days old and of mixed sex, were collected from laboratory-reared cocoons. Two trifoliate leaves were placed in glass vials measuring 10 mm x 25 mm. Older leaves were used as these were assumed to have been present at the time of chemical application. One to two parasitoids were introduced into each vial, and then capped with gauze. The vial was then laid on moist cotton wool, and placed in a controlled environment cabinet set at 15 °C. A fan built into the cabinet ensured continuous air movement. Mortality was measured after 24 hours and the percentage mortality was calculated and corrected using Abbott's formula (Abbott 1925) to account for natural mortality. The first bioassay used nine parasitoids/treatment. Subsequently between 16-20 parasitoids were used per treatment up to 22 days post-application.

RESULTS

Topical dosing

The LD_{50} values shown in Table 1 indicate that both chemicals were more toxic to the *M. aethiopoides* adults, than to its host *S. discoideus*. Both parasitoid and host exhibited similar susceptibility to each of the chemicals. The selectivity ratio derived from LD_{50} non-target/LD_{50} of pest (Table 1), further reinforced the observation that chlorpyrifos and fenitrothion were highly toxic to the parasitoid adult.

An attempt to determine LD_{50} values for *M. aethiopoides* cocoons proved difficult. Treatment of cocoons with up to 800 mg/litre and 900 mg/litre chlorpyrifos and fenitrothion respectively did not produce a significant mortality compared to the control. Although numbers of test subjects at the highest rates tested were low (7 and 9), it provided an indication of likely response.

TABLE 1: Toxicity of topically applied chlorpyrifos and fenitrothion to *M. aethiopoides* and *S. discoideus* adults.

Species	Insecticide	Numbers tested	LD_{50} µg/g	95% C.I. (weighted)	Selectivity ratio
M. aethiopoides	chlorpyrifos	415	4.33	3.27 - 5.59	0.81
	fenitrothion	311	4.29	3.78 - 5.22	0.74
S. discoideus	chlorpyrifos	399	5.36	5.02 - 5.72	
	fenitrothion	204	5.80	5.29 - 6.32	

Residue exposure

Figure 1 shows the corrected percentage mortality of parasitoid adults at selected days after treatment. At the recommended field rate of 0.3 kg/ha chlorpyrifos mortality was high for the first 3 days after spraying, with 78% mortality recorded on the first day, falling to 65% after 3 days. Mortality dropped significantly ($P<0.05$) by day 6 to 6.0%; after 15 days insecticide residues on the foliage were no longer toxic to *M. aethiopoides* adults. At 1.0 kg/ha the toxicity of residues remained high (> 80.0% mortality) for up to 15 days after treatment although by day 22 this had dropped to 50.0%.

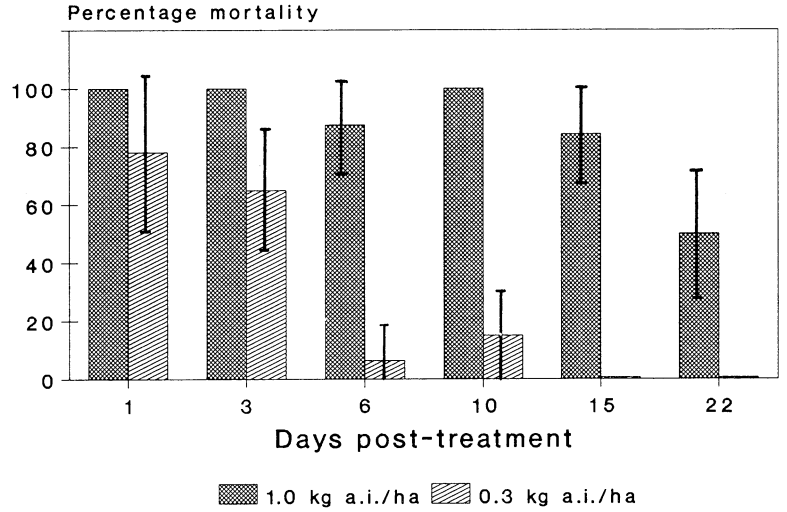

Fig. 1: Residual activity of chlorpyrifos on lucerne relative to contact mortality of adult *M. aethiopoides* (confidence intervals shown).

DISCUSSION

The results of laboratory experiments using topical application of chlorpyrifos and fenitrothion on adults of *S. discoideus* and its parasitoid *M. aethiopoides* suggest that current control measures would eliminate both weevil and its enemy. Likewise parasitoid larvae would similarly be affected, as death of the weevil host before larval development is completed would result in death of the internal parasitoid (Dumbre and Hower 1976).

Conversely preliminary tests against the *M. aethiopoides* cocoons indicate that this stage of the life cycle would probably survive a field application of insecticide. In the field, 5th instar *M. aethiopoides* emerge from weevils and fall into the litter layer to pupate. The pupae, as well as being protected by the cocoon case, use leaf litter as a

further cover/substrate. This probably protects the developing adult further from environmental extremes and possible natural enemies and may also provide protection from insecticide. Additionally, given that a small number of weevils may survive a field insecticide application, survivors could act as hosts for adult parasitoids emerging in spring, and thus ensure the continued survival of the parasitoid in the field albeit at low population densities.

The effect of field residues on the parasitoid adult was not as marked as that shown by toxicological studies. At field rates (0.3 kg/ha), high mortality occurred when parasitoids were exposed to 1 and 3 day old chlorpyrifos residues but activity declined rapidly thereafter. A similar rapid fall off in toxicity was reported by Dumbre and Hower (1977) who tested four chemicals for residual toxicity against *M. aethiopoides* adults exposed to alfalfa, grown and treated in a greenhouse. The prolonged activity of foliage treated with the high rate of chlorpyrifos (1.0 kg/ha) is to be expected, given that this concentration is 3.1 times the recommended field rate.

Insecticides can have a significant impact on biological control agents depending on their phenology and timing of the spray. The spray recommendations against alfalfa weevil were found to coincide with the time when the parasitoid *Microctonus colesi* were predominately larvae in the adult alfalfa weevils (Hower and Luke 1975). Insecticide application resulted in a 98% reduction in weevil populations as opposed to untreated paddocks, with a consequent decline in adult *M. colesi* densities. Conversely where all life stages of a parasitoid are present in the field, chemicals have been shown to have minimal impact on a thriving field population of parasitoids (Aeschlimann 1983).

Goldson (1984) recommended interim insecticidal control of sitona weevil at the end of the post-aestivatory flights in late summer — early autumn. At this time temperatures in the field are beginning to drop with a consequent slowing of insect development. Detailed study showed that in autumn 1988, degree day accumulations over this critical period meant that *M. aethiopoides* had only reached the larval stage within the weevil at the time of spraying (Goldson *et al* in prep.). Therefore insecticide treatment was unfortunately timed at the most vulnerable stage of parasitoid development and must be seen as being detrimental to parasitoid survival.

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