BIOASSAY OF SOME NOVEL INSECTICIDES AGAINST LIGHTBROWN APPLE MOTH LARVAE

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SUMMARY

Several novel insecticides were incorporated into artificial diet and sprayed onto potted apple trees in preliminary assessments against lightbrown apple moth (*Epiphyas postvittana*) larvae. Diet incorporation yielded LC₅₀ values in descending order of toxicity: chlorfluazuron, avermectin, XRD 473, teflubenzuron, triflumuron and azinphos-methyl after 21 days. Mortality after 7 days was comparable for most of the insecticides, when potted apple trees infested with larvae were sprayed at field rates. At 36% and 10% of field rate, significant differences between other insecticides became apparent. Seven day old larvae were significantly more susceptible to chlorfluazuron than older larvae on both artificial diet and potted apple trees.

INTRODUCTION

A limited case of azinphos-methyl resistance in lightbrown apple moth (LBAM) has been identified in the Nelson region (Suckling *et al* 1984), and consequently several new and selective insecticides have been investigated for LBAM control. Laboratory bioassays can usefully supplement field trials of these insecticides, for example, by yielding information on changes in susceptibility with larval age, or on the comparative toxicity of given insecticides. This paper reports on laboratory studies using LBAM larvae exposed to insecticides in artificial diet and on potted apple trees.

The majority of the insecticides used were insect growth regulators in various stages of commercial development, hence much of the following limited information is gleaned from unpublished technical reports. Most of these insecticides are highly selective with low mammalian toxicities, and good persistence. They tend to be slow acting and in some cases are reported to be more toxic to particular larval stages. Assessment must take account of the time required for them to work, usually several days. Their mode of action is different from that of organophosphorus insecticides, and this could therefore render them useful for controlling resistance, either alone or in combination with other insecticides. No account has been taken here of possible effects on eggs, pupae, or adults.

MATERIALS AND METHODS

Insecticides used

Chlorfluazuron (Atabron = IKI 7899) 5EC is a relatively broad spectrum benzoyl urea chitin inhibitor, that relies largely on ingestion, with some reported contact activity. Teflubenzuron (Nomolt) 15EC and triflumuron (SIR 8514) 25WP are also benzoyl urea chitin inhibitors, while XRD 473 5EC is a contact and stomach active moulting inhibitor, that has been reported to be toxic to early instars. Fenoxycarb (Ro 13-5223) 25WP is a non-neurotoxic carbamate with juvenile hormone activity and proven efficacy against leafrollers (Charmillot 1983); Avermectin B1 (MK 936) 1.8EC is a macrocyclic lactone with wide spectrum activity, that is thought to act by blocking signal transmission from interneurons to excitatory motor neurons (Campbell *et al* 1983), and is thus very different from the other insecticides tested. Azinphos-methyl 50WP and chlorpyrifos 50WP and 40EC have been included as standards because of

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their widespread use in leafroller control. In the first apple tree experiment, both wettable powder and emulsifiable concentrate formulations of chlorpyrifos were used, because all the other test chemicals were emulsifiable concentrates except for the standard, azinphos-methyl. This allowed for a comparison of the effects of formulation in the standards.

Unless otherwise stated, Fishers' protected LSD tests were carried out using the angular transformation on percentage mortality to assign Duncan's letters at the significance level of 5%.

Diet incorporation: Probit response

Newly hatched larvae were added to artificial diet (Singh 1983) in Petri dishes, and maintained at 20°C and 16L:8D for 7 days. They were then transferred to Petri dishes containing a range of five to seven insecticide concentrations, with ten larvae per dish and three replicates per concentration. The dishes were maintained for a further 21 days at 20°C before mortality assessment, so that even the slowest acting insecticides would have had a chance to affect all larval stages after the first instar. Insecticide was incorporated into the diet by distributing 1 ml of aqueous solution evenly over 10 g of diet, which was then pressed flat into a 2 mm thick layer covering the bottom of the dish. Any excess liquid was allowed to evaporate. Concentration ranges were established from preliminary experiments and are expressed as $\mu\text{g/g}$ (ppm) of diet. Controls were run with diet treated with distilled water only. Probit analysis was carried out using POLO (Robertson *et al* 1980).

Diet incorporation: Effect of larval age

Neonate larvae were established as above, with 10 larvae added to 10 g of diet per dish. For each insecticide, five replicate dishes were assigned to each treatment date, with treatments at larval ages of 0, 7, 14, 21 and 28 days. Insecticide concentrations were chosen to provide maximum resolution after 7 days, (i.e. 3.0 ppm for all insecticides tested except fenoxycarb and teflubenzuron, which were used at 6.0 ppm). Mortality was assessed after 7 days at 20°C, and larvae were then transferred to untreated diet until pupation, to determine any residual effects arising from the 7 day exposure interval.

Potted apple trees: Mortality at field rates, 36% and 10% field rates

Neonate larvae were allowed to establish for 7 days on 20-25 cm tall 2 year old potted 'Sturmer' apple seedlings caged under perspex cylinders with a gauze top, and held at 20°C and 16L:8D. The number of established larvae was determined, and the trees were then sprayed while rotating on a turn-table. The sprayer consisted of two Potter Precision Spray Tower nozzles (Burkard Mfg. Co. Ltd., Rickmansworth, U.K.) directed to provide even coverage over the trees, with 10 mls sprayed per nozzle at approximately 101 kPa from a distance of 0.4 m. Three replicate trees for each of three concentrations (recommended field rate, 36% field rate, and 10% field rate) were tested for each chemical, with nine trees in the control. Mortality was assessed after 7 days at 20°C, and again at 21 days.

Potted apple trees: Effect of larval age

Neonate larvae were established for 7 days on potted apple trees, as above. For each chemical, four replicate trees were assigned to each treatment date, corresponding to larval ages of 7, 14 and 21 days. The trees were sprayed as above, and maintained at 20°C before assessment after 7 days, and again at 21 days. All trees were sprayed with 100 ppm aqueous solutions, except for the controls which received water only.

RESULTS AND DISCUSSION

Bioassay by incorporation of insecticides into artificial diet gave a greater than 100 fold difference in the LC_{50} s between most and least toxic insecticides although further replication would be desirable to improve the accuracy of these estimates (Table 1). Chlorfluzuron and avermectin were together the most toxic chemicals, followed by XRD 473, teflubenzuron, triflumuron and azinphos-methyl. Fenoxycarb was also included in this series, but dosage-mortality regression could not be carried out on the data, as inconsistent mortality occurred during pupation. Fenoxycarb retarded larval development at a concentration as low as 0.1 ppm in the diet. Additional larval moults added about 3 weeks to the time taken to 90% pupation, compared to control larvae

TABLE 1: Probit response of 1 week old lightbrown apple moth larvae to insecticides (ppm) incorporated in artificial diet after 3 weeks of exposure.

Insecticide	N	b $\pm$ s.e.	LC ₅₀	95% C.L.	LC ₉₀
chlorfluazuron	150	1.41 $\pm$ 0.36	0.09	*—*	0.74
avermectin	150	1.36 $\pm$ 0.30	0.22	*—*	1.9
XRD 473	120	4.19 $\pm$ 0.80	1.0	*—*	2.0
teflubenzuron	210	0.88 $\pm$ 0.20	3.4	0.55 – 11	90
triflumuron	170	2.16 $\pm$ 0.46	9.9	6.3 – 14	39
azinphos-methyl	300	2.80 $\pm$ 0.44	10	7.1 – 12	29

N = Total number tested with insecticide; b = slope in probits; s.e. = standard error of the slope; LC₅₀ or LC₉₀ = concentration causing 50% or 90% mortality; 95% C.L. = 95% confidence limits.

(5-6 weeks). Most fenoxycarb treated pupae were clearly abnormal in appearance, and less than 10% of pupae from 0.1 ppm emerged as normal adults, and no adults emerged from higher concentrations.

When 7 day old larvae were sprayed with insecticides at field rates on potted apple trees, there was no significant difference between most treatments after 7 days (Table 2), or 21 days (not shown). Teflubenzuron and XRD 473 were the least toxic chemicals tested with this method. At 36% of field rate, only chlorpyrifos and chlorfluazuron were as effective as at full field rates, while teflubenzuron and XRD 473 induced significantly lower mortality than the other treatments (Table 2). After 3 weeks, mortality for 36% of field rates of these chemicals was still significantly lower than those produced by chlorpyrifos and chlorfluazuron.

TABLE 2: Comparative response of lightbrown apple moth larvae to insecticides sprayed on potted apple trees, after 1 week of exposure.

Insecticide Formulation Field Rate (ai) Concentration	Mean percent mortality (s.e.m.)		
	10% Field rate	36% Field rate	Field Rate
azinphos-methyl 50WP, 500 ppm	53 (7) bc	83 (9) ab	92 (8) a
chlorpyrifos 50WP, 375 ppm	100 (0) a	100 (0) a	94 (6) a
chlorpyrifos 40EC, 375 ppm	100 (0) a	100 (0) a	100 (0) a
avermectin 1.8EC, 10 ppm	23 (13) c	68 (16) bc	91 (6) ab
chlorfluazuron 5EC, 100 ppm	82 (14) ab	96 (2) ab	96 (2) a
XRD 473 5EC, 100 ppm	26 (7) c	51 (8) c	76 (5) bc
teflubenzuron 15EC, 100 ppm	37 (10) c	46 (20) c	56 (9) c
control	2 (0.7) d	2 (0.7) d	2 (0.7) d

Column entries not sharing common letters are significantly different, according to Fisher's protected LSD tests on angular transformed data.

At 10% of field rate, larval mortality from chlorpyrifos was significantly higher than all other treatments except chlorfluazuron (Table 2). However, these results should be viewed with caution due to the high variance resulting from testing only

approximately 45 larvae at each concentration. Nevertheless, these results suggest that there appears to be a larger margin of safety in the recommended rates of currently used organophosphates than in some of the other candidates tested. It was also noted that mortality in the lower rates of avermectin, teflubenzuron and XRD 473 continued to rise between 7 and 21 days post-treatment (results not shown).

While fenoxycarb consistently delayed pupation, it also caused severe defoliation of the potted trees at all three rates (recommended field rate = 125 ppm ai). Moreover, fenoxycarb treated larvae appeared to cause more leaf damage than untreated larvae, although this was not quantified. Larval mortality 55 days after treatment (i.e. 62 day old larvae, and counting unsuccessful pupation) was 84%, 91% and 94% at the 10%, 36% and 100% field rates respectively, although several potted trees had little or no foliage left at the time. Control mortality at that date was approximately 30%.

When larvae were reared on untreated artificial diet for different time intervals before being transferred to treated diet, or placed on apple trees sprayed after different time intervals, the influence of larval age on susceptibility to the insecticides became apparent (Table 3). Some care must be taken in comparisons between the two methods, due to the different larval growth rates on apple trees and on diet in these experiments, and the generally high variance. There was no difference in mortality between age groups in the control for either experiment.

TABLE 3: The influence of lightbrown apple moth larval age on mortality 7 days after treatment from insecticides in artificial diet and on potted apple trees. (Teflubenzuron was at 6 ppm in the diet, all others at 3 ppm. All trees were sprayed with 100 ppm ai solutions).

Chemical	Larval Age (days)	Artificial Diet	Potted apple trees
		%Mortality (s.e.m.)	%Mortality (s.e.m.)
teflubenzuron	0	40(10)a	—
	7	42(20)a	0 (0)b
	14	66 (7)a	71 (6)a
	21	70 (6)a	51 (7)a
	28	66(12)a	—
chlorfluazuron	0	70(24)b	—
	7	100 (0)a	96 (4)a
	14	80 (7)b	100 (0)a
	21	68 (8)b	60(10)b
	28	62(13)b	—
XRD 473	0	60 (5)b	—
	7	38(11)c	50 (9)b
	14	66 (4)b	97 (3)a
	21	84 (5)a	65(10)b
	28	88 (6)a	—
control	0	16(11)a	—
	7	36(17)a	8 (8)a
	14	22(19)a	20(12)a
	21	22(18)a	20 (9)a
	28	14(17)a	—

Column entries not showing common letters are significantly different according to Fisher's protected LSD tests on angular transformed data.

At a concentration of 6.0 ppm in the diet, teflubenzuron was equally toxic to all ages tested ($P > 0.05$), but at a lower significance level ($P < 0.10$), it was less toxic to 0 and 7 day old larvae than to older larvae. This trend was also apparent on the apple trees, on which no 7 day old larvae died but 14 and 21 day old larvae were susceptible.

With chlorfluazuron, 7 day old larvae were more susceptible than other age groups reared on treated diet, while on the apple trees, 7 and 14 day old larvae were more susceptible than 21 day old larvae ($P < 0.05$).

In the case of XRD 473, 21 and 28 day old larvae exposed to treated diet were significantly more susceptible than younger larvae ($P < 0.05$) but no trend was apparent on the treated trees.

Elsewhere, fenoxycarb has been shown to be most toxic to last instar leafroller larvae, and in Europe fruit damage can be kept low with two spring applications timed to coincide with development of late instars (Charmillot *et al* 1983; de Reede *et al* 1983). Control is largely achieved by depressing over-wintering populations. This is less likely in New Zealand, as there is considerable overlap in leafroller generations and orchard infestation often occurs from mated females flying in from hedgerows and scrub.

CONCLUSION

The nil tolerance of damage on export fruit could present serious problems for slow-acting chemicals if these were used by themselves, and spray timing would therefore be of the utmost importance in their application in New Zealand. However, further investigation of these insecticides for use in horticultural spray programmes would appear to be justified, as indications are that insecticidal activity against lightbrown apple moth can be achieved.

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