LABORATORY EVALUATIONS OF INSECT GROWTH REGULATORS AGAINST LIGHTBROWN APPLE MOTH

R.A. McGHIE and A.R. TOMKINS

*Ruakura Agricultural Centre, MAFTech North
Private Bag, Hamilton*

SUMMARY

Larvicidal and ovicidal activity of a range of Insect Growth Regulators (IGR) against lightbrown apple moth (*Epiphyas postvittana* Walker) was determined. Ranking of chemical activity using EC50 values in continuous feeding bioassays was: flufenoxuron > fenoxycarb > XRD-473 > teflubenzuron > diflubenzuron. Only flufenoxuron and XRD-473 had significant activity against the early larval instars. Fenoxycarb significantly increased the larval weight and length of the larval period compared with untreated insects. Flufenoxuron, XRD-473 and fenoxycarb had strong ovicidal activity when moths oviposited on dry residues on foliage. Direct treatment with fenoxycarb reduced percent egg hatch of young eggs. All eggs were unaffected by direct treatment with the other IGRs.

INTRODUCTION

Lightbrown apple moth (LBAM) is a pest of a number of export fruit crops in New Zealand. Generally this pest is controlled by multiple applications of broad-spectrum organophosphate insecticides.

IGRs, a relatively new class of insecticide, have lower mammalian toxicities than many organophosphate insecticides used in orchards and have been reported to be less disruptive to predators and parasites (Preleg 1983; Westigard 1979). However, the delayed larval knockdown of the first IGRs developed has limited their use in protecting fruit crops against insect damage (Novak and Sehnal 1973; Sbragia *et al* 1983). Some more recently developed IGRs have been shown to have faster larval knockdown and greater contact activity against a number of insect species than the earlier developed chemicals (Komblas and Hunter 1986; Neumann and Guyer 1986; Robertson and Preisler 1986).

With increased activity against early larval stages and/or significant ovicidal activity, an IGR would have potential for inclusion in export fruit spray programmes. This paper reports on laboratory bioassays comparing the larvicidal and ovicidal activity of a range of IGRs against LBAM.

METHODS

All bioassay insects were obtained from a laboratory colony of LBAM reared on a lucerne-protein-dust and agar based diet. The colony and all bioassays were held in a controlled environment with a 16:8(L:D) photoperiod, a temperature of 20°C ± 0.2°C and a relative humidity of 70-90%. Adults were provided with a 10% sucrose solution via cotton wicks.

Continuous Feeding Larval Bioassays

Preliminary bioassays were conducted for each chemical using a wide range of concentrations (0.001-100 ppm) to determine the activity range of each chemical. From these preliminary tests, bioassays each comprising seven to ten concentrations and an untreated control were performed.

For the bioassays a known quantity of each IGR, suspended in a constant volume of water, was incorporated into a known weight of rearing diet while still in a liquid form by thorough mixing. The same volume of water was added to liquid diet for the untreated controls. Insecticide formulations and concentrations of the IGRs used in the

Proc. 41st N.Z. Weed and Pest Control Conf.

diet, expressed as ppm ($\mu\text{g/g}$), are listed in Table 1. The diet was set in 5 ml test tubes, inoculated with one neonate LBAM larva (<24 h old) and stopped with cotton wool. Seventy-five larvae were used per concentration.

Larvae were checked for mortality or pupation at intervals. Pupae were removed from the test tubes and sexed. Any abnormal pupae were noted and held separately. The success of adult emergence was noted, with abnormal adults recorded as unsuccessful adult emergence. Only deformed structures and abnormalities visible to the naked eye were recorded.

EC50 values were calculated by Logit analysis after 13-14 days exposure on diets and again after all insects had died or emerged as adults.

Larval Weight Bioassay

An additional bioassay was performed to assess weight increase of larvae fed fenoxycarb treated diet. Eight concentrations of fenoxycarb (Table 2) plus an untreated control were tested. A maximum of 30 larvae per concentration were individually weighed and then discarded at 18, 28, 32, 42 and 49 days after neonate larvae were initially placed on the diet.

Ovicide Bioassays

Two bioassays were conducted, firstly, by treating egg masses laid on an untreated surface and secondly, by allowing oviposition on treated foliage.

In bioassays where egg masses were treated directly, the masses were laid on sheets of plastic as in the colony. Egg masses of a range of ages from <1 day to 9 days old were selected (20 to 24 egg masses per age group) and dipped for 30 seconds in constantly stirring suspensions of IGRs (Table 3). Untreated egg masses were dipped in water. All egg masses were air-dried at room temperature before being placed in individual containers. Larvae were removed daily until the completion of eclosion, and hatched and unhatched eggs were then assessed by stereo-microscope.

In bioassays where moths oviposited on a treated surface, feijoa (*Feijoa sellowiana*) foliage was dipped for 30 seconds in constantly stirring suspensions of IGRs and then air-dried. Previously paired moths were placed in oviposition cages with the dried foliage on the day it was treated and the moths were allowed to oviposit for 2 nights. Leaves on which egg masses had been laid were removed from the cages after the first night. Egg masses on the leaves from both nights were pooled and placed in open containers until completion of eclosion, with larvae being removed daily. Hatched and unhatched eggs were then assessed by stereo-microscope from 20 egg masses per chemical. Egg masses had an average of 61 eggs per mass.

RESULTS AND DISCUSSION

Continuous Feeding Larval Bioassays

Mortality of untreated LBAM was less than 9% in all bioassays except the fenoxycarb bioassay where it was 25%.

From a comparison of overall EC50 values (larval and pupal mortalities plus partial adult emergence and adult deformities) diflubenzuron was the least active IGR tested. The other chemicals were from 44 to over 3000 times more active than diflubenzuron. The ranking of the chemicals from the most active to the least was: flufenoxuron (0.063 ppm) > fenoxycarb (0.55 ppm) > XRD-473 (1.3 ppm) > teflubenzuron (3.8 ppm) > diflubenzuron (189 ppm). Suckling *et al* (1985) similarly found an approximate three-fold difference between the effectiveness of XRD-473 and teflubenzuron in larval feeding bioassays.

Mortality after 13-14 days exposure gave an indication of the IGR's activity against early larval stages. Flufenoxuron and XRD-473 achieved over 90% mortality of larvae at concentrations of 1.0 ppm and 10 ppm respectively, indicating significant activity against early larval instars. EC50 values were 0.23 and 5.2 ppm respectively. The differential susceptibility of the different larval instar stages was not determined. Suckling *et al* (1985) has reported XRD-473 more active against later LBAM instars and Leonard *et al* (1987) reported XRD-473 equally active against all lepidopterous larval stages. Diflubenzuron and fenoxycarb at all concentrations had mortality levels similar to those in the untreated and teflubenzuron achieved 26% mortality at 1000 ppm (the

highest concentration tested) after 13-14 days exposure. Because of this lack of activity against early larval instars, EC50 values could not be calculated for these chemicals at this stage.

The larval period for insects fed untreated diet is normally 47 days from placement of neonate larvae on the diet. Diflubenzuron, teflubenzuron, flufenoxuron and XRD-473 extended this larval period by 20 to 50 days. During this extended larval period larval development was inhibited relative to the untreated control. These abnormal larvae may have slowly died from starvation as many were noted to have an old head capsule or skin still attached, or to have abnormal mouth parts. No measurement was made of the amount of feeding by larvae. Despite the extended period, these chemicals may greatly reduce crop damage in the field.

Fenoxycarb also extended the larval period but to a greater extent, with larvae still present at higher concentrations (>10 ppm) 120 days after they were placed on the diet. Larvae were noted to be larger in size and less mobile than untreated larvae with areas of cuticle hardening and abnormal mouth parts. They were considered to be supernumerary instar stages.

The IGRs tested differed in their effect on LBAM's ability to pupate and on the success of adult emergence. Fulfenoxuron and XRD-473 did not markedly disrupt pupation, but caused many adults to emerge with crumpled deformed wings from apparently normal pupae (Table 1). Singh and Dugdale (1979) also noted this type of adult deformity when larvae were fed low concentrations of a juvenile hormone analogue (JHA). No adults emerged from the few abnormal pupae formed.

TABLE 1: Percentage adult emergence from normal pupae (female and male pupae pooled) and deformed pupae (fenoxycarb and diflubenzuron only).

flufenoxuron (WL115110 5% WDL)		XRD-473 (5% EC)		fenoxycarb (Insegar 25% WP)		
ppm	Normal	ppm	Normal	ppm	Normal	Deformed
0	96	0	92	0	84	-
0.01	96	0.05	92	0.1	94	100
0.03	94	0.1	97	0.5	86	43
0.06	67	0.5	97	1	100	35
0.1	35	1	67	5	-	0
0.15	10	2.5	33	10	-	0
0.3	-	5	-	50	-	0
1	-	10	-	100	-	0
3	-			500	-	0

diflubenzuron (Dimlin 25% WP)			teflubenzuron (CME-134 15% SC)	
ppm	Normal	Deformed	ppm	Normal
0	99	-	0	98
1	97	0	0.1	95
5	96	33	0.3	89
10	97	0	0.6	97
50	77	0	1.0	91
75	80	6	1.3	87
100	91	3	10	91
250	90	3	30	71
500	83	8	100	71
1000	80	0	1000	100
1500	66	0		

Fruit Crops

Fenoxycarb, diflubenzuron and teflubenzuron disrupted pupation to varying degrees, causing larval-pupal intermediaries and pupae with deformed structures to be formed, similar to those described by Singh and Dugdale (1979). Fenoxycarb showed the most activity with no normal pupae being formed at concentrations of 5 ppm fenoxycarb and above, although some abnormal pupae did successfully emerge as adults at lower concentrations (Table 1). Suckling *et al* (1985) also observed marked pupal abnormalities and reduced adult emergence from fenoxycarb treated larvae. Because fenoxycarb was effective at preventing LBAM from reaching the reproductive stage it would reduce size of the next generation.

The proportion of abnormal pupae increased with increasing concentrations of both diflubenzuron and teflubenzuron to a maximum of 60% and 67% respectively. A small proportion of abnormal pupae from all diflubenzuron concentrations emerged successfully as adults (Table 1). No adults successfully emerged from abnormal pupae formed on teflubenzuron treated diet.

Larval Weight Bioassay

Results from the larval weight bioassay were similar to those found by Singh and Dugdale (1979). Larvae at all fenoxycarb concentrations were of similar weight to the untreated larvae after 18 days exposure (Table 2). By day 28 (the first day of pupation for untreated insects) weight of larvae from all fenoxycarb concentrations were similar and heavier than the untreated. Larvae at concentrations >0.5 ppm increased in weight after this, and were, at 49 days about 2 to 2.5 times heavier than the untreated larvae just before pupation (Table 2). There was no difference in larval weight at fenoxycarb concentrations of between 50 to 500 ppm. Thus, although the previous bioassay showed that fenoxycarb could prevent LBAM from reaching the reproductive stage, its effects in lengthening the larval period and increasing larval weight (and therefore feeding) means that it is unlikely to protect fruit from feeding damage.

TABLE 2: Mean weight (mg) of LBAM larvae fed fenoxycarb treated diet.

Fenoxycarb concentration ppm	Days after placement on diet					
	18	28	32	35	42	49
0	12.4	56.5	-	-	-	-
0.1	-	72.7	74.6	70.4	-	-
0.5	-	74.5	82.3	83.3	87.7	111.2
1	11.7	73.7	85.7	86.5	100.9	98.2
5	11.4	77.7	93.3	86.5	99.2	102.2
10	7.8	73.6	89.1	85.0	103.4	104.3
50	10.4	89.9	91.5	106.2	126.6	135.7
100	12.2	81.4	95.1	113.3	115.7	141.3
500	14.3	79.9	92.1	112.0	122.4	129.2

Ovicide Bioassays

As reported by Gelbic and Sehnal (1973), JHAs can inhibit embryogenesis in some insect species when eggs are treated during the first half of the incubation period. Fenoxycarb, a JHA, significantly ($P < 0.01$) decreased percent hatch of young eggs (<1 day and 3 day old) when treated directly (Table 3). Percent hatch of older eggs was unaffected.

Direct treatment of eggs of all ages by diflubenzuron, teflubenzuron, fenoxycarb, flufenoxuron and XRD-473 did not reduce the percentage egg hatch compared with the untreated egg masses (Table 3). However, these chemicals, and particularly fenoxycarb, flufenoxuron and XRD-473, all showed significant ($P < 0.01$) ovicidal activity compared with the untreated when oviposition occurred on the dried residues (Table 3). This differential ovicidal activity has also been reported by Broadbent and Pree (1984) for Oriental fruit moth (*Grapholitha molesta*) eggs topically treated or laid on a diflubenzuron treated surface.

TABLE 3: Percentage egg hatch from egg masses treated either directly (egg age < 1 day to 9 days old) or indirectly by oviposition on treated foliage.

Chemical Rate g ai/100 litres	Direct Treatment Egg Mass Age (days)							Oviposition on treated foliage
	<1	2	3	4	6	7	9	
Untreated	86	79	89	84	84	68	91	95
diflubenzuron 10	82	-	76	-	82	-	86	86
fenoxycarb 37.5	20	-	22	-	90	-	82	1
teflubenzuron 15	82	68	-	86	-	68	-	50
XRD-473 10	84	65	-	85	-	60	-	2
flufenoxuron 1	94	-	89	-	86	-	76	7

CONCLUSIONS

Flufenoxuron and XRD-473 were the most effective of the range of IGRs tested having strong ovicidal activity and larval knockdown ability. Fenoxycarb also showed strong ovicidal activity but increased the duration and weight of the larval stage and hence the period of possible feeding. Diflubenzuron was the least active of the IGRs against LBAM in these bioassays having little ovicidal or larvicidal activity.

The relatively rapid knockdown of larvae by flufenoxuron and XRD-473 indicates they have potential for protecting export fruit crops from feeding damage by LBAM. Future field trials will be conducted to better determine the efficacy of these chemicals by timing spray applications to coincide with periods of major adult flight and egg laying.

ACKNOWLEDGEMENTS

To Ms C. Thomson for assistance with insect colony maintenance and Ms B. Dow for statistical analyses. To Ivon Watkins - Dow Ltd., Shell Chemical Co., May and Baker Ltd., Yates Chemical Co. and Fernz Ltd. for supplying the chemicals.

REFERENCES

- Broadbent, A.B. and Pree, D.J., 1984. Effects of diflubenzuron and BAY SIR 8514 on the Oriental fruit moth (Lepidoptera : Olethreutidae) and the obliquebanded leafroller (Lepidoptera : Tortricidae). *J. Econ. Entomol.* 77: 194-197.
- Gelbic, I. and Sehnal, F., 1973. Effects of juvenile hormone mimics on the codling moth *Cydia pomonella* (L.) (Lep., Olethreutidae). *Bull. Ent. Res.* 63: 7-16.
- Komblas, K.N. and Hunter, R.C., 1986. A benzoylphenyl urea for rational control of pests on fruit and vegetables. *Brit. Crop Prot. Conf. — Pests and Diseases.* 907-914.
- Leonard, P.K., Riches, M.N. and Howard, M., 1987. XRD-473 Environment/Pest Interactions. *Pestic. Sci.* 20: 157-158.
- Neumann, R. and Guyer, W., 1987. Biochemical and toxicological differences in the modes of action of the benzoylureas. *Pestic. Sci.* 20: 147-156.
- Novak, V. and Sehnal, F., 1973. Effects of a juvenoid applied under field conditions to the Green Oak leaf roller, *Tortrix viridana* L. (Lep., Tortricidae). *Z. Ang. Ent.* 73: 312-318.
- Peleg, B.A., 1983. Effect of a new insect growth regulator, RO 13-5223, on hymenopterous parasites of scale insects *Entomophaga*. 28 (4): 367-372.
- Robertson, J.L. and Preisler, H.K., 1986. XRD-473 (An experimental benzoylphenylurea) : Rates and optimal time of application to Western spruce budworm. *J. Entomol. Sci.* 21 (1): 16-20.
- Sbragia, R.J., Bisabri-Ershadi, B., Rigtarnik, R.H., Clifford, D.P. and Dutton, R., 1983. XRD-473, a new acylurea insecticide effective against *Heliothis*. *Int. Congr. Plant Prot. Proc. Conf.* 10th.1: 417-24

- Singh, P. and Dugdale, J.S., 1979. Morphogenetic and physiological effects of a juvenile hormone analogue on the lightbrown apple moth, *Epiphyas postvittana* (Lepidoptera : Tortricidae). *N.Z. J. Zoo.* 6: 381-387.
- Suckling, D.M., Kuijpers, L., and Rogers, D.J., 1985. Bioassay of some novel insecticides against lightbrown apple moth larvae. *Proc. 38th N.Z. Weed and Pest Control Conf.*: 45-49.
- Westigard, P.H., 1979. Codling Moth : Control on pears with diflubenzuron and effects of nontarget pest and beneficial species. *J. Econ. Entomol.* 72: 552-554.