LABORATORY TESTS WITH THE INSECT GROWTH REGULATOR, DIFLUBENZURON, AGAINST WHITE-FRINGED WEEVIL ADULTS AND ARMY CATERPILLAR LARVAE

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Summary

The hatch of eggs of white-fringed weevil (*Graphognathus leucoloma*) adults fed daily on leaves of white clover (*Trifolium repens*) dipped in 10, and 1000 ppm diflubenzuron solutions was reduced by 95 and 98% respectively. If weevils were fed for 8 days on lucerne (*Medicago sativa*) treated with 10, 50, and 200 ppm diflubenzuron solutions, the eggs laid subsequently showed reduced viability, with the highest concentration inducing almost complete mortality in eggs laid over the 8 days immediately following treatment. Complete mortality was produced in army caterpillar (*Pseudaletia separata*) by feeding fourth instar larvae on maize sprayed with 10 ppm diflubenzuron solution; most mortality occurred at the pre-pupal stage.

INTRODUCTION

Part of our research programme involves screening of juvenile hormone analogues and insect growth regulators against some of New Zealand's major insect pests. Among the latter compounds are a group of benzoyl-phenyl ureas, discovered by Philips-Duphar B.V. of the Netherlands. The first reported compound of this type, code-named DU19111 (van Daalen *et al* 1972) was synthesised in our laboratory. Subsequently, a related difluoro compound, diflubenzuron, became available as a water dispersible powder (25% active ingredient) from Philips-Duphar B.V. This formulation was used in all our tests.

Diflubenzuron is a moulting inhibitor which interferes with the synthesis of chitin (Post and Vincent 1973). The chemical has low mammalian toxicity (acute oral LD_{50} to rats is 4 640 - 10 000 mg/kg) but is very effective when ingested by insects. It has been used successfully in the United States in field tests against foliage-feeding insects such as gypsy moth larvae (*Porthetria dispar*) (Granett and Dunbar 1975) and the boll weevil (*Anthonomus grandis*) (Taft and Hopkins 1975). In the former case, the affected insects exhibited both non-feeding activity and a number of moulting abnormalities, while in the latter case, diflubenzuron prevented hatch of weevil eggs.

Some of the insect pests of pasture and crops in New Zealand which may be amenable to control with diflubenzuron include white-fringed weevil (*Graphognathus leucoloma*) and a variety of lepidopterous insects such as corn earworm (*Helicoverpa armigera conferta*), greasy cutworm (*Agrotis ypsilon*), army caterpillar (*Pseudaletia separata*) and porina caterpillar (*Wiseana* spp).

This paper reports the results of tests of diflubenzuron against white-fringed weevil adults and larvae of army caterpillar.

White-fringed weevil is an important pest of lucerne (*Medicago sativa*). The larvae are difficult to kill with insecticides (Todd 1964, 1969) and present research into chemical control is aimed at controlling the adult by repeated foliar sprayings with organo-phosphate or carbamate insecticides (East *et al* 1975).

While the army caterpillar is no longer a major problem on maize (Valentine 1975), we had suitable supplies of the larvae, and used them to determine the effect of diflubenzuron on a lepidopterous insect.

METHODS

White-fringed weevil

Experiment 1. Fresh white clover (*Trifolium repens*) leaves were dipped into 0, 10, and 1000 ppm aqueous diflubenzuron solutions respectively and after the excess solution had drained away, the leaves were transferred to 9 cm diameter plastic petri dishes in which weevils were held individually. Ten weevils were used for each treatment.

Clover leaves were replaced daily, and fresh diflubenzuron solutions prepared every alternate day. Faecal material within the petri dishes was removed every few days with damp tissue paper. After 24 days, all the weevils were fed on untreated clover for a further 11 days.

Three days after the treatment commenced, the amount of feeding by each weevil was estimated and recorded daily and egg batches were counted and transferred individually to petri dishes containing damp filter paper. Eggs were held at laboratory temperature conditions (about 15 - 20° C) for between 50 to 90 days to test their viability.

Experiment 2. Diflubenzuron solutions were sprayed onto lucerne plants 25 - 30 cm high potted individually in 17 × 17 × 18-cm plastic liver pails. Treatments were 0, 10, 50, and 200 ppm diflubenzuron solutions, applied as a fine mist, at 50 ml/m^2, with a laboratory pressure-pack spray unit. When the lucerne had dried, it was covered with a fine terylene mesh supported by a wire frame. There were three replicates of each treatment and 10 weevils were introduced onto each plant and confined there for 8 days. The weevils were then transferred to petri dishes and fed on untreated clover for 20 days. Eggs laid over 3-day intervals were collected at 4 times during this period and observed for hatch.

Army caterpillar

Diflubenzuron was applied to maize (*Zea mays*) plants *ca.* 20 cm high growing in 17 × 17 × 18-cm plastic liver pails. Treatments were 50 ml/m^2 of 0, 10, 50, and 200 ppm diflubenzuron solutions. The maize plants were sprayed and covered as described above. There were three replicates of each treatment and 10 fourth instar larvae were used in each replicate.

Larvae were confined on the maize plants for 5 days. After this period, larvae were collected and fed for 7 days on untreated maize leaves. The inability of larvae to pupate plus morphological abnormalities were used to assess mortalities.

RESULTS

White-fringed weevil

As the results of experiment 1 show, diflubenzuron, when fed daily to white-fringed weevil, was highly effective at preventing egg hatch (Table 1). The overall egg hatches from weevils fed on clover leaves dipped in 10, and 1000 ppm diflubenzuron solutions, was reduced by about 95 and 98% respectively, when compared with that from weevils fed on untreated clover.

TABLE 1: HATCH OF EGGS LAID BY WHITE-FRINGED WEEVIL ADULTS DURING AND AFTER 24 DAYS FEEDING ON DIFLUBENZURON-TREATED CLOVER

Treatment	*% Hatch of eggs laid* during treatment (24 days)	after treatment (11 days)	overall (35 days)
untreated	52.0 A	27.4 A	43.4 A
10 ppm diflubenzuron	1.6 B	4.0 B	2.4 B
1000 ppm diflubenzuron	0.13 B	2.4 B	0.77 B

Weevils laid few viable eggs while feeding on 1000 ppm diflubenzuron-treated clover. When these weevils were transferred to untreated clover, the viability of their eggs increased but was still about 90% lower than that of eggs from weevils fed throughout the experiment with untreated clover. A similar situation was apparent for eggs laid by weevils fed on clover treated with 10 ppm diflubenzuron. Diflubenzuron did not exert antifeeding activity on weevils at any stage, nor did it reduce the numbers of eggs laid.

Examination of the eggs showed that the effect of diflubenzuron on eggs laid by females feeding on treated clover was similar to that reported by Moore and Taft (1975) for the boll weevil. Some of the eggs contained larvae which appeared normal and could be seen moving inside the egg but were unable to break out of the egg. Other eggs developed a black, white, or orange appearance and eventually became infected with fungus.

The ovicidal effect of diflubenzuron on newly laid eggs was also examined. Fourteen batches of eggs, containing a total of about 400 eggs, were each treated with 10 μl of 10 ppm diflubenzuron solution within 24 hours of the eggs being laid. Only 0.5% of these eggs hatched while the hatch of 625 untreated eggs, taken from the same group of weevils, was about 15%.

Experiment 2 was designed to obviate the possible ovicidal effect present in experiment 1; any observed effects were due only to the diflubenzuron ingested by the weevils.

The results show that the effect of diflubenzuron ingested by weevils is temporary (Table 2). For example, feeding on lucerne treated with a 50 ppm solution, caused the weevils to lay non-viable eggs for at least 3 days. However, no residual effect remained by the eighth day. With the more concentrated treatment (200 ppm), weevils laid non-viable eggs for almost 8 days, with some residual effect remaining until about 13 days ($P < 0.05$).

TABLE 2: HATCH OF EGGS LAID BY WHITE-FRINGED WEEVIL ADULTS AFTER 8 DAYS FEEDING ON DIFLUBENZURON-TREATED LUCERNE

		% Hatch of eggs laid after removal from treatments		
Days after treatment	*Untreated*	*10 ppm diflubenzuron*	*50 ppm diflubenzuron*	*200 ppm diflubenzuron*
2 - 3	34.6	8.4	0	0
6 - 8	22.1	10.8	17.9	0.8
10 - 13	42.5	57.6	58.5	20.1
17 - 20	39.2	28.1	39.3	28.2

It appears that diflubenzuron could be used to produce a permanent effect on eggs laid by weevils provided the weevils were fed either continuously on low doses or at intervals on higher doses.

Army caterpillar

Effects were assessed after 5 days feeding on maize plants. Larvae on untreated maize had eaten virtually all the available foliage while feeding activity was reduced on treated maize (Fig. 1). Approximately 65, 75 and 55% of maize treated with 10, 50, and 200 ppm diflubenzuron respectively, remained after the 5-day feeding period. This effect was reflected in the average weight of the larvae which was 0.6 g for the untreated maize and 0.44, 0.45, and 0.45 g for the 10, 50, and 200 ppm diflubenzuron-treated maize respectively. All the larvae fed on untreated maize had moulted successfully as had the majority of larvae feeding on the diflubenzuron-treated maize (Table 3). The live fifth instar larvae were then transferred to fresh untreated maize.

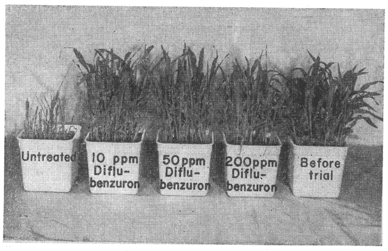

Fig. 1. Maize eaten in 5 days by 10 fourth instar army caterpillar larvae.

TABLE 3 : POST-TREATMENT EFFECTS OF DIFLUBENZURON ON ARMY CATERPILLAR LARVAE

Treatment	*No. fifth instar larvae*	*Area of fresh maize eaten*	*No. pupae*	*No. moths emerged*	*% Mortality*
untreated	30	*ca.* 21 cm^2	25	24	20 A
10 ppm diflubenzuron	29	*ca.* 27 cm^2	0	0	100 B
50 ppm diflubenzuron	21	*ca.* 10 cm^2	0	0	100 B
200 ppm diflubenzuron	23	*ca.* 2 cm^2	0	0	100 B

The post treatment effects of diflubenzuron on the larvae are shown in Table 3. Larvae from untreated maize continued to feed for a further 3 - 4 days before pupation. The anti-feeding effect on larvae fed on 10 ppm diflubenzuron-treated maize was only temporary, and these larvae commenced active feeding when placed on fresh maize. Larvae fed on higher concentrations of diflubenzuron continued to feed at a reduced rate after removal from the treated maize.

All larvae fed on treated maize died; 19% at the pre-moult stage, the remainder at the pre-pupal stage. In comparison, 80% of the larvae fed continuously on untreated maize eventually pupated and emerged as moths (Table 3).

DISCUSSION

In the experiments with white-fringed weevil adults, the lowest rate of diflubenzuron used on lucerne (10 ppm solution applied at 50 ml/m^2) would represent an application rate of 5 g/ha. This is encouraging from the point of view of controlling field populations.

One of the difficulties of controlling white-fringed weevil with this material could be the length of time the adults are present. The persistence of diflubenzuron on foliage in the field and the minimum amount required to be ingested by weevils to prevent their eggs from hatching needs to be determined.

The experiments with the army caterpillar larvae demonstrated the potential of diflubenzuron in the control of a foliage-feeding lepidopterous insect. In these experiments, the lowest rate used, equivalent to a concentration of 0.3 ppm on the maize foliage, induced complete mortality. Granett and Dunbar (1975) found an EC_{50} of 0.013 ppm in experiments with fourth instar gypsy moth larvae. Therefore, it is possible that even lower concentrations of diflubenzuron could be used for army caterpillar also.

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