Effect of the chitin-synthesis-inhibitor, chlorfluazuron, on immature development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

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Abstract

Chlorfluazuron, one of the chitin-synthesis-inhibitor group of insect growth regulators, was tested against a primary pest of stored grain which develops inside the grain. Its effect on the survival and development time of the immature stages of *Rhyzopertha dominica* (F.), the lesser grain borer, was assessed by rearing individual larvae on kibbled wheat either untreated or treated with 0.5 mg/kg chlorfluazuron. The difference between rates of survival in treated and untreated insects was due to differences in larval mortality. Most of the differential mortality occurred during or shortly after the first or second instar moults. At 30°C, rate of development from egg hatch to adult through four larval instars was a few days longer for treated (33 days) than untreated (31 days). A few larvae, both untreated and treated, went through a fifth instar and a smaller number of treated larvae included a sixth instar. In contrast to juvenile hormone analogues which usually cause mortality during metamorphosis to pupae or adults, chlorfluazuron killed the early developmental stages of *R. dominica* and extended its development time only slightly, thus minimising damage to the commodity.

Introduction

Although the stored product industry is moving away from its former reliance on chemical protectants towards non-chemical methods, insect control still largely depends on chemical protectants or fumigants. In order to stay ahead of the continual emergence of insecticide resistance in insects, and to lower toxic residues in the grain, potential new fumigants and protectants with lower residual toxicities need to be assessed.

Insect growth regulators (IGRs), which have low mammalian toxicity and degrade rapidly in the environment (Eisler 1992), have been shown to very effective at low doses. However, the juvenile hormone analogues, such as methoprene, have major weaknesses: they are poor at controlling the important primary pests which develop inside the grain, such as the rice weevil, *Sitophilus oryzae,* and the lesser grain borer, *Rhyzopertha dominica* (Bengston 1987). In addition, they are most effective during metamorphosis of larvae to pupae and pupae to adults and do not kill the early larval stages which cause the greatest damage to the commodity (Moser et al. 1992). The chitin-synthesis inhibitor group (CSIs) of IGRs, while also having low mammalian toxicities, high specificity and rapid degradation in the environment (Eisler 1992), were found to be very effective against the major stored-product pest species, including the internal grain feeders (e.g. Carter 1975; Nawrot et al. 1987). Of five CSIs tested, chlorfluazuron was found to be the most effective at controlling four major pest species (Elek and Longstaff 1994).

The toxicity of CSIs is caused by interference with the synthesis of chitin which is critical to the formation of cuticular structures and successful moulting of immature stages. Therefore, it is to be expected that all development stages of the target insects would be susceptible to the effect of CSIs. In fact, the toxicity of CSIs is often tested against a variety of pests by applying the chemicals topically (Omatsu et al. 1991) or in the diet of different larval instars. The responses vary according to the age of instar treated, but generally CSIs are more potent the longer larvae feed on a treated diet (Rajendran and Shivaramaiah 1983). Several of the major stored product pests feed exclusively inside the grain, making assessment of larval mortality very difficult. However, in assessing the potential of a new protectant, the proportions of adults which emerge from treated grain should not be the only index of the effectiveness of the insecticide. If the larvae eat the heart out of the grain before dying as pupae, considerable damage could be done to the commodity. It is important to determine at which stage during development the pest is killed. This paper describes the effect of one of the CSIs, chlorfluazuron (Helix®), on the survival and rate of development of immature stages of *R. dominica*, a primary pest species which normally develops within the grain.

Methods

General

R. dominica (RD 2 susceptible strain) was cultured on whole wheat ('Rosella' variety, ASW grade soft wheat) at 56–60% r.h. and 30°C. Kibbled wheat was treated with 0.5 mg/kg of chlorfluazuron [1–(3,5-dichloro-4–(3-chloro-5-trifluoro-methyl-2-pyridyloxy)phenyl)-3-(2,6-difluorobenzoyl) urea] 984 g/kg a.i. w/w (ICI Cropcare, Australia), using the method of Elek and Longstaff (1994), and held for two weeks at 30°C for the insecticide to equilibrate throughout the wheat.

Assay of immature development of *R. dominica*

Eggs were collected on filter papers from untreated cultures of *R. dominica* (Elek 1994), and incubated at 30°C, 60% r.h. Newly emerged 1st instar larvae were transferred individually into wells of ELISA 96-well microtitre trays for their subsequent development (Elek 1994). To each of 48 wells in four consecutive rows were added tiny pieces of untreated kibbled wheat (at least one of endosperm and one of germ) and to a further 48 cells were added pieces of kibbled wheat treated with chlorfluazuron. Three replicates were set up similarly, each incubated in the same incubator at 30°C, 60% r.h. Insects

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were inspected daily, at approximately the same time of day, to ensure a food surplus and record moulting, loss (i.e. when the larva had escaped) or death. Pupae were sexed before adult eclosure (Potter 1935). The survival rate for each developmental stage was determined, and comparisons of the numbers which were lost and died for each developmental stage and treatment were made using analysis of variance (ANOVA). The length of development time for each developmental stage was determined as the period between successive moults. Total development time (the time from when eggs hatched to adult eclosure) was compared for untreated and treated insects using ANOVA.

Results

There was an average of 70% survival (from 3 replicates of 48 larvae) for 1st instar to adult for those reared on untreated kibbled wheat compared with 19% survival for those reared on kibbled wheat treated with 0.5 mg/kg of chlorfluazuron. Figure 1 illustrates that, on both untreated and treated kibbled wheat, most deaths and losses occurred during early development, and a few adults (4 and 5%, respectively) died after eclosure.

The 12% of larvae which were 'lost' were mostly 1st and 2nd instars. It was observed that many of these two stages spent considerable time (up to several days) wandering before they started to feed, perhaps looking for a more substantial food source. The effect of treatment had no effect on numbers lost, but there was a significant difference in stage of development of the lost larvae (ANOVA $p<0.01$).

The greatest mortality in untreated larvae occurred during the 1st instar. The difference in survival between treatments was caused by higher mortalities in the 1st and 2nd instars on treated kibbled wheat (Figure 1). Most larvae died because they were unable to complete the moulting process. The mortality over all developmental stages was 17% for untreated and 69% for treated *R. dominica*. Using Abbott's formula to correct for mortality of untreated individuals (Finney 1980), there was an overall mortality of 63% of larvae reared on treated kibbled wheat. There were significant differences in treated and untreated, and in the stage of development of larvae which died (all $p<0.01$, Table 1).

Of a total of 101 untreated and 28 treated insects which survived to the adult stage, 96% had only 4 instars. A 5th instar was included by nine untreated and five treated larvae and a 6th instar by 2 treated larvae. The cumulative mean development time for all larval instars (Fig. 2), shows that the proportion of time taken by each developmental stage remained the same in both treatments, except for the addition of a 6th instar by two treated individuals. Treatment lengthened the total development time by slightly lengthening each stage. The mean total development time at 30°C (from egg hatch to adult eclosure), when larvae with instars were excluded, was still slightly longer but not significantly so (Table 2, ANOVA $p=0.1$, excluding one insect which took 49.8 days to develop).

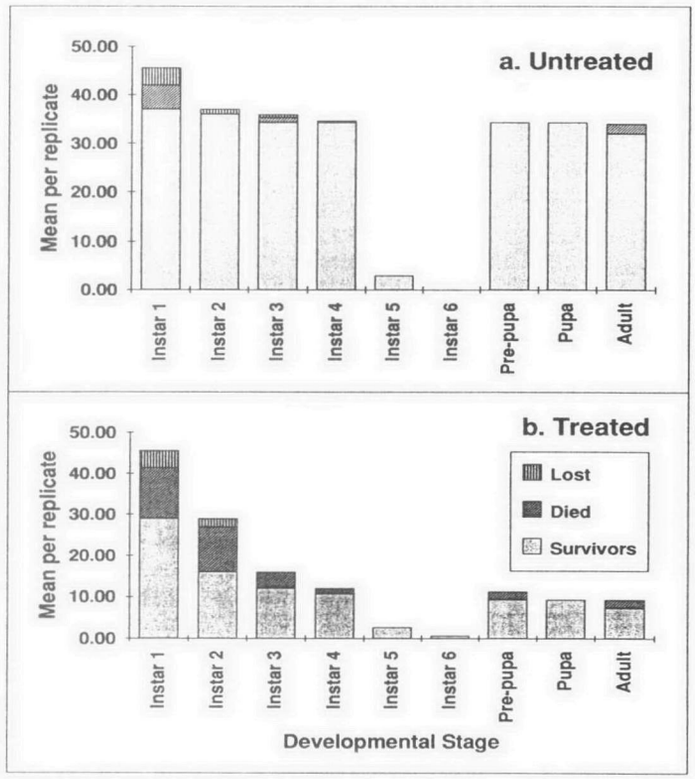

Fig. 1. Mean numbers of *Rhyzopertha dominica* which survived, died or were lost at each developmental stage at 30°C, 56% r.h.: a. reared on untreated kibbled wheat; b. reared on kibbled wheat treated with 0.5 mg/kg chlorfluazuron.

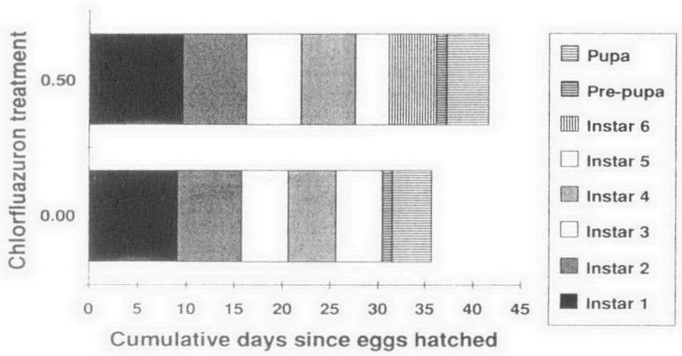

Fig. 2. Mean cumulative development time (days) for each developmental stage of *Rhyzopertha dominica* reared at 30°C, 56% r.h. on kibbled wheat either untreated or treated with chlorfluazuron (mg/kg). See Figure 1 for numbers which survived each development stage. Note that only a few larvae completed 5th and 6th instars.

Table 1. Analysis of variance table for numbers of larvae of each developmental stage (instar) of *Rhyzopertha dominica* which died when reared on kibbled wheat untreated or treated with 0.5 mg/kg chlorfluazuron.

Source of variation	Sum of squares	df	Mean square	F–ratio	P
Treatment	7.137	1	7.137	41.508	<0.01
Instar	23.490	7	3.356	19.517	<0.01
Treatment*instar	8.725	7	1.246	7.429	<0.01
Error	5.502	32	0.172		

Table 2. Analysis of variance table for total development time for *R. dominica* (egg hatch to adult eclosure) at 30°C reared on kibbled wheat untreated or treated with 0.5 mg/kg chlorfluazuron (excluding one outier).

Source of variation	Sum of squares	df	Mean square	F–ratio	P
Block	206.00	2	102.00		
Treatment	52.67	1	52.67	2.75	0.10
Error	2125	111	19.14		

Discussion

A mortality of 63% of all immature stages which developed on kibbled wheat treated with 0.5 mg/kg chlorfluazuron is considerably lower than the 95% mortality of progeny expected for this dose on whole wheat (Elek, unpublished data). Most of the difference could be accounted for by egg mortality, since 30% mortality can occur in eggs laid by adults reared on treated wheat. It could be the result of doses being less effective on kibbled wheat. The chemical may be broken down more rapidly on kibbled wheat compared with whole wheat. Alternatively, it may be due to the larvae feeding exclusively on the exposed endosperm during their first two instars (Elek 1994) without the first instar having to bore through the germ or bran layer, which usually absorb most of the insecticide (Rowlands and Bramhall 1977). Although the 3rd and 4th instars fed on the germ particles (Elek 1994), which would be expected to have higher levels of chlorfluazuron than the endosperm, the mortality during the first two instars was significantly higher than that during the 4th and later stages (Fig. 1). This reinforces the data presented in this paper that demonstrate that the early developmental stages of *R. dominica* are more susceptible to chlorfluazuron than later stages. Other CSIs have been shown to be more effective on early rather than later stages of development; for example, diflubenxuron, teflubenzuron and triflumuron were all more effective on young larvae of *Spodoptera litteralis* (Lepidoptera) when larvae were treated both topically and by feeding (Ascher and Nemny 1984). Diflubenzuron was more effective on early instars of *Culex quinquefasciatus* (Diptera) while methoprene was more effective on later stages (Amin and White 1984). Diflubenzuron was more effective at preventing progeny of *Trogoderma granarium* (Coleoptera) when it was fed to younger instars so that they had a longer feeding time. However, a few insects appear to be more susceptible at later stages; *Locusta migratoria* (Orthoptera) and *Dysdercus ungulatus* (Hemiptera) were more susceptible to diflubenzuron when it was applied to 5th instars (Onyeocha and Fuzeau-Braesch 1991; Ahmed 1992).

The numbers lost were the same for untreated and treated larvae (Fig. 1), which suggests that chlorfluazuron does not alter the wandering behaviour of the early instars.

The total development time of 31 days for untreated *R. dominica* on kibbled wheat in this study is shorter than the 34.2 days recorded for egg hatch to adult eclosure by Elek (1994) at 30°C using the same method. The estimate for untreated times in this study should be the more reliable since larger numbers of larvae reached maturity (101 vs. 24). Nevertheless, it is still longer than the development times on whole wheat (26–28 days) recorded by Howe (1950). Slightly longer development times following treatment with other CSIs also occurred in *S. littoralis* and *T. castaneum* (Emam et al. 1988; Estal et al. 1990). Egg hatch rates or development times were not included in this study because the eggs were not treated in any way.

IGRs have low toxicity to mammals, high specificity and rapid degradation in the environment. The CSIs show promise as grain protectants, not only because they are effective at preventing progeny of those pests which develop inside the grain, but also because they kill immature stages early in their development, thus minimising damage to the commodity.

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