CHAPTER I

INFLUENCE OF GROWTH REGULATORS ON LARVAL LIFE IN
T. GRANARIUM
1.1 PRECOCENE-I ON LARVAL DEVELOPMENT

INTRODUCTION

Precocenes (Bowers et al., 1976) and their synthetic analogues (Ohta and Bowers, 1977, Brooks et al., 1979a) induce symptoms of juvenile hormone deficiency in insects (Bowers et al., 1976; Pener and Orshan, 1977; Chenevert et al., 1978; Tarrant and Cupp, 1978; Farag and Varjas, 1981; Pener et al., 1981). These compounds selectively affect the corpora allata and cause atrophy and necrosis of the glands in several species (Unnithan et al., 1977; Pener et al., 1978; Schooneveld, 1979a,b). At sufficiently high doses, these compounds also have a lethal action on susceptible and non-susceptible species (Feyereisen et al., 1981a; Fridman-Cohen et al., 1984). The mode of action of precocenes in sensitive species involves a tissue specific conversion of these compounds within the corpora allata to highly reactive epoxides which alkylates cellular components and destroys the ability of the glands to produce juvenile hormone (Brooks et al., 1979a; Pratt et al., 1980). It is proposed that the specificity of precocene action on corpora allata of susceptible insects may be largely the result of a very high specific activity of epoxidase in that tissue (Pratt et al., 1980; Pratt, 1983). Instar dependent differences in the anti-allatin action of the precocenes were related to the activity of the glands; inactive corpora allata were considered to be insensitive (Unnithan and Nair, 1979; Masner et al., 1979; Farag and Varjas, 1981). However, in Diploptera punctata precocene is effective on both active and inactive glands (Feyereisen et al., 1981a)
and in *Locusta*, drastic reduction of the biosynthetic activity of the corpora allata by electrocoagulation of the pars intercerebralis does not prevent the glands to be as susceptible to precocene as the fully active glands of the controls (Pratt and Pener, 1983).

The precocenes terminate allatal function in both immature and adult stages affecting a variety of physiological functions depending upon juvenile hormone (Bowers, 1976). The juvenile hormone of insects regulate metamorphosis of the larval stages and the development of eggs in adult females following metamorphosis (Wigglesworth, 1964; Engelmann, 1979). Treatment of several insect species with precocenes induces precocious metamorphosis of the immature stages and prevents ovarian development in the adult stage (Bowers and Martínez-pardo, 1977). In the larval instar the continued presence of juvenile hormone assures the developing insect of maintaining larval characteristics (Retnakaran et al., 1985). In treated larvae, the compounds reduce the level or activity of endogenous juvenile hormones causing premature pupation (Edwards et al., 1985). Precocene induced premature metamorphosis has been reported in some hemiptera and orthoptera while in holometabolous insects, precocenes appear to be ineffective in inducing precocious development. Young nymphs of *Oncopeltus fasciatus* topically treated or exposed to vapours of precocene skip one or more instars and become tiny sterile short-lived adults (Bowers, 1976). First instar larvae of *Eurygaster integriceps* exposed to filter paper deposits of precocene-II rarely induces protetelic development.
(Polivanova, 1985). In the oriental chinch bug *Cavelerius saccharivorus okajima* exposure to precocene-II results in very high mortality as well as precocious metamorphosis (Yamada and Yagi, 1984). The precocious forms also show several adultoid characters. In *Spodoptera mauritia* larvae, repeated treatments with precocene-II during the fifth and sixth instars have no effect on the larval period but prolonged larval-pupal ecdysis and precocious metamorphic changes in the resulting pupae are observed (Mathai and Nair, 1984). In *Rhodnius prolixus*, Tarrant and Cupp, (1978) found that precocious metamorphosis could be induced from virtually all of the treated nymphal stages. Precocene-I applied to fourth instar nymphs of *Schistocerca gregaria*, induces precocious metamorphosis as well as sterilization and pigmentation changes (Chenevert et al., 1978). In *Locusta*, topical treatment of precocene-I was most effective in inducing precocious fifth instar adultiforms when applied in the fourth instar within 24 hour of ecdysis (Miall, 1980). In the bug *Oxycarenus lavaterae*, precocious metamorphosis is induced by the application of precocenes on third, fourth as well as fifth instars (Belles and Baldellou, 1983). Precocenes-I and II applied topically to fourth and fifth instar nymphs of the grasshopper *Heteracris littoralis*, causes different degrees of precocious metamorphosis (Alrubeai, 1986). Induction of precocious metamorphosis by prococenes is more useful in controlling agricultural pests destructive during the larval stages as it would considerably curtail crop damage by larval feeding (Retnakaran et al., 1985). Usual
attempts to induce precocious metamorphosis in holometabolous species have consistently failed (Bowers, 1983). However, precocene-II fed to larvae of the silkworm Bombyx mori, successfully induced precocious pupation eliminating the last larval stage (c.f. Bowers, 1983). In some other holometabolous insects such as Galleria mellonella, Heliothis virescens and Tenebrio molitor, topical treatment or feeding of the precocenes causes a delay in development (Bowers, 1983).

This study examines the influence of precocene, fed to fourth instar larvae of T. granarium, in moulting, pupation and subsequent adult development.

MATERIALS AND METHODS

Trogoderma larvae used for the experiments were obtained from cultures freshly started with a large number of eggs and maintained at 35±1°C.

The synthetic precocene used was precocene-I, 7-methoxy-2, 2-dimethylchromene (Sigma chemicals company, USA). Development and metamorphosis of fourth instar larvae fed on a wheat flour diet mixed with precocene-I was studied. 2500 mg of wheat flour was weighed into separate containers. To the experimental media, precocene-I dissolved in acetone was added and mixed well to give a final concentration of 10 µg/mg of wheat flour. To the control medium acetone alone was added. Both the control and experimental feed were kept for complete evaporation of the acetone. Fifty fourth instar larvae
were then introduced into each tube. For further observations the cultures were maintained at 35±1°C. The pupae obtained were removed and the resultant adults were examined for any developmental abnormalities. A total of 100 larvae were used in the experimental and an equal number in the controls.

RESULTS

In the precocene mixed wheat flour-diet, the percentage of pupation was observed to be 78 at the end of 53 days (19-53 days). Mortality percentage obtained was 22 at the end of 68 days. All the larvae had attained full growth but the (22%) larvae which died showed stunted growth. The occurrence of larval ecdyses was observed to be more in the precocene-treated culture than the controls. In the control culture, pupation was 100% at the end of 25 days.

Pupae (78%) from the experimental culture developed into stage I, (7.69%), III (5.13%) and VI (53.85%) individuals (Chapter II .3) respectively while 33.33% of the pupae formed normal adults. All the pupae obtained from the control culture formed normal adults.

DISCUSSION

The rate of development of an insect is affected essentially by the abundance and quality of food (Peters and Barbosa, 1977). The growth retarding effect observed in the Trogoderma larvae is not entirely due to the antifeedant effect of precocene, because 78% of the larvae could develop upto pupal stage. Moreover, 12.82% of
the pupae had formed adultoids showing severe morphogenetic defects and 53.85% emerged showing small deviations from normal adult development. This clearly suggested that feeding did occur in larvae which affected their subsequent adult development. Although precocenes induce precocious metamorphosis in a number of paurometabolous species, topical treatment or feeding of the precocenes to immature holometabolous insects have more generally failed to cause precocious development (Bowers, 1983). Similar effect of precocene i.e. lack of premature pupation is observed also in T. granarium larvae. However, a delay in development typically induced by precocenes in holometabolous insects like Galleria mellonella, Heliothis virescens and Tenebrio molitor is also found in Trogoderma larvae. In T. granarium larvae that underwent pupal moults, the schedule was found to be greatly lengthened and little change in the size of the pupae compared to that formed from normally fed larvae was observed.

In Trogoderma larvae that failed to pupate and eventually died, the stimulation of larval ecdyses is similar to the starvation induced moults reported in other insect species. The number of starvation-induced moults far exceeded the frequency of larval ecdyses that occurred in identically aged normally fed larvae. The starved larvae were also incrementally smaller in size. Larvae of the family Dermestidae are reported to undergo indeterminate number of larval instars prior to larval-pupal ecdysis (c.f. Baker, 1977). This variation has been observed to occur despite controlled rearing conditions with
optimal laboratory diets but has been determined to be even more apparent under conditions of starvation (Burges, 1960). Beck (1971 a,b; 1972; 1973 a,b) studied in detail the factors affecting regressive development in larvae of T. glabrum. Starved mature larvae of this species was shown to undergo a series of remoults in which the post-ecdysis larvae were increasingly smaller in size and weight. In larvae of the black carpet beetle Attagenus megatoma (Dermestidae), starvation was not found to affect the rate of pupation although moulting increased the weight loss and resulted in substantially smaller pupae for both males and females (Baker, 1977). In the army worm Leucania separata intense crowding in the early larval stage tends to induce supernumerary moults (c.f. Masayuki and Tojo, 1985). Additional larval ecdysis during starvation is hypothesized to better adapt the larvae to continued starvation. A few larvae of T. granarium (22%) showed a continuation of larval ecydyses and lived for a long time (30-68 days). The supernumerary moults possibly compensated for the reduced size due to food avoidance but the factor(s) that cause pupal moults in these larvae are absent or limited. All the control larvae completed pupation in 25 days.

Antifeedant action of precocene-II on Rhodnius prolixus was reported by Azambuja et al (1982). However, in the insect other precocenes including precocene-I was not observed to elicit antifeedant activity at dosages capable of inducing precocious metamorphosis. Since oxidative activation of precocenes into cytotoxins is the ascertained mode of action, temporary antifeedant action of precocene is explained to be due to cytotoxic effects on oxygenase-containing cells of the gut (Bowers, 1983).
I.2 MARGOSAN-O ON LARVAL DEVELOPMENT

INTRODUCTION

Neem seed extracts are effective against a large number of insect pests with satisfactory non-toxic side effects. Margosan-O, a commercial preparation of neem seed extract, was registered for non-food crops by Environmental Protection Agency in the U.S.A. in 1985. This preparation can be effectively used for ornamental plants either as a foliar spray or as a soil irrigant with systemic action (Larson, 1987).

Toxicity, growth inhibitory and repellent properties of Margosan-O (containing 340 μg of azadirachtin per ml) was tested against six species of cockroaches - Blatta orientalis, Blattella germanica, Byrsotria fumigata, Gromphadorhina portentosa, Periplaneta americana and Supella longipalpa (Adler and Uebel, 1985, 1987). All first instar nymphs of B. orientalis, B. germanica and S. longipalpa were reported to die after consuming treated lab-chow pellets while last instar nymphs of all the cockroach species tested showed increased mortality and retarded development after feeding on pellets impregnated with the neem extract. Margosan-O topically applied as well as injected into the abdomens of last instar B. orientalis nymphs resulted in reduction in growth and increased mortality. Placing first instar nymphs of B. orientalis on a surface treated with Margosan-O was not found to produce a notable effect. Antifeedant activity of Margosan-O applied to corn leaf squares was tested against three
orthopteran insects — *Dissosteira carolina, Gryllus pennsylvanicus* and *Diapheromera femorata*. Concentrations of 10% and 5% were found necessary to produce significant reduction in feeding on the mixed sexes of grasshoppers, female crickets and female walking sticks (Adler and Uebel, 1984).

In this study, growth and developmental effects of Margosan-O through feeding at different concentrations was tested against second instar and fourth instar larvae of *T. granarium*.

**MATERIALS AND METHODS**

Experimental larvae were obtained from cultures freshly started with large number of eggs and maintained at 35±1°C on crushed wheat. In this way, larvae of desired age could be collected for various experiments.

Margosan-O (Vikwood Botanicals Inc, Wisconsin, U.S.A.) used contained 340 μg azadirachtin per ml.

I. Margosan-O on second instar larva of *T. granarium*.

About 2500mg of wheat flour was weighed into separate containers. To these 500, 300, 50, 10 and 5 μl of Margosan-O was added together with a solvent (95% ethanol) and mixed well to give a final concentration of 0.2, 0.12, 0.02, 0.004 and 0.002 μl per mg of wheat flour respectively. To the control media 95% ethanol alone was added. The solvent was evaporated and the feed was kept for 2-3 days to
get rid of the traces of alcohol. Fifty second instar larvae were then transferred into each container. The experimental set up was maintained at 35±1°C and observations were made at regular intervals. In a batch of experiments, 250 larvae were used in the experimental set and an equal number in the controls.

Development of second instar larvae in the presence of Margosan-O was also studied. For this, 10 and 20 µl of Margosan-O was applied on separate filter papers together with ethanol to facilitate even distribution. Larvae were transferred to containers with sufficient untreated wheat flour and the treated filter paper. In the controls, a filter paper treated with ethanol alone was used. For further examination the cultures were kept at 35±1°C. A total of 100 larvae were used in the experimental and an equal number in the controls.

II. Margosan-O on fourth instar larva of _T. granarium_.

To study the development of fourth instar larvae fed on Margosan-O mixed wheat flour, 2500 mg of the feed was weighed into separate containers. In the experimental cultures 500, 10 and 5 µl of Margosan-O together with 95% ethanol was added and mixed well to give a final concentration of 0.2, 0.004 and 0.002 µl per mg of wheat flour respectively. In the control 95% ethanol alone was added and both the experimental and control media were kept for complete evaporation of the alcohol. Larvae were then transferred into the wheat-flour and maintained at 35±1°C. A total of 300 larvae in the experimental set and an equal number in the controls were used.
RESULTS

I. Margosan-O on second instar T. granarium larva.

At the doses 0.2, 0.12, 0.02, 0.004 and 0.002 µl per mg of wheat flour, mortality was 100% (9, 11, 27, 27 and 30 days respectively after introduction of the larvae to the Margosan-O mixed food). In the controls, no mortality was observed. In the higher dosages - 0.2, 0.12 and 0.02 µl moulting was severely inhibited. 6-15 exuviae were observed in cultures containing 50 larvae. In the lower dosages namely 0.004 and 0.002 µl, 63-70 moults were observed and in 10-16% of the larvae, death occurred in the moult from the second to the third instar. In the controls 78-108 larval moults were observed. Pupation was completed in control cultures within 28 days.

In the second instar Trogoderma larvae fed on untreated wheat flour in the presence of treated filter paper (10 and 20 µl), pupation was observed to be 100% at the end of 27 days.

II. Margosan-O on fourth instar larva.

In the dosages 0.2, 0.004 and 0.002 µl per mg of wheat flour, mortality was 88, 76 and 56% after 15, 28 and 19 days respectively in the experimental cultures. Mortality was not observed in the controls. In the dosage 0.004 µl, the incidence of larval moults was increased when compared to the controls. At the other dosages the occurrence of larval ecdyses was similar to the controls, though all the control larvae eventually showed pupal moults. Pupation was 12,
24 and 44% in the dosages 0.2, 0.004 and 0.002 µl per mg of wheat flour. These larvae pupated synchronously with the controls which showed 100% pupation. Pupae obtained from the larvae fed on 0.2 µl Margosan-0 per mg wheat flour diet, formed stage IV (33.33%), stage V (33.33%) and stage VI (33.33%) individuals. Of the total number of pupae obtained in the medium containing 0.004 µl/mg of wheat flour, 8.33% formed stage I adults, 8.34% stage III, 25% stage V and 25% stage VI individuals while 33.33% of the pupae emerged into normal adults. In the dose 0.002 µl, 22.72% of the pupae emerged as normal individuals while 9.09, 4.56, 9.09 and 54.54% developed into stage I, stage IV, stage V and stage VI individuals respectively. In the controls all the pupae emerged into normal adults.

DISCUSSION

Margosan-0 has lethal effects on second instar Trogoderma larvae at all the dosages tested. At the three higher concentrations used (0.2, 0.12 and 0.02 µl per mg of wheat flour) severe growth retardation and moult inhibition was observed, mortality occurred at 9, 11 and 27 days respectively after transferrence of the larvae into the treated media. At these dosages lethal effects of the compound on the larvae was therefore more conspicuous. At lower concentrations of Margosan-0 (0.004 and 0.002 µl per mg of wheat flour) in the wheat flour diet, the larvae showed typical starvation-induced moults though the survival period (27 to 30 days) was not increased. The incidence of larval ecdyses nearly equalled that observed in similarly
aged normally fed larvae though control larvae eventually pupated. Margosan-O was also found to cause ecdysis failures for 10-16% of the larvae which died in the moult from the second to the third instar. Margosan-O fed first instar nymphs of *B. orientalis*, *B. germanica* and *S. longipalpa* were also prevented from developing into second instar nymphs and survival and moulting was reported to be zero in these young forms (Adler and Uebel, 1987) and was attributed to the growth disturbing and moult inhibiting properties of azadirachtin. First instar nymphs of *B. orientalis* placed on a surface treated with the neem extract was not found to have any effect (Adler and Uebel, 1987). Filter paper deposits of Margosan-O on fresh wheat flour failed to produce any growth and developmental abnormalities in young larvae of *T. granarium*.

Growth-disruptive effects of Margosan-O on fourth instar *Trogoderma* larva was comparably less severe. However, mortality was 88% in the highest dosage tested and since 12% of the larvae pupated, feeding possibly did occur with adverse consequences. In larvae fed on a lesser amount of Margosan-O mixed wheat flour diet (0.004 μl per mg of wheat) the occurrence of larval ecdyses was increased suggestive of starvation induced moults while pupal moults comparable to controls occurred in 24% of the larvae. However, subsequent pupal morphogenesis was found to be severely affected in some of the individuals. When the Margosan-O content of the diet was reduced further (0.002 μl per mg of wheat), 44% pupation and
moulting comparable to controls was observed, though mortality was quite high. In fifth instar nymphs of several cockroaches, feeding on Margosan-O impregnated pellets was also reported to increase mortality and retard development to adulthood (Adler and Uebel, 1987).

Comparable studies with Margosan-O on storage pests have not been reported. Azadirachtin is a general insect ecdysis inhibitor and presumably the effects of Margosan-O on *Trogoderma* larva are due to the hormonal disturbances added to the antifeedant effects of the active ingredients as reported in other insects.