Embryo-larvicidal activities of lufenuron on selected lepidopteran pests

by

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Declaration

This thesis reports the results of original research I conducted under the auspices of the Zoology and Entomology Department, University of Pretoria, between September 1997 and January 2001. All the assistance that I received has been fully acknowledged. This work has not been submitted for a degree at any other university.

Edomwande O Emmanuel

Dedication

I dedicate this thesis to the memory of my father, Mr. Richard Edo Edomwande who showed me that there is more pleasure in carrying the pen rather than the gun, and to my beautiful mother, Madam Cecilia Adah, without whose love and strength I would not have survived the rigours and traumas of the Nigerian civil war.

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ABSTRACT

Studies on the embryo-larvicidal activities of lufenuron on immature stages of the American bollworm, Helicoverpa armigera (Hübner); potato tuber moth, Phthorimaea operculella (Zeller); diamondback moth, Plutella xylostella (L.); and the false codling moth, Cryptophlebia leucotreta (Meyrick) under laboratory conditions were carried out between September 1997 and January 2001. In the embryo-larvicidal (ovo-larvicidal) bioassays, mortality of eggs placed on treated substrates (plant materials - leaves, fruits and tubers), was 1.0 – 6.4 % for American bollworm eggs, 0.3 – 5.1 % for potato tuber moth eggs, 2.8 – 6.9 % for diamondback moth eggs and 4.7 – 21.1 % for false codling moth eggs. These results show that lufenuron has a low effect on the embryonic stages of the American bollworm, potato tuber moth, diamondback moth and the false codling moth.

Mortality of first instars of the American bollworm and the potato tuber moth larvae after emergence from the treated substrates was very high (> 90.3 %) but mortality of the first instars of false codling moth larvae was low (< 5.0 %). In the diamondback moth, mortality of first instars from the treated cabbage leaf discs was 37.0 – 80.5 % and 1.8 – 4.5 % for eggs that emerged from untreated cabbage leaf discs. In the four insect pests studied, mortality was most common during or after larval moult and nearly all the dead and dying larvae had typical symptoms of acylurea poisoning, such as black shrivelled body, ruptured exoskeleton, leaking haemolymph and failure to shed the old larval skin.

Emergence of adult C. leucotreta from lufenuron-treated orange fruits was high (75.8 – 90.0 %), but the emergence of adult H. armigera, P. operculella and P. xylostella from the treated plant substrates was very low (< 4.3 %). In the residual activity bioassays, lufenuron was highly effective against first and second instar stages of the American bollworm, potato tuber moth and the diamondback moth over the 10-day trial period, but had no significant effect on the larval and post larval stages of the false codling moth. Histological examination of the integument of larval instars that emerged from eggs placed on lufenuron-treated substrates showed a complete distortion or disorganization of the endocuticular layers. This distortion of larval endocuticule and the degeneration of the epidermal layer suggest that larval death during or after moult could have been caused by a defect in the process of cuticle deposition.

Findings from this study shows that the topical application of lufenuron prior to egg oviposition would reduce the amount of damage caused by the larval instars of H. armigera, P. operculella and P. xylostella.

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CHAPTER 1: INTRODUCTION

The need for alternatives to conventional insecticides for the control of lepidopteran pests have been sought by farmers and agriculturists all over the world because of the problems of insecticide resistance, high insecticide costs as well as the harmful effects of these conventional or traditional insecticides on non-target organisms such as pollinators, predators and parasitoids (Chandler et al. 1992; Nagesh & Verma 1997; Marco et al. 1998). Resistance to most of these traditional insecticides (organochlorines, organophosphates, pyrethroids and carbamates) have been reported in the American bollworm, Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae); the potato tuber moth, Phthorimaea operculella (Zeller) (Lepidoptera: Gelechiidae); the diamondback moth, Plutella xylostella (L.) (Lepidoptera: Yponomeutidae) and the false codling moth, Cryptophlebia leucotreta (Meyrick) (Lepidoptera: Tortricidae) (Talekar & Shelton 1993; Armes et al. 1996; Llanderal-Cazares et al. 1996; Hofmeyr & Pringle 1998). Due to the phenomenon of resistance in these pest populations as well as the risk of these broad spectrum insecticides to non-target organisms, insect growth regulators have become the choice pesticides for the management of the above mentioned pests (Retnakaran & Wright 1987; Newton 1987; Kim et al. 2000).

Insect growth regulators and insect development inhibitors include an array of substances such as chitin synthesis inhibitors (acylureas and buprofezin), juvenile hormone analogues (juvenoids and azadiractins) and ecdysone agonists (Darvas et al. 1992; Pons et al. 1999). These substances interfere with the develpoment and growth of

insects (Darvas et al. 1992; Retnakaran et al. 1985). Chitin synthesis inhibitors, inhibit the synthesis, polymerization, or deposition of chitin in insect eggs or larvae (Cohen 1987; Meola et al. 1999). The inhibition of chitin deposition in treated insects often causes a high mortality during moulting, when the procuticle is subjected to the stresses of ecdysis and cuticular expansion (Dean et al. 1998). The mechanism by which this inhibition occurs is still poorly understood (Soltani et al. 1984).

Lufenuron (N-[2,5-dichloro-4-(1,1,2,3,3,3-hexa-fluoropropoxy)-phenylaminocarbonyl] - 2,6-difluorobenzamide) is a relatively new member of the acylurea class of chitin synthesis inhibitors and it has been shown to be highly effective against the embryonic and larval stages of many insect pests (Hink et al. 1991; Anonymous 1997; Su & Scheffrahn 1996; Wilson & Cryan 1997; Kaakeh et al. 1997; Dean et al.1998; Jay & Cross 2000; Brunner & Skillman 2000). Lufenuron has a low impact on the environment, wild life, and beneficial insects as well as on human beings (Anonymous 1997; Brunner & Skillman 2000).

Susceptibility to insecticidal treatment varies in the different insect life stages and in order to achieve a good insect control measure, the relative susceptibility of the different developmental stages must be determined (Smith & Salkeld 1966). In most insect groups, the egg stage is the weakest link or the most susceptible developmental stage and this also happens to be the least studied stage by entomologists (Smith & Salkeld 1966; Chalfant et al. 1979). Also, in insect pests such as the American bollworm, potato tuber moth and the false codling moth; where the destructive stage (larval stage) is often hidden

inside the food substance, and are thus protected from mortality factors such as pesticides, predators and parasitoids; the egg stage therefore becomes the obvious stage to be targeted in a control program (Chaudhary et al. 1983).

Aims and hypotheses of this study

The aims of this study are: (1) to carry out a laboratory based evaluation on the embryocidal activities of lufenuron on three identifiable egg stages [white stage or young eggs (<12 h old), ring stage or older eggs (>48 h old), and the black head stage (toward the end of the incubation period)] of the American bollworm, potato tuber moth, diamondback moth and the false codling moth; (2) to determine the impact of lufenuron on the newly emerged first instars; (3) to evaluate the residual activities of lufenuron on larval instars that emerged from eggs placed on lufenuron-treated substrates; (4) to determine the mode of action of lufenuron by examining the cuticular structure of larval instars that emerged from eggs placed on treated and untreated substrates under the light microscope. Information from this study would be of benefit to farmers, agriculturists and entomologists as it would shed light on the importance of pesticide spray timing during a control program. It would also shed more light on the mode of action of this chitin synthesis inhibitor against lepidopteran pests.

The hypotheses that will be looked at in this study are: (1) younger embryos of the American bollworm, potato tuber moth, diamondback moth and the false codling moth are more susceptible to lufenuron; (2) increasing the concentration of lufenuron in the bioassay would lead to an increase in embryonic mortality; (3) the residual toxicity of

lufenuron to the developing embryos will decrease as the duration (in days) of exposure increases; (4) larval death is caused by the inhibitory action of lufenuron on cuticle deposition.

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CHAPTER 2: EMBRYO-LARVICIDAL ACTIVITIES OF LUFENURON ON IMMATURE STAGES OF THE AMERICAN BOLLWORM, HELICOVERPA ARMIGERA (HÜBNER) (LEPIDOPTERA: NOCTUIDAE)

2.1: INTRODUCTION

The American bollworm, Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is one of the most destructive agricultural pests in the world (Han et al. 1999). It is a major pest of most field and horticultural crops in Africa, Australia, the Indian subcontinent and southeast Asia (Firempong & Zalucki 1990; Cameron 1989). Worldwide, at least 60 crops and 93 uncultivated plants in a large number of families have been listed as hosts of the American bollworm (Zalucki et al. 1994). Due to its wide distribution and very catholic taste in food plants, the American bollworm is probably the most polyphagous and injurious pest of agricultural and home gardens in South Africa (Annecke & Moran 1982).

The wide hosts of Helicoverpa armigera include cotton, maize, citrus, tomato, sunflower, chickpea, pigeon pea, sorghum, groundnut, soya bean and tobacco (Rivnay 1962; Annecke & Moran 1982; Cunningham et al. 1999). On citrus, a heavy bollworm attack may destroy more than 80 % of developing fruits and damage as much as 50 % of late hanging Valencia fruits if not controlled (Bedford 1968). The newly hatched larval instars feed on the petals as well as on young fruits and this may lead to the drying out of the fruitlets (Vermeulen & Bedford 1998). In some cases, small feeding areas on the green fruit may become enlarged as the fruit grows and this feeding areas may show up

on mature fruit as uniformly shallow circular or oval sunken areas with brownish coloration (Vermeulen & Bedford 1998). In the sunflower plant, the larvae feed on the involucral bracts, while on cotton plants, the buds and bolls are fed on and this often leads to the entry of decay causing microorganisms into the bolls (Rivnay 1962; Annecke & Moran 1982; Cameron 1989). Large clear circular holes are bored into the pods of beans and peas and the leaves of these leguminous plants are also fed on by all the larval instars (Vermeulen 1976; Annecke & Moran 1982).

Adult H. armigera have brown, yellowish-brown or greyish-brown fore-wings with dark brown markings, while the hind wings are pale, greyish-white with dark veins and a broad dusky apical band that has two distinct pale spots (Annecke & Moran 1982). The moths feed on nectar and feeding is necessary before mating and egg laying can occur (Vermeulen 1976; Vermeulen & Bedford 1998). Mating occurs 2 – 4 days after adult emergence and egg maturation lasts about 2 days after mating has occurred (Rivnay 1962; Vermeulen & Bedford 1998). Oviposition occurs on the third night after mating and each female lays an average of 730 eggs during the 2 – 3 weeks of oviposition (Rivnay 1962; Annecke & Moran 1982). The ability of ovipositing females to locate and utilize a wide range of host plants from a number of families is one of the major factors contributing to the pest status of the American bollworm (Zalucki et al. 1986; Fitt 1989). Eggs are laid singly and scattered over the plant or near the flowers, usually on the upper rather than the lower side of leaves (Annecke & Moran 1982; Cameron 1989). On maize plants, the eggs are oviposited on the inflorescence or tassels, while on cotton plants, the eggs are laid on the soft squares, buds or bolls (Rivnay 1962; Cameron 1989).

At oviposition, the eggs measure about 0.5 mm in diameter and are pale-yellow at first, but as the eggs mature, the colour gradually darkens and part of it appears black due to the black head of the developing embryo (Vermeulen 1976; Annecke & Moran 1982). The eggs hatch in about 3 – 4 days in late spring or summer months at temperatures of 22 – 29 °C (Rivnay 1962; Annecke & Moran 1982).

The newly hatched larva is about 2.2 – 3.8 mm long with a whitish-yellow colour and a black head capsule. The second instar larva is yellowish-brown to brick-red with dark head capsule and dark brown prothoracic and supra-anal shields (Vermeulen 1976). The markings of the later instars begin to appear at the third instar and this colour variation ranges from green to reddish brown stripes or spots on the dorsal and lateral surfaces (Vermeulen 1976). Under favourable conditions of temperature, the larva completes development in 2 – 3 weeks (Rivnay 1962). There are six larval instars and they are extremely active, voracious and cannibalistic (Annecke & Moran 1982; Vermeulen & Bedford 1998). At the end of the larval development, the fully grown larva ceases feeding and drops to the ground where it then burrows into the soil for pupation . In South Africa, the duration of the pupal stage may exceed 2 weeks because most of the pupae enter diapause with the onset of cool weather in late summer, autumn and early winter (Annecke & Moran 1982). The colour of the pupa changes from brown to mahogany brown prior to adult emergence (Vermeulen & Bedford 1998). Adults emerge in spring in large numbers at a time when many of the host plants are flowering and this offers attractive sites for egg oviposition (Annecke & Moran 1982). The total development

period from the egg stage to adult emergence is about 40 days under favourable temperature range of 22 – 29 °C (Annecke & Moran 1982).

Control of the American bollworm involves the use of conventional insecticides such as organochlorines, organophosphates, carbamates and pyrethroids (Cameron 1989; Armes et al. 1996; Kranthi et al. 2001). Worldwide, farmers rely heavily on the use of these conventional insecticides for the control of the bollworm, and this has led to problems of resistance development in the pest populations as well as the accumulation of pesticide residues which are toxic to non-target organism (Wolfenbarger et al. 1981; Ahmad & McCaffery 1988; Cameron 1989; Armes et al.1996; Pree & Daly 1996). Resistance of American bollworm to different chemical insecticides have been reported from different parts of the world (Forrester et al. 1993; Armes et al. 1996; Van Jaarsveld et al. 1998; Han et al. 1999; Kranthi et al. 2001).

The objectives of this study were to evaluate the embryo-larvicidal and residual effects of lufenuron on embryonic and post embryonic stages of the American bollworm. Histological studies were also carried out to determine whether lufenuron caused apparent structural abnormalities in the cuticular layers of the newly hatched larval instars.

2.2: MATERIALS AND METHODS

2.2.1: Insects and chemicals

Newly emerged H. armigera adults were collected from a colony maintained at the Plant Protection Research Institute in Pretoria. The moths were put in plastic oviposition chambers (11 cm in diameter and 25 cm high) with nylon screened tops for ventilation and fed 5 % sugar solution (Gu & Walter 1999). The chambers were labelled and kept at 28 ± 1 °C and 12L: 12D photoperiods for oviposition. Eggs oviposited on the screened tops were collected every 12 h for bioassays (Wu et al. 1997). Lufenuron 050 EC as an emulsifiable concentrate containing 50 g a.i./l was supplied by Novartis SA (Pty) Ltd. For all the bioassays, dilutions of 0.02, 0.04 and 0.12 g a.i./l were prepared in distilled water. Fresh dilutions were made immediately prior to each assay and the controls were treated in distilled water.

2.2.2: Bioassay procedures

2.2.2.1: Embryo-larvicidal bioassays

Exposure of American bollworm eggs to different substrates

The activities of lufenuron on embryonic and post embryonic stages of the American bollworm were evaluated on three substrates: tomato leaves, tomato fruits and filter paper discs.

Tomato leaf bioassay

Freshly cut tomato leaves were dipped for 30 s in the appropriate test solutions (0.02, 0.04 and 0.12 g a.i./l), air-dried and transferred to Petri dishes (10 cm diameter) containing moist filter papers (Whatman no. 1, Hillsboro, OR.). The effect of lufenuron

was tested on three different age groups (1 – 3d old) of H. armigera eggs. Eggs (n = 400 per age group) oviposited on nylon nettings were transferred to the treated tomato leaves with a fine camel-hair brush. Each concentration plus its control (in distilled water) was replicated four times and the Petri dishes were kept at a constant temperature of 28 ± 1 °C and a photoperiod of 12L: 12D until larval hatch. To allow for delayed hatching due to pesticide effects, data on egg and first instar mortalities were determined with a stereomicroscope (16X) 48 h after the controls have hatched. First instars were identified by the width of their head capsules (Van der Walt et al. 1993). Newly hatched larvae (< 12 h old) were transferred to individual rearing test tubes (2 cm diameter by 9 cm) containing treated larval diet to avoid cannibalism (Armes et al. 1996; Singh & Rembold 1992). Controls were fed untreated diet and the tubes were stoppered with cotton wool plugs and kept at 28 ± 1 °C and 12L: 12D photoperiods for adult emergence. The experiments were monitored daily and the various forms of morphogenetic deformities caused by lufenuron on the larval and post larval stages were observed (Retnakaran & Smith 1975; Retnakaran & Wright 1987; Clark & Jewess 1990; Darvas et al. 1992). Data on adult emergence were recorded 5 days after controls have emerged.

Tomato fruit bioassay

Tomato fruits were dipped into the appropriate test solutions for 5 minutes, air-dried and transferred to rearing chambers (plastic containers 7 cm high by 12 cm diameter). Eggs (1 - 3 d old; n = 400/age) were transferred from the oviposition substrates to the treated tomato fruits with a fine camel-hair brush. Each concentration plus its control was replicated four times and the plastic containers were covered with nylon netting and kept

at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Data on egg and first instar mortalities were determined under a stereomicroscope (16X) 48 h after controls have hatched. Newly hatched larvae (< 12 h old) were transferred to individual rearing test tubes containing treated larval diets to avoid cannibalism. Controls were fed untreated diets and the test tubes were stoppered with cotton wool plugs. The experiment was monitored daily and the number of dead larvae and pupae were recorded and observed under a stereomicroscope for morphological abnormalities. Data on adult emergence were recorded 5 days after the controls have emerged.

Filter paper bioassay

To determine whether an aritificial substrate would influence the activities of lufenuron against the developing embryos, filter paper discs (10 cm diameter, Whatman no. 1, Hillsboro, OR) were dipped in the appropriate test solutions for 5 seconds, air-dried and placed in plastic containers (7 cm high by 12 cm diameter). Eggs (1 - 3 d old; n = 400/age) were transferred from the oviposition substrates to the treated filter paper discs using a fine camel-hair brush. Each concentration plus its control was replicated four times and the plastic containers were covered with nylon netting and kept at a constant temperature of 28 ± 1 °C and 12L:12D photoperiods until larval hatch. Data on egg and first instar mortalities were recorded under a stereomicroscope (16X) 48 h after the controls have hatched.

Transovarial bioassay

Helicoverpa armigera pupae collected from the Plant Protection Research Institute in Pretoria were kept in individual plastic containers (12 cm high by 5 cm diameter) for

adult emergence. The effects of ingestion of lufenuron by adults were studied by allowing newly emerged moths to feed on 5 % sugar solution containing different concentrations of lufenuron for two days (Plusckell et al. 1998; Marco et al 1998; Jay & Cross 2000). Controls were fed distilled water containing 5 % sugar solution, and the moths (5 males and 5 females) that fed on same test solution or concentration were put in the same breeding chamber (plastic container, 25 cm high by 11 cm diameter) for mating and oviposition. The experiment was replicated four times and the moths were further fed 5 % sugar solution until the end of the experiment (7 days). The sugar solutions were changed every second day to prevent contamination and the chambers were kept at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods (Pluschkell et al. 1998). Eggs (n = 400/concentration) from each breeding chamber were transferred to individual rearing tubes (2 cm diameter by 9 cm high) containing untreated larval diets using a fine camel-hair brush. Data on egg viability (percentage larval hatch) and first instar mortality were determined under a stereomicroscope (16X) 48 h after controls have hatched (Rup & Chopra 1985; Yokoyama & Miller 1991). Hatched larval instars were further reared to the adult stage on the untreated diet. The experiment was monitored daily and data on adult emergence were recorded 5 days after controls have emerged.

Data analyses

The embryocidal effect of lufenuron was calculated as the percentages of embryos that died in the eggs, while postembryonic mortality was determined by the percentage of larvae or pupae that were unable to reach the next life stage. Data on percentage mortality were corrected for natural mortality (Abbott 1925). Where appropriate the data were arcsine transformed before analysis of variance (ANOVA 1) (Statsoft 1995; Pluschkell et

al.1998). Duncan's Multiple Range test was used to evaluate the differences between the treatment groups ($P = 0.05$) (Broadbent & Pree 1984; Moffitt et al. 1984; Statsoft 1995).

2.2.2.2: Residual activity bioassay

The duration over time (in days) that lufenuron was effective on eggs of the American bollworm was evaluated by exposing different age groups of eggs (1-3 d old) to lufenuron residues. Tomato plants grown on plastic containers (7 cm high by 9 cm diameter) were sprayed to run off with the appropriate test solutions (0.02, 0.04 and 0.12 g a.i./l) using a hand sprayer (Abro et al. 1988). The plants were air-dried and kept outdoors for the residue to age.

On the day of treatment and each day thereafter for 10 days, leaves were excised from the treated plants and placed on a moist filter paper disc in a Petri dish (10 cm diameter) (Daly et al. 1988). Eggs (n = 400/age) oviposited on nylon screened tops were transferred to the treated tomato leaves with a fine camel-hair brush. Each concentration plus its control was replicated four times and kept at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Data on egg mortality were determined under a stereomicroscope (16X) 48 h after the controls have hatched. Newly hatched larval instars were allowed to feed on the treated tomato leaves for two days before transferring them to individual rearing tubes (9 cm high by 2 cm diameter) containing untreated larval diet (Singh & Rembold 1992). The experiment was monitored daily and data on adult emergence were recorded 5 days after the controls have emerged.

Data analysis

Data on percentage egg mortality were corrected for natural mortality (Abbott 1925). Where appropriate, the data were arcsine transformed before analysis of variance (ANOVA 1) (Statsoft 1995). Duncan's Multiple Range test was used to evaluate the differences between the treatment groups ($P = 0.05$) (Statsoft 1995; Marshall et al. 1988; Rehimi & Soltani 1999).

2.2.2.3: Histological bioassay

Tomato leaves were dipped in the appropriate test solutions (0.04 and 0.12 g a.i./l) for 30 s, air-dried and transferred to Petri dishes (10 cm diameter) containing moist filter papers. Eggs (< 12 h old; n = 10) oviposited on nylon netting were transferred to the treated leaf discs with a fine camel-hair brush. The experiment was kept at 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Hatched larval instars were allowed to feed on treated leaves for two days after which they were killed by decapitation (Hassan & Charnley 1987). To determine whether lufenuron caused apparent structural abnormalities in the cuticular layers of these neonates, the decapitated larvae were fixed in 2.5 % glutaraldehyde for 1 h, rinsed three times (15 minutes each) in 0.075 M phosphate buffer (pH 7.4 - 7.6). Post-fixation was carried out in 0.25 % aqueous osmium tetraoxide for 1 h at room temperature and the tissues were rinsed three times (15 minutes each) in distilled water before dehydration in 50, 70, 90 % ethanol for 15 minutes each. Further dehydration in 100 % ethanol (3 changes, 15 minutes each) was carried out and the fixed tissues were infiltrated with 30 and 60 % quetol (epoxy resin) for 1 hr, and in pure quetol for 4 h (Kushida 1974). The tissues were polymerized for 24 – 36 h at 65 °C

after which semi-thin sections (2 μm) were cut and stained with toludine blue (Trump et al. 1961). The sections were viewed under a Nikon optiphot microscope and photographed with a Nikon digital camera (DXM 1200).

2.3: RESULTS AND DISCUSSION

2.3.1: Embryo-larvicidal bioassays

Embryocidal effects

Embryonic development was not affected in all the eggs (treated and controls) exposed to the leaf and fruit substrates. Neither young (white stage, 1 d old) nor older eggs at the midpoint of development (ring stage, 2 d old) or eggs at the black head stage (3 d old) were affected by the different concentrations of lufenuron. This result is in agreement with previous studies on the activities of acylureas on developing embryos (Moffitt et al. 1984; Dean et al. 1998; Marco et al. 1998; Pons et al.1999). Also, the incubation period for all the eggs was not affected, and larval hatch (treated and controls) was completed between 3 – 4 days.

Low embryocidal effect was recorded in the three age groups of American bollworm eggs exposed to the leaf and fruit substrates (Tables 1 and 2). The low egg mortality recorded in this bioassay is contrary to results from previous studies on acylureas (Faragalla et al. 1980; Elliott & Anderson 1982). Also, this result is not in agreement with the studies on the activities of lufenuron against embryonic stages of the fruit fly, Drosophila melanogaster (Diptera: Drosophilidae) (Wilson & Cryan 1997). The differences in this embryocidal studies and those reported from other insect groups, could

be because the contact activities of lufenuron, like that of other acylureas is species-dependent (Grosscurt & Jongsma 1987).

In the filter paper bioassay, younger eggs (white stage; < 12 h old) were more sensitive to lufenuron than the older eggs (black head, > 60 h old) (Table 3). This age-related sensitivity could be because cuticle deposition in the older eggs has proceeded to an advanced stage at the time of pesticide treatment (Grosscurt & Jongsma 1987). Similar results on the sensitivity of younger eggs to acylureas have been reported by Ravi & Verma (1997). Marco et al. (1998) found that the exposure of eggs of the sugar beet weevil, Aubeonymus mariaefranciscae (Coleoptera: Curculionidae), to hexaflumuron-impregnated filter papers led to a high mortality of younger eggs, and according to the authors, the high sensitivity of younger eggs to acylureas could be because the critical stage for the penetration of chitin synthesis inhibitors into the developing embryos is the first 24 hours after oviposition. Although there were no investigations on why younger eggs exposed to lufenuron-impregnated filter papers had such a high mortality than eggs of similar age groups exposed to lufenuron-treated fruits or leaf discs, one could however, assume that this substrate effect may have been caused by the properties of the filter paper discs such as the texture and pH. These properties could have led to a high amount of lufenuron been absorbed or retained in the filter paper discs, thus causing high mortality in the younger age group of eggs. Also, the duration of exposure of younger eggs to lufenuron-treated discs was longer than those of older eggs and this could have led to the absorption of more residues into the developing embryos and hence, a high egg mortality prior to eclosion (Coppen & Jepson 1996).

Transovarial effects

The embryocidal activities of lufenuron through adult moths was low (Table 6). Larval hatch from eggs laid by adults fed lufenuron-treated sugar solutions did not differ significantly from the control experiments. Embryonic development as well as the incubation period in all the eggs (treated and controls) were not affected. Dean et al. (1998) reported similar transovarial effects in their studies on the mode of action of lufenuron on larval cat fleas, Ctenocephalides felis, (Siphonaptera: Pulicidae). But Wilson & Cryan (1997), found that when adult fruitflies, Drosophila melanogaster, (Diptera: Drosophilidae) were fed high concentrations of lufenuron, the eggs laid by the females were able to develop to the black head stage, but the overall larval emergence was very low. Also, Haynes & Smith (1993) found that egg hatch in the female boll weevil, Anthonomus grandis grandis (Boheman), (Coleoptera: Curculionidae) was reduced to zero when females were dipped or fed different concentrations of lufenuron (CGA-184699); while Casaña-Giner et al. (1999), reported that lufenuron caused a complete egg suppression in female Ceratitis capitata, (Diptera: Tephritidae). Other results of high embryocidal effects which are contrary to the results obtained in this study, have been reported in various insect groups (Moffitt et al. 1983; Leonard et al. 1987; Horowitz et al. 1992; Brunner & Skillman 2000).

Larvicidal effects

Delayed embryocidal effects in which the inhibitory action of the chemical often leads to the death of a newly emerged larval instar is a common phenomenon in acylureas (Retnakaran & Wright 1987). This phenomenon has been reported in embryocidal as well

as in transovarial bioassays, and mortality of larval instars often occurs during eclosion or soon after larval emergence from the eggshell (Miura et al. 1976; Chang & Borkovec 1980; Elliott & Anderson 1982; Marco et al. 1998; Dean et al. 1998). In the leaf, fruit and transovarial bioassays, mortality of first instars soon after hatch or during moult to the next larval instar was high (> 82%) and larval mortality was not concentration-dependent (Tables 4, 5 and 6). In the filter paper bioassay, data on larval and post larval mortalities were discarded due to the high mortality recorded amongst the younger age group of eggs. Results on delayed ovicidal effects due to chitin synthesis inhibitors have been reported by Van Laecke et al. (1989) and Marco et al. (1998).

Larval mortality was most common during the moult to the second instar stage and nearly all the dead larvae exhibited typical symptoms of acylurea poisoning such as black shrivelled body, failure to shed the old cuticle, reduced body size, ruptured exoskeleton and leaking haemolymph (Figs. 1 and 2). Failure to initiate or complete ecdysis could be due to the impairment of muscle action required for ecdysis (Fogal 1977). Also, the inability to shed the old larval skin could be due to the lack of skeletal rigidity which is caused by reduced amount of chitin in the newly formed cuticle (Clark & Jewess 1990). Some of the surviving second instars that emerged from lufenuron-treated substrates, had balloon-like distortions filled with fluid on their thoracic and abdominal regions (Fig. 3). Abdominal and thoracic distortions could be caused by an increase in internal body pressure or a deterioration of the mechanical properties of the cuticle (Mulder & Gijswijt 1973). Affected larval instars appeared lethargic, were unable to feed and remained at one spot for many days before death. Reports of suppressed larval feeding or feeding

deterrence have been recorded in other insect species and this could be due to the formation of poorly developed and unsclerotized mouth parts (Granett et al. 1983; Neuman & Guyer 1987; Darvas et al. 1992; Pszczolkowski & Smagghe 1999). Larval mortality in the control experiment was very low and there were no records of the above mentioned symptoms.

Post-larval effects

The effect of egg or larval treatment could also be manifested on the pupal stage (Retnakaran et al. 1985; Wilson & Cryan 1997). In the leaf, fruit and transovarial bioassays, the few first instars that survived were able to develop to the pupal and adult stages after a prolonged delay. However, most of the pupae had various forms of morphological deformities such as ruptured cocoons and leaking haemolymph as well as pupae with larval heads and limbs (larviform pupae) (Figs. 4 and 5). Haemolymph leakage was observed mainly from depressions in the middle of the thorax. Retnakaran and Smith (1975) reported similar developmental abnormalities due to acylureas in the spruce budworm, Choristoneura fumiferena, (Lepidoptera: Tortricidae). Also, Wilson & Cryan (1997) reported various forms of pupal abnormalities caused by lufenuron in the fruit fly, D. melanogaster, while Ravi & Verma (1997) found that the treatment of the final instar of the American bollworm with different concentrations of diflubenzuron caused a great reduction in the pupal weight as well as a prolonged delay in the pupal-adult developmental period. In some of the normal cocoons, adult moths did not emerge at the end of the pupal period and closer examination of these pupae showed that the cocoons were empty. Reed & Bass (1980) found similar results in the soybean looper,

Pseudoplusia includens (Walker) (Lepidoptera: Noctuidae). There were no records of deformed or abnormal pupae in the control experiments, and development to the adult stage was not delayed.

Most of the adults that emerged from lufenuron treated bioassays had deformed wings and were unable to fly (Figs. 6 and 7). Similar reports on wing abnormalities due to the effects of lufenuron have been reported in newly emerged fruit fly adults (Wilson & Cryan 1997). Adult emergence in the control experiments was high, but the cumulative mortality at the larval and pupal stages led to a significant decrease in emergence (0.5 – 4.3 %) of adults from the treated substrates (Figs. 8 – 10).

2.3.2: Residual bioassay

The residual activity of lufenuron against American bollworm eggs over the 10 days trial period was low. Larval hatch was high (79.0 – 99.5 %), and there was no significant difference between larval hatch from eggs at the white or ring stage and those at the black head stage in both the treated and control experiments (Tables 7 – 9). This result is not in agreement with the studies of Jay & Cross (2000), who found that lufenuron inhibited egg hatch over an 8 week period in the vine weevil, Otiorhynchus sulcatus, (Coleoptera: Curculionidae). Also, Elliott & Anderson (1982), reported a persistence of up to 10 days against the eggs of the codling moth, Cydia pomonella (Lepidoptera: Tortricidae).

Present result shows that residual mortality to neonates was high over the 10 days trial period and most of the larval instars died at the first instar stage. Marshall et al. (1988)

reported that the exposure of the spotted tentiform leaf miner, Phyllonorycter blancardella (Fabr.) (Lepidoptera: Gracillariidae), to different concentrations of diflubenzuron, triflumuron and teflubenzuron led to a high mortality of the neonates over a 19 week period, while Broadbent & Pree (1984) recorded a persistence of up to 10 days against the larval instars of the Oriental fruit moth, Grapholitha molesta (Busck) (Lepidoptera:Olethreutidae), exposed to different concentrations of diflubenzuron. Also, Herbert & Harper (1985) found that the first instars of the corn ear worm, Heliothis zea (Boddie) (Lepidoptera: Noctuidae) died more rapidly over a 7 day period when exposed to the acylurea CME 134.

Dead and dying larval instars exposed to the different concentrations of lufenuron had typical symptoms of acylurea poisoning such as reduced body size, ruptured exoskeleton and leaking haemolymph (Retnakaran & Wright 1987; Dean et al. 1998). The drastic effects of lufenuron on the survival of the larval and pupal stages led to a low (0.5 – 7.2 %) adult emergence in the treated experiments compared to the high (> 86.0 %) adult emergence in the controls (Figs. 11 – 13).

2.3.3: Histological bioassay

Histological sections through larval tissues (treated and controls) showed different cuticular profiles (Fig. 14A, B). In the treated larva, the major tissue affected was the cuticle, and various degrees of cuticular distortions were observed. There was no difference in the epicuticle (thin outer layer) of lufenuron-treated larva and those of control experiments. This shows that lufenuron had no effect on the epicuticle. This result

is consistent with previous studies on the effects of acylureas on the epicuticle (Retnakaran et al. 1985; Dean et al. 1998). The epicuticle according to Retnakaran & Wright (1987), contains only protein and no chitin, and this could be the reason why it is not affected by chitin synthesis inhibitors. The exocuticular layer (layer beneath the epicuticle) or the outer layer of the procuticle also was not greatly affected by lufenuron. The outer layer of the procuticle in most insect species is often deposited before ecdysis and this could be the reason why the exocuticle is also not affected by chitin synthesis inhibitors (Reynolds 1987; Dean et al. 1998).

The endocuticular layer (inner layer of the procuticle) of lufenuron-treated larva was severely affected or distorted, and scattered globules were observed in most parts of the disorganized layer. Endocuticular distortions have been reported in previous studies on the activities of acylureas on the insect integument (Retnakaran & Wright 1987; Dean et al. 1998; Perez-Farinos et al. 1998). Ascher and Nemny (1976), suggested that acylureas interfered with moulting by softening and reducing the cellular and tissue contents of the endocuticle, while Dean et al. (1998), found that fleas treated with different concentrations of lufenuron, produced abnormal endocuticle which consisted of protein globules embeded in an amorphous chitin matrix. Lim & Lee (1982), reported that diflubenzuron-treated nymphs of Oxya japonica (Willemse) (Orthoptera: Acrididae) had severe endocuticular lesions as well as abnormalities in the deposition and growth of the endocuticle. The endocuticle forms the bulk of the procuticle and any disturbances in the formation of this layer would lead to a serious disruption of the exoskeletal functions of the insect (Grosscurt & Jongsma 1987). Hassan & Charnley (1987), found that the

globular materials in the distorted endocuticle contains protein but no chitin and that the production of chitin microfibrils was completely prevented in tissues exposed to acylureas.

These results suggest that the death of larval instars exposed to lufenuron-treated substrates could have been caused by the distortion of the endocuticular layers during or after ecdysis. This assumption is supported by the studies of Grosscurt & Jongsma (1987), who found that apolysis (separation of the epidermis from the old cuticle) and cuticular resorption occurred in tissues of diflubenzuron-treated larvae; but that the new larval instars died at ecdysis or shortly after, due to lack of rigidity in the exoskeleton, which was caused by disruptions in endocuticle deposition. The balloon-like appearances in larval instars treated with acylureas could have been caused by the poor formation of the endocuticle (Retnakaran et al. 1985). The effects of chitin synthesis could also be seen on the epidermal layer and this may lead to the loss of the epidermal cells as well as a decrease in cytoplasmic organelles including mitochondria and ribososmes (Retnakaran et al. 1985, Hassan & Charnley1987; Dean et al. 1998). Dean et al. (1999) found that lufenuron caused a high mortality in adult fleas and the authors attributed this high mortality to a weakened endocuticle.

Other parts of the larvae that contain chitin such as the foregut, hindgut, peritrophic memberane, trachea, tracheoles and cuticular glands may also be affected by the activities of acylureas (Retnakaran & Wright 1987; Dean et al. 1999). The effect of acylureas on the foregut, hindgut and the peritrophic membrane could lead to a disruption of the larval

digestive system and death of the larval instars (Fogal 1977; Clark et al. 1977; Retnakaran et al. 1985).

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Table 1. Mortality of different-aged eggs of Helicoverpa armigera (Hübner) after exposure to lufenuron treated tomato leaves.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	2.3 ± 0.8a	5.1 ± 1.1a	4.2 ± 1.1a	6.4 ± 1.5a
2	3.0 ± 0.9a	2.0 ± 0.4a	4.2 ± 0.9a	3.1 ± 0.7a
3	2.0 ± 0.8a	3.2 ± 1.5a	1.0 ± 0.4a	2.0 ± 0.7a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft, 1995]). Arcsine transformation was carried out before statistical analysis.

Table 2. Mortality of different-aged eggs of Helicoverpa armigera (Hübner) after exposure to lufenuron treated fruits.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	0.3 ± 0.3a	4.0 ± 1.5a	5.3 ± 0.9a	3.3 ± 1.4a
2	2.8 ± 1.4a	1.6 ± 1.6a	4.4 ± 0.8a	3.8 ± 1.8a
3	0.8 ± 0.5a	2.3 ± 0.7a	4.3 ± 1.8a	1.5 ± 0.7a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 3. Mortality of different-aged eggs of Helicoverpa armigera (Hübner) after emergence from lufenuron treated filter paper discs.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	3.3 ± 1.5a	98.2 ± 1.5c	97.6 ± 1.3c	99.2 ± 2.4c
2	1.0 ± 1.0a	26.5 ± 5.0b	30.8 ± 6.7b	24.5 ± 1.9b
3	1.8 ± 1.0a	13.7 ± 7.1b	8.5 ± 0.9b	15.1 ± 3.3b

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 4. Larvicidal activity of lufenuron against first instars of Helicoverpa armigera (Hübner) after emergence from eggs placed on lufenuron treated tomato leaves.

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	0.0 ± 0.0a	95.0 ± 1.1b	93.0 ± 1.3b	95.8 ± 1.0b
2	0.9 ± 2.8a	94.0 ± 1.2b	94.3 ± 1.3b	92.3 ± 1.4b
3	0.7 ± 1.0a	94.3 ± 1.0b	90.5 ± 1.0b	92.5 ± 2.3b

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 5. Larvicidal activity of lufenuron against first instars of Helicoverpa armigera (Hübner) after emergence from eggs placed on lufenuron treated fruits.

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	6.5 ± 1.8a	91.5 ± 1.9b	93.0 ± 2.3b	93.8 ± 1.3b
2	2.0 ± 2.5a	90.5 ± 0.6b	93.4 ± 1.0b	95.2 ± 1.0b
3	5.5 ± 1.3a	90.8 ± 1.7b	90.5 ± 0.5b	92.0 ± 1.3b

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 6. Viability of eggs laid by female moths fed lufenuron-treated sugar solution (transovarial bioassay).

Concentration (g a.i./l)	% Egg hatch (mean ± SE)	% Mortality of first instars (mean ± SE)
0	90.3 ± 1.3b	0.0 ± 0.0a
0.02	88.5 ± 3.0b	82.0 ± 3.1b
0.04	91.3 ± 4.0b	82.5 ± 2.9b
0.12	85.0 ± 6.1b	84.3 ± 5.7b

Means within a column followed by the same letter are not significantly different (P = 0.05, Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 7. Residual toxicity of lufenuron to 1 day old Helicoverpa armigera (Hübner) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	5.3 ± 1.7a	10.9 ± 1.5a	8.8 ± 2.8a	9.7 ± 2.8a
2	7.0 ± 4.2a	7.8 ± 2.5a	11.8 ± 4.9a	14.2 ± 2.7a
3	10.5 ± 1.7a	13.0 ± 6.5a	15.5 ± 2.4a	9.5 ± 6.2a
4	2.0 ± 4.1a	3.0 ± 1.9a	10.7 ± 6.8a	9.3 ± 5.3a
5	3.5 ± 3.5a	10.9 ± 2.7a	12.9 ± 4.5a	10.8 ± 6.2a
6	2.0 ± 3.5a	9.4 ± 2.9a	8.3 ± 4.5a	11.5 ± 6.9a
7	9.5 ± 3.8a	16.7 ± 2.0a	16.0 ± 5.6a	15.1 ± 6.4a
8	7.5 ± 5.7a	9.2 ± 6.8a	8.7 ± 5.1a	14.1 ± 5.4a
9	6.0 ± 5.6a	11.5 ± 7.1a	8.0 ± 4.7a	12.8 ± 8.9a
10	6.5 ± 5.6a	14.9 ± 4.8a	12.8 ± 4.2a	13.9 ± 5.2a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 8. Residual toxicity of lufenuron to 2 day old Helicoverpa armigera (Hübner) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	12.4 ± 2.9a	8.3 ± 1.6a	10.1 ± 4.2a	14.0 ± 4.2a
2	6.0 ± 4.4a	9.6 ± 6.6a	14.3 ± 3.9a	13.8 ± 2.8a
3	8.5 ± 4.1a	11.5 ± 5.2a	11.3 ± 5.5a	17.4 ± 2.6a
4	5.0 ± 1.8a	13.1 ± 5.5a	12.5 ± 3.2a	8.9 ± 3.0a
5	4.5 ± 3.9a	4.7 ± 1.7a	4.5 ± 2.1a	8.4 ± 3.2a
6	7.0 ± 3.0a	13.9 ± 8.9a	12.4 ± 3.7a	16.2 ± 2.9a
7	6.0 ± 5.6a	8.5 ± 2.6a	9.6 ± 4.3a	12.2 ± 5.6a
8	5.0 ± 2.4a	12.1 ± 6.3a	10.5 ± 5.0a	12.1 ± 7.0a
9	6.5 ± 6.6a	4.2 ± 3.8a	6.8 ± 2.8a	4.8 ± 4.2a
10	8.0 ± 6.5a	13.5 ± 5.3a	13.6 ± 5.3a	9.8 ± 0.9a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 9. Residual toxicity of lufenuron to 3 day old Helicoverpa armigera (Hübner) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	7.5 ± 5.2a	13.0 ± 6.6a	11.2 ± 3.0a	14.5 ± 2.7a
2	7.5 ± 5.9a	9.0 ± 5.5a	13.5 ± 2.5a	12.9 ± 2.8a
3	6.0 ± 4.1a	4.1 ± 4.8a	4.6 ± 3.6a	8.1 ± 7.1a
4	2.0 ± 2.1a	3.1 ± 4.0a	5.5 ± 5.8a	9.5 ± 5.2a
5	4.0 ± 3.4a	4.6 ± 3.6a	2.1 ± 2.4a	5.1 ± 5.0a
6	5.5 ± 3.3a	5.5 ± 3.9a	12.4 ± 3.8a	7.4 ± 2.9a
7	8.5 ± 2.9a	5.6 ± 3.8a	6.6 ± 6.3a	5.9 ± 3.6a
8	2.0 ± 3.5a	3.1 ± 3.4a	4.6 ± 3.7a	7.2 ± 2.3a
9	0.5 ± 2.0a	3.5 ± 3.5a	6.5 ± 6.3a	3.5 ± 4.4a
10	5.0 ± 4.1a	4.1 ± 4.8a	6.1 ± 5.9a	8.1 ± 0.9a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

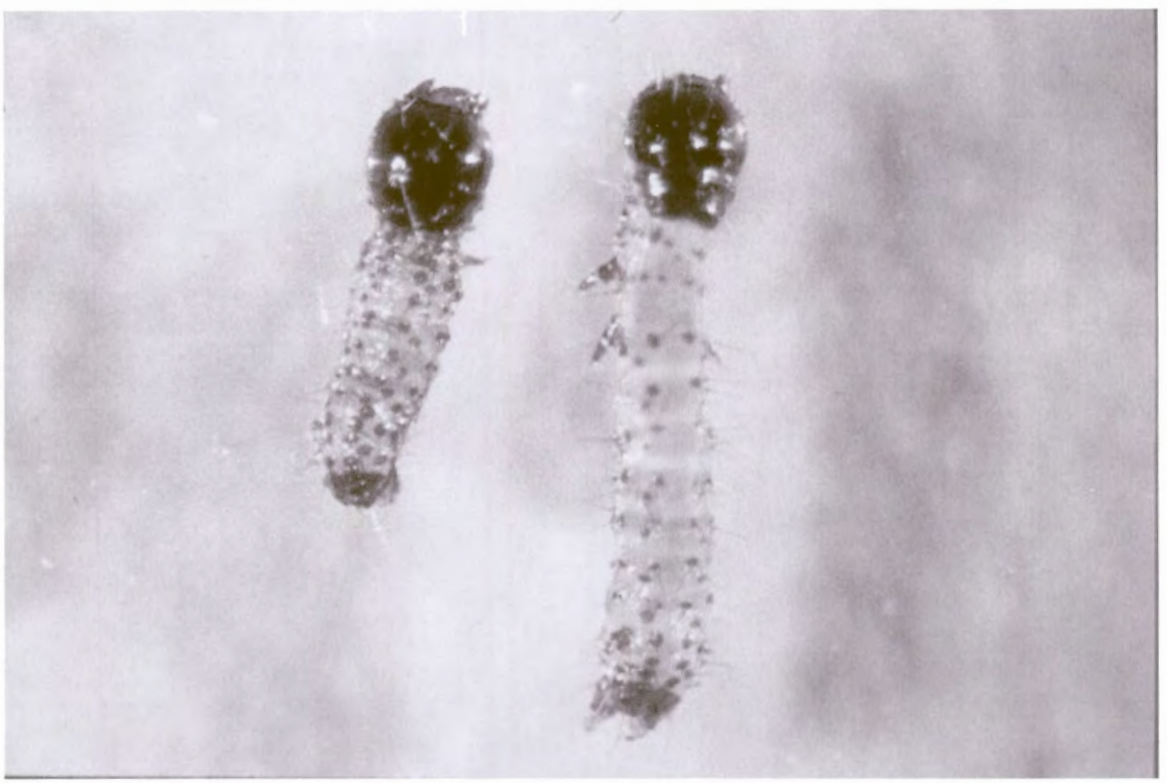

Fig. 1. Effects of lufenuron on first instar Helicoverpa armigera (Hübner) larvae: Left = small sized larva that emerged from lufenuron-treated substrate; Right = normal larva that emerged from untreated substrate).

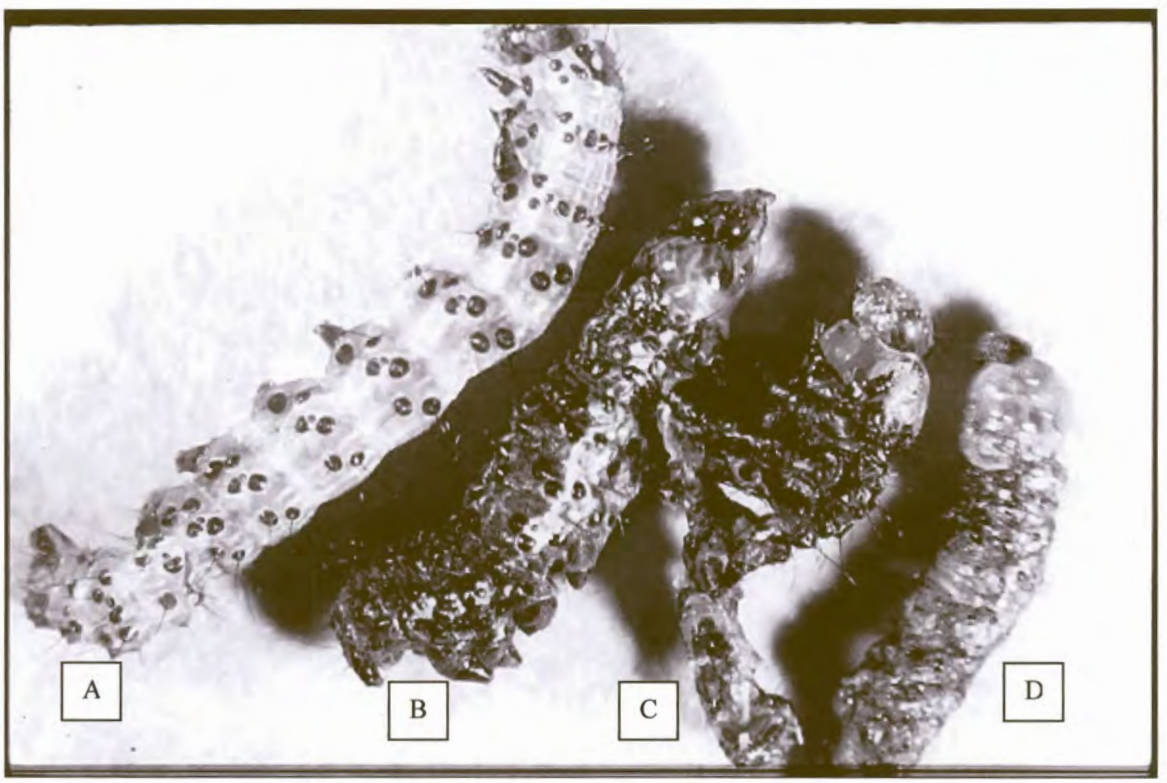

Fig. 2. Effects of lufenuron on second instar Helicoverpa armigera (Hübner) larvae: A = normal larva that emerged from untreated substrate; B – D = dark shrivelled larvae that emerged from lufenuron-treated substrates.

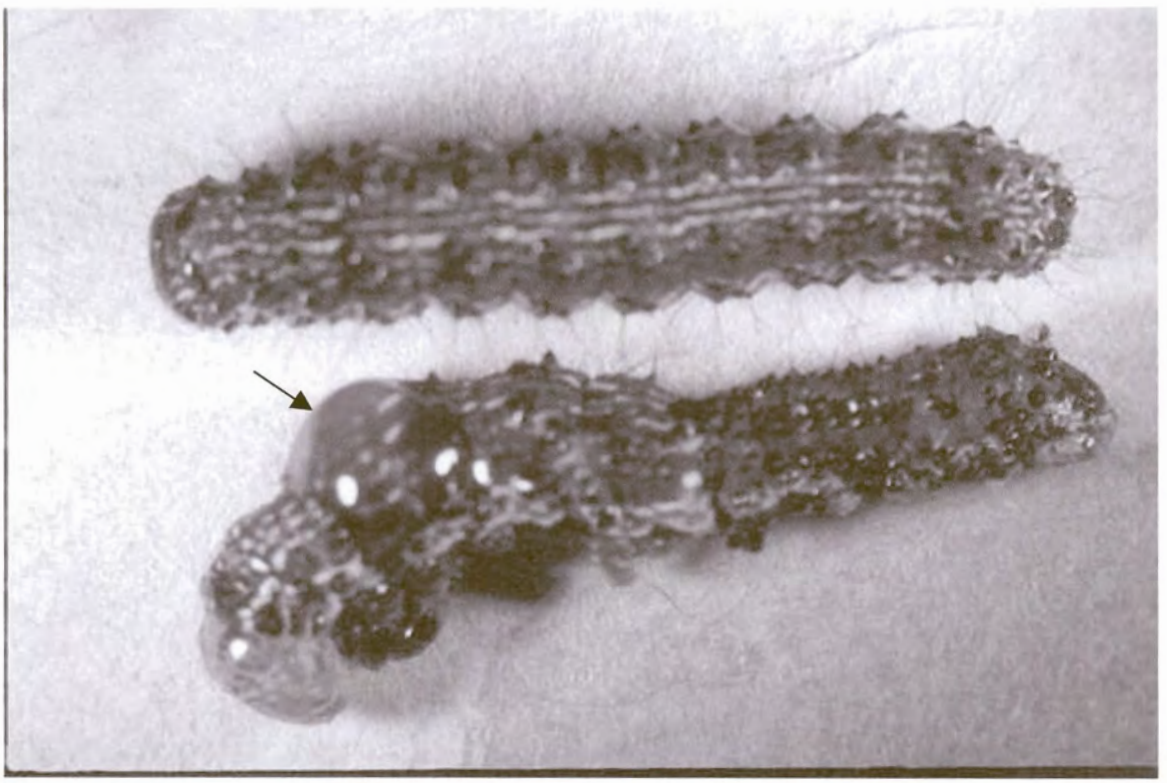

Fig. 3. Dorsal view of second instar Helicoverpa armigera (Hübner) larvae after emergence from eggs placed on treated leaves: top = normal larva that emerged from untreated substrate; bottom = larva with fluid-filled, balloon-like distortion that emerged from lufenuron-treated leaf.

Fig. 4. Morphological deformity caused by lufenuron at the larval-pupal (prepupal) stage.

Fig. 5. Larviform pupa (pupa with larval head and limbs) that emerged from lufenuron-treated substrate.

Fig. 6. Abnormal Helicoverpa armigera (Hübner) adult that emerged from lufenuron-treated substrate

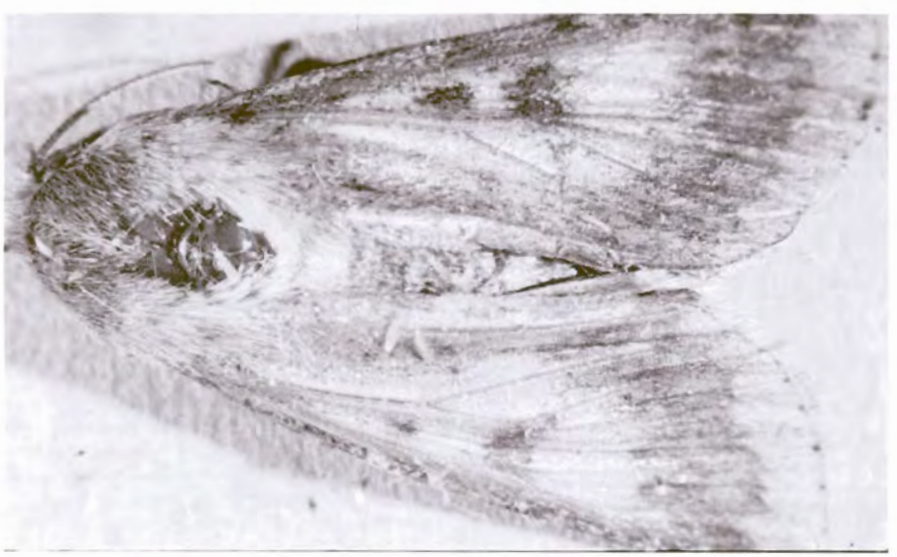

Fig. 7. Normal Helicoverpa armigera (Hübner) adult that emerged from control experiment

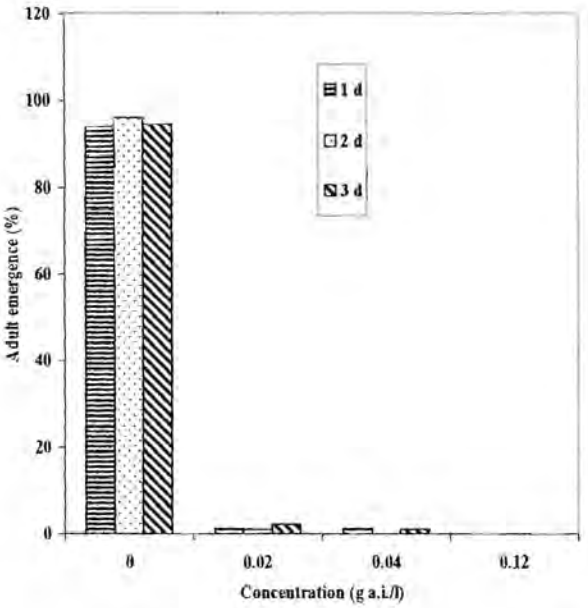

Fig. 8. Helicoverpa armigera (Hübner) adult emergence from treated tomato leaf bioassay.

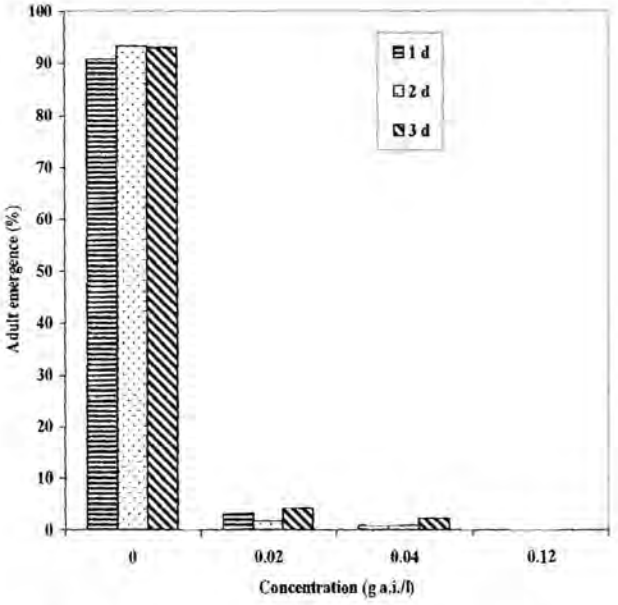

Fig. 9. Helicoverpa armigera (Hübner) adult emergence from treated tomato fruit bioassay.

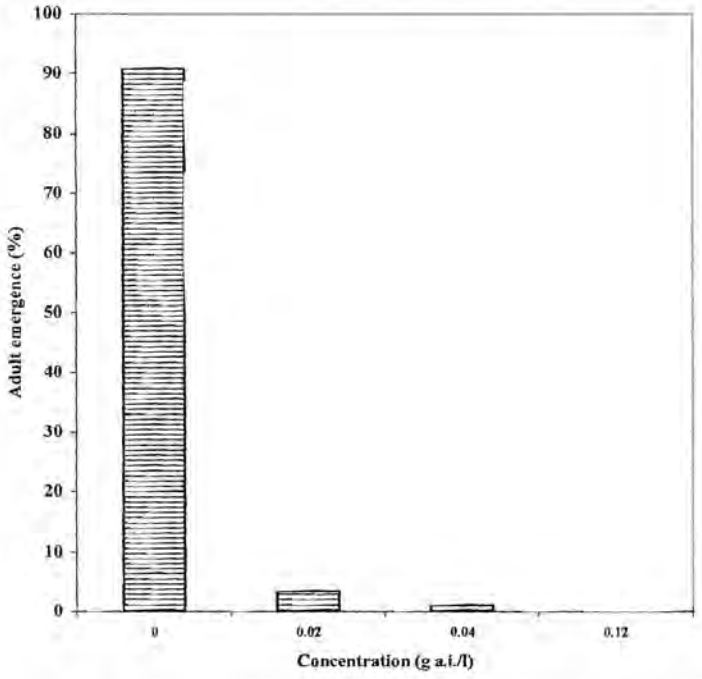

Fig.10. Helicoverpa armigera (Hübner) adult emergence (transovarial bioassay).

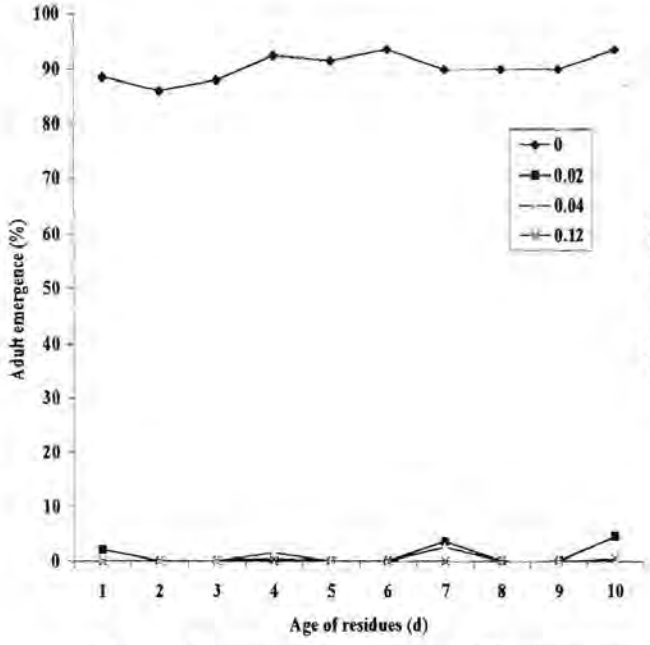

Fig. 11. Percentage emergence of Helicoverpa armigera (Hübner) adults from 1 day old eggs in in the residual activity bioassay.

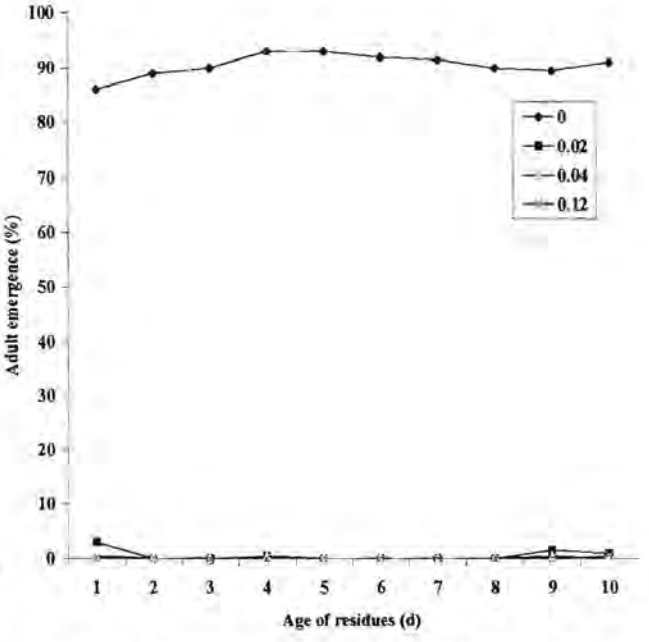

Fig. 12. Percentage emergence of Helicoverpa armigera (Hübner) adults from 2 day old eggs in the residual activity bioassay.

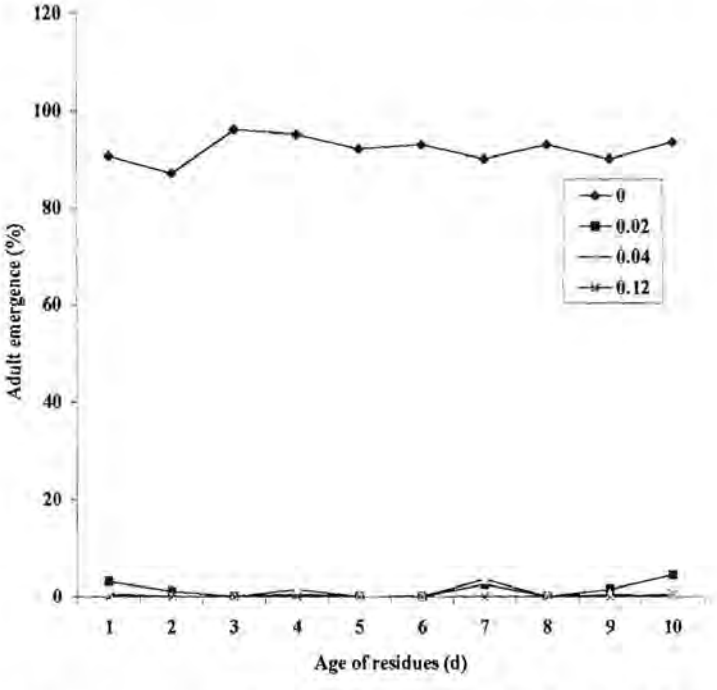

Fig.13. Percentage emergence of Helicoverpa armigera (Hübner) adults from 3 day old eggs in the residual activity bioassay.

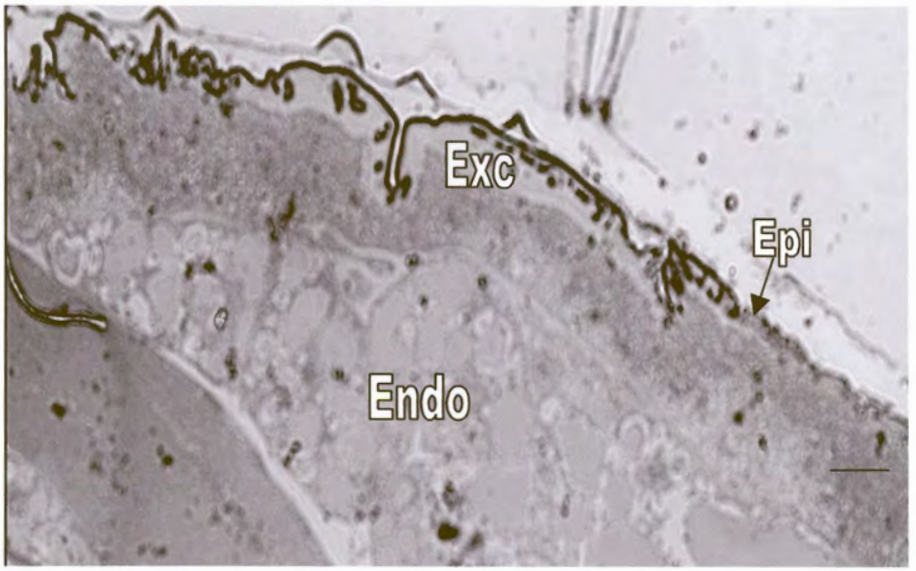

Fig. 14A. Cross section through the integument of untreated Helicoverpa armigera (Hübner) larva showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 48.3 μm.

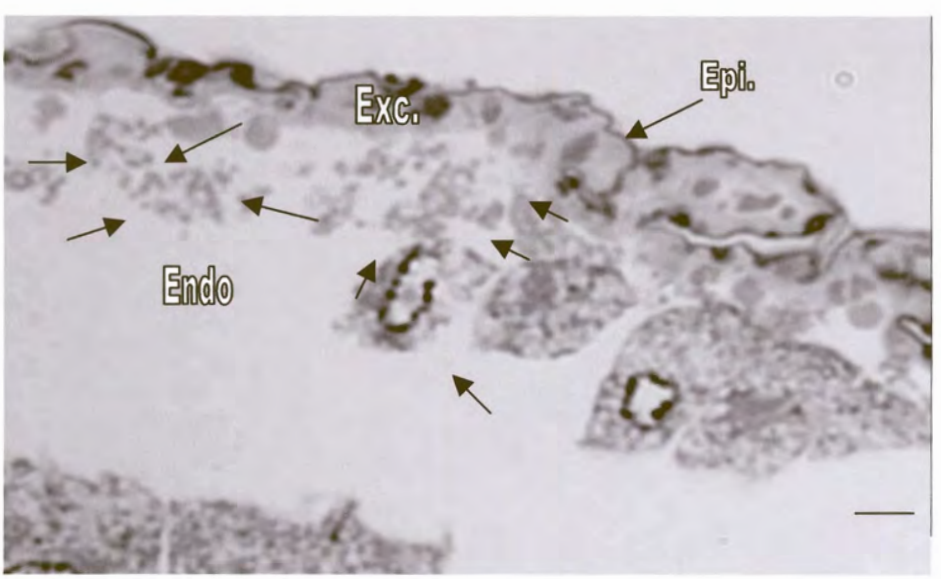

Fig. 14B. Cross section through the integument of lufenuron-treated larva showing a distorted endocuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 48.3 μm.

CHAPTER 3: EMBRYO-LARVICIDAL ACTIVITIES OF LUFENURON ON IMMATURE STAGES OF THE POTATO TUBER MOTH, PHTHORIMAEA OPERCULELLA (ZELLER) (LEPIDOPTERA: GELECHIIDAE)

3.1: INTRODUCTION

Potato tuber moth, Phthorimaea operculella (Zeller) (Lepidoptera: Gelechiidae) is a major pest of potato, tobacco and tomato worldwide, especially in warm temperate, and subtropical climates (Fenemore 1988; Jansens et al. 1995; Mohammed et al. 2000). In potato plants, the females deposit eggs on the underside of the leaves as well as on exposed tubers, and the emerging larvae mine the mesophyll layer of leaves (Radcliff 1982). As the larvae grow older, they tunnel through the tubers causing more damage to the plant (Radcliff 1982; Jansens et al. 1995). Also, when infested tubers enter storage without chemical treatment, the larvae continue to develop, tunneling extensively, filling the tuber with frass and permitting entry of decay causing microrganisms (Radcliff 1982; Gurr & Symington 1998).

On tomato plants, the larvae attack the tomato foliage as well as the fruits which they penetrate through the stem end, thus presenting a serious threat to the quality of the fruits (Fenemore 1988; Gilboa & Podoler 1995). In South Africa, the potato tuber moth is also a major pest of tobacco, and high pest population densities have been reported in areas where potato and tomatoes are cultivated together with tobacco (Bennett et al. 1999). Other host plants of the potato tuber moth are Cape gooseberry, brinjal, wild gooseberry and other solanaceous plants (Broodryk 1967; Ferguson 1989).

The potato tuber moth is about 8 mm in length and 15 mm across spread-wings, with greyish-brown coloration (Fig. 5) (Annecke & Moran 1982). The female lays about 170 – 300 soft pearly-white eggs on the soil near the potato tubers or on the underside of potato leaves (Rivnay 1962; Traynier 1975; Annecke & Moran 1982). The newly laid eggs are about 0.5 mm in length and 0.41 mm in width with a smooth or wrinkled chorion (Broodryk 1967). At oviposition, the egg content is white, but turns to yellow, then to reddish brown and finally dark as the larvae prepares to emerge from the eggshell (Broodryk 1967). Larval hatch occurs within 3 – 5 days under favourable temperature conditions (Rivnay 1962; Broodryk 1967). The newly hatched larva measures about 1 mm in length and has a dark sclerotized head with two antero-lateral groups of six stemmata (Broodryk 1967). The neonate larvae eat their way into the leaf veins, petioles or tubers where they grow and cause damage to the plant (Rivnay 1962; Broodryk 1967; Annecke & Moran 1982; Ferguson 1989). The larva passes through four larval instars and under favourable conditions, larval development is completed within two weeks (Broodryk 1967; Annecke & Moran 1982). When ready to pupate, the larva leaves its food and pupates in a suitable place in the soil or any sheltered spot covered with debris (Rivnay 1962; Annecke & Moran 1982). The newly formed pupa is initially white, but turns brown as pupation continues (Broodryk 1967). Adults emerge from the pupa after about one week under temperature range of 25 – 32 °C (Broodryk 1967).

Within tubers, potato tuber moth larvae are protected from mortality factors such as parasitoids, predators and insecticides, it is therefore important to monitor and control the potato tuber moth populations while larvae are still on foliage or outside the tubers

(Chaudhary et al. 1983). Current control measures for the potato tuber moth involve the use of both cultural techniques and the suppression of the foliar population by biological and chemical methods (Radcliff 1982; Shelton et al. 1981; Ferguson 1989). Farmers in South Africa and other parts of the world rely heavily on conventional insecticides such as carbamates, organochlorines and organophosphates for the control of the potato tuber moth. The over use of these insecticides has led to the problems of resistance development in the pest populations as well as the accumulation of pesticide residues in the environment (Findlay 1975; Shelton & Wyman 1979; Radcliff 1982; Llanderal-Cazáres et al. 1996; Bennett et al.1999).

This study focused on the embryo-larvicidal as well as the residual activities of lufenuron on immature stages of the potato tuber moth. The effects of this chitin synthesis inhibitor on the tissues of the newly hatched larval instars were also evaluated.

3.2: MATERIALS AND METHODS

3.2.1: Insects and Chemicals

Adult potato tuber moths collected from a colony maintained at the Vegetable and Ornamental Plant Institute, Roodeplaat, Pretoria, were put in oviposition chambers (25 cm high by 11 cm diameter). The moths were fed 10 % honey solution to increase egg production and the chambers were then covered with nylon screened tops and kept at a constant temperature of 28 ± 1 ^{0}C and photoperiods of 12L: 12D for oviposition. Filter paper discs (10 cm diameter, Whatman no. 1, Hillsboro, OR.) were used as oviposition substrates and oviposited eggs were collected every 12 h for bioassay. Lufenuron 050 EC

as an emulsifiable concentrate containing 50 g a.i./l was supplied by Novartis SA (Pty) Ltd. Serial dilutions of 0.02, 0.04 and 0.12 g a.i./l were prepared with distilled water for all the bioassays. Control experiments were treated with distilled water.

3.2.2: Bioassay procedures

3.2.2.1: Embryo-larvicidal bioassays.

Two bioassays were carried out to evaluate the embryo-larvicidal activities of lufenuron on the immature stages of the potato tuber moth.

Treated tuber bioassay

Potato tubers were dipped in the appropriate test solutions for 30 s, air-dried, and transferred to rearing chambers (plastic containers, 7 cm high and 12 cm diameter). Eggs (1 – 4 d old; n = 400 per age group) oviposited on the filter papers were transferred to treated potato tuber substrates with a fine camel-hair brush and the rearing chambers were covered with a nylon netting. Each concentration plus its control was replicated four times. To allow for delayed hatching as well as for the penetration of the larvae into the tubers, the percentage of eggs from which larvae hatched and the number of dead first instars were recorded after 7 days. Egg count, hatch determination and the identification of dead first instars on each potato tuber were carried out under a stereomicroscope (16 X) (Broodryk 1967). Surviving larvae in each plastic container were reared to the adult stage on the treated tubers. Controls were fed untreated tubers and the experiment was monitored daily until adult emergence. The various types of morphological disorders induced by lufenuron on the larval and post larval stages were also observed and

photographed. After 32 days, all the potato tubers were dissected and dead larval instars were retrieved and identified (Broodryk 1967).

Egg dip bioassay

Potato tuber moth eggs (1 – 4 d old; n = 400 per age group) oviposited on filter papers were dipped in the appropriate test solutions for 5 minutes, air-dried and transferred to untreated potato tuber substrates using a fine camel-hair brush. Each concentration plus its control (dipped in distilled water) was replicated four times and the tubers were kept in rearing chambers until larval hatch. Egg and first instar mortalities were determined under a stereomicroscope (16X) 7 days post treatment. Surviving larval instars were reared to the adult stage on untreated potato tubers and the experiment was monitored daily until adult emergence. Data on adult emergence were recorded 32 days post treatment and dead larval and post-larval stages with morphological deformities were observed and photographed.

Data analysis

Data on percentage egg and first instar mortalities were corrected for natural mortality (Abbott 1925). Where appropriate, the data were arcsine transformed before analysis of variance (ANOVA) (Statsoft 1995). Duncan's multiple range test was used to evaluate the differences between the treatment groups ($P = 0.05$) (Broadbent & Pree 1984; Statsoft 1995; Rehimi & Soltani 1999).

3.2.2.2: Residual activity bioassay

The residual activity of lufenuron on immature stages of the potato tuber moth was evaluated over a 10 day trial period by dipping potato tubers into the appropriate test solutions for 30 seconds, and the pesticide residues were allowed to age at normal room temperatures (Moffitt et al. 1984). On the day of treatment and each day thereafter, potato tuber moth eggs (1 – 4 d old; n = 400 per age group) oviposited on filter papers were transferred to the treated potato tuber substrates with a fine camel-hair brush. Each concentration plus its control (dipped in distilled water) was replicated four times and the tubers were kept in rearing chambers (plastic containers 7 cm high by 12 cm diameter) and covered with nylon netting.

To allow for delayed hatching as well as for the penetration of the larvae into the tubers, data on percentage egg mortality were recorded under a stereomicroscope (16X) 7 days post treatment. Surviving larval instars in each plastic container were reared to the adult stage on the treated tubers. Data on percentage adult emergence were recorded after 32 days to allow for delayed development due to pesticide effects.

Data on percentage egg mortality were corrected for natural mortality (Abbott 1925). Where appropriate the data on egg mortality as well as the data on adult emergence were arcsine transformed before analysis of variance (Statsoft 1995). Duncan's multiple range test was used to evaluate the differences between the treatment groups ($P = 0.05$) (Statsoft 1995; Rehimi & Soltani 1999).

3.2.2.3: Histological bioassay

Eggs (< 12 h old; n = 10) to be used for histological studies were transferred from the oviposition substrates to potato tubers treated with 0.04 and 0.12 g a.i./l of lufenuron. Controls were transferred to potato tubers treated with distilled water, and the tubers were kept in plastic containers (7 cm high by 12 cm diameter) and covered with nylon netting. Hatched larval instars were allowed to feed on the treated tubers for 2 days after which they were killed by decapitation. The tissues were fixed in 2.5 % gluteraldehyde for 1 h, rinsed 3 times (15 minutes each) in 0.075 M phosphate buffer (pH 7.4 – 7.6). Post fixation was carried out in 0.25 % aqueous osmium tetraoxide for 1 h at room temperature, and the tissues were rinsed 3 times (15 minutes each) in distilled water before dehydration in 50, 70 and 90 % ethanol for 15 minutes each. Further dehydration in 100 % ethanol (3 changes, 15 minutes each) was carried out and the fixed tissues were infilterated with 30 and 60 % quetol for 1 h and in pure quetol for 4 h (Kushida 1974). The tissues were polymerized for 24 – 36 h at 65 °C and semi-thin sections (2 μm)were cut and stained with toludine blue (Trump et al. 1961). Sections were viewed under a Nikon Optiphot light microscope and photographed with a Nikon digital camera (DXM 1200).

3.3: RESULTS AND DISCUSSION

3.3.1: Embryo-larvicidal bioassays.

Embryocidal effects

Egg mortality from both bioassays was very low and there was no significant difference between larval hatch from the treated and untreated experiments (Tables 1 and

2). Embryonic development in all the eggs (treated and control) reached the final black head stage and eggs exposed to the different concentrations of lufenuron were able to hatch on the fourth or fifth day after oviposition. The duration of development or incubation period recorded in this study is in agreement with the results of Broodryk (1971). The lack of embryocidal activities reported in both bioassays shows that lufenuron has no contact activity on the eggs of the potato tuber moth. This result is contrary to previous reports on the embryocidal activities of acylureas against lepidopteran, dipteran and coleopteran pests (Hoying & Riedl 1980; Assal et al. 1983; Casaña-Giner et al. 1999; Jay & Cross 2000). Ammar (1984), reported a high ovicidal effect in younger eggs of the Colorado potato beetle, Leptinotarsa decemlineata (Say) (Coleoptera: Chrysomelidae) exposed to different concentrations of diflubenzuron and triflumuron while Young et al. (1987), found that diflubenzuron significantly reduced larval hatch in the eggs of the cabbage maggot, Delia radicum, (L.) (Diptera: Anthomyiidae).

Larvicidal effects

Mortality of first instars from the lufenuron-treated tubers was very high (> 90 %) compared with the low (< 8.5 %) first instar mortality in the egg dip bioassay (Tables 3 and 4).The differences in first instar mortalities between eggs exposed to lufenuron-treated tubers and the eggs immersed in the different concentrations of lufenuron could have been due to the duration of contact between the eggs and the chemical residues. The eggs that were exposed to the treated tubers were in contact with lufenuron residues through out the incubation period, while eggs that were immersed in the various

concentrations of lufenuron were only in contact with the chemical for 5 minutes; and this difference in duration of contact or exposure may have led to the differences in the mortality of first instars in both bioassays. In other words, the cumulative effects of lufenuron absorbed into the embryos during development as well as the ingestion of treated potato tubers after larval hatch could have led to a high mortality of first instars in the treated tuber bioassay. This view is supported by the studies of Coppen & Jepson (1996), who found that the exposure of second instar Schistocerca gregaria, (Orthoptera: Acrididae) to benzoylphenyl ureas over a four day period resulted in higher mortality of the larvae than when similar larval instars were exposed to the same active ingredients over one or two day periods.

Eggshell consumption is not a very common phenomenon amongst emerging first instars of the potato tuber moth, and this may also have contributed to the low larval mortality in the egg dip bioassay. The fewer the eggshells consumed during emergence, the lower the amount of lufenuron residues in the body of the developing larvae and hence the low larval mortality at the first or second instar stages. This is consistent with the findings of Visser (1998), who reported a high number of unconsumed eggshells after the emergence of potato tuber moth larvae from different concentrations of lufenuron.

In the treated tuber bioassay, larval mortality was most common during the moult to the second instar stage and microscopic examination of the dead and dying larvae showed that ecdysis was initiated but not completed, and most of the dead larval instars were unable to escape from the old cuticle. The inability to shed the old cuticle could be due to

the lack of skeletal rigidity which is caused by the reduced amount of chitin in the new cuticle (Clark & Jewess 1990, Perez-Farinos et al. 1998). In other cases, the new cuticle appeared very weak and ruptured easily resulting in the desiccation of the larvae. Other symptoms such as reduced body size as well as black shrivelled body were observed amongst dead larvae (Fig. 1). The darkening of the larval body could be due to the exposure of the haemolymph to the surrounding air (Retnakaran et al. 1985).

In the treated tuber bioassays, few (< 2 %) of the surviving first instars were able to penetrate the tubers and developed to the pupal and adult stages after a long delay. This delay in larval development could have been caused by the effects of the pesticide residues in the body of the developing larvae (Darvas et al. 1992; Clark & Jewess 1990). Larval mortality was very low in both the egg dip and control bioassays.

Post larval effects

Moult disruptions also occurred during the larval-pupal (prepupal) stage and most of the newly emerged pupae had larval features such as larval abdomen and limbs (larviform) (Fig. 2). Other types of morphological abnormalities such as pupa with reduced body size, ruptured cocoons and leaking haemolymph were also observed at the pupal stage (Fig. 3). Haemolymph leakage occurred mainly in the thoracic region, and some malformed pupae had larval skin retained on the lower part of the abdomen (Figs 2 & 3). Similar results have been reported in other acylureas (Retnakaran & Smith 1975, Fogal 1977; Retnakaran & Wright 1987). Few cases of pupal malformations were

reported in the egg dip bioassay and there were no records of malformed pupae in the control experiments.

Morphological deformities such as adults with reduced abdominal size and stubby wings were observed amongst adults emerging from the treated tuber bioassays. Also, some emerging adults were unable to escape from the cocoons (Fig. 4). The inability of adults to free themselves from the cocoons could be due to the weakening of the wings by the residues of lufenuron or because the body parts of the emerging adults were stuck to the pupal sacs by dried haemolymph which leaked into the cocoons during pupal-adult development (Grosscurt & Anderson 1980; Reed & Bass 1980). Soltani et al. (1984), found that diflubenzuron does not inhibit pupal development in the mealworm, Tenebrio molitor, (L.) (Coleoptera: Tenebrionidae), but that it affects adult ecdysis, leading to the formation of adults with abnormal elytra. Also, Vennard et al. (1998) found that adult malformations such as incomplete wing rotation, soft wings, soft cuticle, twisted femur and tibia, and the inability of eclosed adults to fly were due to the activities of insect growth regulators. In the spruce budworm, Choristoneura fumiferena, (Clemens) (Lepidoptera: Tortricidae), delayed moulting effects such as retention of pupal cuticle, lethargy, loss of fluids, and death shortly after adult emergence were observed by Brushwein & Granett (1977).

Cumulative mortality at the larval and pupal stages led to a low (< 2%) adult emergence in the treated tuber bioassay, while high adult emergence (> 72.5%) was recorded in the egg dip bioassay as well as in the control experiments (Figs. 6 and 7).

The high number of dead first instars as well as the low adult emergence recorded in the treated tuber bioassays suggests that lufenuron could be valuable in reducing the damage caused by the potato tuber moth.

3.3.2: Residual activity bioassay

Potato tuber moth eggs were not sensitive to the three concentrations of lufenuron used in this bioassay. Embryonic development was not affected and egg mortality over the 10 days trial period was low (< 5.6%) (Tables 5 – 8). The low embryocidal effect reported in this bioassay is contrary to previous reports on the residual activities of acylureas against lepidopteran pests (Berry et al. 1980; Ascher et al. 1982). Elliott & Anderson (1982) found that egg hatch in the codling moth, Laspeyresia pomonella (Lepidoptera: Olethreutidae) was inversely related to the length of time that the chorion was in contact with diflubenzuron solution. Also, these authors reported that the residual activity of diflubenzuron against the eggs of the codling moth was excellent when the compound had dried on the apple and leaf substrates. Present results however, does not agree with the findings of the above authors and also, there was no significant difference in embryonic development in the different residue ages used in this study.

The ingestion of lufenuron-treated tubers by the emerging neonates led to a high mortality of the first instars and this greatly reduced the damage done to the potato tubers. At the highest concentration (0.12 g a.i./l), larval mortality at the first instar stage was very high and damage to the potato tubers were not visible to the naked eye. However, few of the larval instars that emerged from the lower concentrations (0.02 and 0.04 g

a.i./l) were able to develop to the pupal and adult stages after a prolonged delay. Elliott & Anderson (1982), found that the incorporation of diflubenzuron to the diet of larval instars of the codling moth, L. pomonella, led to a high mortality of the first and second instars over a 10 day trial period, while Dean et al. (1998) reported a high mortality of first instars of the cat fleas, C. felis after emergence from eggs exposed to different concentrations of lufenuron. Su & Scheffrahn (1996), recorded a 100 % mortality and a persistence of up to 19 weeks against subterranean termites (Isoptera: Rhinotermitidae) exposed to different concentrations of lufenuron.

The delayed effects of lufenuron was also manifested at the pupal and adult stages and this led to a low adult emergence in all the concentrations over the 10 day trial period (Figs. 8 – 11). Similar results of low adult emergence due to the inhibitory effects of acylureas have been reported in other pests (Fogal 1977; Retnakaran & Wright 1987; Ishaaya et al. 1988; Wilson & Cryan 1997; Rehimi & Soltani 1999).

3.3.3: Histological bioassay

The spectrum of effects of benzoylphenyl urea treatment follows a consistent pattern which reflects the primary site of action, namely the disruption of chitin synthesis (Retnakaran et al. 1985). Histological examination of the tissues of the potato tuber moth larvae showed severe lesions or distortions in the exo - and endocuticular layers. Both the exo- and endocuticles were affected, but the endocuticle had more tissue and cellular disorganization than the exocuticle (Fig. 12A, B). Globular materials were also observed in the endocuticle of lufenuron-treated tissues, while the exo- and endocuticular layers of

untreated tissues were continuous and undamaged. This result agrees with the studies of Perez-Farinos et al. (1998), who found that the exo- and endocuticular layers of the integument of the beet weevil, Aubeonymous mariefranciscae (Roudier) (Coleoptera: Curculionidae) were totally disorganized or eroded when exposed to the chitin synthesis inhibitor, hexaflumuron. This irregular deposition of chitin-protein layers in the exo- and endocuticular layers could be responsible for the mechanical weakness and death of the embryos soon after hatch (Perez-Farinos et al. 1998; Dean et al. 1999). Reports of endocuticular distortions as well as the obliteration of the cytoplasmic organelles and other parts of the integument due to acylureas have been reported by Mulder & Gijswijt (1973). According to Fogal (1977), failure in the formation of the endocuticle of larval tissues treated with acylureas could lead to a reduction in the strength of muscular attachments and this might lead to an interference with muscular activities during ecdysis, larval movement and feeding.

The reason why lufenuron-treated larvae succeed only partly or not at all in casting off their exuvium is because the newly formed cuticle contains only the epicuticular and exocuticluar tissues which are not properly attached to the epidermis, and this therefore makes the cuticle to be very delicate and unable to resist the muscular traction and increased tugor needed during larval moult (Mulder & Gijswijt 1973; Ker 1977). Dean et al. (1999), reported a decrease in the amount of epidermal cytoplasm as well as lytic changes in cytoplasmic organelles in the integument of adult cat fleas, C. felis, fed different concentrations of lufenuron; while Retnakaran et al. (1997) reported a complete degradation of the old cuticle as well as changes in the organelles of the epidermal cells

in the sixth instar larvae of the spruce budworm, Choristoneura fumiferena, (Clemens) (Lepidoptera: Tortricidae) exposed to different concentrations of RH-5992 insect growth regulator.

Other results of endocuticular distortions, obliteration of cytoplasmic organelles as well as the inhibition of chitin biosynthesis in larval instars treated with acylureas have been reported by Post et al. (1974); Grosscurt & Anderson (1980) and Meola et al.(1999). Histological findings from this study suggest that distortions of the procuticular layer (exo- and endocuticle) could have been caused by the incorrect deposition of the chitin-protein layer or the complete inhibition of chitin biosynthesis in the integument of the emerging larval instars. This view is supported by the studies of Retnakaran et al. (1987) and Perez-Farinos et al. (1998). The high mortality of first instars from the lufenuron-treated bioassays before, during or immediately after larval moult has been attributed to the fact that chitin biosynthesis is highest during the early part of the larval stadium, and the distortion of the procuticular layers by acylureas at this stage, could lead to the incorrect deposition of chitin or a complete inhibition of chitin synthesis (Grosscurt & Jongsma 1987). This incorrect deposition of chitin during chitin synthesis often leads to an increase in the death of the neonates (Grosscurt & Jongsma 1987; Retnakaran et al. 1987).

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Table 1. Mortality of different-aged eggs of Phthorimaea operculella (Zeller) after exposure to lufenuron-treated tubers.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	2.5 ± 0.6a	5.1 ± 1.1a	2.5 ± 1.7a	2.3 ± 1.4a
2	1.0 ± 0.7a	2.3 ± 0.4a	2.8 ± 2.1a	1.8 ± 1.8a
3	1.5 ± 0.6a	3.4 ± 1.5a	1.3 ± 0.9a	0.3 ± 0.3a
4	0.3 ± 0.3a	1.8 ± 0.9a	1.5 ± 0.6a	1.8 ± 0.5a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 2. Mortality of different-aged eggs of Phthorimaea operculella (Zeller) after exposure to different concentrations of lufenuron (egg dip bioassay).

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	3.6 ± 3.5a	5.8 ± 3.4a	7.0 ± 1.5a	7.0 ± 3.8a
2	2.7 ± 1.5a	6.4 ± 5.4a	4.2 ± 1.4a	2.6 ± 1.9a
3	0.5 ± 0.5a	3.1 ± 1.9a	2.5 ± 1.9a	1.0 ± 0.6a
4	1.5 ± 1.5a	1.0 ± 0.6a	1.5 ± 1.5a	1.5 ± 1.8a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 3. Larvicidal activity of lufenuron against first instars of Phthorimaea operculella (Zeller) after emergence from eggs placed on treated potato tubers.

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	0.8 ± 0.8a	91.8 ± 1.0b	94.8 ± 3.4b	96.5 ± 2.1b
2	3.5 ± 1.7a	94.5 ± 1.6b	90.5 ± 2.9b	95.3 ± 2.1b
3	2.8 ± 1.2a	90.5 ± 0.3b	93.5 ± 1.9b	97.3 ± 2.1b
4	2.5 ± 1.6a	90.3 ± 3.5b	97.3 ± 0.9b	97.5 ± 1.2b

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 4. Larvicidal activity of lufenuron against first instars of Phthorimaea operculella (Zeller) (egg dip bioassay).

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	0.5 ± 0.3a	1.0 ± 0.7a	2.3 ± 1.1a	8.5 ± 3.5a
2	0.8 ± 0.5a	1.5 ± 1.2a	1.0 ± 0.4a	6.3 ± 1.6a
3	1.5 ± 0.6a	1.3 ± 0.3a	1.3 ± 0.6a	2.0 ± 1.4a
4	0.3 ± 0.3a	1.3 ± 0.3a	1.0 ± 0.7a	0.3 ± 0.3a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 5. Residual toxicity of lufenuron to 1 day old Phthorimaea operculella (Zeller) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	1.0 ± 2.9a	4.7 ± 3.6a	3.8 ± 2.0a	3.8 ± 3.2a
2	1.5 ± 3.5a	0.5 ± 2.0a	3.7 ± 4.2a	5.6 ± 3.8a
3	0.5 ± 3.4a	0.0 ± 0.0a	3.6 ± 4.4a	2.1 ± 3.3a
4	0.5 ± 2.0a	3.2 ± 3.1a	2.1 ± 3.4a	3.2 ± 3.1a
5	0.0 ± 0.0a	1.6 ± 1.5a	0.5 ± 0.6a	1.5 ± 2.9a
6	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 2.9a	0.5 ± 2.0a
7	0.0 ± 0.0a	0.5 ± 2.0a	1.0 ± 2.3a	2.6 ± 3.7a
8	0.0 ± 0.0a	0.5 ± 2.0a	0.5 ± 2.0a	2.5 ± 3.8a
9	0.0 ± 0.0a	1.0 ± 2.9a	0.0 ± 0.0a	0.5 ± 2.0a
10	1.0 ± 2.9a	1.9 ± 3.5a	0.0 ± 0.0a	1.5 ± 2.9a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 6. Residual toxicity of lufenuron to 2 day old Phthorimaea operculella (Zeller) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	0.5 ± 2.0a	3.2 ± 3.1a	0.5 ± 3.5a	2.6 ± 3.8a
2	2.0 ± 2.5a	2.1 ± 3.3a	1.6 ± 3.5a	2.6 ± 2.0a
3	2.0 ± 3.4a	1.6 ± 2.9a	2.9 ± 2.7a	0.0 ± 0.0a
4	0.5 ± 2.0a	1.5 ± 2.9a	2.6 ± 3.9a	1.5 ± 2.9a
5	0.5 ± 2.0a	0.5 ± 2.0a	0.5 ± 2.7a	2.6 ± 3.8a
6	0.5 ± 2.0a	0.5 ± 2.0a	0.0 ± 0.0a	0.0 ± 0.0a
7	0.0 ± 0.0a	0.5 ± 2.0a	0.5 ± 2.0a	1.5 ± 2.9a
8	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 2.9a	1.0 ± 2.9a
9	0.0 ± 0.0a	0.0 ± 0.0a	0.5 ± 2.0a	0.0 ± 0.0a
10	0.5 ± 2.0a	0.0 ± 0.0a	0.5 ± 2.0a	2.6 ± 3.8a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 7. Residual toxicity of lufenuron to 3 day old Phthorimaea operculella (Zeller) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	0.0 ± 0.0a	1.1 ± 2.1a	0.5 ± 2.0a	2.1 ± 2.5a
2	0.5 ± 2.0a	1.0 ± 2.3a	3.6 ± 3.5a	0.5 ± 2.0a
3	1.0 ± 2.9a	2.6 ± 3.8a	3.2 ± 3.3a	2.1 ± 3.3a
4	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 2.9a	0.5 ± 2.0a
5	0.0 ± 0.0a	0.0 ± 0.0a	1.5 ± 2.9a	1.0 ± 3.8a
6	1.0 ± 2.8a	0.5 ± 2.0a	0.5 ± 2.0a	0.0 ± 0.0a
7	0.5 ± 2.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
8	0.5 ± 2.0a	0.5 ± 2.0a	0.0 ± 0.0a	0.0 ± 0.0a
9	0.5 ± 2.0a	0.0 ± 0.0a	0.5 ± 2.0a	1.0 ± 2.9a
10	1.0 ± 2.9a	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 2.9a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 8. Residual toxicity of lufenuron to 4 day old Phthorimaea operculella (Zeller) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)			
	0	0.02	0.04	0.12
1	0.0 ± 0.0a	1.0 ± 2.9a	1.0 ± 2.9a	0.5 ± 2.0a
2	0.5 ± 2.9a	1.1 ± 2.8a	3.6 ± 3.5a	0.5 ± 2.0a
3	1.0 ± 2.9a	0.0 ± 0.0a	1.5 ± 2.9a	0.0 ± 0.0a
4	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
5	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.5 ± 2.0a
6	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 2.9a
7	0.0 ± 0.0a	1.0 ± 2.9a	0.5 ± 2.0a	0.0 ± 0.0a
8	1.0 ± 2.9a	0.0 ± 0.0a	0.0 ± 0.0a	0.5 ± 2.0a
9	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
10	0.0 ± 0.0a	1.0 ± 2.9a	0.0 ± 0.0a	0.0 ± 0.0a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Fig.1A. Normal first instar Phthorimaea operculella (Zeller) larva that emerged from untreated tuber

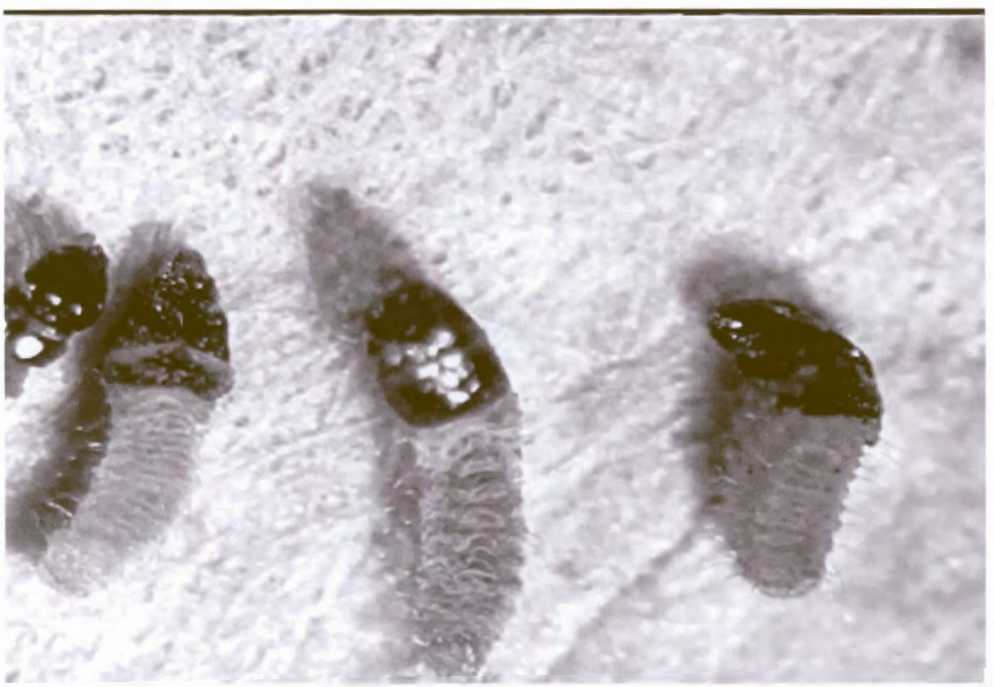

Fig.1B. Effect of lufenuron on first instar Phthorimaea operculella (Zeller) larvae: Dark shrivelled first instars that emerged from eggs placed on lufenuron-treated tubers.

Fig. 2. Dorsal view of malformed Phthorimaea operculella (Zeller) pupae: Left = incompletely spun cocoon (larviform pupa) with haemolymph leakage in the mid-section; Center = normal pupa from control experiment; Right = small-sized pupa with larval skin attached to abdominal end.

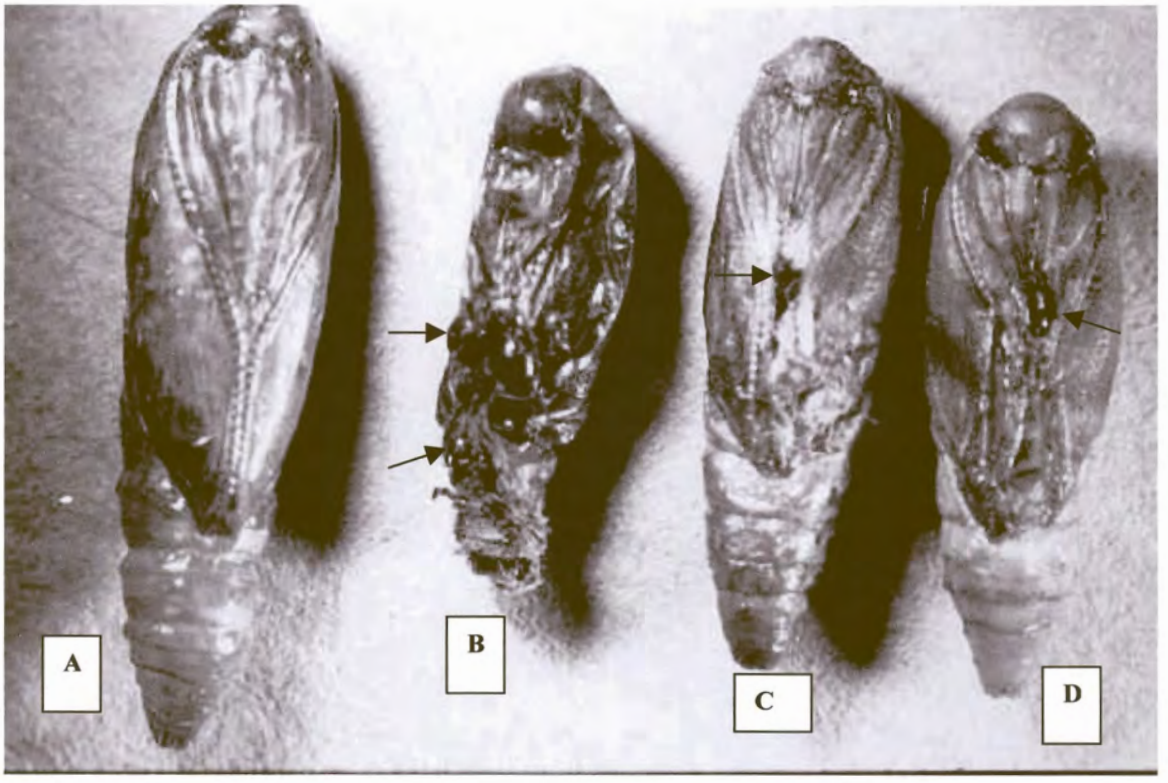

Fig. 3. Effects of lufenuron on Phthorimaea operculella (Zeller) pupae: A = normal pupa that emerged from untreated tuber; B – D = pupae with ruptured cocoons and leaking haemolymph that emerged from lufenuron-treated tubers.

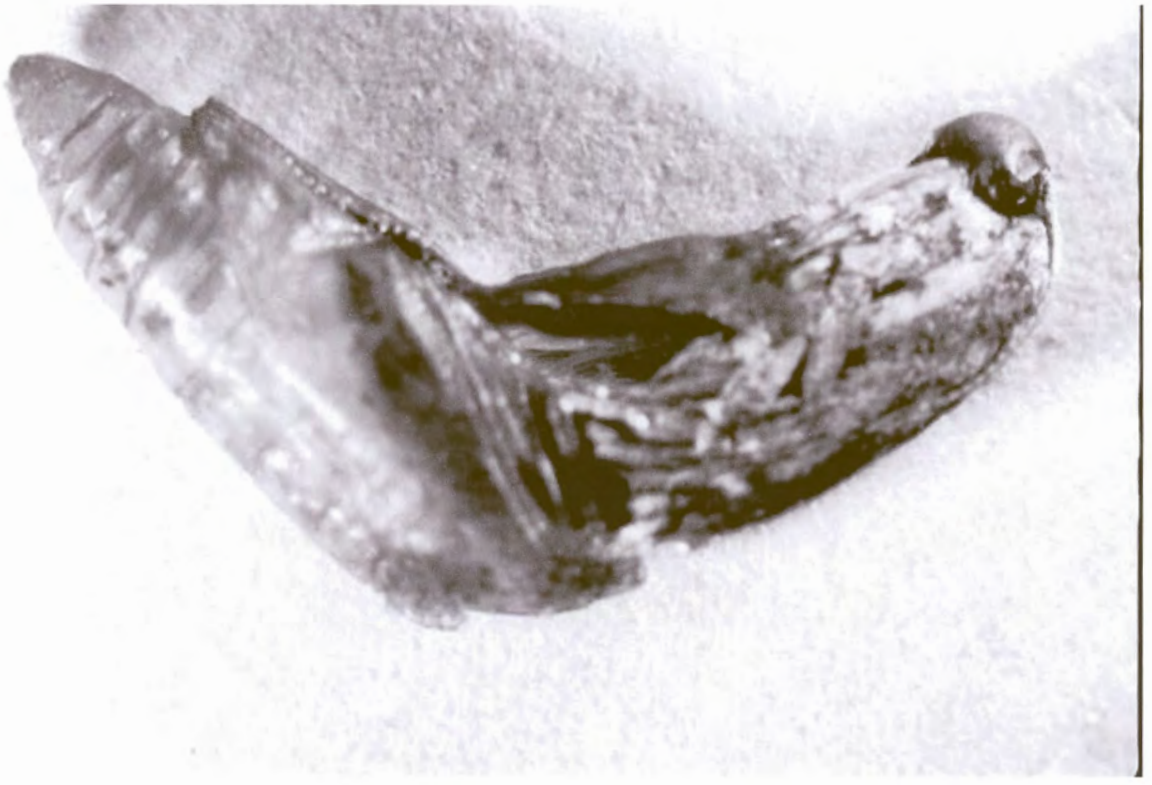

Fig. 4. Phthorimaea operculella (Zeller) adult unable to escape from pupal case at emergence.

Fig. 5. Adult potato tuber moth

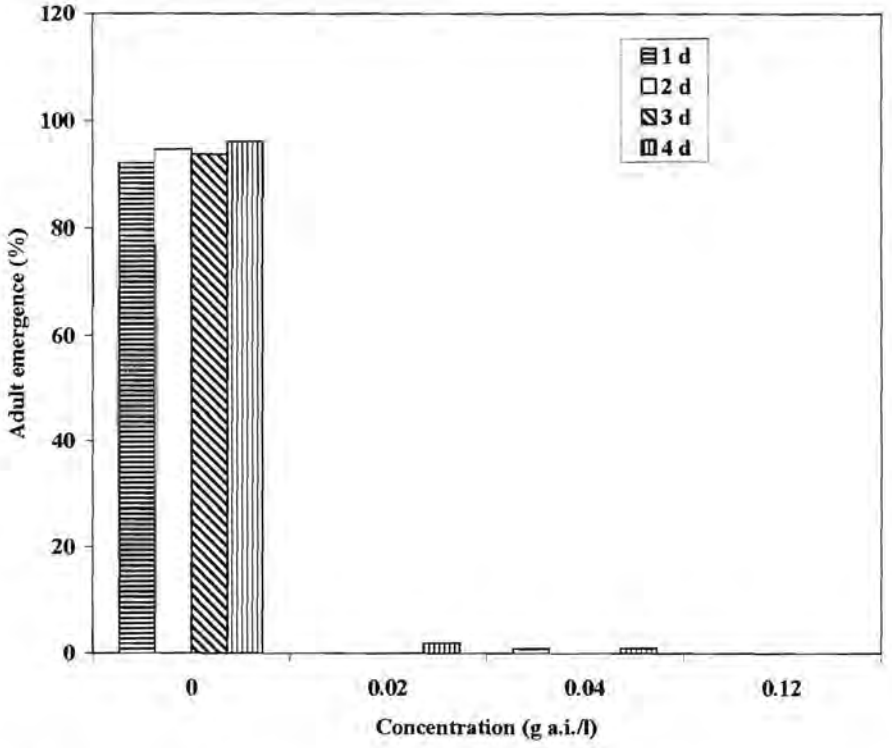

Fig. 6. Phthorimaea operculella (Zeller) adult emergence from lufenuron-treated tubers.

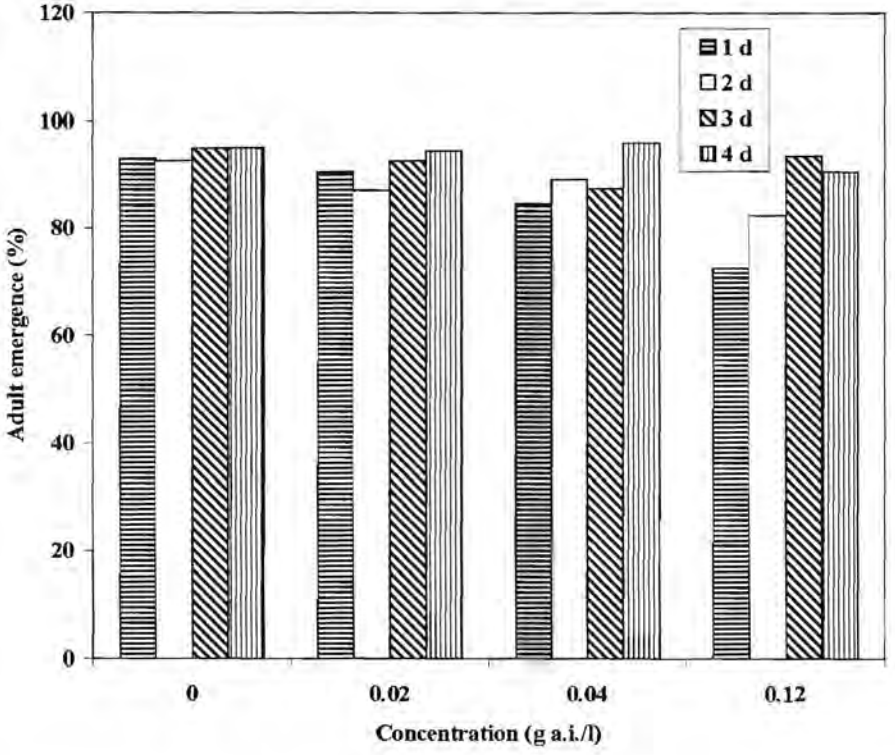

Fig. 7. Phthorimaea operculella (Zeller) adult emergence from the egg dip bioassay

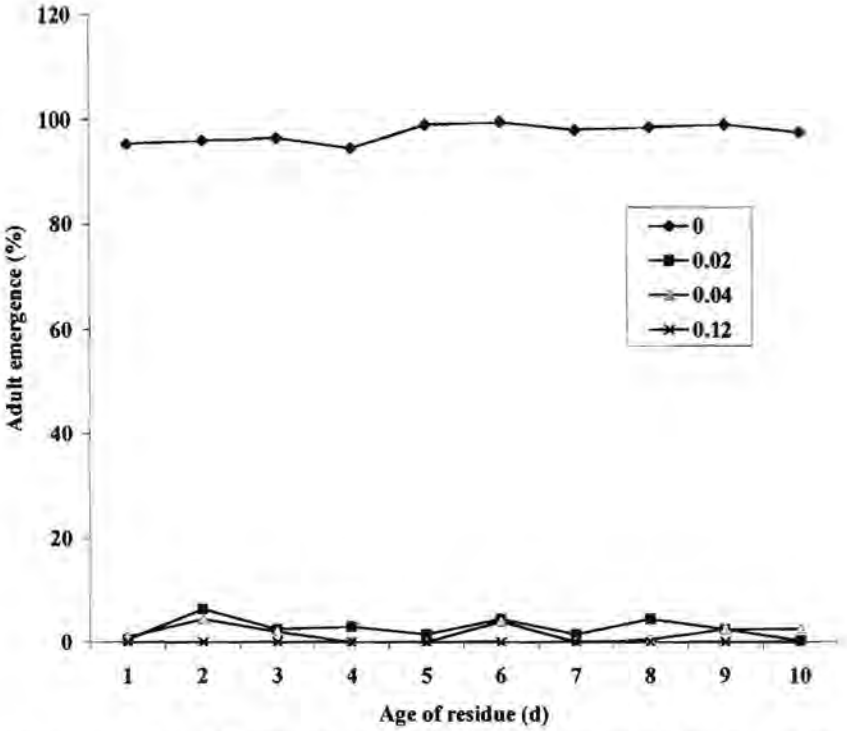

Fig.8. Percentage emergence of Phthorimaea operculella (Zeller) adults from 1 day old eggs in the residual activity bioassay.

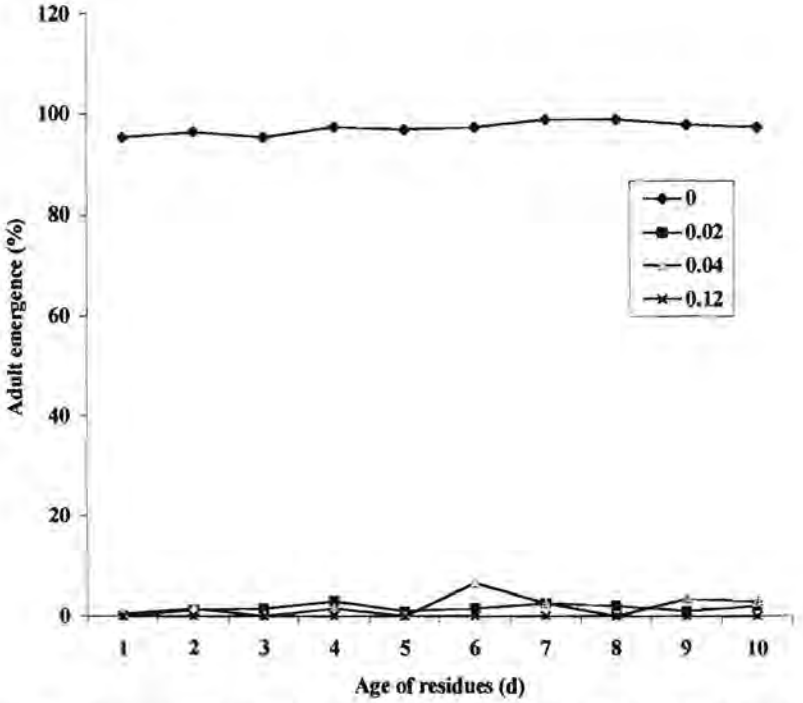

Fig. 9. Percentage emergence of Phthorimaea operculella (Zeller) adults from 2 day old eggs in the residual activity bioassay.

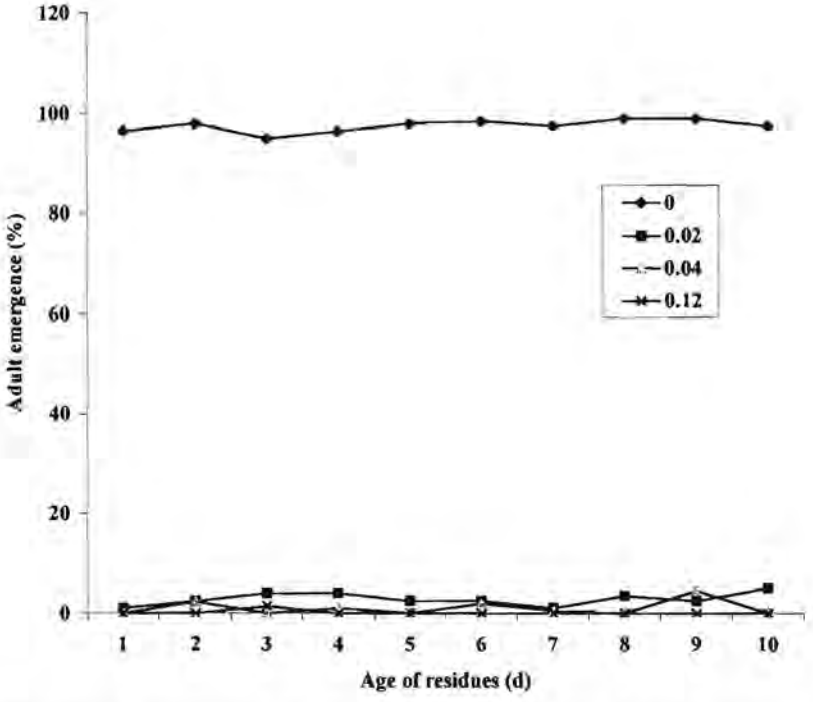

Fig. 10. Percentage emergence of Phthorimaea operculella (Zeller) adults from 3 day old eggs in the residual activity bioassay.

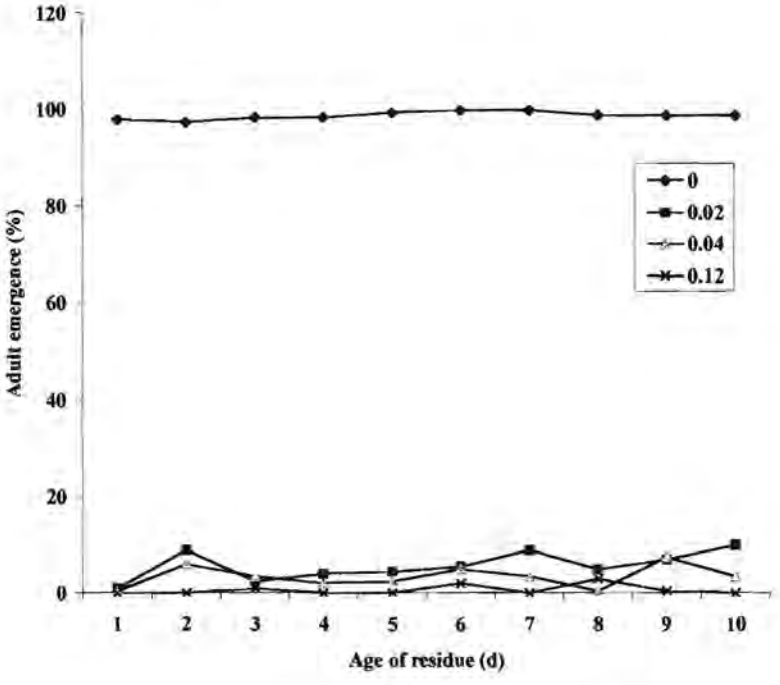

Fig. 11. Percentage emergence of Phthorimaea operculella (Zeller) adults from 4 day old eggs in the residual activity bioassay.

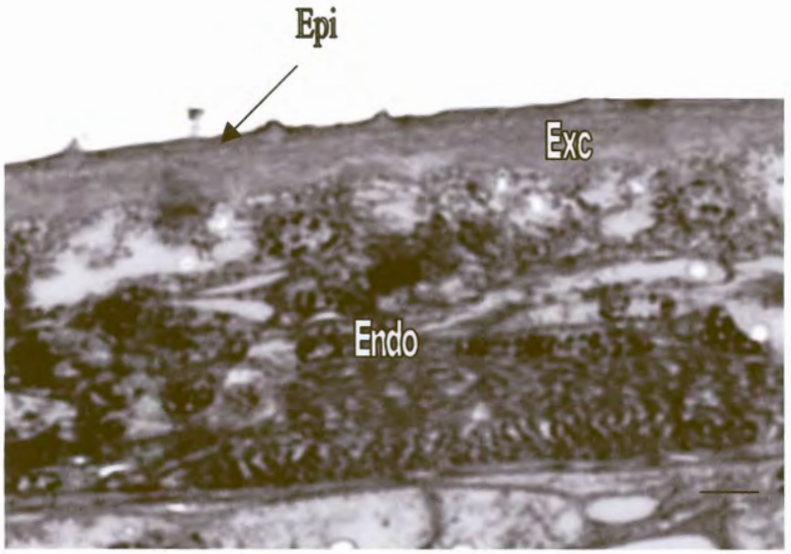

Fig. 12A. Cross section through the integument of untreated second instar Phthorimaea operculella (Zeller) larva, showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 51.2 μm.

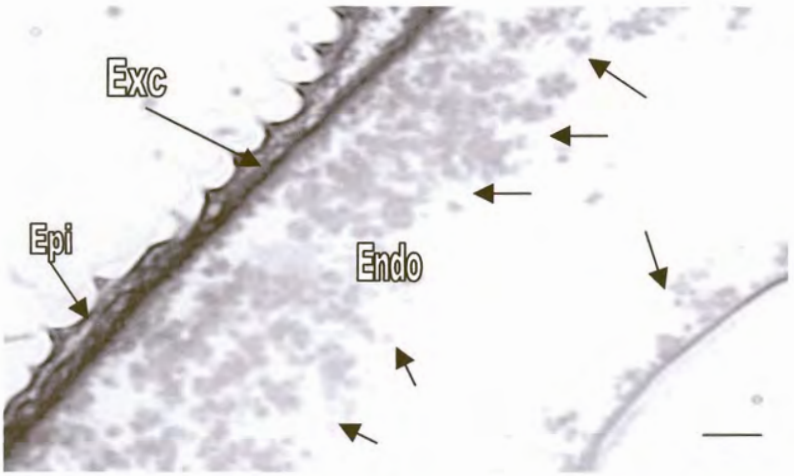

Fig. 12B. Distorted endocuticle of lufenuron-treated larva with scattered globular materials (arrows). Epi. = epicuticle; Exc = exocuticle; Endo = endocuticle.

Bar = 51.2 μm.

CHAPTER 4: EMBRYO-LARVICIDAL ACTIVITIES OF LUFENURON ON IMMATURE STAGES OF THE DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA (L.) (LEPIDOPTERA: YPONOMEUTIDAE).

4.1: INTRODUCTION

The diamondback moth, Plutella xylostella (L.) (Lepidoptera: Yponomeutidae) is one of the most destructive cosmopolitan pests of cruciferous crops (Shelton et al. 1993; Talekar & Shelton 1993). The diamondback moth is cosmopolitan in distribution because of its extreme adaptability to varied ecological and climatic conditions (Thomas & Ferguson 1989). In South Africa, climatic conditions are never unfavourable for the growth of diamondback moth populations and the moth is active throughout the year (Annecke & Moran 1982).

The host plants of the diamondback moth includes cultivated crucifers such as cabbage, mustard, broccoli, cauliflower, brussels sprouts and turnips as well as uncultivated or alternate host plants such as wild mustard, wild radish, pepper cress and water cress (Jones & Jones 1981; Annecke & Moran 1982; Reddy et al. 1996). The newly emerged larval instars mine the leaves of the host plant, leaving the upper epidermis intact, while the older instars feed on the tissues leading to the skeletonization of the leaf blades (Jones & Jones 1981). Most of the tissues between the major veins are removed thus causing a severe damage to the plants (Jones & Jones 1981; Thomas & Ferguson 1989).

In tropical and subtropical countries, crucifer production have been severely affected by the destruction caused by the diamondback moth and it is estimated that 42.2 million tons of crucifer is destroyed annually all over the world (Shelton et al.1993; Kfir 1996). The greatest damage occurs when the infestation takes place in young plants (Rivnay 1962; Workman et al. 1980). Sears et al. (1985) reported that the larval instars may move into newly formed cabbage head, or the larvae may develop on wrapper leaves first, then invade the head, thus making it more difficult to control the pest. In older plants, when diamondback moth larvae invade the outer leaves, the quality of the crop is reduced and this often leads to poor market returns for the farmer (Sun et al. 1995).

The adult moth is grey in colour with three pale triangular markings on the inner edge of each fore-wing that form a diamond pattern when the wings are folded (Fig. 4) (Annecke & Moran 1982). Mating occurs a day or two after adult emergence and the female oviposits small, yellowish-green eggs singly or in small clusters on the underside of the leaves of the host plant (Rivnay 1962; Annecke & Moran 1982). The eggs are oval in shape and the incubation period lasts about 3 – 4 days at temperatures of 26 – 29 ^{o}C (Rivnay 1962). The eggs hatch into small, pale-green larvae about 2 mm in length and the neonates start feeding on the internal tissues of the leaves immediately after emergence (Rivnay 1962; Annecke & Moran 1982). The larva is very active and when disturbed, it wriggles rapidly and may fall off the leaf on which it is feeding, remaining suspended to it by a silken thread (Jones & Jones 1981). The feeding period lasts for about 7 – 10 days under favourable temperatures and when fully grown, the larva constructs a delicate open cocoon made of a fine network of white, silken threads for the development of the pupal

stage (Jones & Jones 1981; Annecke & Moran 1982). After one or two days of quiescence in the prepupal stage, a light yellow or green pupa is formed (Jones & Jones 1981). Pupation may occur on the underside of the leaves of the plants or beneath fallen leaves and debris on the ground beneath the plant (Annecke & Moran 1982). The developmental period from eggs to adult is about 2 – 3 weeks under constant temperatures of 28 °C and the newly emerged adult is active mainly in the evenings (Jones & Jones 1981; Annecke & Moran 1982).

Control of the diamondback moth involves the use of insecticides such as organophosphates, organochlorines, carbamates, pyrethroids and acylureas (Ismail & Wright 1991). Biological control measures involving the use of biopesticides such as Bacillus thuringiensis, as well as biocontrol agents such as parasitoids have been used in different parts of the world to control the diamondback moth (Thomas & Ferguson 1989; Kfir 1996; Selman et al. 1997).

A particular problem with the diamondback moth is its marked ability to develop resistance to insecticides (Shelton et al. 1993). Resistance to organochlorines, organophosphates, carbamates, pyrethroids, and some acylureas as well as to biological pesticides such as B. thuringiensis has been reported from different parts of the world (Denholm & Rowland 1992; Shelton et al. 1993; Tabashnik 1994; Perez & Shelton 1996; Lasota et al. 1996; Baker & Kovaliski 1999). The recent development of resistance to some acylureas and B. thuringiensis products is of great concern, as these products are

usually regarded as compatible with natural enemies in integrated pest management programs (Ismail & Wright 1991).

This study focused on the embryo-larvicidal and residual activities of lufenuron on immature stages of the diamondback moth. Histological studies were also carried out to evaluate the effects of this chitin synthesis inhibitor on the newly emerged larval instars.

4.2: MATERIALS AND METHODS

4.2.1: Insects and chemicals

Adult diamondback moths were collected from the colony maintained at the Plant Protection Research Institute in Pretoria. The moths were put in oviposition chambers (25 cm high and 11 cm diameter) and fed 10 % sugar solution (Moore et al. 1992). The chambers were labelled and kept at 28 ± 1 °C and a photoperiod of 12L: 12D for oviposition. Eggs laid on waxed paper discs were collected every 12 h for bioassays. Lufenuron 050 EC as an emulsifiable concentration containing 50 g a.i./l was supplied by Novartis SA (Pty) Ltd. Dilutions of 0.02, 0.04; 0.08 and 0.12 g a.i./l were prepared with distilled water. Fresh dilutions were made immediately prior to each assay and controls were treated with distilled water.

4.2.2: Bioassay procedures

4.2.2.1: Embryo-larvicidal bioassay

Two bioassays were carried out to determine if the susceptibility of diamondback moth eggs to lufenuron varied with changes in ages of the developing embryos.

Leaf-dip bioassay

Cabbage leaf discs (6 cm diameter) were dipped into appropriate test solutions for 30 seconds, air-dried and transferred to plastic cages (7 cm high by 12 cm diameter) (Ismail & Wright 1991; Shelton et al. 1993; Zhao & Grafius 1993; Edelson et al. 1997). Eggs (1 – 3 d old; 100 per leaf disc) were transferred from the waxed paper substrate to the treated cabbage leaf discs with a fine camel-hair brush. The brushes were cleaned in distilled water between each treatement to avoid contamination and each concentration plus its control was replicated four times. The plastic cages were covered with nylon netting and kept at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. The experiment was monitored daily and data on egg and first instar mortalities were determined with a stereomicroscope (16X) 48 h after the controls have hatched. First instars were identified by the width of the head capsules (Fauziah 1990).

Hatched larval instars were allowed to feed on the treated cabbage leaf discs for 2 days and the treated leaf discs were replaced by fresh clean untreated leaf discs (Ismail & Wright 1992; Perez et al. 1997). Surviving larval instars were reared to the adult stage on fresh untreated cabbage leaf discs and the experiment was monitored daily until adult emergence (Ismail & Wright 1992). The various forms of morphological deformities caused by lufenuron on the larval and post larval stages were also observed. Data on adult emergence were recorded 3 weeks post treatment.

Waxed paper bioassay

Discs (4 cm diameter) were cut from waxed paper (Cut-Rite, Reynolds Metal, Richmond, VA) and immersed into the appropriate test solutions for 30 seconds, air-dried and transferred to plastic cages (7 cm high by 12 cm in diameter) (Pons et al. 1999). Eggs (1-3 d old; n = 400 per age group) were transferred to the treated waxed papers with a fine camel-hair brush. Each concentration plus its control was replicated four times and the plastic cages were covered with a nylon netting and kept at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Data on egg and first instar mortality were recorded with a stereomicroscope (16X) 48 h after the controls have hatched. Surviving larval instars were reared to the adult stage on untreated cabbage leaves and the experiment was monitored daily until adult emergence. Data on adult emergence were recorded 3 weeks post treatment.

Data analyses

The embryocidal effect of lufenuron on developing embryos was calculated as the % of embryos that died in the eggs. Data on percentage mortality (egg and first instar mortalities) as well as the percentages of adult emergence, were corrected for natural mortality (Abbott 1925). Where appropriate, the data were arcsine transformed before analysis of variance (Statsoft 1995). Duncan's multiple range test was used to evaluate the differences between the treatment groups ($P = 0.05$) (Statsoft 1995; Rehimi & Soltani 1999).

4.2.2.2: Residual activity bioassay

Canola plants grown in plastic cups (4 cm high by 6 cm diameter) were sprayed to run-off with the appropriate test solutions (0.02, 0.04, 0.08 and 0.12 g a.i./l) using a hand held sprayer (Abro et al. 1988). Controls were sprayed with distilled water and the residues were allowed to age at room temperatures. To determine the duration over time (in days) that the residues of lufenuron will be effective against embryonic and post embryonic stages of the diamondback moth, eggs (1 – 3 d old; n = 400 per age group) were transferred from the oviposition substrates to marked sections on the leaves of the treated plants (Hofmeyr & Pringle 1998; Pons et al. 1999). Each concentration plus its control was replicated four times and the plants were kept at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Data on egg mortality were determined with a stereomicroscope (16X) 48 h after controls have hatched. Surviving larval instars were reared to the adult stage on the treated canola plants and data on percentage adult emergence were recorded 3 weeks post treatment.

Data analyses

Data on percentage mortality and adult emergence were corrected for natural mortality (Abbott 1925). Where appropriate, the data were arcsine transformed before analysis of variance (ANOVA) (Statsoft 1995). Duncan's multiple range test was used to evaluate the differences between the treatment groups (P = 0.05) (Statsoft 1995; Trisyono & Chippendale 1998).

4.2.2.3: Histological bioassay

Eggs (< 12 h old; n = 10) oviposited on waxed paper substrates were transferred to

treated (0.08 and 0.12 g a.i./l) cabbage leaf discs (6 cm diameter) in Petri dishes (10 cm diameter). Controls were transferred to untreated cabbage leaf discs and the Petri dishes were kept at 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Hatched larval instars were allowed to feed on the treated leaf discs for 2 days and the larvae were collected for histological studies. The larvae were decapitated and fixed in 2.5 % gluteraldehyde for 1 h, rinsed three times (15 minutes each) in 0.075M phosphate buffer, (pH 7.4 – 7. 6). Post fixation was carried out in 0.25 % aqueous osmium tetraoxide for 1 h at room temperatures and the tissues were rinsed three times in distilled water before dehydrating in graded ethanol series (50, 70, 90 %) for 15 minutes each. Further dehydration was carried out in 100 % ethanol (three changes, 15 minutes each) and the tissues were infiltrated with 30 % epoxy resin for 1 h . Further infiltration with 60 and 100 % epoxy resin were carried out for 1 and 4 h respectively (Kushida 1974). The tissues were polymerized at 65 ° C for 24 h and semi-thin sections (2 μm) were cut onto gold grids and stained with toludine blue (Trump et al. 1961). Stained sections were viewed under a Nikon Optiphot light microscope and photographed with a Nikon digital camera (DXM 1200).

4.3: RESULTS AND DISCUSSION

4.3.1: Embryo-larvicidal bioassays.

Embryocidal effects.

Low embryocidal effect was recorded in the cabbage leaf disc bioassay as well as in the waxed paper bioassay (Tables 1 and 2). Embryonic development was not affected in both bioassays and egg mortality was low (< 6.9 %). Also, the incubation period in both

assays were not affected and larval hatch in both controls and treated was completed between 3 – 4 days after oviposition. This result is not in agreement with reports from previous studies on the embryocidal activities of acylureas (Ascher et al.1986; Purcell & Granett 1986; Tuttle & Ferro 1988; Hayens & Smith 1993). Kohyama (1986), found that the exposure of diamondback moth eggs to different concentrations of teflubenzuron led to a significant inhibition in egg hatch, while Perng et al.(1988) found that the embryos of diamondback eggs exposed to different concentrations of teflubenzuron were able to develop to the black head stage but were unable to break out of the chorion, thus leading to a high egg mortality. Ascher et al. (1980), attributes the inconsistency in ovicidal bioassay results to the differences in the crystal sizes of the particles of these chemicals after dilutions, while Purcell and Granett (1986) suggested that the differences in the sensitivity of eggs to acylureas could be caused by the differences in the pharmacodynamics of the acylurea used in the bioassay.

Larvicidal effects

In the cabbage leaf bioassay, first instar mortality was high (61.8 – 80.5 %) at the higher concentrations (0.08 and 0.12 g a.i./l) used, compared to the low (< 1.3%) first instar mortality recorded at similar concentrations in the waxed paper bioassay (Tables 3 and 4). In the cabbage leaf bioassay, the ingestion of lufenuron residues from the treated cabbage leaf discs as well as the exploratory activities of the larvae over the treated leaf surfaces could have led to the high mortality of first instars (Pons et al. 1999). On the other hand, larval instars emerging from the inert waxed paper discs, had access to clean

untreated cabbage leaves as food and this could have contributed to the low larval mortality at the first instar stage.

This result is consistent with the report of Pons et al. (1999), who found that contact exposure of larval instars to residues on inert materials such as plastic Petri dishes and waxed paper discs did not affect subsequent larval survival as much as exposure to residues on natural substrates such as leaves and fruits. Also, newly emerged larval instars have been shown to do more exploratory feeding on natural substrates than on plastic or other artificial surfaces and this often leads to an increase in the pesticide residues in the body of the larvae (Jackson 1982; Pons et al. 1999). Knapp & Cilek (1988), reported that the mortality of larval instars of the housefly, Musca domestica (L.) (Diptera: Muscidae) was directly proportional to the duration of exposure to triflumuron, while Grosscurt & Jongsma (1987), found that the exposure of larval instars of some insect species to acylureas for a brief period often led to low larval mortalities.

Mortality of larval instars in the cabbage leaf bioassay was highest during ecdysis and most of the dead larvae were dark and shrivelled and the old exoskeletons were still attached to the lower part of their abdomen (Figs. 1 and 2). Prior to death, larval instars exposed to the treated cabbage leaf discs remained motionless and were unable to feed on the cabbage leaves. The inability to feed on the leaf discs could have been caused by the displacement of the mandibles and labrum or the blockage of the gut (Retnakaran et al. 1985). Fogal (1977), reported that the incomplete clearance of the larval gut at moult as well as the reduced amount of chitin in the newly moulted mouth parts could prevent the

larval instars from feeding after ecdysis. Similar reports of feeding deterrence caused by acylureas have been reported by Peleg (1983). Tuttle & Ferro (1988), found that the anti-feedant effect caused by acylureas could provide a relief from pest pressures in the field, while Nagesh & Verma (1997), reported that the application of lufenuron to cabbage farms in India led to a great increase in cabbage yield as well as a high percentage mortality of the diamondback moth larvae. Also, studies by Kim et al. (2000) have shown that lufenuron was highly effective (> 80 % efficacy) against the diamondback moth larvae in South Korea.

Larval development to the pupal and adult stages occurred after a prolonged delay in the lufenuron-treated leaf bioassay. In contrast, there was no delay in the development of larval instars after emergence from the waxed paper discs. This delay in larval development is consistent with the studies of Ismail & Wright (1991), who found that a significant proportion of diamondback moth larvae exposed to acylureas were able to survive the next larval moult, but subsequently appeared to feed and develop at a much slower rate than larval instars that emerged from control experiments.

Post-larval effects

In the cabbage leaf bioassay, only few larval instars were able to develop to the pupal and adult stages after emerging from lufenuron-treated leaf discs, and a high number of these post larval stages, died at the larval-pupal (prepupal) stage (Fig. 3). There were only few records of death at the prepupal stage in the waxed paper as well as control bioassays. Cumulative mortalities at the larval, prepupal and pupal stages led to a very

low (< 3 %) adult emergence in the lufenuron-treated leaf bioassay compared to the high (> 74.5 %) adult emergence recorded in the waxed paper bioassay (Figs. 5 and 6).

The production of sublethal chronic effects such as delayed larval growth, reduced larval movement and feeding deterrence in the lufenuron-treated leaf bioassay suggests that if lufenuron is applied before larval hatch or during the early larval instar stage, the amount of damage caused by the diamondback moth could be greatly reduced.

4.3.2: Residual activity bioassay

The four concentrations of lufenuron used in this study had no effect on embryonic development. Eggs treated at the white stage (1 d old) were able to develop to the black head stage and larval hatch was high in all the residue ages (Tables 5 – 7). The low egg mortality recorded in all the egg age groups could have been caused by the weak contact activity of lufenuron (Anonymous 1997). This result is consistent with the studies of Perng et al. (1988) who found that chlorfluzuron and diflubenzuron had practically no effect on the eggs of the diamondback moth.

Larval emergence from both treated and untreated canola plants was completed 3 – 4 days after oviposition, but the ingestion of lufenuron-treated leaves by the neonates led to a high larval mortality at the first instar stage. Also, mortality at subsequent larval and post larval stages was high. Smith et al. (1985), reported that the exposure of the rice water weevil, Lissorhoptrus oryzophilus, (Coleoptera: Curculionidae) to diflubenzuron-treated foliage led to high larval mortality as well as a great reduction in damage to the

rice plants. In this study, the amount of damage to lufenuron-treated canola plants was very low compared to the numerous holes or windows on untreated canola leaves.

The residual activity of lufenuron against larval and post-larval stages of the diamondback moth did not decrease over the 10 days trial period and this duration of effectiveness agrees with reports from previous studies (Herbert & Harper 1985; Marshall et al. 1988). Kim et al. (2000) and Nagesh & Verma (1997), reported a persistence of up to 14 days against larval stages of the diamondback moth exposed to different concentrations of lufenuron. Very low adult emergence (< 1 %) was recorded in the lufenuron-treated experiments compared with the high (> 94.5 %) adult emergence in the control experiment (Figs. 7 – 9). Based on the low adult emergence as well as the high larval mortality recorded in all the concentrations used in this bioassay, it appears that lufenuron could provide longer residual control of the diamondback moth in IPM programs.

4.3.3: Histological bioassay

Under the light microscope, the integument of untreated diamondback moth larvae consists of a well-defined epicuticle, exocuticle and a well arranged endocuticle (Fig. 10A). In the lufenuron-treated integument (Fig. 10B), the thin epicuticle was not affected by the lytic activities of lufenuron, but the exocuticle appeared partially eroded. The epicuticle does not contain chitin and this could be the reason why it was not affected by lufenuron. This result on the impact of lufenuron on the epi and exocuticular layers is in agreement with the studies of Perez-Farinos et al. (1998) and Dean et al. (1999).

In the endocuticle of lufenuron-treated integument, there were clear symptoms of cellular degeneration and disorganization as well as the presence of scattered globular materials. In contrast, there were no cellular degenerations or distortions of the endocuticular layer in the integument of control larvae, and this suggests that the lamellar structure of the procuticle was not affected by lufenuron (Filshie 1982; Perez-Farinos et al. 1998; Dean et al. 1999). The lamellar structure reflects a regular pattern of deposition of the chitin-protein layers and the lack of this structure could lead to an alteration in the deposition process and this may in turn lead to the death of the embryo prior to eclosion or immediately after moulting (Leopold et al. 1985; Retnakaran & Wright 1987; Perez-Farinos et al. 1998). The migration or movement of cytoplasmic contents into the procuticle as seen in lufenuron-treated integument could be as a result of the destruction of the epidermal layer due to the lytic activities of lufenuron. Similar results have been observed in the larval instars of other insect species (Binnington et al. 1987; Hassan & Charnley 1987; Perez-Farinos et al. 1998).

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Table 1. Mortality of different-aged eggs of Plutella xylostella (L.) after exposure to lufenuron-treated leaf discs.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	6.8 ± 2.4a	4.3 ± 1.3a	4.5 ± 1.3a	5.4 ± 2.2a	5.7 ± 2.0a
2	2.7 ± 1.7a	3.6 ± 0.8a	2.8 ± 1.6a	6.9 ± 2.5a	4.9 ± 1.6a
3	3.5 ± 1.7a	2.9 ± 1.4a	5.2 ± 2.9a	3.1 ± 1.8a	3.6 ± 2.5a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 2. Mortality of different-aged eggs of Plutella xylostella (L.) after exposure to treated waxed paper discs.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	3.1 ± 1.3a	4.0 ± 2.3a	3.6 ± 3.5a	7.2 ± 5.9a	5.5 ± 3.6a
2	5.3 ± 3.3a	4.2 ± 5.0a	4.4 ± 5.4a	4.2 ± 4.3a	3.5 ± 5.3a
3	1.5 ± 0.9a	3.6 ± 2.4a	5.7 ± 5.2a	2.6 ± 1.5a	5.4 ± 3.3a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried before statistical analysis.

Table 3. Larvicidal activity of lufenuron against first instars of Plutella xylostella (L.) after emergence from eggs placed on treated cabbage leaf discs.

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	4.5 ± 1.3a	63.0 ± 2.0c	65.5 ± 3.4c	80.5 ± 2.9c	72.5 ± 2.5c
2	4.5 ± 1.3a	37.0 ± 1.7b	73.4 ± 1.8c	76.8 ± 3.0c	73.0 ± 1.9c
3	1.8 ± 3.5a	38.5 ± 1.0b	69.4 ± 1.8c	72.0 ± 1.1c	77.0 ± 3.2c

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 4. Larvicidal activity of lufenuron against first instars of Plutella xylostella (L.) after emergence from eggs placed on treated waxed paper discs.

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	0.3 ± 0.2a	2.0 ± 0.9a	0.8 ± 0.7a	1.3 ± 0.6a	0.5 ± 0.3a
2	0.5 ± 0.3a	1.3 ± 1.0a	1.0 ± 0.4a	7.0 ± 0.4a	0.3 ± 0.2a
3	0.0 ± 0.0a	0.5 ± 0.3a	0.5 ± 0.3a	0.3 ± 0.2a	0.5 ± 0.3a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 5. Residual toxicity of lufenuron to 1 day old Plutella xylostella (L.) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	0.5 ± 0.3a	1.6 ± 2.9a	4.1 ± 1.9a	3.6 ± 3.4a	5.6 ± 4.2a
2	2.3 ± 1.7a	3.5 ± 3.4a	1.0 ± 2.3a	2.1 ± 2.4a	1.6 ± 2.9a
3	2.0 ± 2.3a	3.1 ± 3.4a	3.6 ± 3.5a	4.2 ± 3.8a	4.1 ± 3.8a
4	1.0 ± 2.2a	2.5 ± 3.9a	3.6 ± 3.5a	5.1 ± 4.2a	5.6 ± 3.8a
5	2.8 ± 2.9a	4.7 ± 3.3a	2.9 ± 3.5a	3.1 ± 3.0a	3.4 ± 3.6a
6	1.5 ± 2.0a	1.6 ± 2.8a	3.1 ± 3.1a	3.7 ± 3.5a	2.1 ± 2.4a
7	1.8 ± 1.8a	2.1 ± 3.4a	3.8 ± 3.6a	2.6 ± 3.9a	3.1 ± 3.0a
8	3.8 ± 2.9a	3.8 ± 2.0a	0.5 ± 0.3a	3.7 ± 3.4a	4.7 ± 3.6a
9	0.5 ± 0.3a	1.6 ± 2.9a	2.6 ± 2.9a	2.1 ± 2.4a	3.6 ± 3.6a
10	2.3 ± 1.8a	1.5 ± 2.0a	2.0 ± 2.1a	3.1 ± 3.0a	2.6 ± 2.9a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 6. Residual toxicity of lufenuron to 2 day old Plutella xylostella (L.) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	2.0 ± 3.4a	3.8 ± 1.8a	2.1 ± 2.4a	0.6 ± 2.0a	2.3 ± 3.0a
2	1.5 ± 2.9a	2.1 ± 2.2a	3.2 ± 3.4a	1.6 ± 1.9a	0.5 ± 2.0a
3	1.0 ± 2.3a	3.6 ± 4.4a	6.1 ± 5.9a	1.5 ± 3.5a	2.0 ± 3.4a
4	0.0 ± 0.0a	4.5 ± 3.6a	4.0 ± 3.4a	2.0 ± 3.3a	1.0 ± 2.8a
5	3.0 ± 4.1a	3.7 ± 3.4a	1.6 ± 2.9a	3.6 ± 3.4a	3.2 ± 4.1a
6	4.0 ± 4.2a	4.2 ± 3.6a	1.5 ± 2.9a	2.2 ± 2.4a	1.6 ± 2.9a
7	1.5 ± 2.9a	2.6 ± 3.9a	2.6 ± 3.9a	3.7 ± 4.4a	1.6 ± 2.9a
8	2.0 ± 2.4a	1.4 ± 2.1a	3.2 ± 3.0a	1.0 ± 2.5a	2.1 ± 2.4a
9	1.2 ± 2.8a	1.5 ± 2.9a	3.1 ± 4.1a	2.0 ± 3.5a	2.6 ± 3.7a
10	2.0 ± 3.4a	2.1 ± 2.4a	2.6 ± 3.9a	2.6 ± 4.1a	3.1 ± 4.1a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 7. Residual toxicity of lufenuron to 3 day old Plutella xylostella (L.) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	1.5 ± 3.6a	2.6 ± 3.4a	2.0 ± 3.4a	1.1 ± 2.8a	3.1 ± 3.0a
2	0.5 ± 2.0a	0.5 ± 2.0a	2.6 ± 2.5a	1.7 ± 3.5a	0.5 ± 2.0a
3	1.6 ± 2.9a	3.8 ± 4.4a	4.1 ± 3.6a	1.6 ± 2.9a	0.5 ± 2.0a
4	1.5 ± 3.5a	4.6 ± 3.9a	3.1 ± 4.7a	3.0 ± 3.1a	2.0 ± 2.5a
5	3.5 ± 4.4a	3.2 ± 4.1a	2.6 ± 2.7a	3.5 ± 3.3a	2.6 ± 3.9a
6	1.5 ± 3.5a	1.6 ± 2.9a	1.0 ± 2.7a	2.1 ± 3.4a	0.5 ± 2.1a
7	2.6 ± 2.0a	1.5 ± 2.9a	1.5 ± 2.9a	1.6 ± 2.9a	0.5 ± 2.0a
8	0.5 ± 2.0a	1.5 ± 2.8a	2.1 ± 3.4a	2.1 ± 3.4a	1.1 ± 2.3a
9	0.0 ± 0.0a	0.5 ± 2.0a	0.5 ± 2.0a	1.0 ± 2.8a	1.1 ± 2.3a
10	0.0 ± 0.0a	1.5 ± 3.0a	1.5 ± 2.9a	1.0 ± 2.8a	0.0 ± 0.0a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis

Fig. 1. Effects of lufenuron on second instar Plutella xylostella (L) larvae: Dark, shrivelled larvae that emerged from eggs placed on lufenuron-treated leaf discs (Top and bottom); Normal larva that emerged from untreated leaf discs (middle). Arrows = old cuticle.

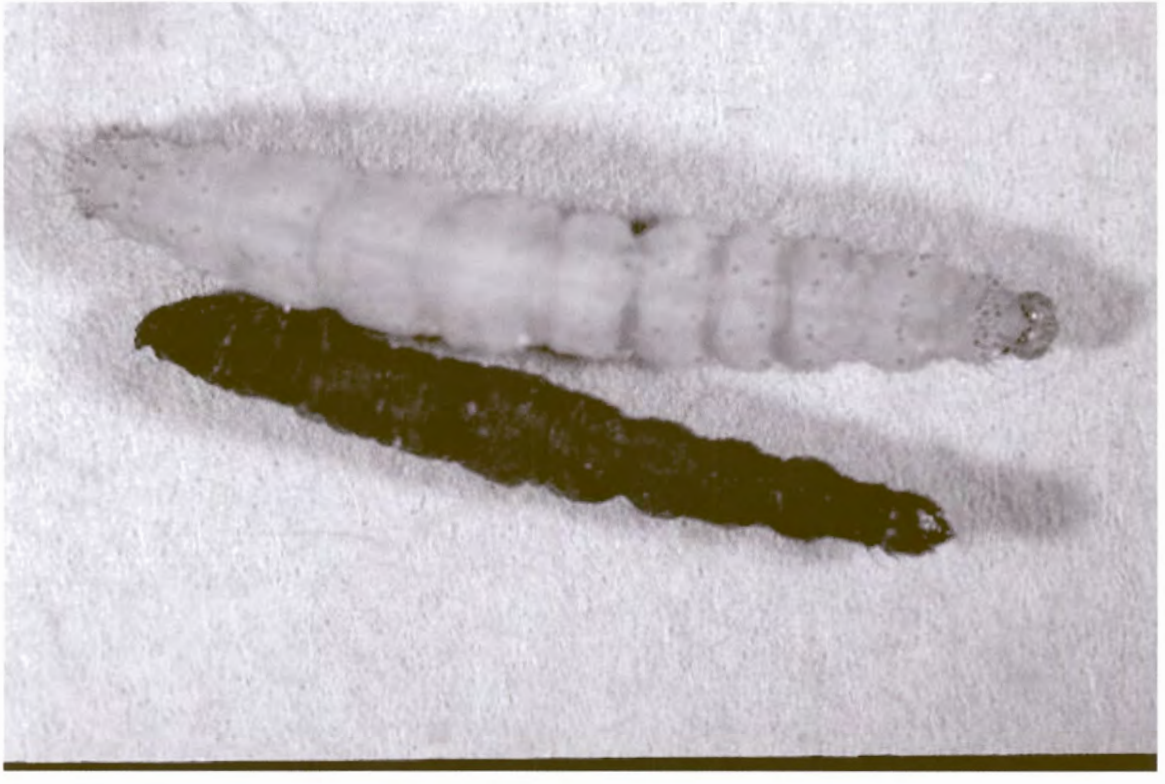

Fig. 2. Effects of lufenuron on final larval instars of Plutella xylostella (L): Top = normal larva that emerged from untreated leaf disc; Bottom = dark larva that emerged from lufenuron-treated leaf disc.

Fig. 3. Morphological deformity caused by lufenuron at the larval-pupal stage: Top = normal pupa that emerged from control experiment; Bottom = dead prepupa that emerged from lufenuron-treated cabbage leaf discs; (pupa and prepupa were removed from the open work cocoon)

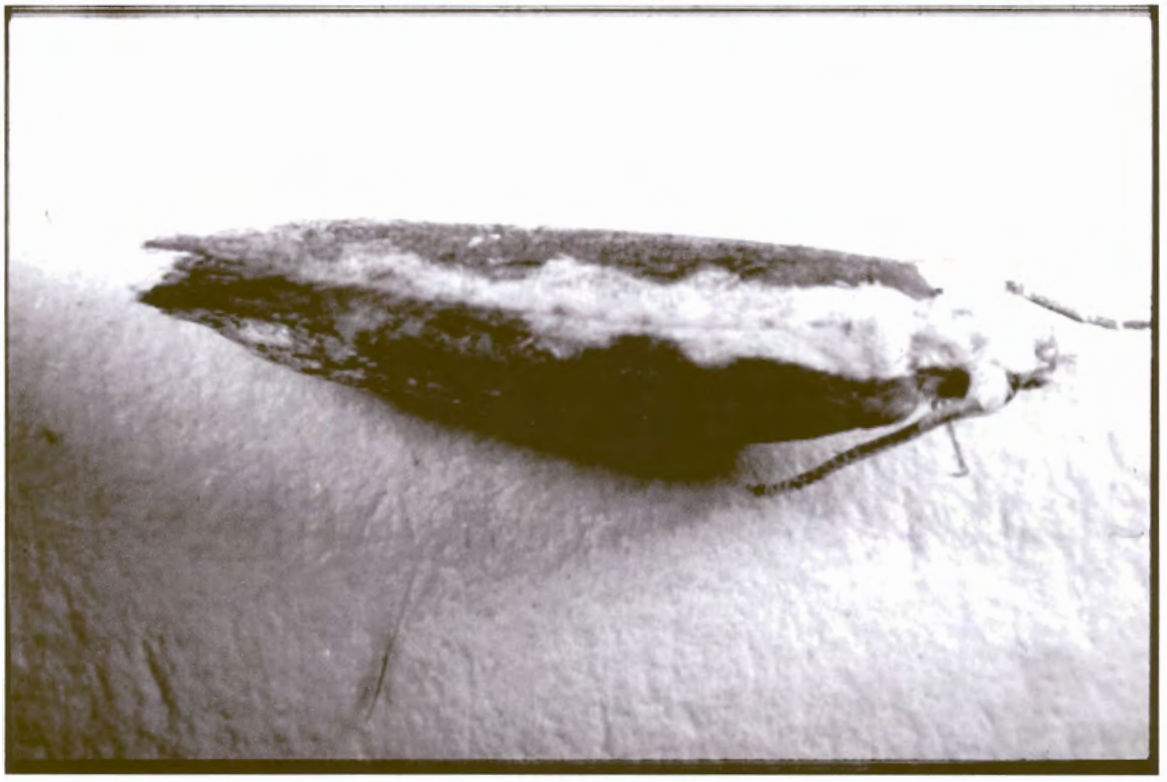

Fig. 4. Adult diamondback moth.

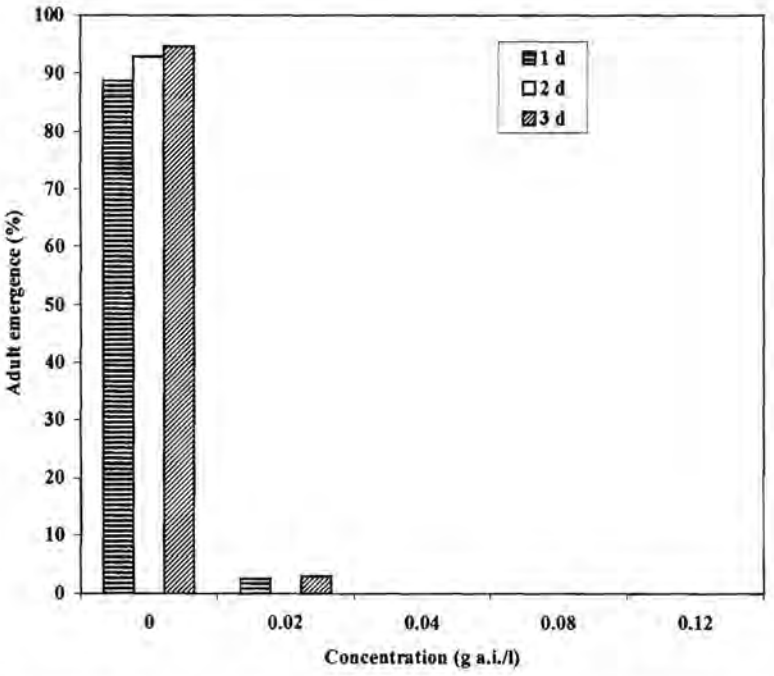

Fig. 5. Plutella xylostella (L.) adult emergence from treated cabbage leaf discs.

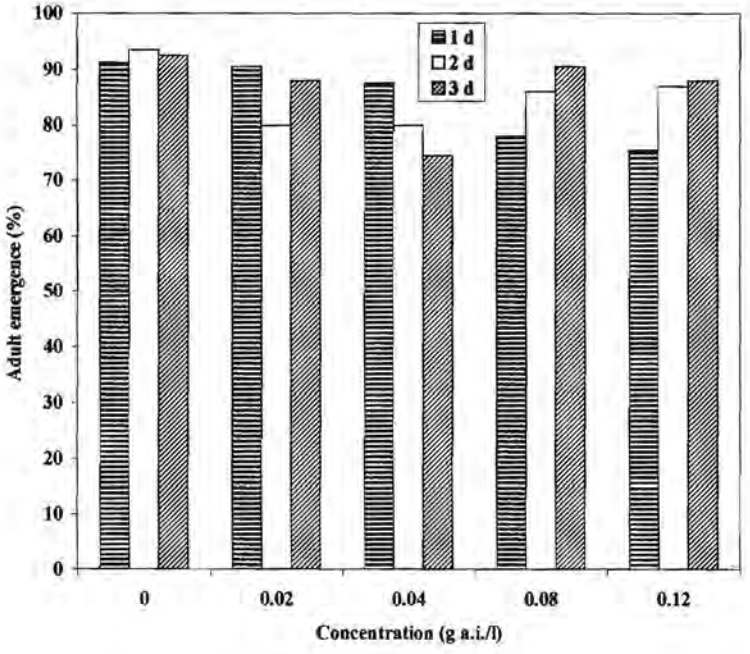

Fig. 6. Plutella xylostella (L.) adult emergence from treated waxed paper discs.

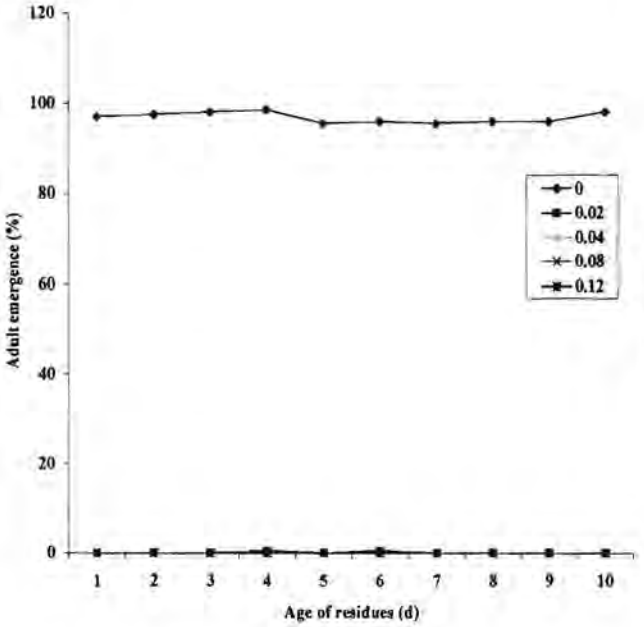

Fig. 7. Percentage emergence of Plutella xylostella (L.) adults from 1 day old eggs in the residual activity bioassay.

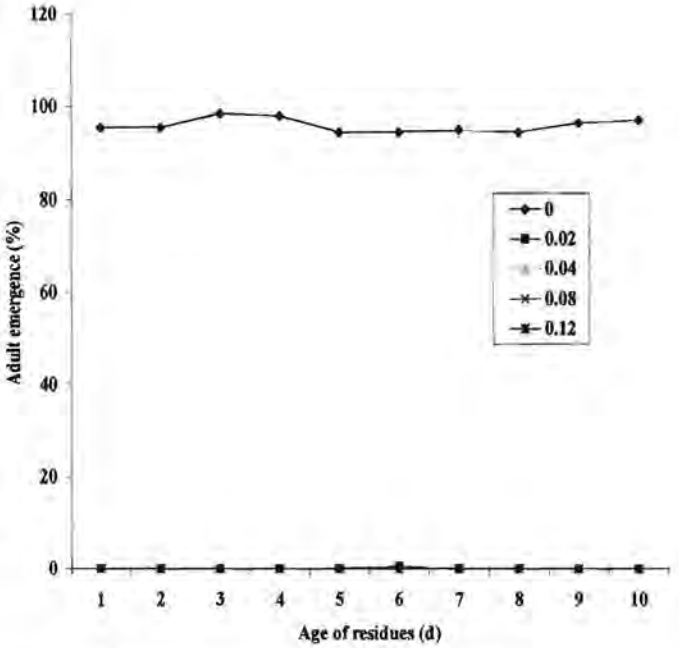

Fig. 8. Percentage emergence of Plutella xylostella (L.) adults from 2 day old eggs in the residual activity bioassay.

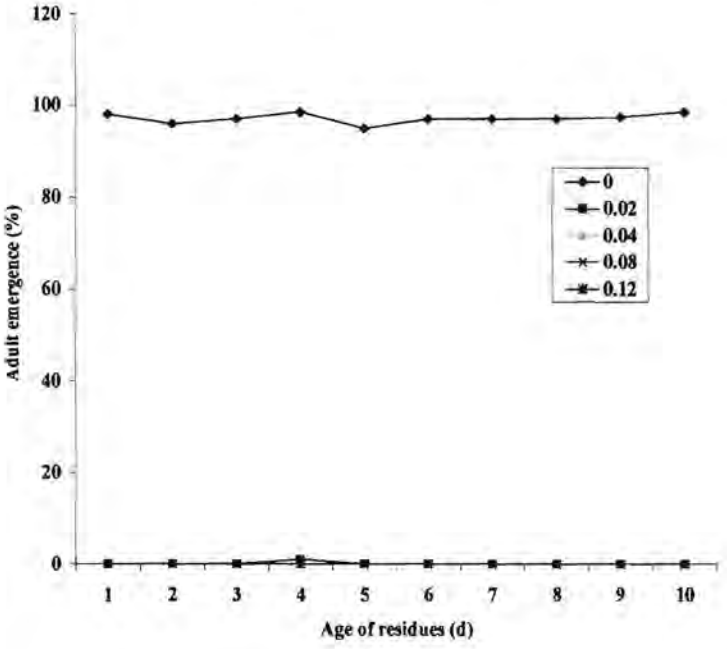

Fig. 9. Percentage emergence of Plutella xylostella (L.) adults from 3 day old eggs in the residual activity bioassay.

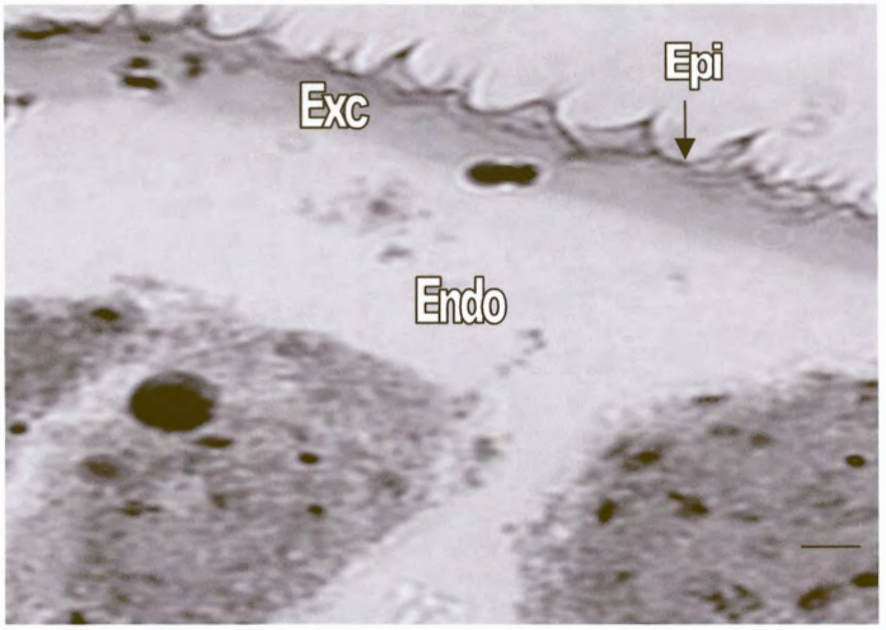

Fig. 10A. Cross section of the integument of untreated Plutella xylostella (L.) larva showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle.
Bar = 46.5 μm.

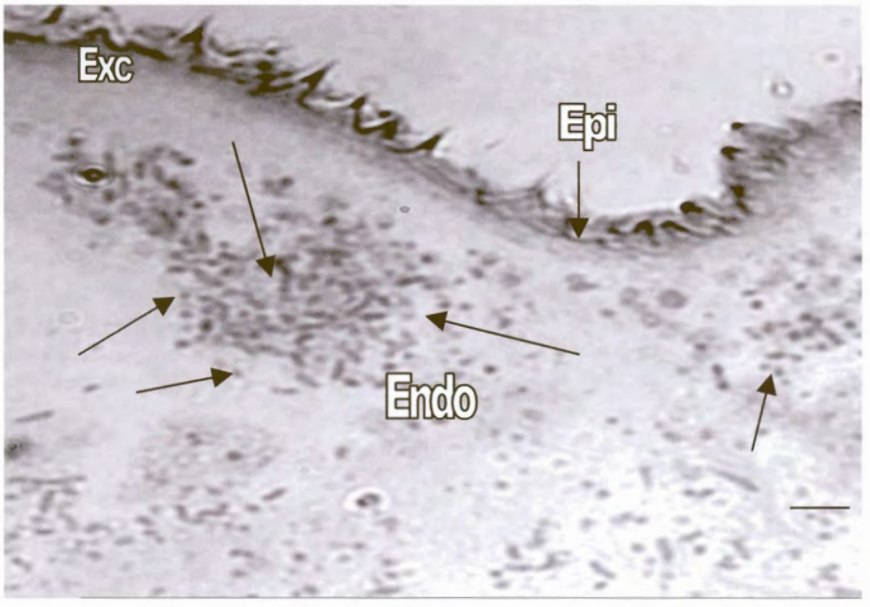

Fig. 10B. Cross section of the integument of lufenuron-treated second instar Plutella xylostella (L.) larva showing distorted endocuticle with scattered globular materials (arrows). Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 46.5 μm.

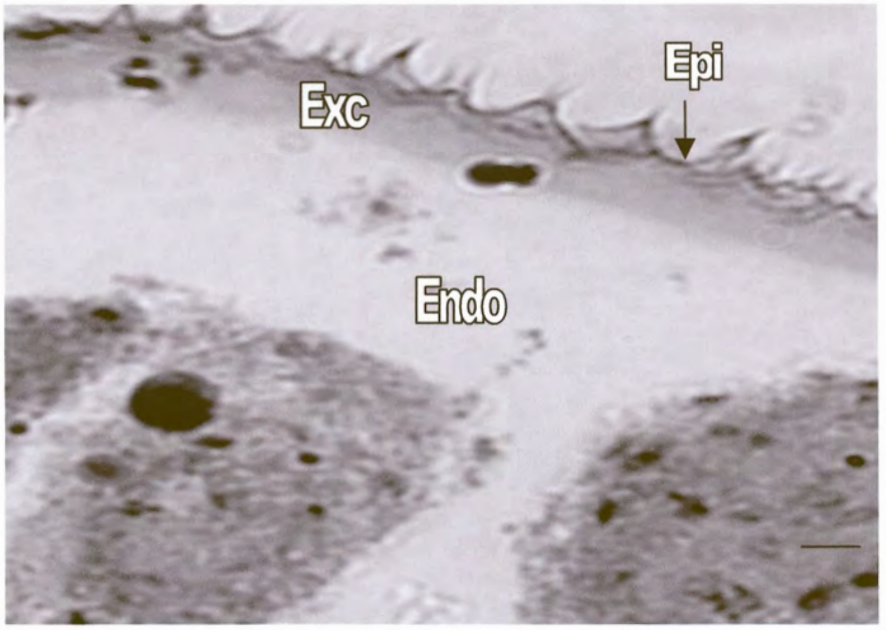

Fig. 10A. Cross section of the integument of untreated Plutella xylostella (L.) larva showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle.
Bar = 46.5 μm.

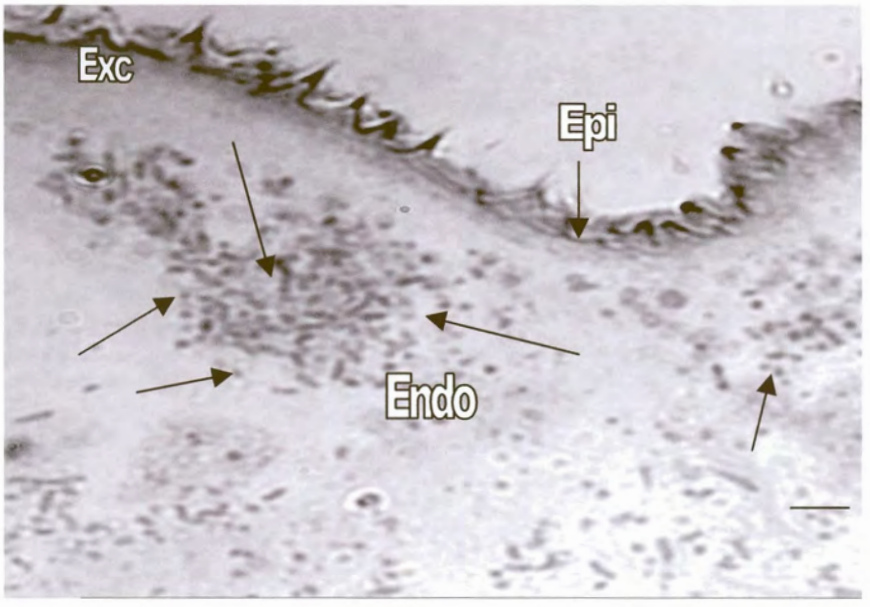

Fig. 10B. Cross section of the integument of lufenuron-treated second instar Plutella xylostella (L.) larva showing distorted endocuticle with scattered globular materials (arrows). Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 46.5 μm.

CHAPTER 5: EMBRYO-LARVICIDAL ACTIVITIES OF LUFENURON ON IMMATURE STAGES OF THE FALSE CODLING MOTH, CRYPTOPHLEBIA LEUCOTRETA (MEYRICK) (LEPIDOPTERA: TORTRICIDAE)

5.1: INTRODUCTION

False codling moth, Cryptophlebia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is widely distributed in the sub-saharan areas of Africa and the surrounding islands of the Indian and Atlantic oceans (Annecke & Moran 1982; CIBC 1984). In West Africa, it is a major pest of cotton and citrus fruits, while in Zimbabwe and Central Africa it attacks cotton and sorghum (Newton 1998; Martin et al. 2000). It has also been recorded as a pest of maize in Central Africa, but in Southern Africa, it is a major pest of oranges, peaches, apricots, almonds, olives, walnuts, macademias and guavas (Annecke & Moran 1982; Newton 1998). In Southern Africa, the false codling moth overlaps in distribution and host range with two relatives: the litchi moth, Cryptophlebia peltastica (Meyrick) and the macadamia nut borer, Cryptophlebia batrochopa (Meyrick) (Newton & Crause 1990). On citrus, guava and macadamia, the eggs of the false codling moth are oviposited directly on the fruit surface and the emerging larvae penetrates the fruits where they cause fruit decay, premature ripening and abscission (Newton 1989). Infested fruits may be shed as early as November, when they are about 15 – 20 mm in diameter (Annecke & Moran 1982). In peaches, the eggs are laid on the leaves, close to the fruits and the newly emerged larvae bore into the fruits where they feed close to the stone of the fruit (Annecke & Moran, 1982, Newton 1998). The packing of infested fruits causes post-harvest decay and this may lead to export rejection of the fruits because of international

phytosanitary regulations (Begemann & Schoeman 1999). Although, a precise evaluation of the extent of crop damage is extremely difficult to make due to variable infestation from tree to tree, orchard to orchard and from season to season, Schwartz (1981), estimated damage to citrus crop to be as high as 90 % in severe attacks. Differences in crop damage have been noted on the various orange types, with the Naval citrus cultivars being the most susceptible variety in South Africa (Newton 1998; Begemann & Schoeman 1999). Estimates of long term crop losses have been reported from Letaba and Zebediela estates in the Northern Province, as well as in Richmond, (Kwazulu Natal Province), Nelspruit (Mpumalanga Province) and Rustenberg (North West Province) (Schwartz 1981; Newton 1986; Begemann & Schoeman 1999, Newton 1998). Crop losses occur at any time from December to January until crop harvest (Hofmeyr & Pringle 1998).

The broad range of host plants, together with the mild tropical and subtropical climates, makes the false codling moth an all-year round pest in different parts of Africa (Newton 1998). Adult moths will breed throughout the year if there is a continous supply of fruits, and the peak infestation period is between November-December and February-March (Schwartz 1981). Adult false codling moths are dark brown in colour with black markings and white dots on the forewings (Fig. 3) (Pinhey 1975; Newton 1998). The moths are active at night and mating occurs shortly after emergence (Newton 1998). The females lay about 100 – 400 eggs near or on the fruits (Newton 1998). The fruit conditions have been shown to have some impact on the oviposition pattern, with more eggs been laid on early ripening fruits as well as on damaged fruits (Newton 1989). False

codling moth eggs measure about 1 mm diameter and are flat, oval and translucent with a reticulate sculpture (Newton 1998). Larval hatch occurs in about 5 – 7 days at 28 ± 1 °C and the newly emerged larval instars are creamy-white with brown heads (Newton 1998). In the field, only one larva completes its development in a fruit and this could be due to the cannibalistic behaviour of the neonates (Schwartz 1981). However, in laboratory cultures, as many as 100 or more larvae could be raised in a single container (Newton 1998). Hatched first instars burrow into fruits where they feed on the inner rind and the pulp, but rarely on the wet juicy flesh (Pinhey 1975). When green fruits are attacked, they ripen prematurely and fall to the ground and this often lead to the control measure known as 'orchard sanitation' which involves the removal and destruction of infested fruits on the trees and on the ground. This method, helps to kill the larvae inside the fruits before they emerge to pupate in the soil (Annecke & Moran 1982; Newton 1998). As the larva ages, the colour changes to pink-red and at maturity, the fully grown larva measures about 15 – 20 mm in length (Annecke & Moran 1982; Newton 1998). A matured larva passes through 5 larval instars and prior to pupation, it leaves the fruit and spins a silk cocoon on the surface of the soil where it pupates (Pinhey 1975). In laboratory cultures, when the larva is ready to pupate, it leaves the artificial diet or orange fruit and spins a cocoon inside the cotton wool plug or under the filter paper. Pupation lasts about 3 weeks in summer, but longer (3 – 4 months) in winter, without a quiescent period or diapause (Annecke & Moran 1982; Newton 1998). According to Schwartz (1981), under constant conditions of 27 °C and 70 % RH, false codling moth development takes about 23 – 26 days from egg to adult and there is no diapause.

False codling moth control is expensive and very difficult because the newly emerged larval instars penetrate the fruits immediately after hatch, thus escaping the toxic effects of pesticides and natural enemies (Newton 1998). Integrated control measures have been recommended for the control of this pest, and these measures involve the use of cultural methods (e.g. orchard sanitation), biological control methods as well as the use of chemical insecticides (Newton 1998). In South Africa, the two insect growth regulators registered for the control of the false codling moth on citrus are teflubenzuron and triflumuron, but resistance to these chitin synthesis inhibitors have been observed in the Citrusdal and Swellendam areas (Krause et al. 1996; Hofmeyr & Pringle 1998).

This study focused on the embryo-larvicidal and residual activities of lufenuron on immature stages of the false codling moth. Also, histological studies were carried out to determine the impact of lufenuron on the newly emerged larval instars.

5.2: MATERIALS AND METHODS

5.2.1: Insects and chemicals

False codling moth pupae collected from the Zebediella citrus estate in the Northern Province, were put in cages (honey jars; 12 cm high by 5 cm diameter; with screened tops for ventilation) for adult emergence. The cages were kept at 28 ± 1 °C and 12L: 12D photoperiods until adult emergence. Orange fruits (Navel or Valencia) and waxed paper discs were used as oviposition substrates, and oviposited eggs were collected every 12 h for bioassays. Lufenuron, 050 EC as an emulsifiable concentrate containing 50 g a.i./l was supplied by Novartis SA (Pty) Ltd. Dilutions of 0.02, 0.04, 0.08 and 0.12 g a.i./l

were prepared with distilled water for all the bioassays. Fresh dilutions were prepared immediately prior to each assay and controls were dipped in distilled water.

5.2.2: Bioassay procedures

5.2.2.1: Embryo-larvicidal bioassays

Two bioassays were carried out to evaluate the effect of lufenuron on the embryonic and post embryonic stages of the false codling moth.

Orange fruit bioassays

Ripe orange fruits (Navel or Valencia) were wrapped in aluminium foils, and a section of the foil was cut open and marked with an ink spot (2 cm diameter) to indicate the area where data were to be collected (Moffitt et al. 1984; Hofmeyr & Pringle 1998). The marked sections were kept face up to allow the moths to oviposit directly on the exposed fruit surfaces. The foils were removed after oviposition and the eggs were counted under a stereomicroscope (16X) (Moffitt et al. 1988; Riedl et al. 1995). Eggs (50 per orange; n = 200 per age; remaining eggs within the marked spot were destroyed) oviposited on the marked spot were dipped in the appropriate test solutions for 30 seconds, air dried and placed in plastic rearing chambers (25 cm high by 11 cm diameter) (Moffitt et al. 1984; Hofmeyr & Pringle 1998; Pons et al. 1999). Each concentration plus its control (in distilled water) was replicated four times and the plastic chambers were covered with nylon netting and kept at 28 ± °C and 12L:12D photoperiods until larval hatch. To allow for delayed hatching as well as for the penetration of the larval instars into the treated fruits, data on egg and first instar mortalities were recorded with a stereomicroscope (16X) 48 h after the controls have hatched. Surviving larval instars in each plastic

chamber were further reared to the adult stage on the treated fruit and the experiment was monitored daily until adult emergence. After 35 days, the oranges were dissected and dead larval and post larval stages were recorded. Also, the various forms of morphological deformities induced by lufenuron on the larval and post larval stages were observed and photographed.

Waxed paper bioassays

Eggs (1, 3 and 5 d old; n = 200 per age group) oviposited on waxed paper discs (5 cm diameter) were dipped into appropriate test solutions for 30 seconds, air-dried and placed in plastic containers (12 cm high by 7 cm diameter) containing untreated navel oranges (Broadbent & Pree 1984). Each concentration plus its control was replicated four times and the plastic containers were covered with nylon netting and kept at 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Data on egg and first instar mortalities were recorded with a stereomicroscope (16X) 48 h after controls have hatched. Surviving larval instars were reared to the adult stage on the untreated orange fruits. The experiment was monitored daily and after 35 days, the oranges were dissected and dead immature stages were recorded.

Data analyses

Data on percentage egg mortality were corrected for natural mortality (Abbott 1925). Where appropriate, the data were arcsine transformed before analysis of variance (Statsoft 1995). Ducan's multiple range test was used to evaluate the differences between the treatment groups ($P = 0.05$) (Statsoft 1995; Rehimi & Soltani 1999).

5.2.2.2: Residual activity bioassay.

To evaluate the residual activities of lufenuron on eggs of the false codling moth, ripe oranges (Valencia and Navel) were dipped in the appropriate test solutions for 30 seconds, air-dried and kept at room temperatures to allow the residues to age. On the day of treatment, each fruit was wrapped in aluminium foil and a section on the foil was cut open to allow the moths to oviposit directly on the treated surface (Riedl et al. 1995; Pons et al. 1999). The cut section was marked with an ink spot (2 cm diameter) and the aluminium foils were removed and the eggs counted under a stereomicroscope (16X). Only eggs (1, 3 and 5 d old; n = 200 per age) oviposited within the marked spot were used for data analysis (Hofmeyr & Pringle 1998). The fruits were placed in plastic chambers (7 cm high by 12 cm diameter) and covered with nylon netting and each concentration plus its control was replicated four times and the experiment was kept at 28 ± 1 °C, 12L: 12D photoperiods until larval hatch. Data on egg mortality were recorded with a stereomicroscope (16X) 48 h after the controls have hatched. Surviving larval instars were reared to the adult stage on the treated fruit and the experiment was monitored daily until adult emergence. Data on adult emergence were recorded after 35 days to allow for delayed larval growth due to pesticide effects.

Data analyses

Data on percentage egg mortality were corrected for natural mortality (Abbott 1925). Where appropriate, the data were arcsine transformed before statistical analysis (Statsoft 1995). Duncan's multiple range test was used to evaluate the differences between the treatment groups ($P = 0.05$) (Statsoft 1995; Rehimi & Soltani 1999).

5.2.2.3: Histological bioassays

To determine the effects of lufenuron on the integument of false codling moth larvae, orange fruits were dipped in the appropriate test solutions (0.08 and 0.12 g a.i./l) for 30 seconds, air-dried and placed in plastic chambers (7 cm high by 12 cm diameter). Eggs (< 12 h old, n = 20) oviposited on waxed papers were transferred to a marked section on the fruit with a fine camel-hair brush. Control experiments were dipped in distilled water and the plastic chambers were covered with nylon netting and kept at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods until larval hatch. Hatched larval instars were allowed to feed on the treated fruits for 3 days after which the fruits were dissected, and dead larval instars were collected for histological studies based on the methods of Kushida (1974) and Trump et al. (1961).

The larvae were fixed in 2.5 % glutaraldehyde for 1 h, rinsed three times (15 minutes each) in 0.075 M phosphate buffer (pH 7.4 – 7.6). Post fixation was carried out in 0.25 % aqueous osmium tetraoxide for 1 h at room temperature, and the tissues were rinsed three times in distilled water before dehydration in graded ethanol series (50, 70, and 90 %). Further dehydration was carried out in 100 % ethanol (three changes, 15 minutes each) and the tissues were infiltrated with 30 and 60 % epoxy resin (1 h each) respectively. Further infiltration with pure epoxy resin was carried out for 4 h and the tissues were polymerized for 24 – 36 h at 65 ° C. Semi-thin sections (2 μm) were cut and stained with toludine blue and the sections were viewed under a Nikon Optiphot light microscope and photographed with a Nikon digital camera (DXM 1200).

5.3: RESULTS AND DISCUSSION

5.3.1: Embryo-larvicidal bioassays.

Egg hatch and subsequent larval entry into the fruits were not affected by lufenuron in the orange and waxed paper bioassays. In both bioassays, eggs treated at the white stage (1 day old) or ring stage (3 days old) developed to the black head stage and were able to hatch successfully. Egg mortality was low in both the fruit (4.7 – 21.1 %) and the waxed paper (0.5 – 6.4) bioassays. There was no significant difference in mortality between the treated and control experiments (Tables 1 and 2). Although previous studies with other tortricid moths have shown that acylureas have high embyrocidal effects against these pests, results from this study suggest the contrary (Elliott & Anderson 1982; Broadbent & Pree 1984; Purcell & Granett 1986). Hofmeyr & Pringle (1998), found that false codling moth eggs collected from the Zebediella estate were more susceptible to triflumuron than eggs collected from the Swellendam and Citrusdal areas, and the authors attributed this differences in susceptibility to severe selection pressure with this chitin synthesis inhibitor. Also, Elliott & Anderson (1982), found that a linear relationship existed between percentage egg hatch and the age of the eggs; with higher mortality occurring at the younger age group.

Larvicidal effects

Mortality of first instars soon after hatch or during moult to the next larval instar was low in both bioassays (Tables 3 and 4). First instar mortality in the fruit bioassay was <4.3 %, while in the waxed paper bioassay, it was < 1 %, thus suggesting that lufenuron has very low larvicidal effect on early instars of the false codling moth. This result is

contrary to reports from previous studies on the activities of acylureas on first instar stages of tortricid moths (Hoying & Riedl 1980; Retnakaran 1982; Purcell & Granett 1986; Newton 1987). However, this result is consistent with the studies of Whiting et al. (2000), who found that the first instars of Epiphyas postvittana (Lepidoptera: Tortricidae) were less sensitive to lufenuron than third and fifth instar stages. Also, Elliott & Anderson (1982), found that first instars of the codling moth, Cydia pomonella (Lepidoptera:Olethreutidae) were able to penetrate apples treated with 500 ppm diflubenzuron and developed normally to the adult stage without exhibiting any signs of delayed larvicidal effects such as slow larval growth.

The low larval mortality recorded in the fruit bioassay could be because the neonates did not consume sufficient amount of lufenuron residues before penetrating the fruits or that these early instars were very tolerant to residues of lufenuron as reported in other tortricid pests (Rappaport & Robertson 1981; Elliott & Anderson 1982; Retnakaran et al. 1985). Larval mortality was also very low in the control experiments. Morphological examination of the dead instars showed that the larvae were smaller and had dark shrivelled body (Fig. 1).

Post-larval effects

In both bioassays, the percentage of larval instars that survived to the pupal and adult stages was high. Moult disruption was observed at the larval-pupal stage, but pupal mortality was very low and only few of the instars died at the prepupal stage (Fig. 2). There were no records of larviform pupa or pupae with other forms of morphological

deformities such as ruptured and leaking haemolymph in the two bioassays. This suggests that the different concentrations of lufenuron had no impact on the development of the post larval stages. This is contrary to the studies of Whiting et al. (2000), who found that the consumption of lower concentrations of lufenuron by larval instars of E. postvittana resulted in the formation of deformed pupae as well as on a prolonged delay in pupal development to the adult stage. Adult emergence in both bioassays was very high (Figs. 4 & 5), and development from the egg stage to the adult stage was completed in less than 32 days in both the treated and control experiments. There were no records of abnormal adults or adults that were unable to escape from the pupal case in the two bioassays. The high adult emergence recorded in the fruit and waxed paper bioassays is a further confirmation of the assumption that the newly emerged larval instars did not consume sufficient lufenuron residues from either the eggshells or from the treated fruit surfaces before entering the orange fruits. This assumption is supported by the studies of Hoying & Riedl (1980), who found that the ingestion of diflubenzuron residues by codling moth larvae during fruit entry did not cause any adverse effect on subsequent larval development and adult emergence. Ascher & Nemny (1974), have suggested that the larvicidal activity of diflubenzuron depends to a large extent on the continuous ingestion of contaminated materials by the developing larvae, however, this is not possible in the false codling moth, because the larval instars only spends a short time on the treated surface before penetrating the inner part of the fruits. This behaviour has made the control of the false codling moth very difficult in the different citrus producing areas of South Africa (Newton 1998).

5.3.2: Residual activity bioassay

The residual activity of lufenuron against eggs of the false codling moth was low over the 10 day trial period (Tables 5 – 7). Egg mortality was low (2.1 – 20.7%) in all the residue ages, and embryonic development was not affected by the four concentrations used in this bioassay. This is contrary to previous studies on the residual activities of acylureas against lepidoteran pests (Gilette et al. 1978; Elliott & Anderson 1982). Hoying & Riedl (1980), showed that diflubenzuron residues remained active against eggs of the codling moth for at least 6 weeks, while Broadbent & Pree (1984) found that triflumuron was active for up to 10 days against the eggs of the Oriental fruit moth, Grapholitha molesta (Busck.) (Lepidoptera: Olethreutidae).

Subsequent entries of the neonates into the treated fruits as well as damage to these fruits was very high in both the treated and control experiments. This is contrary to the studies of Moffitt et al. (1984), who found that egg hatch and subsequent larval entry into treated apples were greatly reduced over a 19 week period when codling moth eggs were deposited on acylurea treated fruits. The residual activity of lufenuron against larval and post larval stages was also very low and this led to a high record of adult emergence in both the treated and control experiments (Figs. 6 – 8).

5.3.3: Histological bioassay

Dead larval instars that emerged from treated fruits were darker and smaller in size than those that emerged from untreated fruits. Histological examinations of these larval instars (treated and untreated) showed that there were no significant differences in the

level of damage to the epi and exocuticular layers (Fig. 9 A,B). Similar results of low histological impact of acylureas on the epi and exocuticular layers have been reported in other insects (Mulder & Gijswijt 1973; Percy et al. 1987; Retnakaran & Wright 1987; Retnakaran et al. 1989, Dean et al. 1998; Dean et al. 1999). However, the integument of larval instars that emerged from the treated fruit had a cuticle that was almost devoid of the endocuticular layer, while the cuticle of larval instars that emerged from untreated fruit showed a uniform, well-defined and undamaged endocuticular layer. Previous reports of severe endocuticular distortion by acylureas have been reported in other tortricids (Lim & Lee 1982; Retnakaran et al. 1997).

According to Retnakaran et al. (1997), the most obvious feature in the cuticle of acylurea-treated larvae is a complete absence of ordered fibrils or distinct lamellae in the endocuticular layer. Also, Lee et al. (1990) and Nakagawa et al. (1992) found that the impairment of chitin incorporation in the integument of larval instars treated with acylureas, was manifested in the endocuticular layers; while Leopold et al. (1985), reported that the endocuticule of the pupae of the cotton boll weevil, Anthonomus grandis (Coleoptera: Curculionidae) was completely eroded after exposure to different concentrations of diflubenzuron. The mortality of larval instars during or shortly after moult could be because of failure in chitin deposition in the endocuticular layer (Reynolds 1987; Retnakran et al. 1997; Dean et al. 1999). Lim & Lee (1982), also found that ecdysial failure and mortality of Oxya japonica (Willemse) (Orthoptera: Acrididae) nymphs after exposure to diflubenzuron was caused by the blocking of chitin deposition in the endocuticule.

The epidermal layer which is responsible for the secretion of the cuticular components have also been shown to be affected by acylureas in some insect species (Dean et al. 1999). Reynolds (1987), found that chitin synthesis as well as the synthetic activity of the epidermis could be inhibited by acylureas and that this might lead to the death of the larval instars during or after ecdysis. In the present study, the death of larval instars after emergence from lufenuron-treated fruits could have been caused by the distortion of the endocuticular layer or by the lytic changes in the epidermis (Percy et al. 1987; Hassan & Charnley 1978; Retnakaran et al. 1997; Dean et al. 1999).

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Table 1. Mortality of different-aged eggs of Cryptophlebia leucotreta (Meyrick) after exposure to lufenuron-treated fruits.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	7.3 ± 2.4a	13.7 ± 1.6a	10.6 ± 5.0a	18.6 ± 4.4a	21.1 ± 1.4a
3	7.3 ± 2.5a	11.3 ± 2.4a	5.9 ± 2.3a	19.3 ± 3.4a	14.8 ± 4.8a
5	3.2 ± 1.5a	4.7 ± 1.9a	6.7 ± 4.5a	5.2 ± 3.0a	11.8 ± 5.5a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 2. Mortality of different-aged eggs of Cryptophlebia leucotreta (Meyrick) after exposure to treated waxed paper discs.

Egg age (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	4.5 ± 3.2a	2.6 ± 1.8a	3.5 ± 1.7a	2.6 ± 1.9a	6.4 ± 3.6a
3	2.6 ± 1.5a	2.6 ± 1.8a	2.1 ± 0.8a	4.7 ± 3.2a	3.6 ± 1.7a
5	1.0 ± 0.6a	1.5 ± 0.9a	2.6 ± 1.9a	2.5 ± 1.9a	2.1 ± 2.0a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 3. Larvicidal activity of lufenuron against first instars of Cryptophlebia leucotreta (Meyrick.) after emergence from eggs placed on treated orange fruits.

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	0.8 ± 0.5a	2.3 ± 1.3a	2.1 ± 0.9a	2.8 ± 1.0a	4.3 ± 1.8a
3	0.0 ± 0.0a	2.0 ± 0.9a	3.0 ± 1.8a	3.7 ± 1.9a	3.8 ± 1.9a
5	0.5 ± 0.3a	1.5 ± 0.9a	1.7 ± 0.9a	3.7 ± 1.9a	2.5 ± 1.8a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 4. Larvicidal activity of lufenuron against first instars of Cryptophlebia leucotreta (Meyrick) after emergence from eggs placed on treated waxed paper discs.

Egg age (days)	% Larval mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	0.3 ± 0.3a	1.0 ± 0.7a	1.0 ± 0.4a	1.0 ± 0.4a	1.0 ± 0.4a
3	1.0 ± 0.6a	0.5 ± 0.3a	0.8 ± 0.5a	1.0 ± 0.7a	0.5 ± 0.3a
5	0.8 ± 0.5a	0.7 ± 0.5a	1.0 ± 0.7a	0.5 ± 0.3a	0.8 ± 0.5a

Means within a column followed by the same letter are not significantly different ($P = 0.05$; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 5. Residual toxicity of lufenuron to 1 day old Cryptophlebia leucotreta (Meyrick) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	4.3 ± 3.1a	9.2 ± 6.1a	7.8 ± 4.4a	10.7 ± 4.3a	10.0 ± 3.6a
2	8.8 ± 4.0a	11.3 ± 1.3a	13.8 ± 2.5a	11.0 ± 3.0a	8.9 ± 1.2a
3	6.2 ± 4.2a	5.3 ± 2.2a	11.5 ± 2.8a	13.5 ± 3.8a	15.9 ± 2.6a
4	7.0 ± 4.3a	13.3 ± 2.4a	12.8 ± 3.7a	10.6 ± 3.1a	9.4 ± 1.4a
5	3.5 ± 2.9a	4.9 ± 2.2a	6.0 ± 4.3a	4.3 ± 3.6a	13.8 ± 2.5a
6	5.0 ± 2.4a	9.5 ± 2.6a	14.2 ± 3.3a	12.4 ± 5.6a	17.8 ± 2.2a
7	4.8 ± 3.2a	4.3 ± 3.1a	6.0 ± 1.5a	9.1 ± 1.4a	8.0 ± 1.1a
8	8.0 ± 0.4a	7.5 ± 1.6a	10.5 ± 2.8a	11.3 ± 3.0a	9.8 ± 1.3a
9	7.5 ± 4.1a	8.3 ± 3.8a	7.3 ± 3.9a	9.5 ± 3.8a	9.0 ± 1.6a
10	10.1 ± 3.7a	12.7 ± 2.6a	10.9 ± 1.6a	8.6 ± 0.6a	12.7 ± 2.2a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 6. Residual toxicity of lufenuron to 3 day old Cryptophlebia leucotreta (Meyrick) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	5.6 ± 3.8a	13.9 ± 4.4a	20.5 ± 1.8a	18.6 ± 4.0a	20.7 ± 1.3a
2	10.5 ± 4.4a	12.8 ± 3.3a	13.7 ± 3.7a	12.3 ± 6.5a	11.3 ± 3.5a
3	7.0 ± 3.2a	12.0 ± 4.5a	12.1 ± 3.5a	8.8 ± 5.5a	9.3 ± 5.2a
4	7.5 ± 5.1a	8.2 ± 6.6a	11.5 ± 3.1a	8.9 ± 2.5a	15.5 ± 3.8a
5	4.0 ± 0.6a	2.8 ± 5.0a	3.9 ± 1.8a	5.7 ± 2.4a	9.8 ± 3.0a
6	3.5 ± 2.7a	7.0 ± 3.3a	6.9 ± 4.0a	9.3 ± 5.2a	7.2 ± 3.7a
7	9.5 ± 2.3a	9.3 ± 3.0a	12.0 ± 3.8a	14.9 ± 3.5a	14.8 ± 1.8a
8	10.5 ± 3.1a	6.8 ± 3.7a	10.8 ± 1.3a	8.3 ± 4.0a	15.0 ± 1.8a
9	8.2 ± 3.3a	14.3 ± 2.6a	12.1 ± 2.9a	13.4 ± 3.4a	14.1 ± 5.7a
10	7.0 ± 4.2a	4.7 ± 3.0a	8.9 ± 1.2a	5.3 ± 2.2a	6.5 ± 3.2a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

Table 7. Residual toxicity of lufenuron to 5 day old Cryptophlebia leucotreta (Meyrick) eggs.

Age of residues (days)	% Egg mortality (mean ± SE) Concentration (g a.i./l)				
	0	0.02	0.04	0.08	0.12
1	8.0 ± 7.6a	7.1 ± 3.1a	8.8 ± 2.3a	8.7 ± 6.2a	9.8 ± 2.7a
2	9.3 ± 5.0a	10.8 ± 6.7a	11.5 ± 6.2a	7.5 ± 1.9a	6.8 ± 4.4a
3	4.3 ± 2.3a	6.3 ± 5.8a	7.5 ± 4.9a	8.1 ± 3.8a	12.1 ± 3.3a
4	3.0 ± 3.2a	4.2 ± 2.9a	3.3 ± 2.9a	4.2 ± 3.3a	2.1 ± 5.5a
5	1.5 ± 2.9a	2.8 ± 3.1a	4.8 ± 1.9a	1.2 ± 4.9a	3.7 ± 1.8a
6	2.1 ± 5.4a	3.2 ± 4.3a	2.1 ± 3.5a	4.5 ± 3.6a	7.4 ± 4.2a
7	2.5 ± 2.3a	3.3 ± 3.3a	4.2 ± 1.7a	8.1 ± 4.9a	6.9 ± 3.8a
8	4.5 ± 4.0a	2.5 ± 2.4a	4.5 ± 3.1a	7.2 ± 3.9a	6.3 ± 3.1a
9	4.3 ± 2.0a	5.8 ± 4.4a	9.5 ± 4.6a	6.7 ± 5.4a	11.0 ± 5.2a
10	4.0 ± 3.7a	8.5 ± 3.0a	6.3 ± 2.9a	4.5 ± 3.2a	5.5 ± 3.2a

Means within a column followed by the same letter are not significantly different (P = 0.05; Duncan multiple range test [Statsoft 1995]). Arcsine transformation was carried out before statistical analysis.

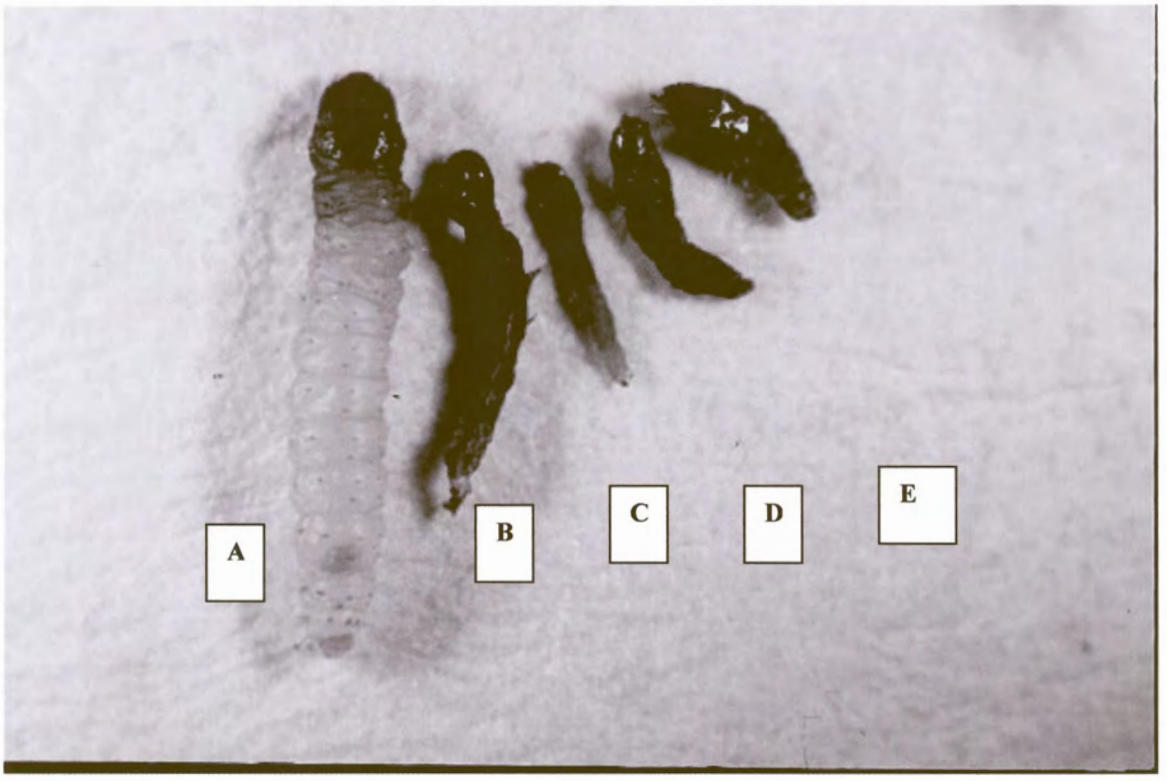

Fig. 1. Effects of lufenuron on first instar Cryptophlebia leucotreta (Meyrick) larvae: A = normal larva that emerged from untreated orange fruit; B – E = dark, shrivelled larvae that emerged from lufenuron-treated fruits.

Fig. 2. Morphological deformity caused by lufenuron at the larval-pupal stage.

Fig. 3. Adult False codling moth

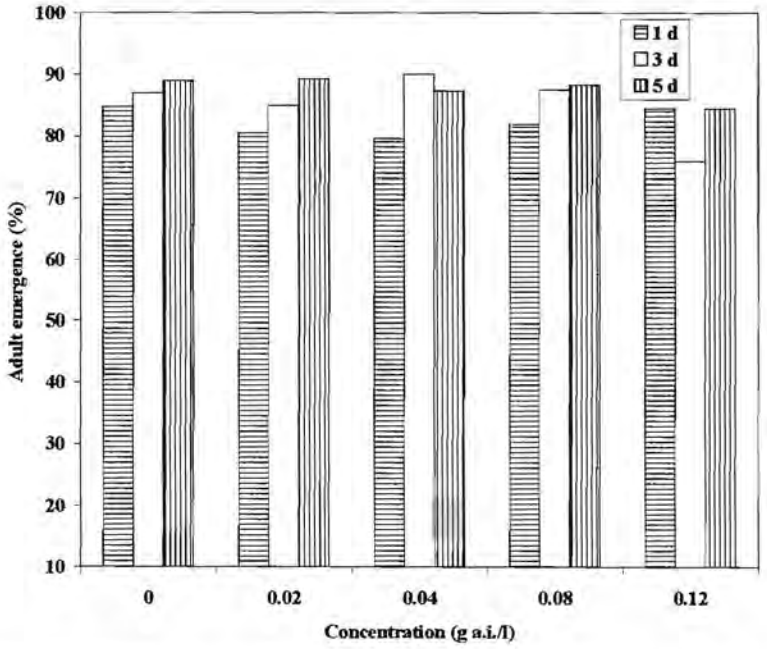

Fig. 4. Cryptophlebia leucotreta (Meyrick) adult emergence from treated fruits.

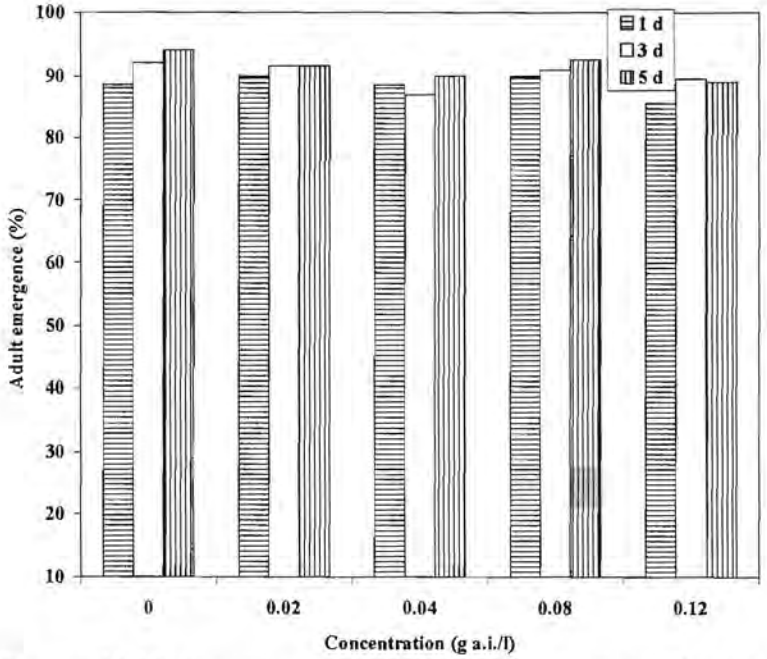

Fig. 5. Cryptophlebia leucotreta (Meyrick) adult emergence from treated waxed paper discs.

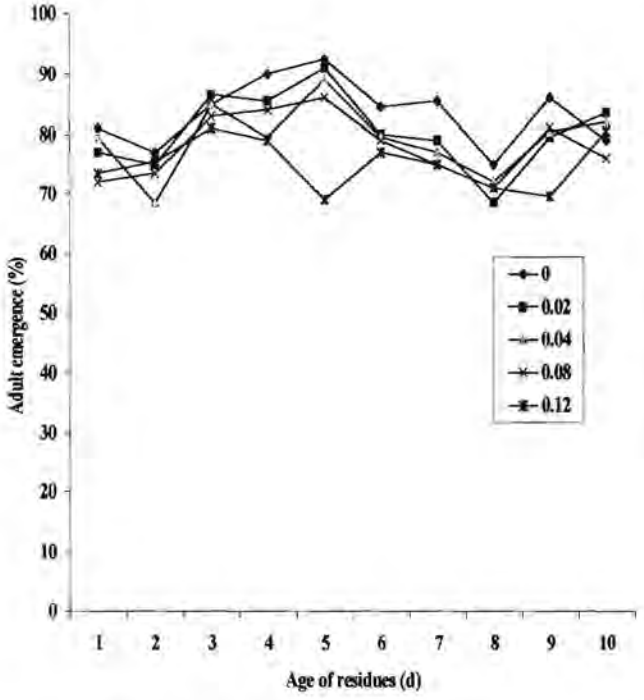

Fig. 6. Percentage emergence of Cryptophlebia leucotreta (Meyrick) adults from 1 day old eggs in the residual activity bioassay.

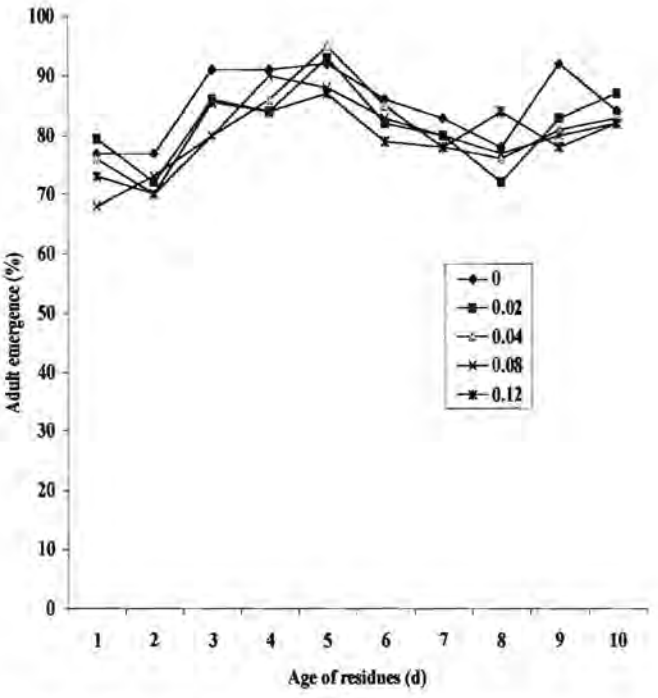

Fig. 7. Percentage emergence of Cryptophlebia leucotreta (Meyrick) adults from 3 day old eggs in the residual activity bioassay.

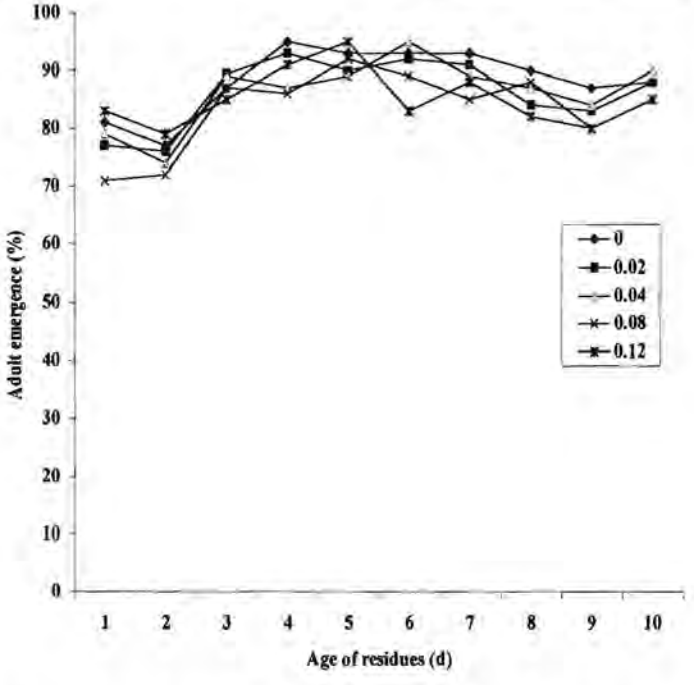

Fig. 8. Percentage emergence of Cryptophlebia leucotreta (Meyrick) adults from 5 day old eggs in the residual activity bioassay.

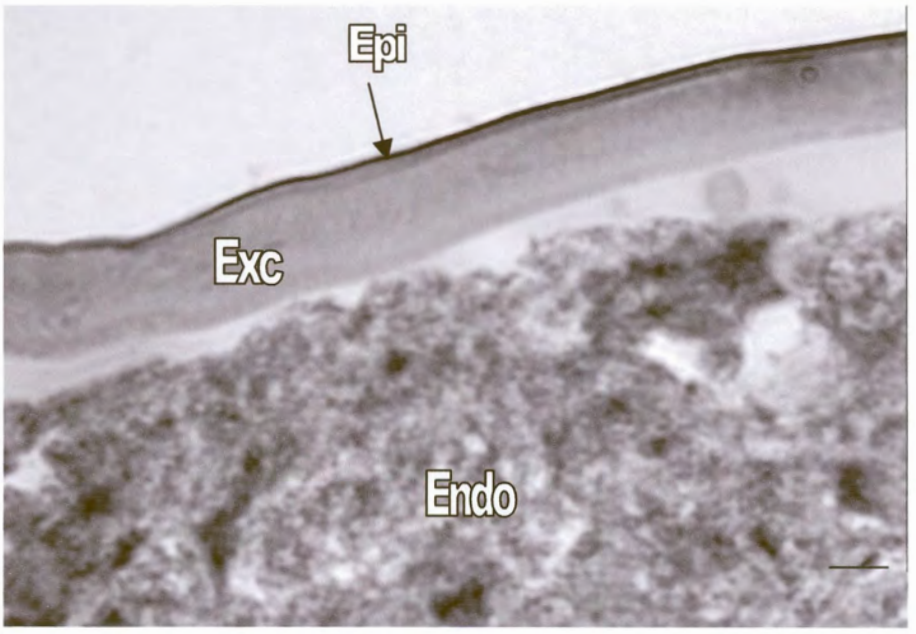

Fig. 9A. Cross section through the integument of untreated Cryptophlebia leucotreta (Meyrick) larva showing normal cuticle. Epi = epicuticle; Exc = exocuticle; Endo = endocuticle. Bar = 43.1 μm.

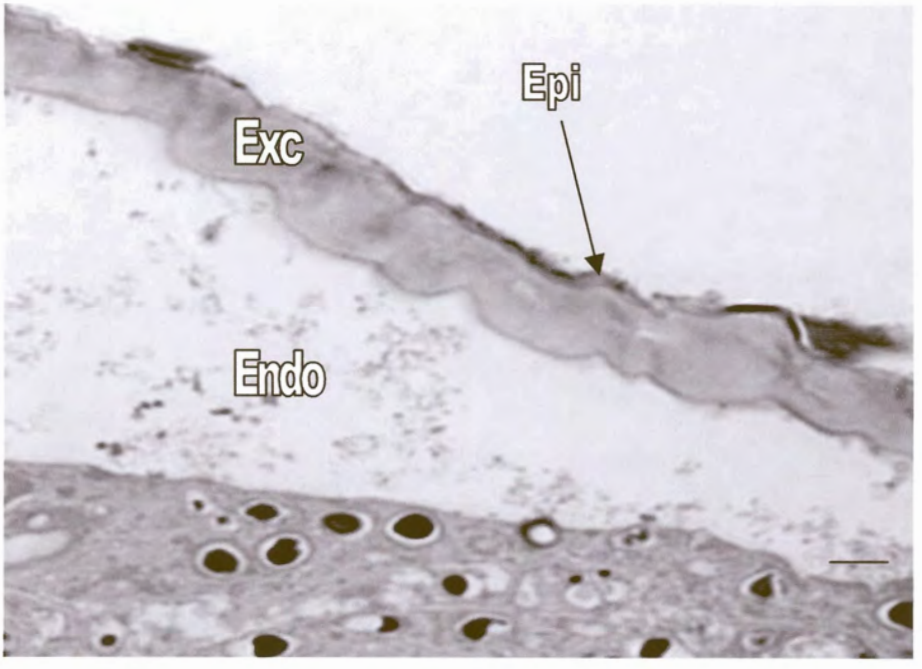

Fig. 9B. Cross section through the integument of lufenuron-treated larva showing a severely eroded endocuticle. Epi = epicuticle; Exc = exocuticle; Endo = endocuticle. Bar = 43.1 μm.

CHAPTER 6: CONCLUSION

The major aim of this study was to determine if lufenuron had a high embryocidal effect on the eggs of H. armigera, P. operculella, P. xylostella, and C. leucotreta; and to determine if younger (white stage) embryos were more susceptible to the inhibitory effects of lufenuron than older (ring stage and the black head stage) embryos. Results from this study indicates that lufenuron has very low embryocidal effect on the developing embryos and that neither young nor older embryos of the above named pests were affected by the inhibitory effects of lufenuron.

Present results also show that the cumulative effects of lufenuron absorbed into the embryos during embryogenesis, and the ingestion of lufenuron residues after larval hatch, could have led to the high mortality of first instars of the American bollworm, the potato tuber moth and the diamondback moth during or immediately after ecdysis. Although the larvicidal effects of lufenuron against first instars of the false codling moth was very low, the residual activities of this chemical against early instars of the American bollworm, the potato tuber moth and the diamondback moth did not decrease over the 10 day trial period, and this led to a low percentage emergence of adult H. armigera, P. operculella and P. xylostella.

In all the pests studied, larval mortality occurred during or shortly after moulting, thus suggesting that normal cuticle deposition was affected by lufenuron. Histological view of lufenuron-treated tissues verified this suggestion, and it was observed that the death of

these larval instars could have been caused by the abnormal deposition of the endocuticular layers.

Findings from this study show that although lufenuron has a weak embryocidal effect, its larvicidal properties against H. armigera, P. operculella and P. xylostella are very strong, and this chemical could be valuable in reducing the damage caused by these pests in integrated pest management programs. However, further studies, under field conditions, are needed to evaluate the embryo-larvicidal activities of this chitin synthesis inhibitor on the American bollworm, the potato tuber moth and the diamondback moth.