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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).

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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 °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 ^{o}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.

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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

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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

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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).

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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.

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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.

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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.

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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.

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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.

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

	% Egg mortality (mean ± SE)				
Age of residues (days)	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.

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

	% Egg mortality (mean ± SE)				
Age of residues (days)	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

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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.

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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.

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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)

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Fig. 4. Adult diamondback moth.

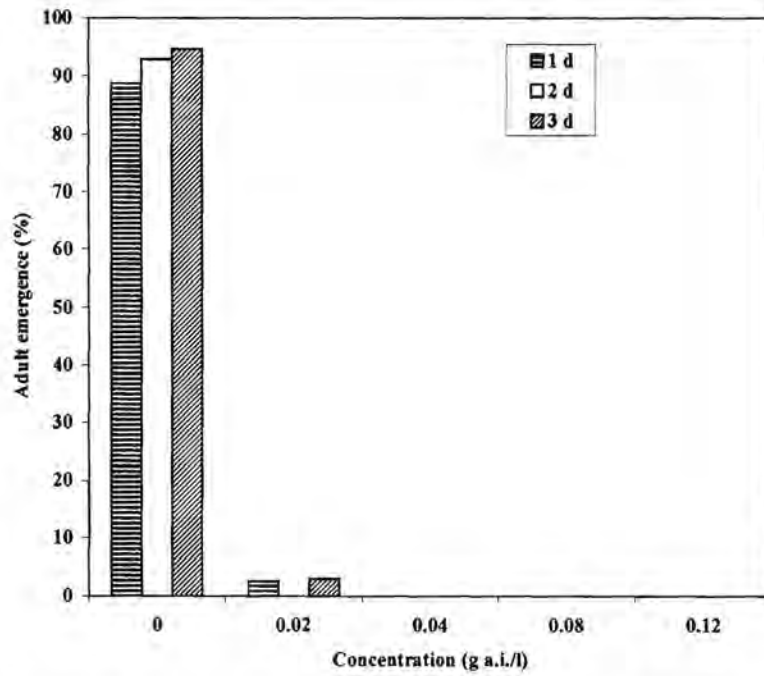

Fig. 5. Plutella xylostella (L.) adult emergence from treated cabbage leaf discs.

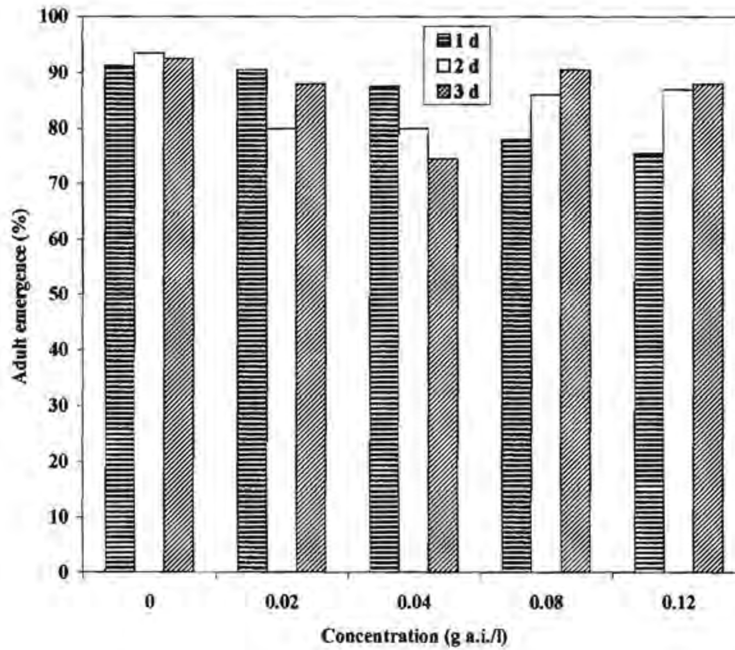

Fig. 6. Plutella xylostella (L.) adult emergence from treated waxed paper discs.

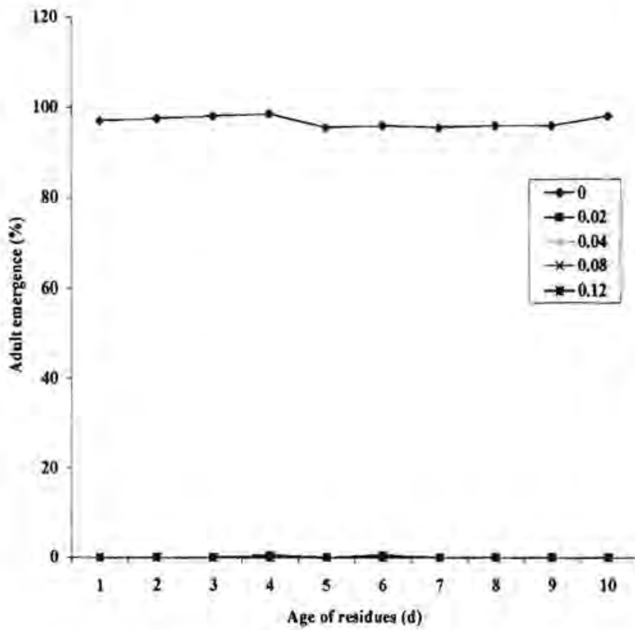

Fig. 7. Percentage emergence of Plutella xylostella (L.) adults from 1 day old eggs in the residual activity bioassay.

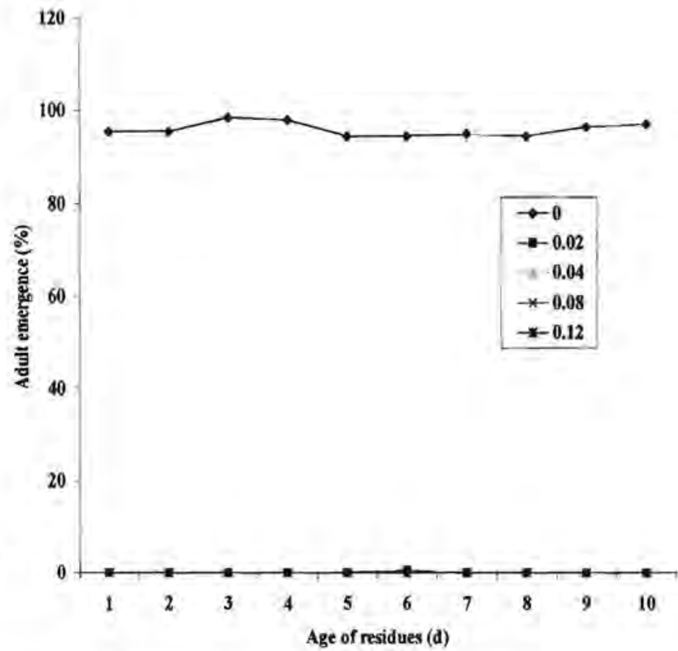

Fig. 8. Percentage emergence of Plutella xylostella (L.) adults from 2 day old eggs in the residual activity bioassay.

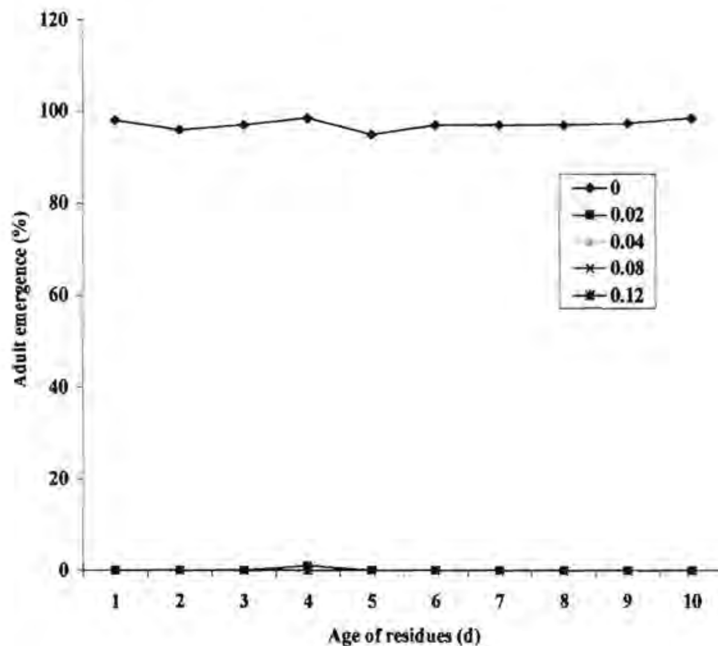

Fig. 9. Percentage emergence of Plutella xylostella (L.) adults from 3 day old eggs in the residual activity bioassay.

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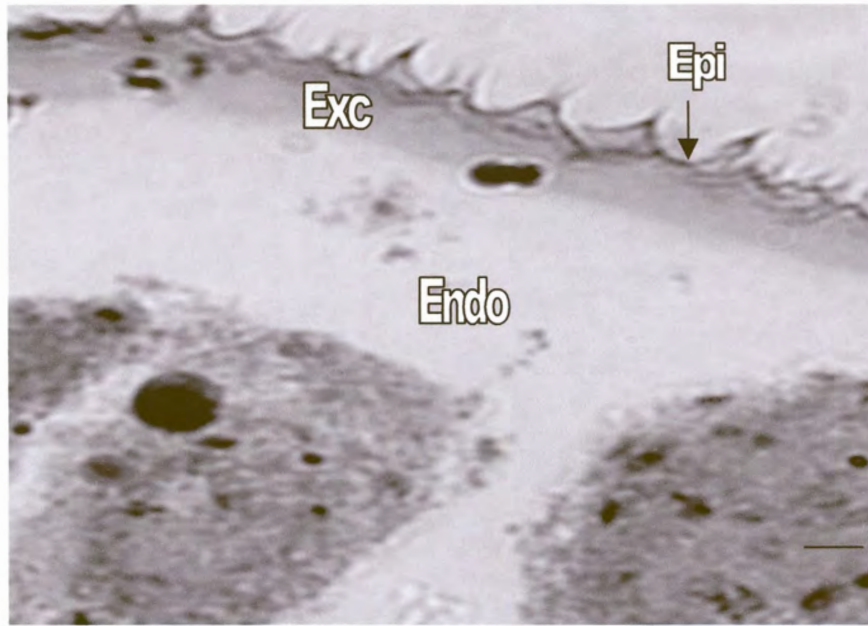

Fig. 10A. Cross section of the integument of untreated Plutella xylostella (L.) larva showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle.
Bar = 46.5 μm.

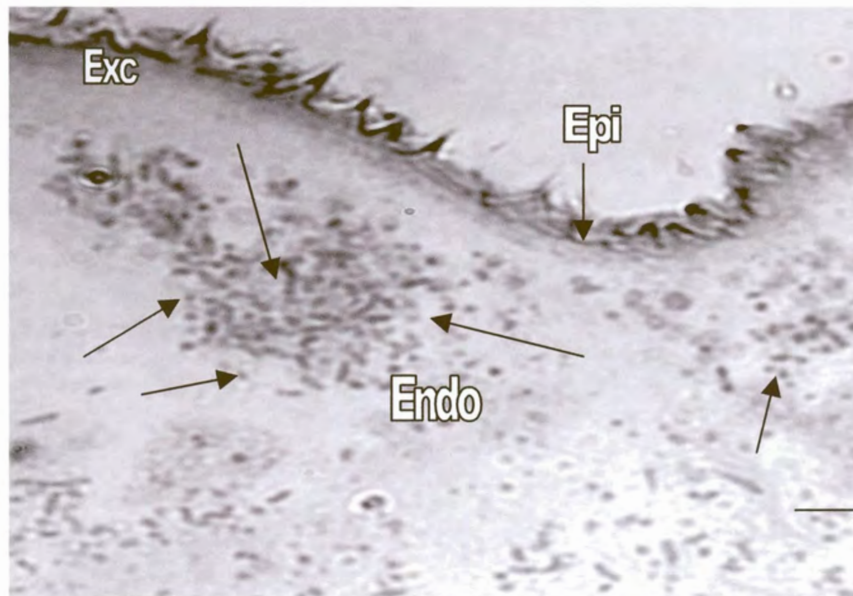

Fig. 10B. Cross section of the integument of lufenuron-treated second instar Plutella xylostella (L.) larva showing distorted endocuticle with scattered globular materials (arrows). Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 46.5 μm.

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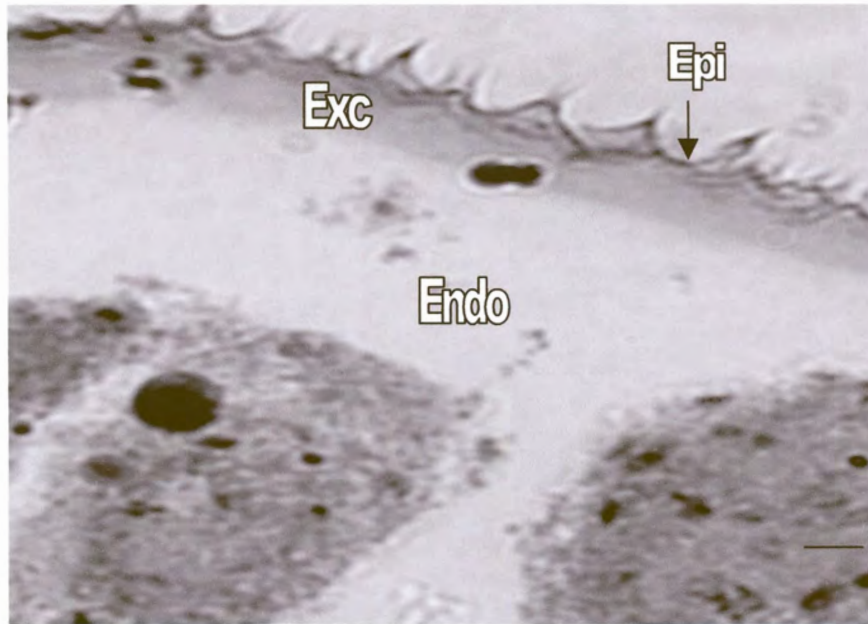

Fig. 10A. Cross section of the integument of untreated Plutella xylostella (L.) larva showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle.
Bar = 46.5 μm.

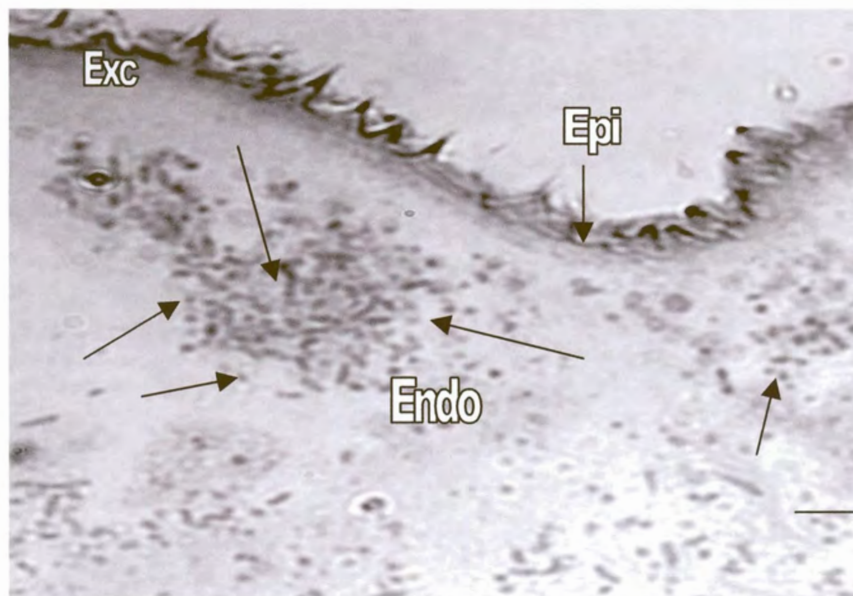

Fig. 10B. Cross section of the integument of lufenuron-treated second instar Plutella xylostella (L.) larva showing distorted endocuticle with scattered globular materials (arrows). Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 46.5 μm.

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

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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

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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.

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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.

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

	% Larval mortality (mean ± SE)				
Egg age (days)	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.

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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.

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

	% Egg mortality (mean ± SE)				
Age of residues (days)	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.

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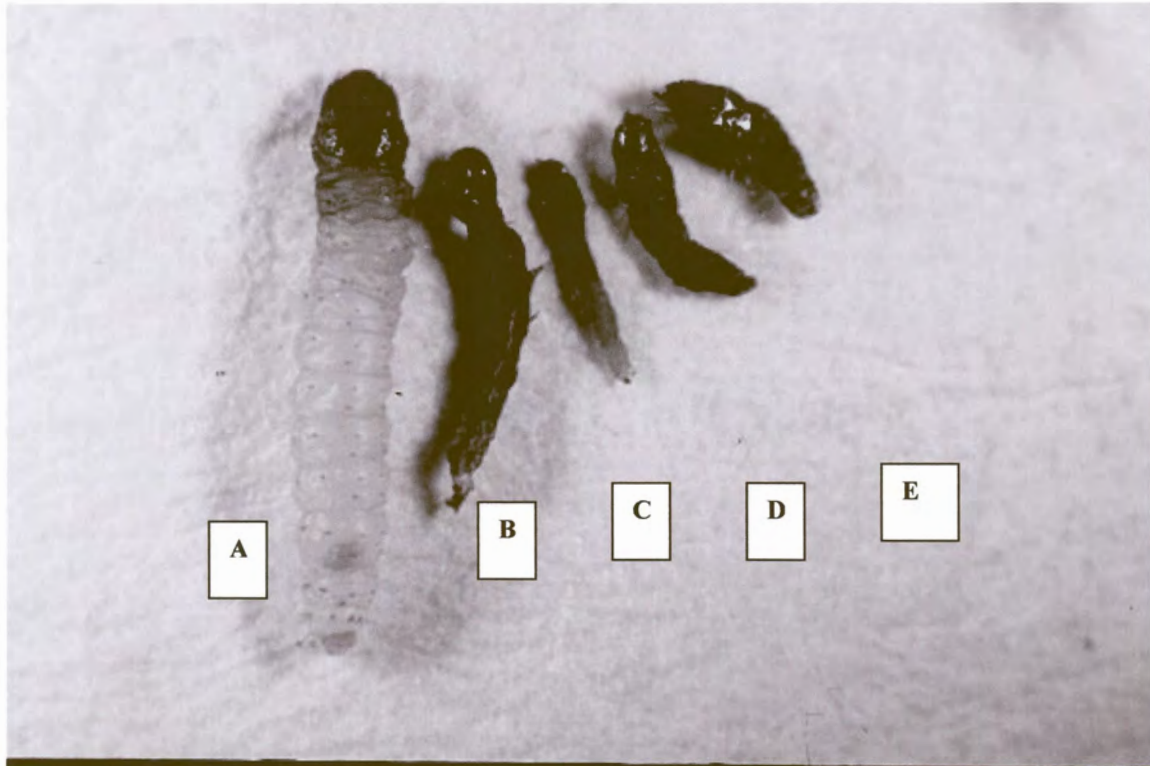

Fig. 1. Effects of lufenuron on first instar Cryptophlebia leucotreta (Meyrick) larvae: A = normal larva that emerged from untreated orange fruit; B – E = dark, shrivelled larvae that emerged from lufenuron-treated fruits.

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

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Fig. 3. Adult False codling moth

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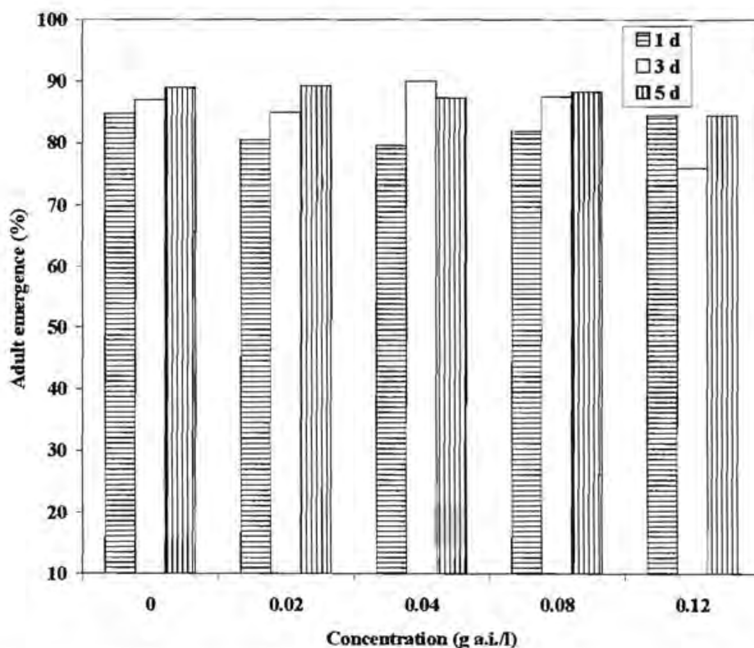

Fig. 4. Cryptophlebia leucotreta (Meyrick) adult emergence from treated fruits.

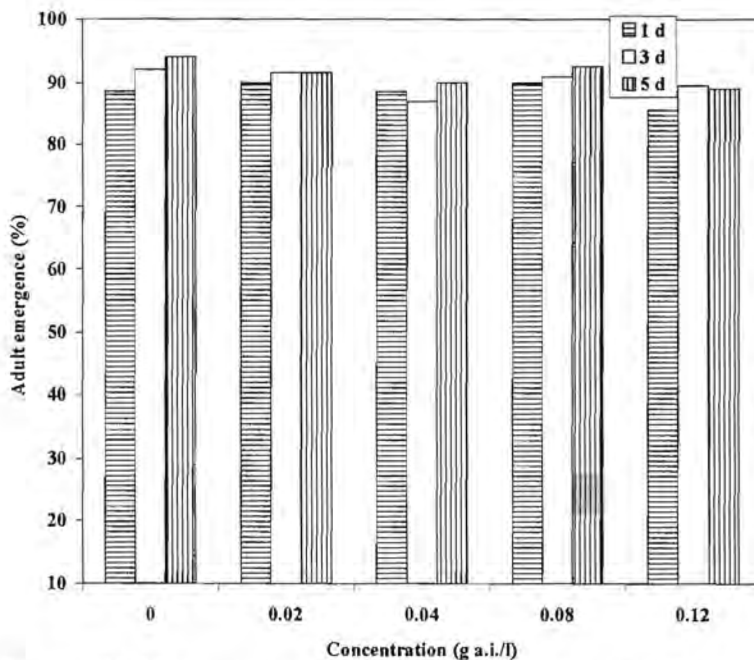

Fig. 5. Cryptophlebia leucotreta (Meyrick) adult emergence from treated waxed paper discs.

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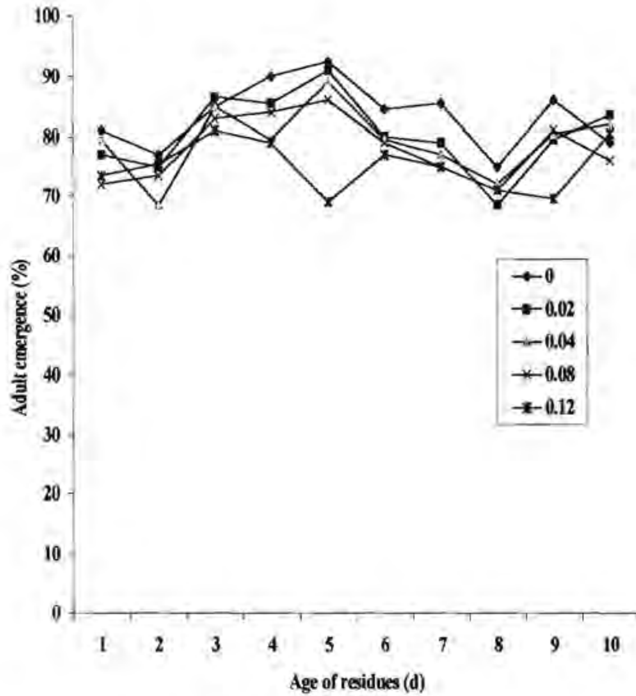

Fig. 6. Percentage emergence of Cryptophlebia leucotreta (Meyrick) adults from 1 day old eggs in the residual activity bioassay.

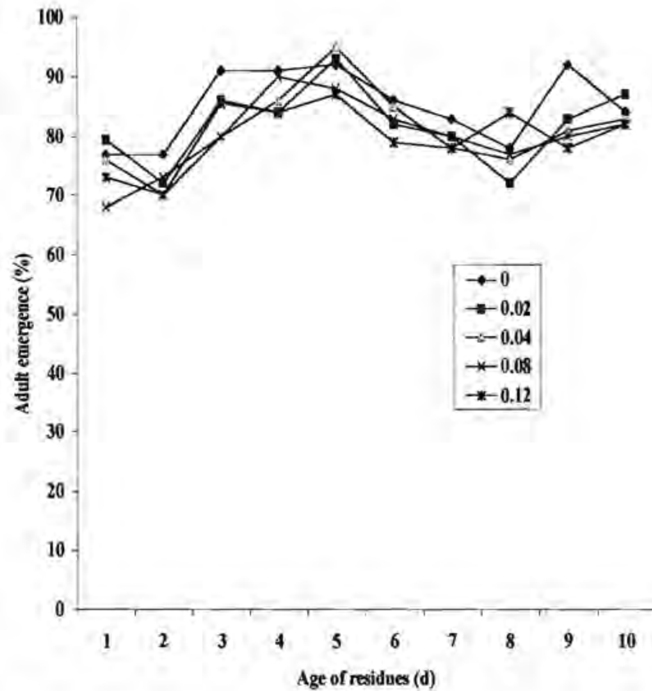

Fig. 7. Percentage emergence of Cryptophlebia leucotreta (Meyrick) adults from 3 day old eggs in the residual activity bioassay.

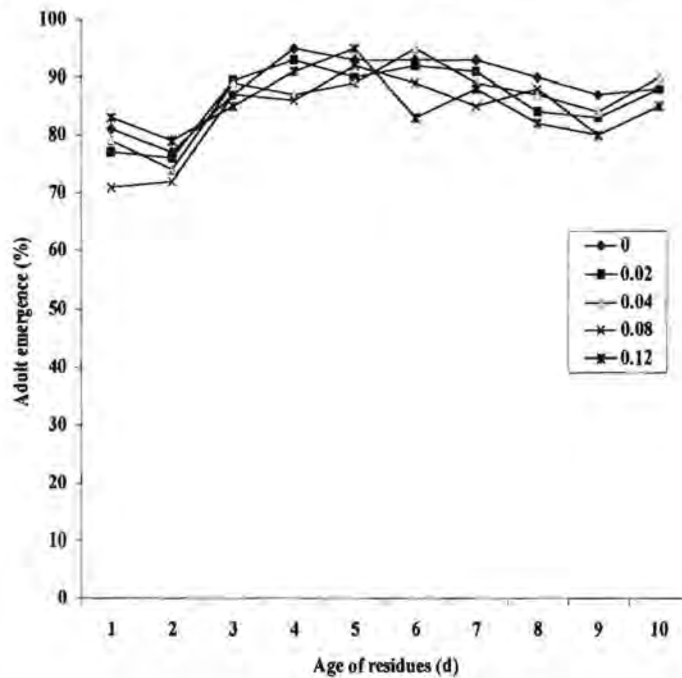

Fig. 8. Percentage emergence of Cryptophlebia leucotreta (Meyrick) adults from 5 day old eggs in the residual activity bioassay.

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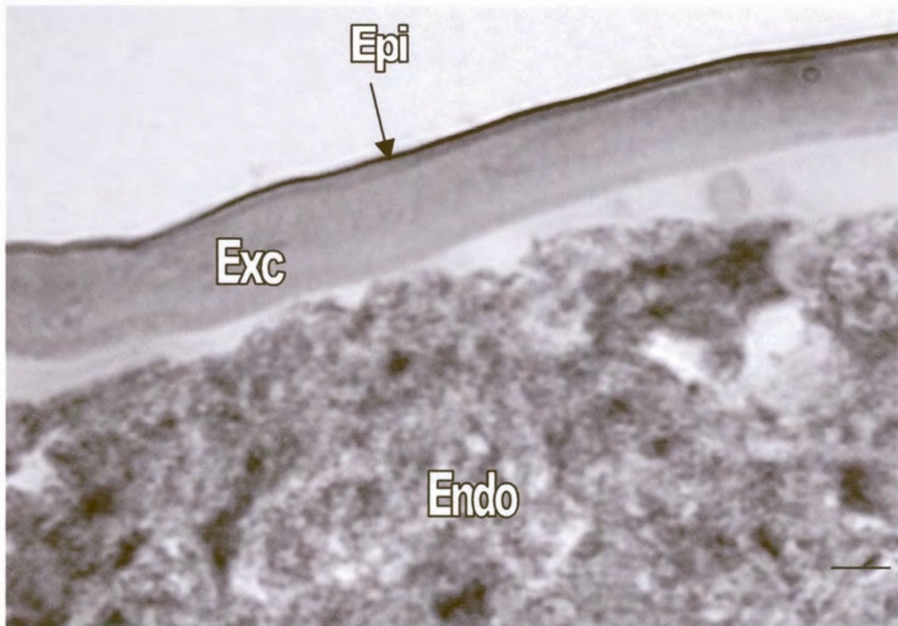

Fig. 9A. Cross section through the integument of untreated Cryptophlebia leucotreta (Meyrick) larva showing normal cuticle. Epi = epicuticle; Exc = exocuticle; Endo = endocuticle. Bar = 43.1 μm.

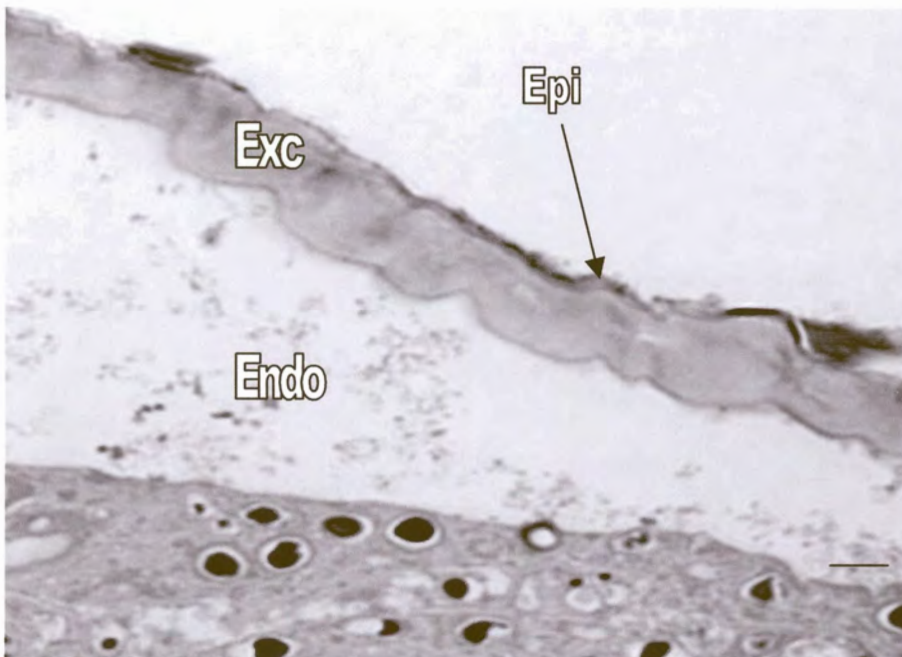

Fig. 9B. Cross section through the integument of lufenuron-treated larva showing a severely eroded endocuticle. Epi = epicuticle; Exc = exocuticle; Endo = endocuticle. Bar = 43.1 μm.

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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

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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.