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.

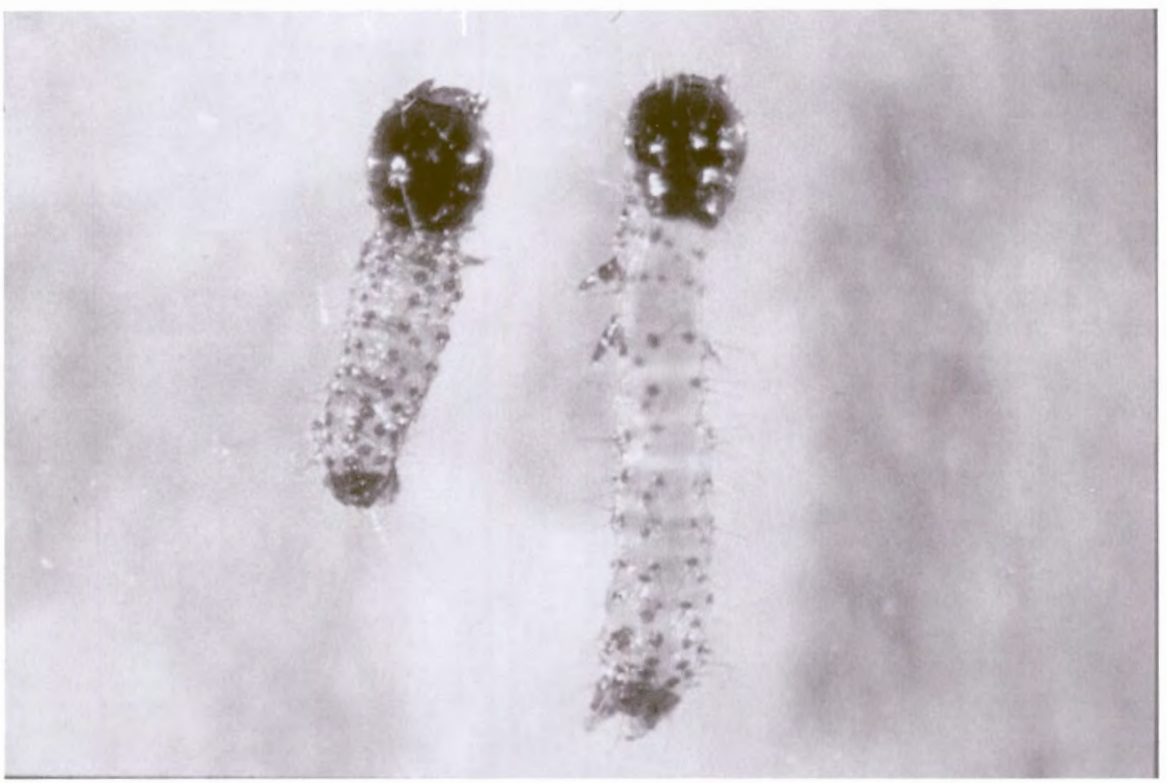

Fig. 1. Effects of lufenuron on first instar Helicoverpa armigera (Hübner) larvae: Left = small sized larva that emerged from lufenuron-treated substrate; Right = normal larva that emerged from untreated substrate).

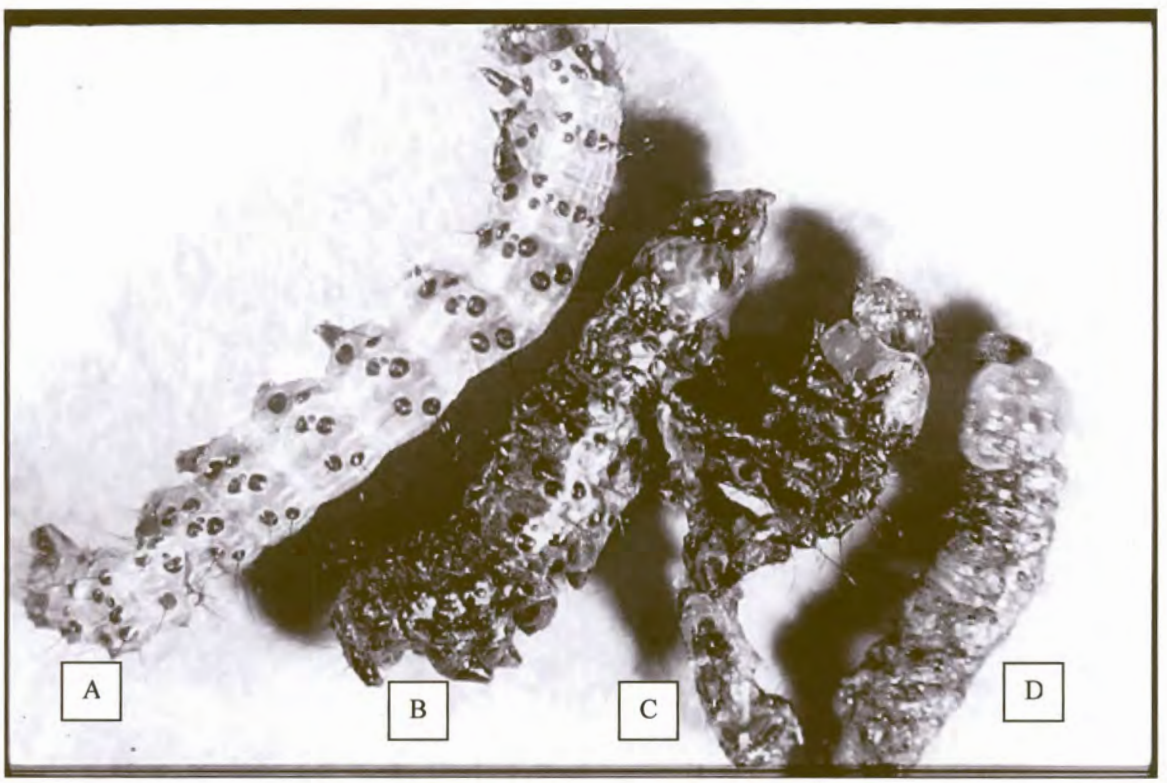

Fig. 2. Effects of lufenuron on second instar Helicoverpa armigera (Hübner) larvae: A = normal larva that emerged from untreated substrate; B – D = dark shrivelled larvae that emerged from lufenuron-treated substrates.

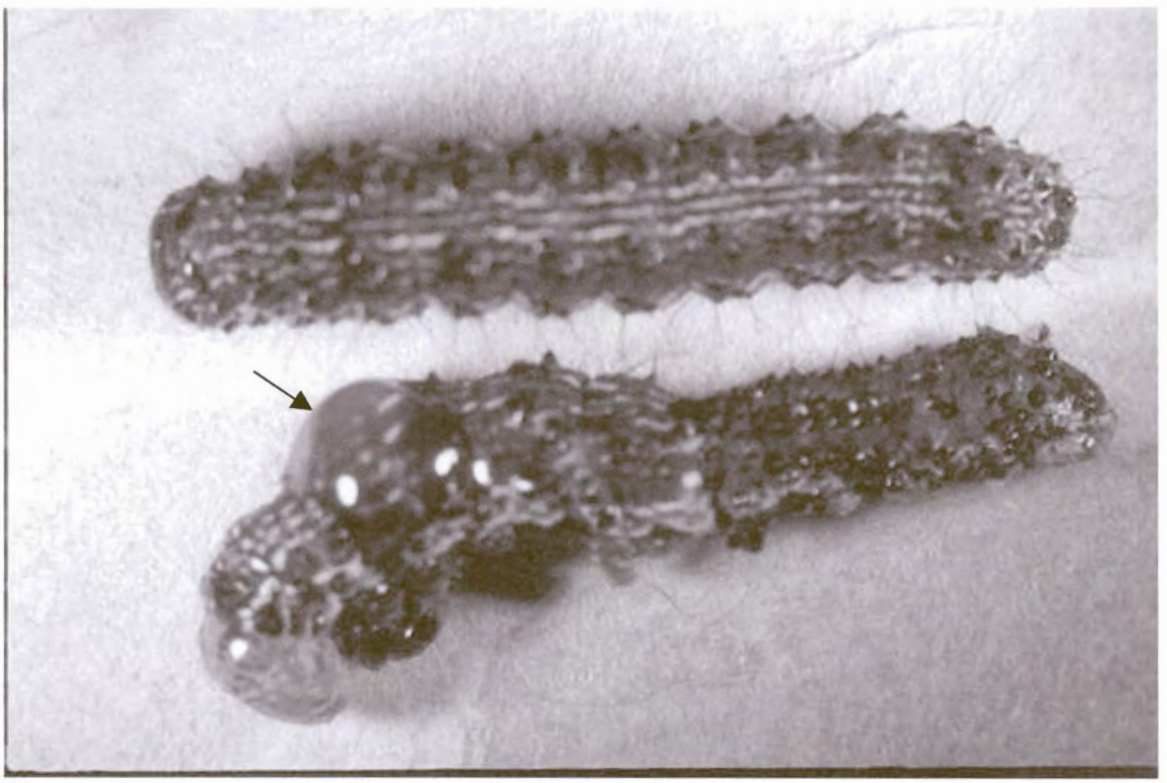

Fig. 3. Dorsal view of second instar Helicoverpa armigera (Hübner) larvae after emergence from eggs placed on treated leaves: top = normal larva that emerged from untreated substrate; bottom = larva with fluid-filled, balloon-like distortion that emerged from lufenuron-treated leaf.

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

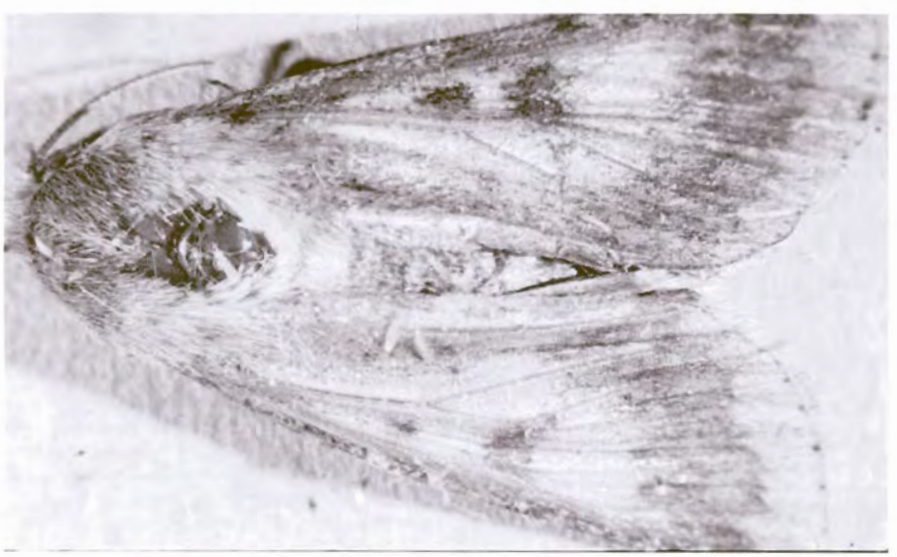

Fig. 7. Normal Helicoverpa armigera (Hübner) adult that emerged from control experiment

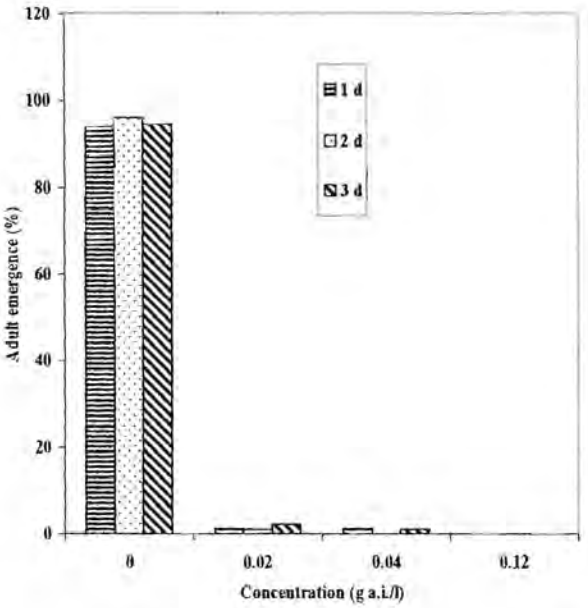

Fig. 8. Helicoverpa armigera (Hübner) adult emergence from treated tomato leaf bioassay.

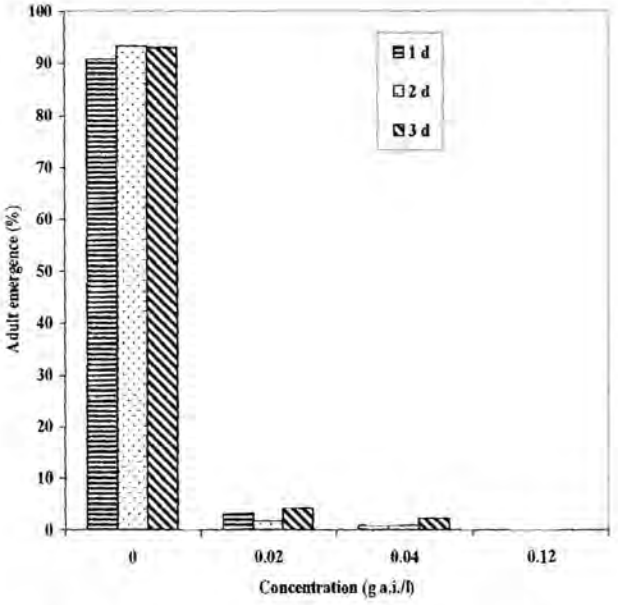

Fig. 9. Helicoverpa armigera (Hübner) adult emergence from treated tomato fruit bioassay.

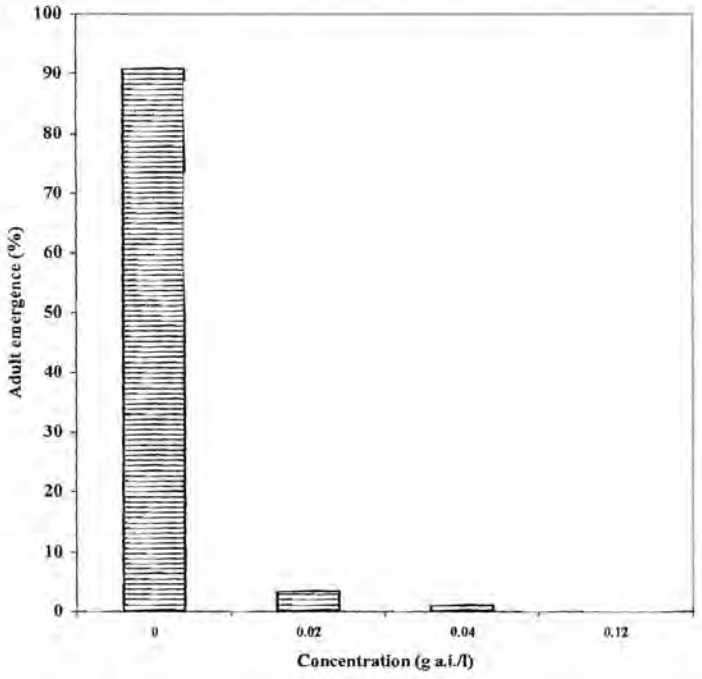

Fig.10. Helicoverpa armigera (Hübner) adult emergence (transovarial bioassay).

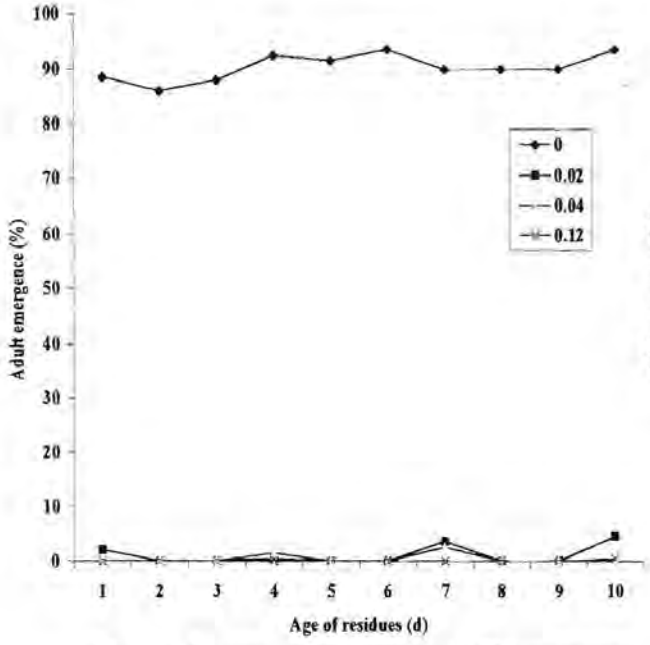

Fig. 11. Percentage emergence of Helicoverpa armigera (Hübner) adults from 1 day old eggs in in the residual activity bioassay.

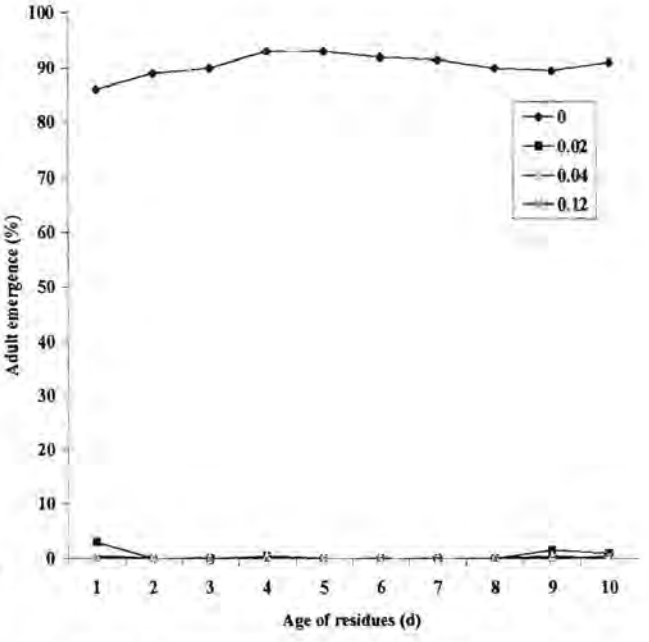

Fig. 12. Percentage emergence of Helicoverpa armigera (Hübner) adults from 2 day old eggs in the residual activity bioassay.

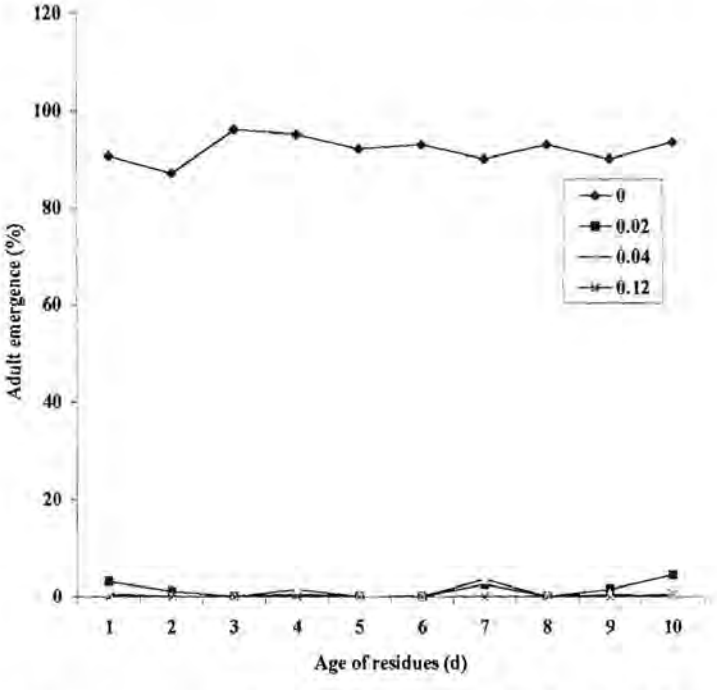

Fig.13. Percentage emergence of Helicoverpa armigera (Hübner) adults from 3 day old eggs in the residual activity bioassay.

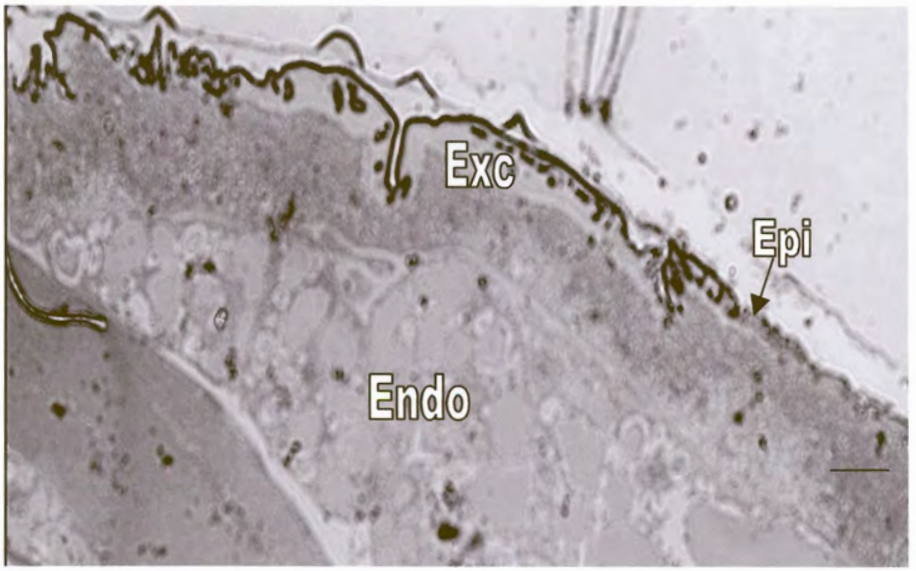

Fig. 14A. Cross section through the integument of untreated Helicoverpa armigera (Hübner) larva showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 48.3 μm.

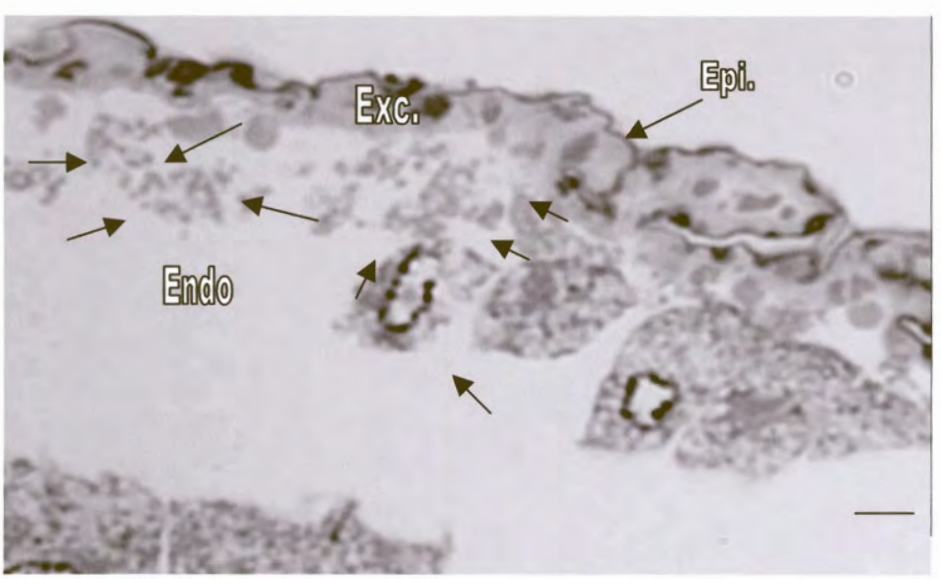

Fig. 14B. Cross section through the integument of lufenuron-treated larva showing a distorted endocuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 48.3 μm.

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

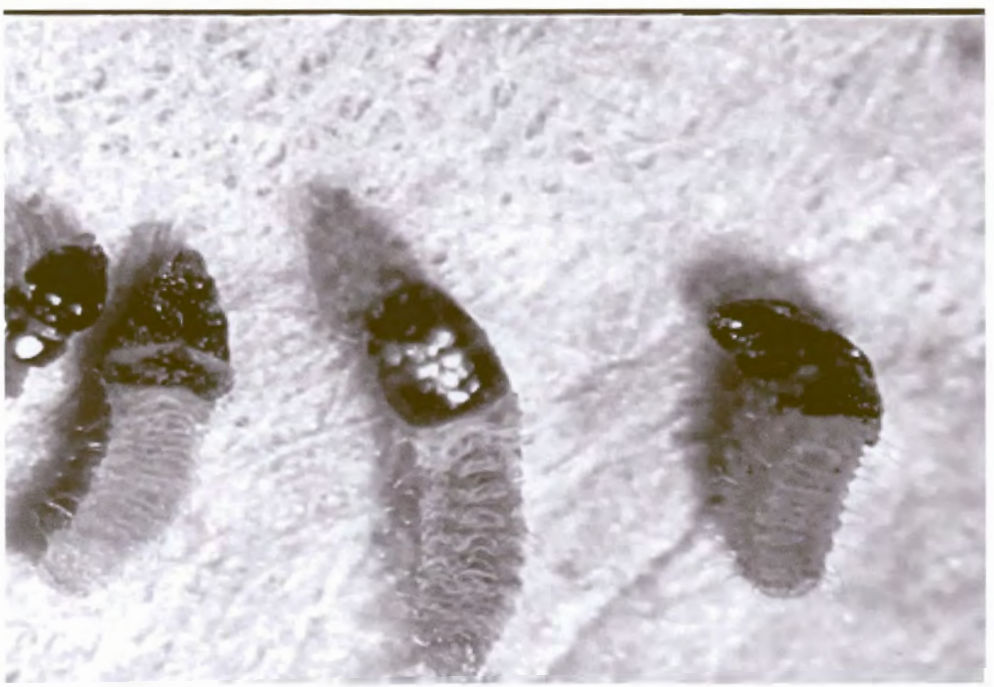

Fig.1B. Effect of lufenuron on first instar Phthorimaea operculella (Zeller) larvae: Dark shrivelled first instars that emerged from eggs placed on lufenuron-treated tubers.

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.

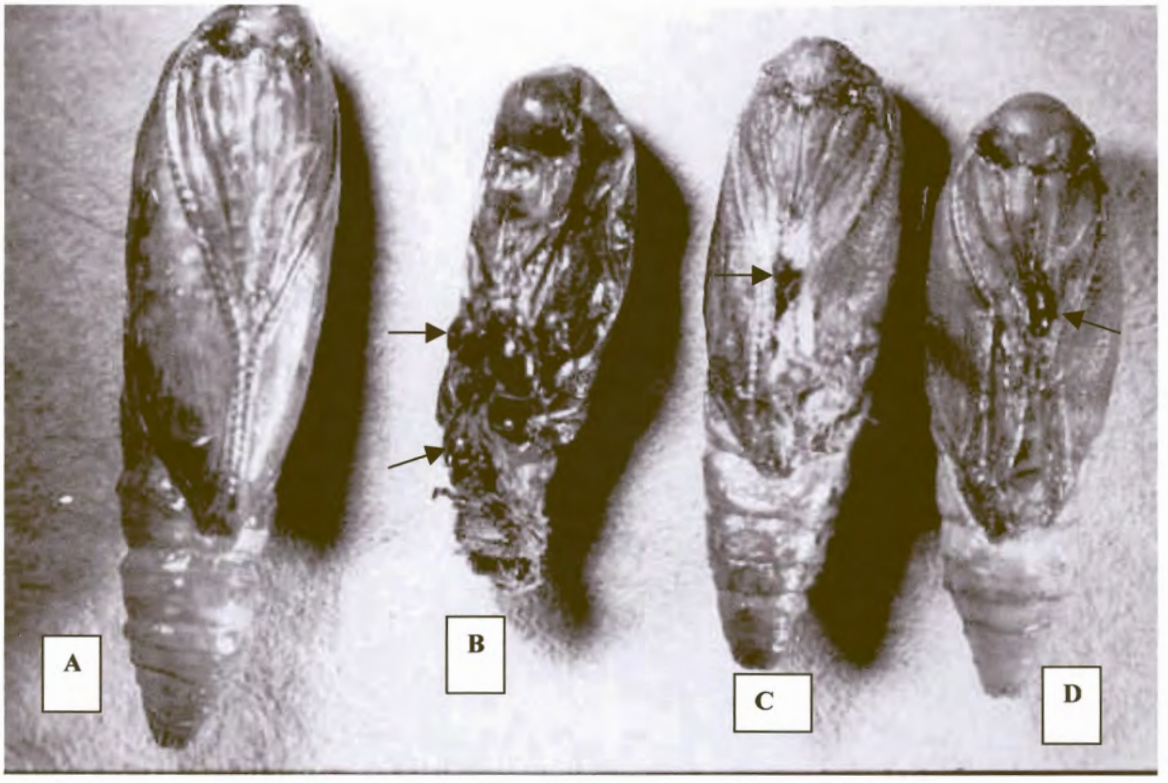

Fig. 3. Effects of lufenuron on Phthorimaea operculella (Zeller) pupae: A = normal pupa that emerged from untreated tuber; B – D = pupae with ruptured cocoons and leaking haemolymph that emerged from lufenuron-treated tubers.

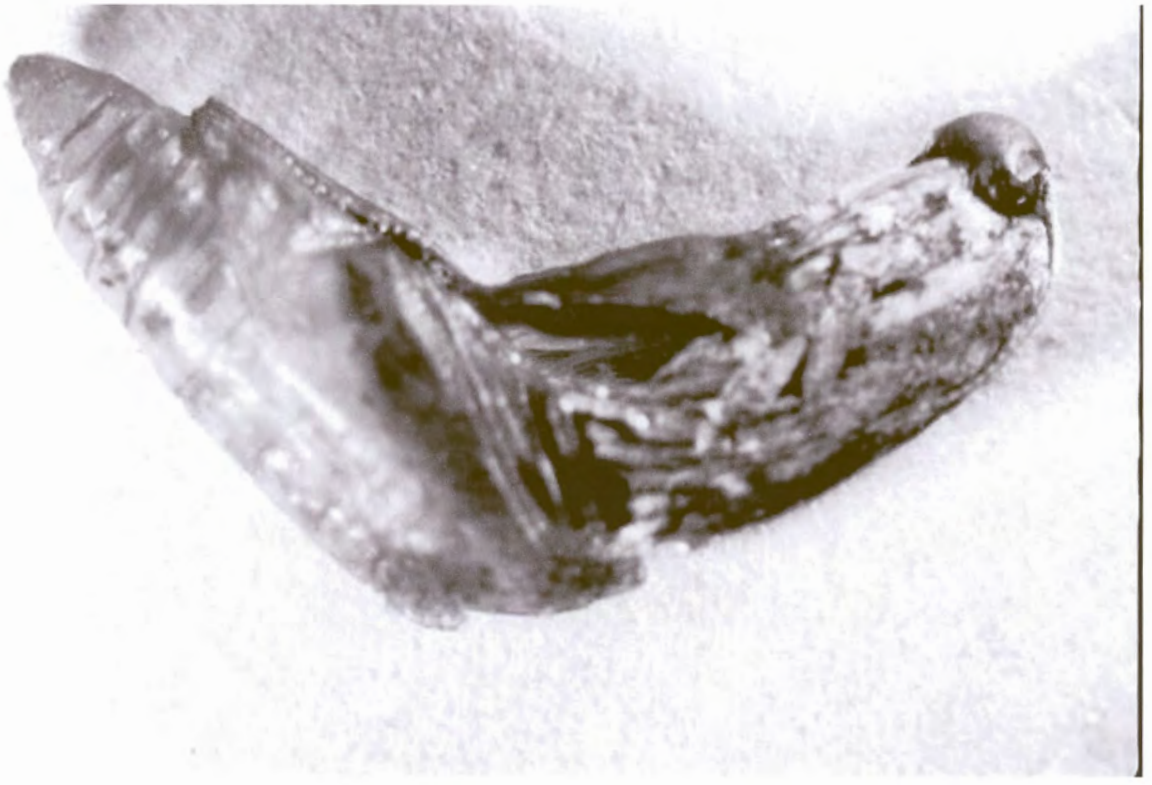

Fig. 4. Phthorimaea operculella (Zeller) adult unable to escape from pupal case at emergence.

Fig. 5. Adult potato tuber moth

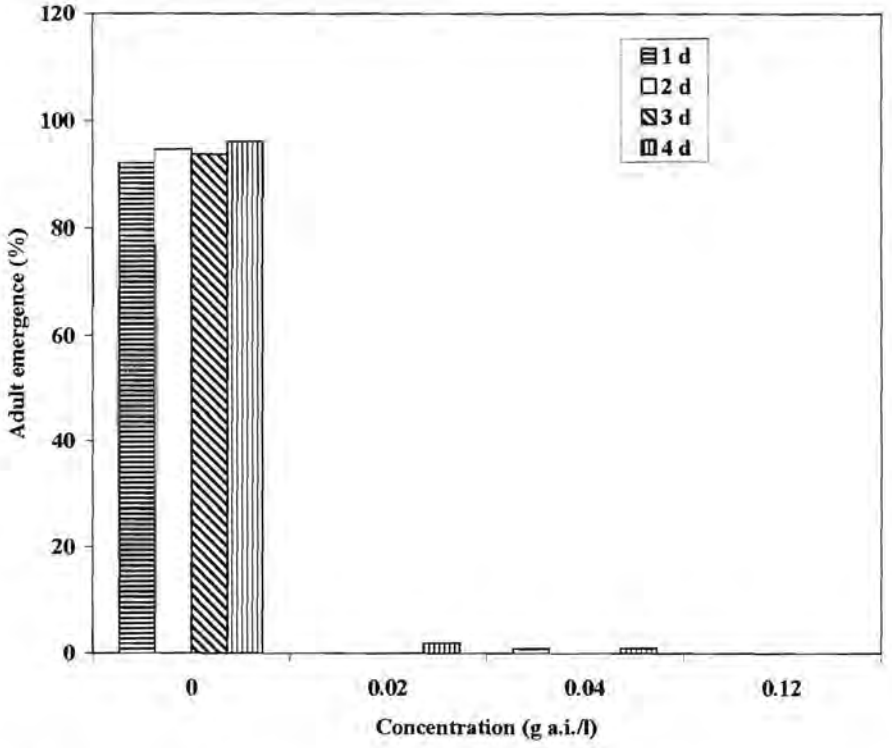

Fig. 6. Phthorimaea operculella (Zeller) adult emergence from lufenuron-treated tubers.

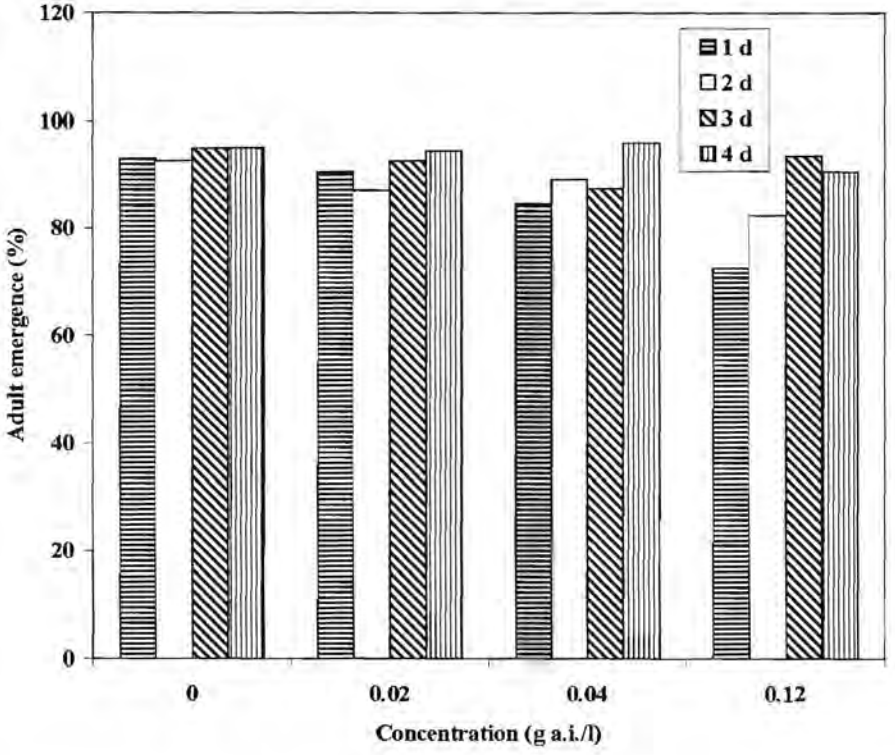

Fig. 7. Phthorimaea operculella (Zeller) adult emergence from the egg dip bioassay

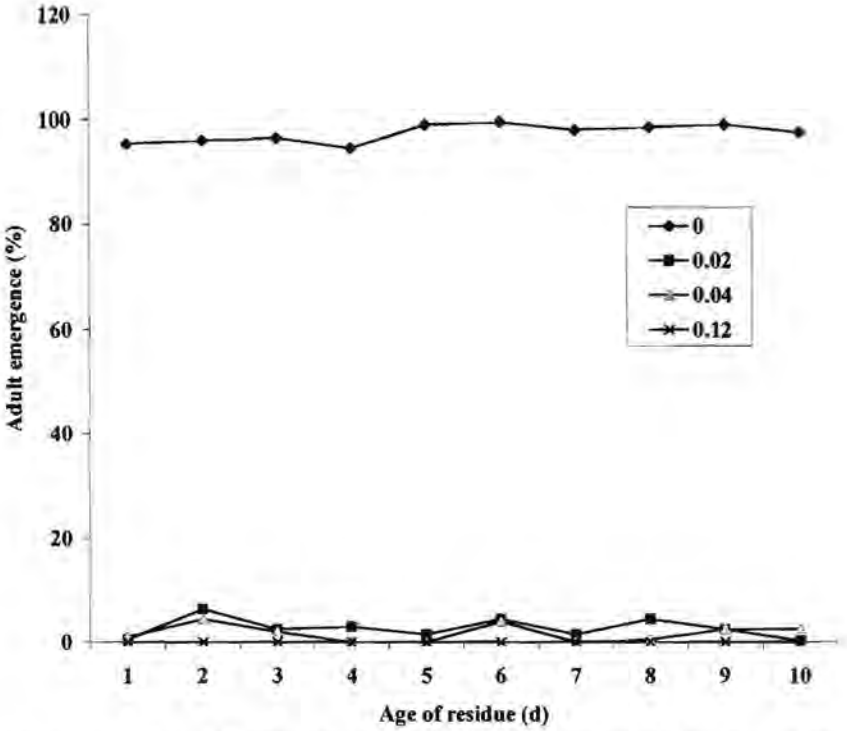

Fig.8. Percentage emergence of Phthorimaea operculella (Zeller) adults from 1 day old eggs in the residual activity bioassay.

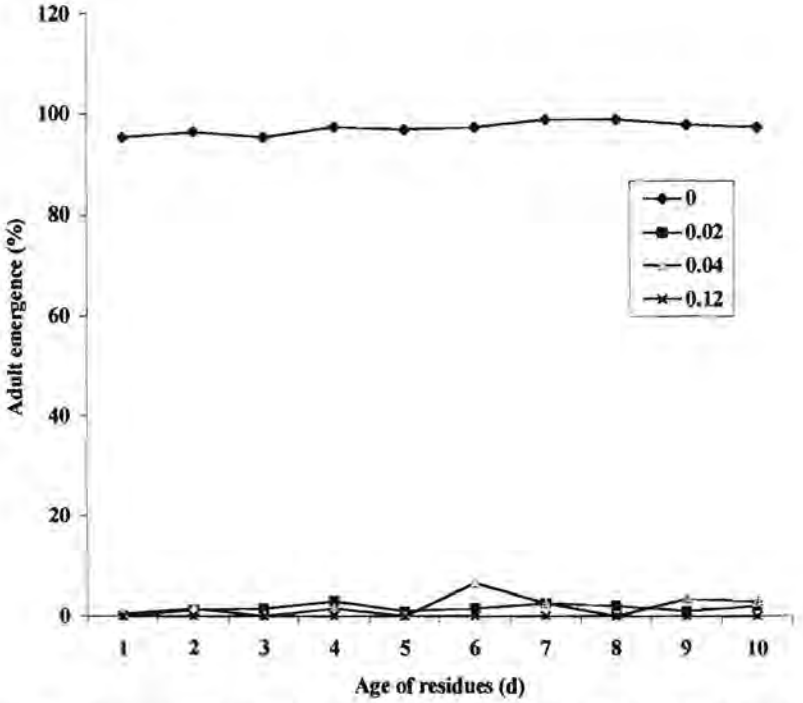

Fig. 9. Percentage emergence of Phthorimaea operculella (Zeller) adults from 2 day old eggs in the residual activity bioassay.

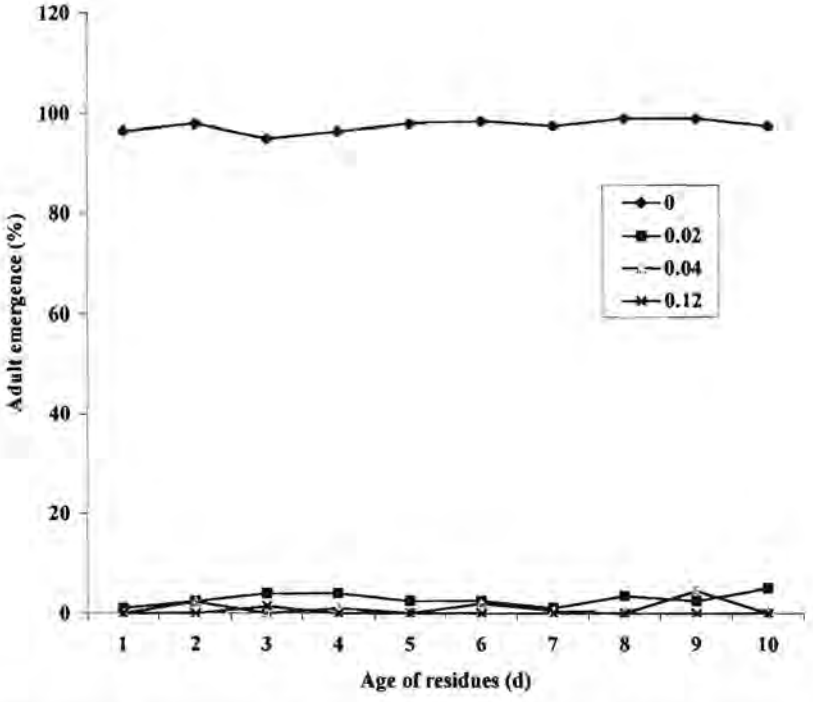

Fig. 10. Percentage emergence of Phthorimaea operculella (Zeller) adults from 3 day old eggs in the residual activity bioassay.

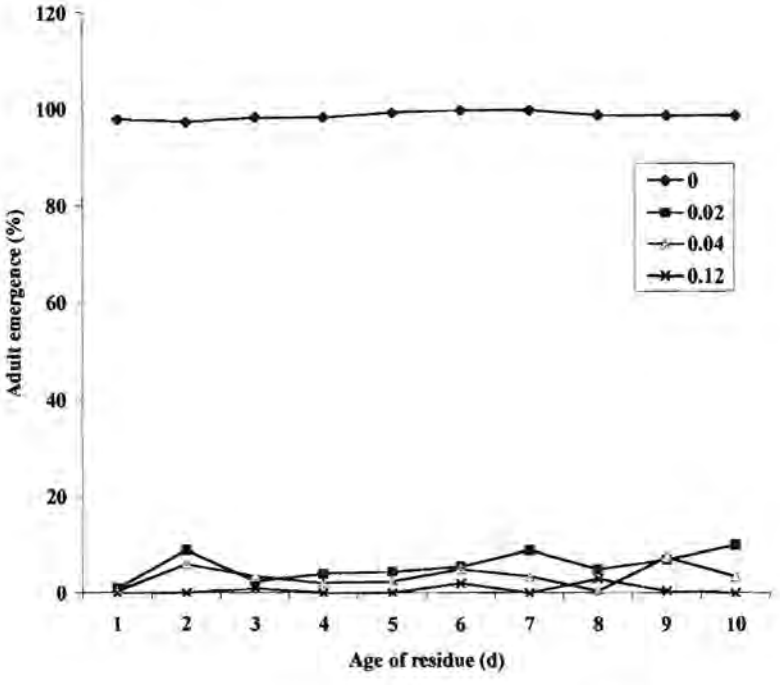

Fig. 11. Percentage emergence of Phthorimaea operculella (Zeller) adults from 4 day old eggs in the residual activity bioassay.

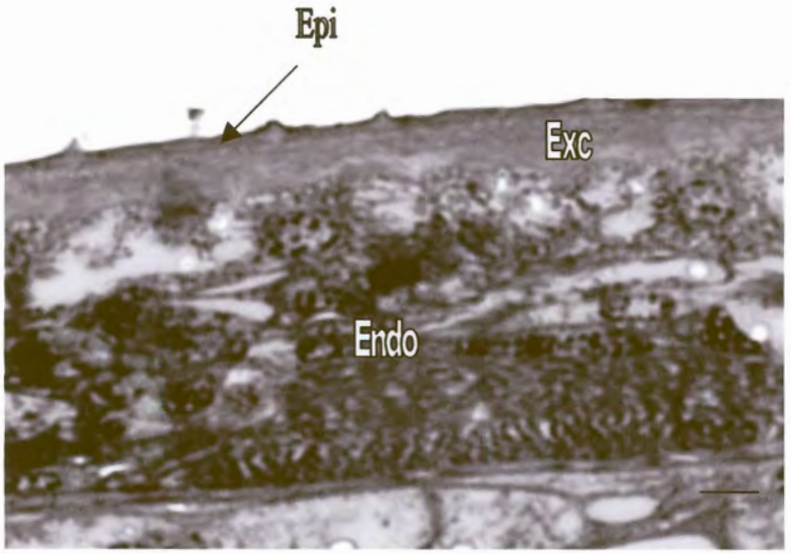

Fig. 12A. Cross section through the integument of untreated second instar Phthorimaea operculella (Zeller) larva, showing normal cuticle. Epi = epicuticle, Exc = exocuticle, Endo = endocuticle. Bar = 51.2 μm.

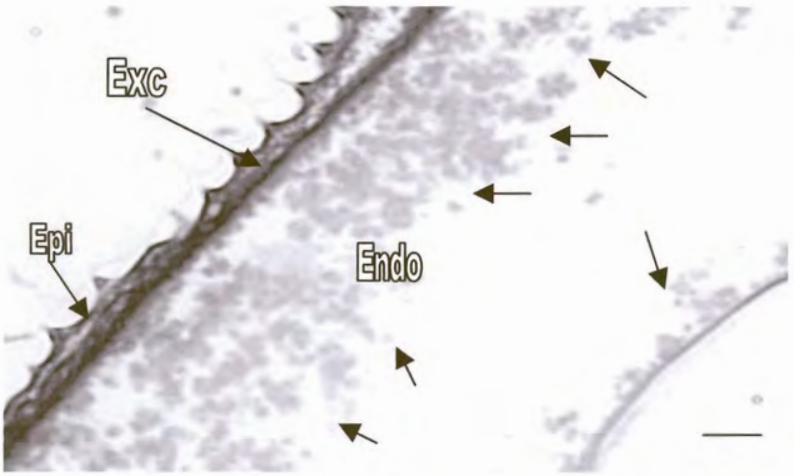

Fig. 12B. Distorted endocuticle of lufenuron-treated larva with scattered globular materials (arrows). Epi. = epicuticle; Exc = exocuticle; Endo = endocuticle.

Bar = 51.2 μm.