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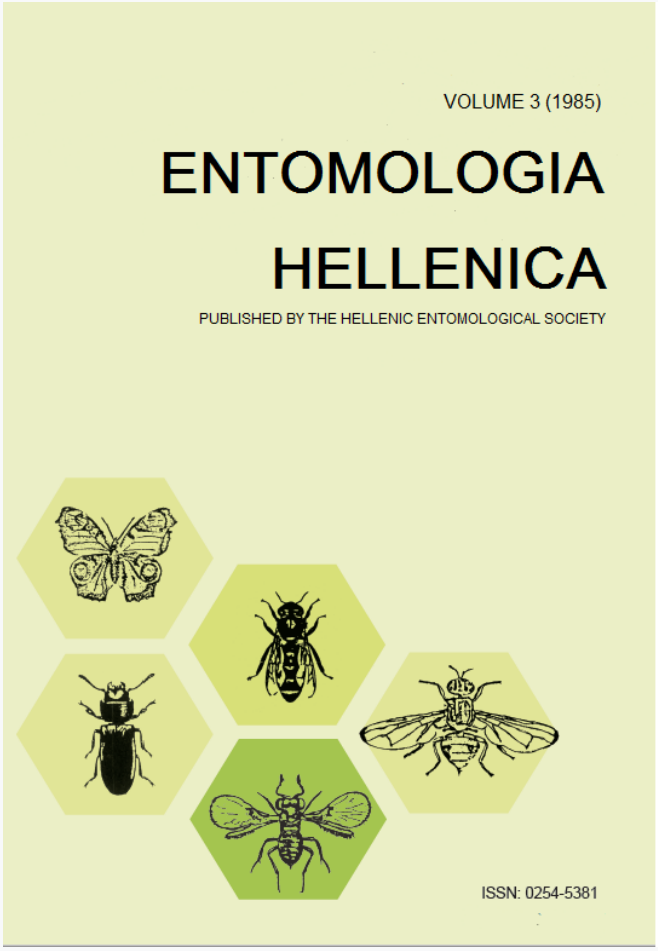

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Effects of a Juvenoid on Lipid Metabolism and Fatty Acid Composition During Growth of *Heliothis armigera*[1]

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ABSTRACT

The effect of the juvenoid ZR-619 (Zoecon Corp.) on lipid metabolism and fatty acid composition of last instar larvae, pupae and pharate adults of *Heliothis armigera* Hbn. (Lepidoptera, Noctuidae) were studied. Treatments at low doses with the juvenoid resulted in an increase of the body weight of larvae and affected the composition of lipids accumulated. In non-treated larvae neutral lipids represent 88.9% of the total lipids, while in treated larvae, the neutral lipids are present at a lower level (80.2%). Fatty acid composition in untreated larvae, pupae and pharate adults is characterized by large proportions of palmitic and oleic acids and higher proportions of linoleic acid than of linolenic acid. Alteration in fatty acid composition is observed in phospholipids of pupae and pharate adults, the proportions of palmitic and oleic acid being lower than in larvae. The juvenoid ZR-619 slightly affected the fatty acid composition of neutral lipids in treated larvae, while fatty acid composition of phospholipids was affected.

Introduction

Juvenoids applied at low or moderate dose to caterpillars of certain insect species frequently extend the period of larval growth (Silhashek and Oberlander 1975; Krypsin et al. 1977; Sehnal et al. 1976; Ciemior et al. 1979). The juvenoid ZR-619 Ethyl-11-methoxy-3,7, 11-trimethyl - dodeca-2,4 - dienethiolate (Zoecon Corp. Palo Alto Ca.), applied to last instar larvae of *Heliothis armigera* Hbn., resulted in an increase of the body weight and prolonged the duration of this instar 6 to 8 times. All larvae died without undergoing pupation (Fytizas 1977). Feeding was stimulated during the first 5 days of treatment and then decreased gradually (Fytizas and Mourikis 1979). The total body metabolism of treated larvae was slightly affected during the first 5 days, but was reduced spectacularly 15 days later (Fytizas and Mourikis 1981).

The importance of lipids in insect development and metabolism is well known (Dadd 1973), especially when great changes occur such as those before and during metamorphosis, diapause and starvation. Since the normal development of insects is disturbed by compounds that mimic juvenile hormone action (Novak et al. 1976), it is possible that lipid metabolism in the last instar of *H. armigera* treated with ZR-619 deviates from the normal metabolic pathway. In this paper we report the results of the effect of ZR-619 on lipid metabolism during the last larval instar of *H. armigera* and changes observed in fatty acid composition of treated larvae.

Materials and Methods

Experimental insects were obtained from a colony maintained at the Benaki Phytopathological Institute

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over many generations. Larvae were reared on a semisynthetic diet (Mourikis and Alexopoulou 1969) and as soon as they underwent the last larval moult, they were transferred individually to 35 ml plastic containers. Food was replaced daily. The juvenoid ZR-619 was topically applied between the abdominal legs of the IVth segment at a dose of 8 μg diluted in 0.8 μl of acetone; treatments started the second day after the last instar moult and were repeated every two days. Dry weight was determined on the whole body of the insects after heating to constant weight at 80°C for 24 hrs.

a. Extraction of lipids

Total lipids were extracted from approx. 2 g wet weight of whole insects based on the procedure of Folch et al. (1957). The extracts were made by grinding the samples with 20 ml of chloroform: methanol (2:1, v/v) using an all glass homogenizer. The mixture was filtered and an additional 10 ml of chloroform: methanol was poured over the residue and added to the filtrate. The filtrate was evaporated to dryness under reduced pressure. Total lipids content was determined by resuspending the lipids in 5 ml pentane and transferring the solution to preweighed test tubes. The pentane extracts were evaporated to dryness under nitrogen, the tubes kept in a desiccator and weighed.

b. Column chromatography

Lipids were separated into neutral lipids and phospholipids by column chromatography on silicic acid (SIL-LC 325 Mesh, Sigma Chem. Comp.): neutral lipids were eluted from the column with 50 ml of chloroform and phospholipids with 100 ml of chloroform:methanol (1:1, v/v). Neutral lipids and phospholipids content was determined by evaporating the chloroform, chloroform: methanol fractions to dryness under reduced pressure, resuspending the lipids in 5 ml of pentane and transferring the pentane extract to preweighed test tubes. The pentane extract was evaporated to dryness under nitrogen, tubes were kept in a desiccator and weighed.

c. Fatty acid analysis

Fatty acid analysis was carried out after derivatization to the methyl esters. Lipids were saponified with transesterification in 1 ml of 0.5 N KOH and 3.5 ml of methanol. The mixture was reacted over night at room temperature and then acidified by addition of 0.5 N H_2SO_4. The fatty acid methyl esters were extracted three times with 3 ml of pentane. Pentane extracts were combined and evaporated to approx. 1 ml. Analysis of fatty acid methyl esters was performed in a Varian 1400 gas-chromatograph equipped with a hydrogen flame ionization detector. The 2m × 1.8 mm (ID) stainless-steel column was packed with 12% Diethylene Glycol Succinate (DEGS) on chromosorb G 80/100 mesh. Column temperature was maintained isothermically at 170°C; injector and detector temperatures were 180°C and 200°C, respectively. Nitrogen was the carrier gas at a flow rate of 20 ml/min. Identification of the fatty acid methyl esters was achieved by comparison with the retention times of a mixture of pure fatty acid methyl ester standards. A Hewlet Packard Model 3370 B integrator fitted to the chromatograph provided quantitative evaluation of the chromatograms and relative quantities of individual fatty acid methyl esters were recorded as percentages of the total peak area.

The data were subjected to arcsin transformation (Steel and Torrie, 1960) prior to statistical analysis. Means comparisons were made with Student's t test.

TABLE 1. Body weight and lipids content (X±SD) of last instar larvae treated with ZR-619 (T) and non-treated (NT) larvae, pupae (P) and pharate adults (Pha) of *H. armigera*.

Stage/treatment		Age*	Mean body weight: Fresh weight (mg)	Mean body weight: Dry weight (mg)	Mean body weight: Dry weight %	Lipids content: % of fresh weight	Lipids content: % of dry weight
Larvae	NT	0(18)	105.8	20.1	19.0±1.7	3.4±0.4	18.0±2.3
"	"	2(6)	193.8	48.0	24.8±0.8	6.1±0.5	24.6±1.9
"	"	4(6)	376.5	104.7	27.8±0.5	8.6±1.1	32.2±3.6
"	"	5(6)	415.3	118.4	28.4±1.4	10.9±1.3	38.5±4.0
Four-day P	"	11(6)	303.8	91.7	30.2±1.2	8.1±0.6	27.0±1.8
Ten-day Pha	"	17(9)	264.3	84.4	31.9±1.8	8.8±0.6	28.0±2.2
Larvae	T	5(12)	525.9	135.1	25.7±1.1	10.9±2.4	42.3±9.4
"	"	15(6)	307.2	81.1	26.4±1.1	9.0±1.0	34.2±3.7

* In days after the last larval moult. Number in parenthesis denotes number of determinations; each determination was on a separate animal.

Results

Larval body weight increased during the last larval moult and reached a maximum level by the 5th day in this stage (Table 1). 5-day-old larvae treated twice with ZR-619 had a higher body weight than that of the same age non-treated larvae; within the following 10 days the weight of the treated larvae decreased gradually. Total lipids of non-treated larvae increased during development. The lipid content of 5-day-old treated larvae was much the same as that of 5-day-old non-treated larvae, expressed as % of fresh and dry body weight (Table 1).

Neutral lipids in last instar larvae, pupae and pharate adults were present in high proportions in the range 86-90% of the total lipids (Table 2), while proportions of phospholipids ranged from 9-14%. The proportion of neutral lipids in treated larvae, representing 80.2% of the total lipids, while phospholipids at 19.8% of total lipids, were higher than in untreated larvae.

a. Total fatty acid composition

Fatty acid analyses of untreated larvae, pupae and pharate adults are shown in Table 3. Palmitic (C16:0) and oleic (C18:1) acids were present at higher levels relative to the other fatty acids; these two fatty acids comprised about 70% of the fatty acid mixture and their proportion remained constant during larval and pupal development. In pharate adults, the level of palmitic and oleic acids decreased sharply and both acids contributed only 41,3% of the total. The level of palmitoleic (C16:1) acid increased during development and almost doubled in pharate adults. Linoleic remained constant in larval and pupal stage, but was doubled in proportion in pharate adults. Stearic (C18:0) and linolenic (C18:3) acids were present in low proportion in the larvae and pupae, but were present in 3-fold higher proportions in pharate adults.

TABLE 2. Per cent composition for neutral lipids and phospholipids of *H. armigera* last instar larvae treated with ZR-619 (T) and non treated (NT) larvae, pupae (P) and pharate adults (Pha).

Stage/treatment	Age*	Neutral lipids**	Phospholipids**
Last instar larvae (NT)	0	87.1 a	12.9 a
Last instar larvae (NT)	5	88.9 a	11.1 a
Four-day P(NT)	11	90.9 a	9.5 a
Ten-day Pha (NT)	17	86.0 a	14.0 ab
Last instar larvae (T)	5	80.2 b	19.8 b
Last instar larvae (T)	15	84.6 ab	15.4 ab

* In days after the last larval moult.
** Data represent average of 5 replicates from 5 independent extractions; in each extraction 6-10 animals were used. Means followed by the same letter in each column are not significantly different (Student's t test P=0.05).

TABLE 3. Relative per cent fatty acid composition of *H. armigera* during larval, pupal and pharate adult development.

	Developmental stage*			
Fatty acids	Last instar larvae**	Last instar larvae***	Pupae****	Pharate adults*****
C 16:0	35.5 a	33.8 a	35.4 a	22.2 b
C 16:1	10.9 a	12.7 a	14.2 ab	16.9 b
C 18:0	2.4 a	2.6 a	trace	6.6 b
C 18:1	36.4 a	36.9 a	39.1 a	19.1 b
C 18:2	10.3 a	10.4 a	9.5 a	21.0 b
C 18:3	4.0 a	4.1 a	2.5 b	14.2 c
Total unsaturated	62.1	63.6	62.0	71.2

* Data represent average of 5 replicates from 5 independent extractions; in each extraction 6-10 animals were used. Means followed by the same letter in each row are not significantly different (Student's t test P=0.05)
** During the last larval moult.
*** 5 days after last larval moult.
**** 4-day pupae.
***** 10-day pharate adults.

b. Fatty acid composition of neutral lipids and phospholipids of treated and non-treated larvae.

Results of fatty acid analyses of neutral lipids and phospholipids in last instar larvae treated with the juvenoid and compared to non-treated ones are shown in Table 4. No considerable difference was found in fatty acid composition of neutral lipids between treated and non treated larvae. Important changes in the fatty acid composition were observed when phospholipids were analyzed: stearic, linolenic and linoleic (C18:2) acids were present in marked and significantly higher proportions.

c. Fatty acid composition of neutral lipids and phospholipids of untreated pupae and pharate adults.

The fatty acid composition of neutral lipids and phospholipids in 4-day-old pupae and 10-day-old pharate adults are shown in Table 5. The pattern for both stages of fatty acid composition in neutral lipids was the same as that of 5-day-old last instar larvae and no significant changes were observed, compared with Table 4. However, changes in fatty acid composition were found in phospholipids: palmitic and oleic acids were reduced by twofold in pupae; palmitoleic and linoleic acids were increased twofold; stearic and linolenic increased threefold. Between pupae and pharate adults smaller changes were observed: stearic and linolenic acids increased, while palmitoleic acid decreased.

TABLE 4. Relative per cent of fatty acid composition of neutral lipids and phospholipids of *H. armigera* last instar larvae non treated and treated with the juvenoid ZR-619.

	Non-treated larvae*				Treated larvae*			
	Neutral lipids		Phospholipids		Neutral lipids		Phospholipids	
Fatty acids	A	B	A	B	B	C	B	C
C 16:0	33.9 a	33.1 a	31.3 a	30.3 a	31.8 a	32.3 a	28.0 ab	26.5 b
C 16:1	8.1 a	13.6 b	12.4 b	12.7 b	8.1 a	13.6 b	15.6 b	11.8 b
C 18:0	2.3 b	1.1 a	1.6 a	1.7 a	trace	1.9 a	2.9 b	6.4 c
C 18:1	39.4 a	38.7 a	38.9 a	39.1 a	41.8 a	43.0 a	37.0 a	28.3 b
C 18:2	13.9 a	9.6 b	12.1 a	12.6 a	8.6 b	8.4 b	15.2 a	19.6 c
C 18:3	2.5 a	3.9 a	3.7 a	3.6 a	2.2 b	2.6 b	6.1 c	7.7 c

* Data represent average of 5 replicates from 5 independent extractions; in each extraction 6 animals were used. Means followed by the same letter in each row are not significantly different. (Student's t test P=0.05).
A= During the last larval moult, B= Five days after the last larval moult, C= Fifteen days after the last larval moult.

TABLE 5. Relative per cent of fatty acids composition in neutral lipids and phospholipids of *H. armigera* pupae and pharate adults.

	Neutral lipids*		Phospholipids*	
Fatty acids	4-day pupae	10-day pha. adults	4-day pupae	10-day pha. adults
C 16:0	33.3 a	33.7 a	17.9 b	19.4 b
C 16:1	10.5 a	14.0 a	24.6 b	17.3 b
C 18:0	2.3 a	0.4 a	6.0 b	7.9 b
C 18:1	39.5 a	39.9 a	20.7 b	20.6 b
C 18:2	12.1 a	9.7 a	22.2 b	22.2 b
C 18:3	2.3 a	2.5 a	8.6 b	12.6 c

* Data represent average of 5 replicates from 5 independent extractions; in each extraction 6 animals were used. Means followed by the same letter in each row are not significantly different (Student's t test P=0.05).

Discussion

The amount of total lipids accumulated in treated larvae was not affected much. However, there was some differentiation in the classes of lipids accumulated. Last instar non-treated larvae accumulated mainly neutral lipids, while in treated larvae of the same age there was a reduced proportion of neutral lipids and an increased proportion of phospholipids. The increased proportion of phospholipids in treated larvae may merely reflect metabolism of storage neutral lipid, which would be necessitated by the decline in feeding during the extended larval stage induced by the treatment.

The fatty acid composition of *H. armigera* total lipids is characterized of the large amount of palmitic and oleic acids and the higher concentration of linoleic over that of linolenic. The concentration of linolenic acid was higher than that of linoleic acid, in most of the Lepidopterous species studied (Gilbert 1967). However, the fatty acid composition of two other Noctuid species, *H. virencens* and *Trichoplusia ni,* (Thompson 1973) had the same pattern as that of *H. armigera.*

The ratio of saturated to unsaturated fatty acids was much the same in larvae and pupae, although there was an icrease of monounsaturated acids and a decrease of polyunsaturated acids. The ratio between saturated and unsaturated fatty acids changes considerably during the pharate adult stage, the amount of palmitic and oleic acids decreases while that of palmitoleic, linoleic and linolenic increases. Important changes in the fatty acids composition of pharate adults of another Lepidopteran, *Hyalophora cecropia,* have been reported by Stephen and Gilbert (1970). They suggested that these changes were probably due to the oxidation of fatty acids to acetyl-CoA and the resynthesis of the fatty acid chains. They also suggested that the fatty acid synthesis depended on the amount of juvenile hormone present and that saturated fatty acids are synthesized when the concentration of juvenile hormone is high.

The juvenoid ZR-619 has no effect on the fatty acid composition in neutral lipids of treated larvae. On the contrary, alteration of fatty acid composition in phospholipids was observed between treated and non-treated larvae: this seems to resemble normal process of fatty acid alteration in phospholipids, which was postponded, because of the prolongation of this instar due to the juvenoid.

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KEY WORDS: *Heliotis armigera,* Lipid metabolism, Fatty acids during growth, Juvenoid effect on larval lipids

Επίδραση μιας Ουσίας Ρυθμιστή Ανάπτυξης Εντόμων στο Μεταβολισμό των Λιπιδίων και τη Σύνθεση των Λιπαρών Οξέων στα Διάφορα Στάδια Ανάπτυξης του *Heliothis armigera*

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ΠΕΡΙΛΗΨΗ

Η επίδραση της ουσίας ZR-619 (Zoecon Corp.) (ρυθμιστή ανάπτυξης εντόμων) στον μεταβολισμό των λιπιδίων και τη σύνθεση του μίγματος των λιπαρών οξέων μελετήθηκε σε τελευταίου σταδίου προνύμφη του *Heliothis armigera* Hbn. Μελετήθηκε επίσης η σύνθεση του μίγματος των λιπαρών οξέων σε νύμφες 4 ημερών και τέλεια έντομα. Επεμβάσεις με ZR-619 είχαν σαν αποτέλεσμα την αύξηση του βάρους των προνυμφών και επηρέασαν τη σύνθεση των λιπιδίων που συσσωρεύονται στην προνύμφη. Η αναλογία των ουδετέρων λιπιδίων, στις προνύμφες που δεν είχαν πάρει ZR-619 ήταν 88.9% των ολικών λιπιδίων, ενώ στις προνύμφες που είχαν πάρει την ουσία ήταν 80.2%. Η σύνθεση του μίγματος των λιπαρών οξέων στις προνύμφες, νύμφες και τέλεια έντομα, χαρακτηρίζεται από τη μεγάλη αναλογία του παλμιτικού και ελαϊκού οξέος και την υψηλότερη αναλογία του λινολεϊκού σε σχέση με αυτή του λινολενικού οξέος. Μεταβολή στη σύνθεση του μίγματος των λιπαρών οξέων βρέθηκε στα φωσφολιπίδια στα στάδια της νύμφης και του τελείου εντόμου. Η αναλογία του παλμιτικού και ελαϊκού οξέος ήταν πολύ χαμηλότερη από αυτή των προνυμφών.

Η ουσία ZR - 619 επηρεάζει πολύ λίγο τη σύνθεση των λιπαρών οξέων στα ουδέτερα λιπίδια της προνύμφης, ενώ η σύνθεση αυτών στα φωσφολιπίδια επηρεάζεται.