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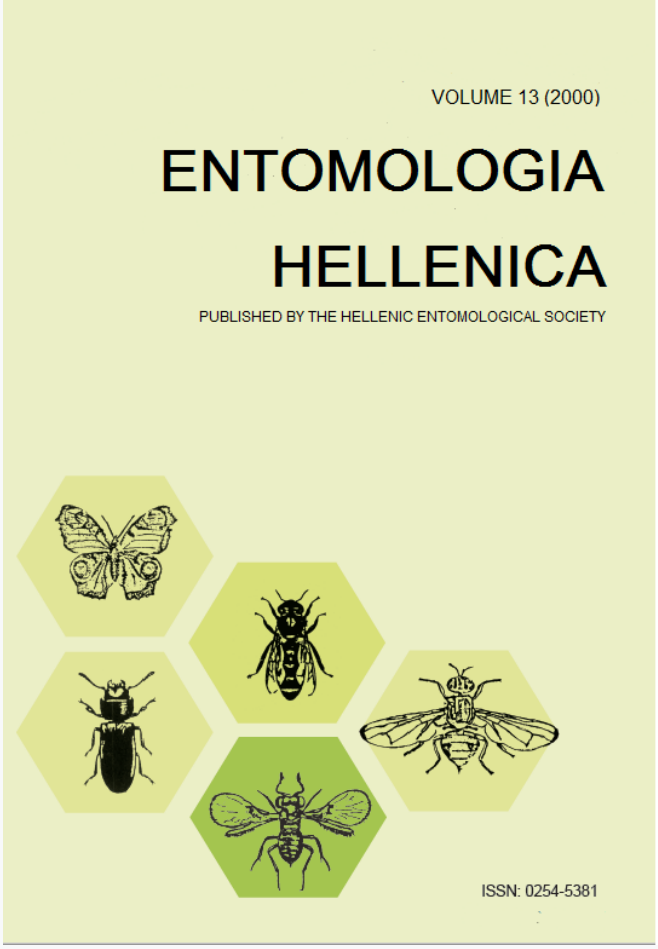

Η επίδραση των C6- μέχρι C10- λιπαρών οξέων στην ανάπτυξη και επιβίωση των προνυμφών του Δάκου της Ελιάς, Bactrocera oleae (Diptera: Tephritidae)

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The effect of C6- to C10- fatty acids on larval growth and survival of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae)[1]

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ABSTRACT

The effects of caproic (C6), amino caproic (C6), caprylic (C8) and capric (C10) acid on larval performance of the olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) were investigated. The acids were added to an artificial larval diet at four different concentration levels, during the mixing of its ingredients. Eggs obtained from a colony of olive fruit flies were placed on these diets and on the control. Caproic acid depressed significantly egg hatchability, larval survival and larval weight at the dietary level of 0.05 and 0.1%. In addition, it did not permit eggs to hatch at the level of 0.2%. On the contrary, aminocaproic acid did not depress any of the parameters recorded up to the level of 0.2%. The levels of 0.4 to 1.6% depressed larval survival larval growth and pupal weight and the level of 3.2% did not permit larvae to survive. Caprylic acid depressed significantly hatchability, larval survival and number of pupae/g diet at the level of 0.0125% and gave no pupae at the level of 0.1%. Finally capric acid depressed egg hatchability, larval survival and larval weight from the level of 0.05% and gave no pupae at the level of 0.2%. Adult emergence was not affected by any of the acids tested.

Introduction

Short and medium carbon chain length fatty acids (C6 to C12) were found to be toxic to beetles, house flies and mosquitoes (Levinson and Ascher 1954, Quaraishi and Thorsteinson 1965, House and Graham 1967) and to inhibit larval survival of fruit flies (Fogleman and Kircher 1986). Presumably they are selective enough to harm insects (House 1967) but would not present a grave hazard to domestic animals and man because they are not stored to any extent in animal fats (Hilditch 1956). They may be environmentally safe insecticide substitutes for more dangerous chemical or biological control agents. Chemical data for these fatty acids are reviewed by Markley (1960).

The effects of fatty acids in insects depend on the species of insect and on the specific fatty acid. A review of these effects in various stages of insects is given by Holyoke and Reese (1987). To my knowledge, there exists no published studies of effects of C6 to C10 fatty acids on the olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) which is the most serious pest of the olive fruit in the Mediterranean basin. Such effects of n-caproic acid ($C_6H_{12}O_2$), -amino-n-caproic acid ($C_6H_{13}NO_2$), caprylic acid ($C_8H_{16}O_2$) and n-capric acid ($C_{10}H_{20}O_2$), upon larval growth and development of the olive fruit fly are presented in this work, which is essential considering further work on the mode of action and on practical applications of these acids.

Materials and Methods

Eggs 48±4 h old were obtained from Laboratory

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Stock T of olive fruit flies originated from the island of Aghia Trias, Attiki, Greece, 1982 and maintained at approximately 25±2o C, 65±5% RH and L16:D8 daily photoperiod according to a rearing technique described by Tsitsipis (1977). The larval diet (medium) used, its composition and preparation is given elsewhere (Manoukas 1975, 1989). This diet was used as a control diet and four additional diets were formulated by adding each fatty acid at four different levels at a geometric sequence. The appropriate quantity of each fatty acid was dissolved in ethyl alcohol except n-amino caproic acid which was dissolved in water and then added to the diet prior to the addition of yeast, soy hydrolysate and cellulose. The appropriate amount of alcohol was added to the control diet. All fatty acids were of analytical grade (Sigma Co., USA). Plastic containers of 4 cm height and 10 cm diameter with covers were used. Forty-five g of diet were placed in each container (replicate) and 4 replicates per each level of acid (treatment) were used with 7 eggs/g diet. Eggs were placed on a filter paper on the surface of the diet and hatchability was checked 3 days later. Hatchability was based on the number of eggs found on the paper and the total eggs set. Number of larvae and their weight were recorded on a 4 g sample obtained randomly on the 10th day following placement of eggs, while pupae were collected and counted from 14th to 21st day. The records on the 10th day were obtained in order to differentiate the effects on larvae from those on pupae. Pupal weight was taken at least 3 days after the last collection and adult emergence was recorded. Statistical procedures employed were those of Steele and Torrie (1960), as indicated in the tables.

Results and Discussion

Table 1 presents the effect of caproic acid and amino caproic acid upon the performance of the olive fruit fly larvae. Hatchability of eggs was significantly depressed by the level 0.05 of caproic acid and further depressed by the level 0.1

TABLE 1. Effect of n-caproic acid and amino-n-caproic acid upon eggs, larvae and pupae of the olive fruit fly (4 replicates/treatment and 7 eggs/replicate)[1].

Expt. no.	Acid% in diet	Egg hatch %	No. of larvae/g diet	Larval weight, mg	No. of pupae/g diet	Pupal weight, mg	Adults, % on pupae
	n-caproic acid						
1	0.0	69^{a}	2.5^{a}	1.7^{b}	2.4^{a}	6.5	87
	0.025	65^{a}	2.9^{a}	2.2^{b}	2.7^{a}	6.6	80
	0.05	20^{b}	1.0^{b}	0.3^{b}	1.0^{b}	6.6	95
	0.1	9^{c}	0.3^{c}	0.2^{b}	0.1^{c}	6.3	100
	0.2	0	—	—	—	—	—
2	0.0	66^{a}	3.3^{a}	2.4^{a}	3.1^{a}	6.8^{a}	92
	0.0125	70^{a}	3.4^{a}	2.5^{a}	3.0^{a}	7.0^{a}	84
	0.025	69^{a}	3.9^{a}	2.0^{a}	3.0^{a}	6.9^{a}	86
	0.05	37^{b}	2.4^{b}	1.5^{b}	2.3**b**	7.0^{a}	90
	0.1	15^{c}	1.3^{c}	0.9^{c}	0.2^{c}	3.8^{a}	90
	amino-n-caproic acid						
1	0.0	72	3.3^{a}	1.3^{a}	2.9^{a}	6.7^{a}	89
	0.2	72	3.1^{a}	1.2^{a}	2.3^{ab}	6.3^{a}	87
	0.4	71	3.4^{a}	0.8^{b}	1.9^{b}	6.2^{a}	92
	0.8	71	2.1^{b}	0.7^{bc}	1.8^{b}	5.3^{b}	71
	1.6	73	1.5^{c}	0.5^{c}	1.4^{c}	5.2^{b}	92
2	0.0	71	2.8^{a}	1.9^{a}	2.0^{a}	7.0^{a}	80
	0.8	73	1.0^{b}	0.6^{b}	0.6^{b}	5.5^{b}	78
	1.6	67	0.5^{c}	0.3^{c}	0.6^{b}	5.7^{b}	70
	3.2	63	0	—	—	—	—
	6.4	61	0	—	—	—	—

[1] Means in the same column in the same experiment followed by the same or no letter do not differ significantly, at the 0.05 level of probability by Duncan's new multiple-range test.

compared to the control (0.00 level), in experiment 1. The same was true for larval survival (larvae/g diet) at 10 day and to pupation (pupae/g diet). Larval weight at 10 day was significantly lower at the level of 0.05 and 0.1 than that of the control but pupal weight did not differ. Pupation at those two levels of caproic acid was greatly delayed (20 days compared to 14 for the control) but the larvae which succeeded to survive and pupate attained normal pupal weight. Adult emergence was also equivalent for all levels. The level 0.2 did not permit any eggs to hatch. Experiment 2 was designed to verify the results of the previous experiment. to include a lower level (0.0125) of caproic acid, and exclude the 0.2 level. The low level of 0.0125 was included to see if there is a beneficial effect of this fatty acid, since a substantial but not significant improvement of number of larvae, larval weight and number of pupae was observed in experiment 1. The results confirmed the results of the previous experiment 1 with the exception that the level of 0.1 depressed pupal weight. The lowest level of 0.0125 failed to have any beneficial effect upon larval performance. The amino caproic acid the level of 0.2 (experiment 1) did not affect any of the parameters recorded. The level of 0.4 depressed both larval weight and number of pupae while the level of 0.8 number of larvae, larval weight, number of pupae and pupal weight. The highest level tested (1.6) depressed number of larvae, larval weight, number of pupae and pupal weight compared to both the control and 0.4 level. Contrary to caproic acid egg hatchability was not affected by any of the levels tested. Experiment 2 in general confirmed the results of the previous experiment for the levels of 0.8 and 1.6. The two additional levels included (3.2 and 6.4), did not permit any larvae to survive. It should be mentioned that developmental time to pupation at the level of 1.6 was approximately 5 days longer than in the control.

Table 2 presents the results of the effect of caprylic acid and n-capric acid. In experiment 1 the level of 0.025 of caprylic acid severely affected

TABLE 2. Effect of caprylic acid and n-capric acid upon eggs, larvae and pupae of the olive fruit fly (4 replicates/treatment and 7 eggs/replicate) [1].

Exp. no.	Acid% in diet	Egg hatch %	No. of larvae/g diet	Larval weight, mg	No. of pupae/g diet	Pupal weight, mg	Adults, % on pupae
	caprylic acid						
1	0.0	70[a]	2.5[a]	1.4[b]	2.4[a]	5.9[a]	82
	0.0250	21[b]	1.1[b]	0.9[b]	0.9[b]	4.8[b]	92
	0.05	9[c]	0.4[c]	0.3[c]	0.2[c]	3.1[c]	91
	0.1	3[d]	—	—	—	—	—
	0.2	0	—	—	—	—	—
2	0.0	75[a]	3.3[a]	1.2[a]	3.4[a]	6.8[a]	84
	0.00625	61[a]	3.4[a]	1.3[a]	2.9[a]	6.3[a]	87
	0.0125	24[b]	3.9[a]	0.7[b]	0.6[b]	6.2[a]	92
	0.025	22[b]	2.4[b]	0.7[b]	0.8b	5.6[b]	93
	0.05	0	1.3[c]	—	—	—	—
	n-capric acid						
1	0.0	71[a]	3.0[a]	1.4[a]	2.5[a]	6.4[a]	92
	0.2	57[b]	2.1[b]	1.1[a]	0.9[b]	5.5[a]	89
	0.4	38[c]	1.2[c]	0.7[b]	0.2[c]	3.5[b]	86
	0.8	9[d]	—	—	—	—	—
	1.6	0	—	—	—	—	—
2	0.0	74[a]	3.3[a]	0.8	2.2[a]	6.5[b]	89
	0.8	61[a]	3.0[a]	0.9	2.3[a]	6.3[a]	88
	1.6	71[a]	2.8[ab]	0.8	2.1[a]	6.3[a]	88
	3.2	54[b]	2.4[b]	0.9	0.5[b]	5.7[a]	83
	6.4	26[c]	1.4[c]	0.8	0.1[c]	4.2[b]	72

[1] Means in the same column in the same experiment followed by the same or no letter do not differ significantly, at the 0.05 level of probability by Duncan's new multiple-range test.

all parameters recorded, with the exception of adult emergence and the level of 0.05 further depressed the same parameters. The level of 0.1 gave negligible hatch. In experiment 2 the level of 0.00625 did not depress significantly larval performance, whereas 0.0125 depressed significantly all parameters with the exception of pupal weight and adult emergence. The level of 0.025 depressed also pupal weight, same as in experiment 1. The level of 0.05 did not permit any eggs to hatch, while in the previous experiment the same level permitted only 9% of eggs to hatch. The levels of n-capric acid used in experiment 1 depressed progressively all parameters with the exception of adult emergence. The level of 0.05 gave lower larval survival than the control and the level of 0.1 further depressed larval survival compared to the 0.05 level and all the other parameters compared to the control. The level of 0.2 gave only 9% hatchability and the level 0.4 did not permit any eggs to hatch. In experiment 2 the levels of 0.0125 and 0.025 did not affect any of the parameters examined. The results of 0.05 and 0.1 level are similar to those obtained in experiment 1 with the exception of the level 0.1, which did not affect larval weight. In general, larvae which succeeded in pupating, even at high levels of fatty acids gave pupal weights equivalent to the control but it took them longer time (2-7 days) to pupate.

The results clearly showed that caproic, amino caproic, caprylic and capric acid inhibited larval growth of the olive fruit fly when incorporated into an artificial diet. The levels used depressed significantly hatchability of eggs, with the exception of amino caproic acid, larval survival and weight as well as pupal weight. This is consistent with the reported effects of the same fatty acids on other insects. Thus, caproic, caprylic and capric acid depressed significantly larval survival of *Pseudosarcophaga affinis* (Fallen) at concentrations of 0.004, 0.8 and 1.8% of the larval medium, respectively (House 1967). Similarly, capric acid depressed larval survival at the dietary level of 3.8 and 2.1% for *Tribolium confusum* (Herbst) (House and Graham 1967) and *Aedes aegypti* (L.) (House 1967), while capric, caproic and caprylic acid acted as sterility agents when added to the diet of *Dermestes maculatus* (Deg.) at the level of 1 to 5% (Cohen and Levinson 1972). Furthermore, different dietary levels of C8-C14 carbon chain length exhibited a differential effect on larval viability of *Drosophila mojavensis* with caprylic acid (C8) having the greatest and myristic acid (C14) having the least effect (Fogleman and Kircher 1986). Evidently the effect of these fatty acids on insects does not follow a simple rule but vary with the specific fatty acid and particular insect. The mode of action of the fatty acids on eggs, larvae, pupae and adults is not known for the olive fruit fly and therefore further work is needed. It seems however, that these fatty acids are detrimental to growth and development of the olive fruit fly in much lower dietary concentration than in other insects. Thus, caproic acid at the level of 0.02 did not permit any larvae of *B. oleae* to survive while this level for *P. affinis* was 2.1% (House 1967). Dietary levels of about 0.35 to 1.4% of caproic, caprylic and capric acid were injurious to larvae of *Musca domestica* L. (Brooks and Fraenkel 1958), compared to the levels of 0.0125 to 0.05 of the present study for B. oleae. Larval survival was also affected by the levels of the fatty acids used and the larvae survived took more days to pupate than the control. In general pupal weight at day 14 was less sensitive to fatty acid than larval survival and weight. The larvae, which survived to pupation, gave normal adult emergence in all treatments, which may indicate that the fatty acids showed no detrimental effects to pupae. Finally, it is of interest to note that the detrimental effect of amino caproic acid occurred at much higher concentrations than that of caproic acid.

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KEY WORDS: Olive fruit fly, C6- to C10- fatty acids, toxic effects, *Bactrocera oleae.*

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ΠΕΡΙΛΗΨΗ

Διερευνήθηκαν οι δυσμενείς επιδράσεις του καπροϊκού (C6), αμινο καπροϊκού (C6), καπρυλικού (C8) και καπρικού (C10) οξέος, στην ανάπτυξη των προνυμφών του Δάκου της ελιάς *Bactrocera oleae* (Gmelin) (Diptera, Tephritidae). Τα οξέα προστέθηκαν στο θρεπτικό υπόστρωμα των προνυμφών σε τέσσερα διαφορετικά επίπεδα συγκεντρώσεων κατά τη διάρκεια της ανάμειξης των συστατικών του υποστρώματος. Τα αυγά πάρθηκαν από την αποικία του δάκου της ελιάς και τοποθετήθηκαν στα πειραματικά υποστρώματα και στο μάρτυρα. Το καπροϊκό οξύ μείωσε σημαντικά και προοδευτικά την εκκολαπτικότητα, επιβίωση και βάρος των προνυμφών στα τροφικά επίπεδα του 0,05 και 0,1%. Επιπλέον το επίπεδο 0,2% δεν επέτρεψε την εκκόλαψη κανενός αβγού. Αντίθετα το αμινο καπροϊκό οξύ δε μείωσε καμία από τις παραμέτρους που μετρήθηκαν μέχρι το επίπεδο του 0,2%. Τα επίπεδα 0,4 μέχρι 1,6% μείωσαν την επιβίωση των προνυμφών το βάρος των προνυμφών και το βάρος των νυμφών και το επίπεδο 3,2% δεν επέτρεψε τη νύμφωση καμίας προνύμφης. Το καπρυλικό οξύ μείωσε σημαντικά την εκκολαπτικότητα, επιβίωση προνυμφών και τον αριθμό των νυμφών ανά gr τροφής στο επίπεδο 0,0125% συγκρινόμενο προς το μάρτυρα και δεν επέτρεψε τη νύμφωση καμίας προνύμφης στο επίπεδο 0,1%. Τέλος, το καπρικό οξύ μείωσε προοδευτικά την εκκολαπτικότητα των αβγών, την επιβίωση των προνυμφών και το βάρος των προνυμφών από το επίπεδο 0,05% και δεν επέτρεψε την νύμφωση καμίας προνύμφης στο επίπεδο 0,2%. Η έξοδος των τελείων δεν επηρεάσθηκε από τα οξέα που δοκιμάσθηκαν.