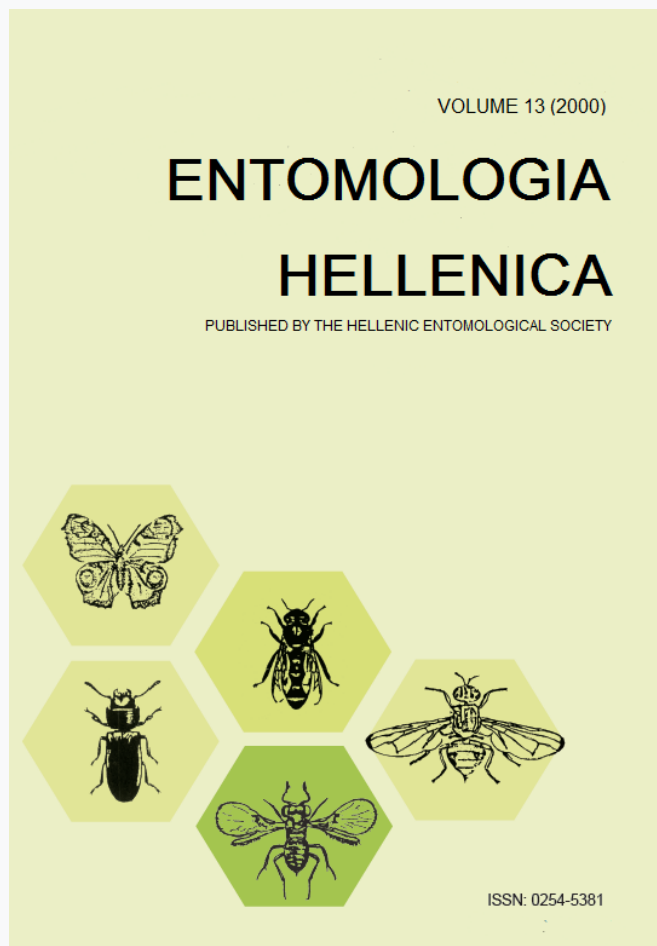


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# The effect of amino acid analogues on larval growth and survival of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae)<sup>1</sup>

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## ABSTRACT

The effects of eight amino acid analogues [L-canavanine, D-cycloserine, allyl-glycine, L-glutamic acid- $\alpha$ -hydrazide, DL-ethionine, L-, -3,4 dihydroxyphenyl-alanine (L-DOPA), DL-, -3,4 dihydroxyphenyl-alanine (DL-DOPA) and thiaproline] added to an artificial diet on egg hatching, larval survival, larval weight, pupal weight and adult emergence of the olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) were investigated. Larval survival and weight were significantly decreased by all amino acid analogues tested. Pupal weight and adult emergence was depressed by L-canavanine, D-cycloserine, L-DOPA, DL-DOPA and allyl-glycine. Of all amino acid analogues tested only L-canavanine inhibited hatching of the eggs. The depression of the parameters affected was increased by increasing the concentration of each analogue tested. The larvae of most experimental diets took longer to pupate than those of the control.

## Introduction

The importance of amino acids in insect nutrition and metabolism has been well documented. The role of amino acids in protein synthesis and in other functions, as neural transmission, detoxification, phospholipid synthesis, energy production and morphogenic processes, has been reviewed by Chen (1985). The free amino acid pool of the olive fruit fly *Bactrocera oleae* Gmelin, (Diptera: Tephritidae) and the requirement of larvae for free amino acid mixtures have been reported (Manoukas 1972, 1989).

Amino acid analogues are non-protein amino acids and appear to be of great ecological importance. They modify insect-plant relationships and they may affect insect behaviour, development and survival with their antimetabolic action

and toxicity. Amino acid analogues and similar compounds are plant secondary metabolites and their biosynthesis, distribution and toxicity have been reviewed by Rosenthal and Bell (1979) and Fowden (1981). Primarily they act as amino acid antagonists preventing normal protein biosynthesis (Dittmer 1950, Fowden et al. 1967). Reese and Holyoke (1987) have reviewed the effect of amino acid analogues and other metabolites upon growth and development of insects and Zografou et al. 1998 have tested some of them upon adult survival and reproduction of the olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae).

In the present work the effect of eight amino acid analogues (L-canavanine, D-cycloserine, L-, -3,4 dihydroxyphenylalanine (L-DOPA), DL-, -3,4 dihydroxyphenylalanine (DL-DOPA), allyl-glycine, DL-ethionine, L-glutamic acid- $\gamma$ -hydrazide and thiaproline) upon hatchability of eggs, larval growth, larval survival, pupal weight and adult emergence of the olive fruit fly was investigated.

<sup>1</sup> Received for publication July 7, 1998.

## Materials and Methods

Eggs  $48 \pm 4$  h old were obtained from olive fruit flies originating from the island of Aghia Trias, Attiki, Greece, 1982 (stock T) and maintained in our laboratory at approximately  $25 \pm 2^\circ\text{C}$ ,  $65 \pm 5\%$  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 1989, 1993). This diet was used as a control diet and four additional diets were formulated by adding each chemical tested at four different levels at a geometric sequence, as shown in the table. The appropriate quantity of each chemical was dissolved in water and then added to the diet prior to the addition of yeast, soy hydrolysate and cellulose. All chemicals were of analytical grade (Sigma Co., USA). Plastic containers of 4 cm height and 10 cm diameter with covers were used (Kris-Pan Co., Greece). Forty-five g of each diet was placed in each container (replicate) and 4 replicates per each level of chemical (treatment) were used with 7 eggs/g diet. Eggs were placed on a filter paper on the surface of the diet and hatchability of eggs was checked 3 days after placement of eggs. The number of larvae and their weight were recorded on a 4 g sample of diet obtained randomly from each replicate on the 10th day following placement of eggs, while pupae were collected and counted from 14th to 21st day. Pupal weight was measured at least 3 days after the last collection of pupae and adult emergence was recorded. Statistical procedures employed were those described by Steele and Torrie (1960) as indicated in the table.

## Results and Discussion

Table 1 presents the results. Hatchability was significantly depressed by the concentration level of 0.02% dietary L-canavanine in both experiments compared to the control (0.00 level). Larval survival (larvae/g diet) was not affected up to the level of 0.02 but it was significantly affected by the level of 0.04 and no larvae survived at the level of 0.08. Larval weight was affected by the level of 0.02. Larval survival to pupation (pupae/g diet) was depressed by the level of 0.01 and pupal weight was depressed by the level of 0.01 (experiment 1) and 0.02 (experiment 1 and 2). Adult emergence was significantly lowered by the level of 0.01. D-cycloserine significantly depressed all parameters recorded from the level of 0.0125 with the exception of hatchability which

was not affected by the levels tested and of adult emergence in experiment 3 which was depressed at the level of 0.025. It is of interest however to note that while the level of 0.05 and 0.1% dietary D-cycloserine did not permit any larvae to survive, hatchability of eggs was not statistically affected by these levels when compared to the control.

Hatchability was not affected by the concentration levels of L-DOPA and DL-DOPA tested. In experiment 1 larval weight was depressed at the level of 0.1 L-DOPA and further at the level of 0.2 when compared to the control. In addition the level of 0.2 depressed number of pupae/g diet, pupal weight and adult emergence. The results of experiment 2, in general confirmed those of experiment 1 and the level of 0.4 did not permit any larvae to pupate. DL-DOPA significantly depressed larval weight, number of pupae, pupal weight and adult emergence at the level of 0.2 (exp. 3 and 4). In experiment 4 the level of 0.4 further depressed larval weight, pupation and pupal weight compared to the level of 0.2, while the level of 0.8 did not permit any larvae to pupate. The level of 0.01 allyl-glycine depressed larval weight and adult emergence while the level of 0.02 depressed all parameters recorded with the exception of hatchability. The level 0.01 and 0.02 of experiment 2 confirmed the results of the experiment 1, while the level of 0.04 further depressed larval survival and weight with zero pupation and the level of 0.08 did not permit any larvae to survive, on the 10th day. In experiment 3 the highest level 0.05 DL-ethionine depressed larval weight when compared to the control. In experiment 4 the level of 0.1 depressed larval weight, larval survival and pupation and the level of 0.2 further depressed the above parameters. Hatchability and adult emergence was not affected.

In experiment 1 L-glutamic acid- $\gamma$ -hydrazide at the level of 0.05 depressed significantly larval survival and larval weight but not pupal weight and adult emergence. It is of importance that hatchability was not affected statistically even by the highest levels used (0.20 and 0.40) when compared to the control. L-glutamic acid- $\gamma$ -hydrazide progressively depressed larval survival from the level of 0.025 (exp. 2) when compared to the control and did not permit any larvae to survive at the level of 0.10. Thiaproline (expt. 3) did not affect statistically any of the parameters recorded up to the level of 0.2 and depressed significantly larval survival, larval weight and pup-

TABLE 1. Effect of the amino acid analogues upon egg hatching, larvae, pupae and adult emergence of the olive fruit fly, when added to a larval artificial diet <sup>1</sup>.

Exp. no.	Analogue, % in diet	Hatchability, %	No. of larvae/g diet	Larval weight, mg	No. of pupae/g diet	Pupal weight, mg	Adults, % on pupae
L-canavanine							
1	Control	75 <sup>a</sup>	3.3	2.5 <sup>a</sup>	2.8 <sup>a</sup>	5.9 <sup>a</sup>	75 <sup>a</sup>
	0.0025	78 <sup>a</sup>	3.5 <sup>a</sup>	2.6 <sup>a</sup>	2.7 <sup>a</sup>	6.0 <sup>a</sup>	71 <sup>a</sup>
	0.005	65 <sup>a</sup>	3.0	2.2 <sup>a</sup>	2.5 <sup>ab</sup>	5.6 <sup>ab</sup>	61 <sup>a</sup>
	0.01	75 <sup>a</sup>	2.8	2.1 <sup>ab</sup>	1.9 <sup>b</sup>	5.3 <sup>b</sup>	40 <sup>b</sup>
	0.02	52 <sup>b</sup>	2.7	1.8 <sup>b</sup>	2.1 <sup>b</sup>	5.3 <sup>b</sup>	39 <sup>b</sup>
2	Control	83 <sup>a</sup>	3.7 <sup>a</sup>	2.8 <sup>a</sup>	3.1 <sup>a</sup>	6.0 <sup>a</sup>	78 <sup>a</sup>
	0.01	76 <sup>a</sup>	3.2 <sup>a</sup>	2.3 <sup>ab</sup>	2.2 <sup>b</sup>	5.7 <sup>ab</sup>	59 <sup>b</sup>
	0.02	55 <sup>b</sup>	3.4 <sup>a</sup>	1.5 <sup>b</sup>	2.0 <sup>b</sup>	5.1 <sup>b</sup>	36 <sup>c</sup>
	0.04	44 <sup>b</sup>	0.7 <sup>b</sup>	1.0 <sup>c</sup>	0.0	—	—
	0.08	41 <sup>b</sup>	0.0	—	—	—	—
D-cycloserine							
3	Control	72	2.6 <sup>a</sup>	1.7 <sup>a</sup>	2.4 <sup>a</sup>	6.2 <sup>a</sup>	83 <sup>a</sup>
	0.003125	75	2.7 <sup>a</sup>	1.6 <sup>a</sup>	2.3 <sup>ab</sup>	6.1 <sup>a</sup>	79 <sup>a</sup>
	0.00625	74	1.9 <sup>ab</sup>	1.2 <sup>ab</sup>	1.6 <sup>ab</sup>	5.6 <sup>ab</sup>	84 <sup>a</sup>
	0.0125	69	1.1 <sup>b</sup>	0.8 <sup>bc</sup>	0.6 <sup>b</sup>	5.4 <sup>b</sup>	75 <sup>a</sup>
	0.025	71	0.4 <sup>c</sup>	0.6 <sup>c</sup>	1.0 <sup>c</sup>	4.5 <sup>c</sup>	35 <sup>b</sup>
4	Control	76	2.7 <sup>a</sup>	1.8 <sup>a</sup>	2.5 <sup>a</sup>	5.8 <sup>a</sup>	91 <sup>a</sup>
	0.0125	75	0.6 <sup>b</sup>	0.9 <sup>b</sup>	0.4 <sup>b</sup>	5.2 <sup>b</sup>	66 <sup>b</sup>
	0.025	68	0.2 <sup>c</sup>	0.5 <sup>c</sup>	0.1 <sup>c</sup>	4.1 <sup>c</sup>	13 <sup>c</sup>
	0.05	71	0.0	—	—	—	—
	0.1	58	0.0	—	—	—	—
L-DOPA							
1	Control	89	3.4	1.9 <sup>a</sup>	2.1 <sup>a</sup>	6.0 <sup>a</sup>	90 <sup>a</sup>
	0.025	83	3.2	1.6 <sup>a</sup>	2.3 <sup>a</sup>	6.2 <sup>a</sup>	92 <sup>a</sup>
	0.05	85	3.0	1.5 <sup>ab</sup>	2.6 <sup>a</sup>	6.1 <sup>a</sup>	91 <sup>a</sup>
	0.1	78	2.8	1.1 <sup>b</sup>	2.0 <sup>a</sup>	5.9 <sup>a</sup>	72 <sup>a</sup>
	0.2	80	2.9	0.7 <sup>c</sup>	0.8 <sup>b</sup>	4.5 <sup>b</sup>	4 <sup>b</sup>
2	Control	81	2.4 <sup>a</sup>	1.8 <sup>a</sup>	2.1 <sup>a</sup>	6.5 <sup>a</sup>	93 <sup>a</sup>
	0.05	75	2.1 <sup>a</sup>	1.6 <sup>a</sup>	1.7 <sup>a</sup>	6.5 <sup>a</sup>	92 <sup>a</sup>
	0.1	70	2.0 <sup>a</sup>	1.1 <sup>b</sup>	1.7 <sup>a</sup>	6.0 <sup>a</sup>	71 <sup>a</sup>
	0.2	84 <sup>b</sup>	2.0 <sup>a</sup>	0.8 <sup>c</sup>	0.9 <sup>b</sup>	4.9 <sup>b</sup>	25 <sup>b</sup>
	0.4	76	0.9 <sup>b</sup>	0.4 <sup>d</sup>	—	—	—
DL-DOPA							
3	Control	80	2.8	2.1 <sup>a</sup>	2.5 <sup>a</sup>	5.9 <sup>a</sup>	78 <sup>a</sup>
	0.025	80	2.3	2.1 <sup>a</sup>	2.1 <sup>a</sup>	6.0 <sup>a</sup>	79 <sup>a</sup>
	0.05	78	2.5	1.9 <sup>a</sup>	2.2 <sup>a</sup>	5.8 <sup>a</sup>	78 <sup>a</sup>
	0.1	78	2.2	1.8 <sup>ab</sup>	2.1 <sup>a</sup>	5.7 <sup>a</sup>	75 <sup>a</sup>
	0.2	68	2.4	1.4 <sup>b</sup>	1.6 <sup>b</sup>	5.2 <sup>b</sup>	54 <sup>b</sup>
4	Control	67	2.7 <sup>a</sup>	1.3 <sup>a</sup>	2.3 <sup>a</sup>	5.7 <sup>a</sup>	74 <sup>a</sup>
	0.1	74	3.0 <sup>a</sup>	1.1 <sup>ab</sup>	2.0 <sup>a</sup>	5.7 <sup>a</sup>	63 <sup>a</sup>
	0.2	70	2.7 <sup>a</sup>	0.9 <sup>b</sup>	1.0 <sup>b</sup>	5.1 <sup>b</sup>	30 <sup>b</sup>
	0.4	67	2.5 <sup>a</sup>	0.4 <sup>c</sup>	0.1 <sup>c</sup>	3.5 <sup>c</sup>	22 <sup>b</sup>
	0.8	72	1.6 <sup>b</sup>	0.1 <sup>d</sup>	—	—	—

Exp. no.	Analogue, % in diet	Hatchability, %	No. of larvae/g diet	Larval weight, mg	No. of pupae/g diet	Pupal weight, mg	Adults, % on pupae
Allyl-glycine							
1	Control	76	2.0 <sup>a</sup>	2.0 <sup>a</sup>	1.9 <sup>a</sup>	5.9 <sup>a</sup>	85 <sup>a</sup>
	0.0025	72	1.9 <sup>a</sup>	1.9 <sup>a</sup>	1.8 <sup>a</sup>	6.1 <sup>a</sup>	96 <sup>a</sup>
	0.005	68	2.1 <sup>a</sup>	1.6 <sup>ab</sup>	2.0 <sup>a</sup>	6.1 <sup>a</sup>	86 <sup>a</sup>
	0.01	67	1.9 <sup>a</sup>	1.4 <sup>b</sup>	1.6 <sup>ab</sup>	5.5 <sup>ab</sup>	54 <sup>b</sup>
	0.02	64	1.7 <sup>b</sup>	0.7 <sup>c</sup>	0.7 <sup>b</sup>	4.9 <sup>b</sup>	—
2	Control	78	2.2 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	6.2 <sup>a</sup>	92 <sup>a</sup>
	0.01	75	2.3 <sup>a</sup>	1.3 <sup>b</sup>	1.9 <sup>a</sup>	5.7 <sup>ab</sup>	37 <sup>b</sup>
	0.02	76	2.2 <sup>a</sup>	0.6 <sup>c</sup>	0.8 <sup>b</sup>	5.1 <sup>b</sup>	0
	0.04	69	0.5 <sup>b</sup>	0.1 <sup>d</sup>	0.0	—	—
	0.08	61	—	—	—	—	—
DL-ethionine							
3	Control	78	2.9	1.9 <sup>a</sup>	2.6	6.4	83
	0.00625	77	2.8	1.8 <sup>a</sup>	2.4	6.3	86
	0.0125	65	3.1	2.1 <sup>a</sup>	2.1	5.9	80
	0.025	70	2.9	1.6 <sup>a</sup>	1.9	6.2	89
	0.05	68	2.7	0.9 <sup>b</sup>	1.8	6.0	89
4	Control	73	3.0 <sup>a</sup>	2.0 <sup>a</sup>	2.7 <sup>a</sup>	6.2	81
	0.025	69	2.8 <sup>a</sup>	1.8 <sup>a</sup>	2.3 <sup>a</sup>	6.3	83
	0.05	64	3.2 <sup>a</sup>	0.7 <sup>b</sup>	1.9 <sup>a</sup>	6.4	78
	0.1	70	1.5 <sup>b</sup>	0.4 <sup>c</sup>	0.8 <sup>b</sup>	6.4	92
	0.2	63	0.8 <sup>c</sup>	0.1 <sup>d</sup>	0.3 <sup>c</sup>	5.8	87
L-glu-γ-hydraz							
1	Control	73	2.8 <sup>a</sup>	1.4 <sup>a</sup>	1.7 <sup>a</sup>	6.7	94
	0.05	61	0.9 <sup>b</sup>	0.8 <sup>b</sup>	0.6 <sup>b</sup>	5.9	71
	0.1	64	0.1 <sup>c</sup>	0.1 <sup>c</sup>	0.0	—	—
	0.2	61	0.0	—	—	—	—
	0.4	57	0.0	—	—	—	—
2	Control	78	2.6 <sup>a</sup>	2.1 <sup>a</sup>	2.4 <sup>a</sup>	5.5	87
	0.0125	82	1.9 <sup>ab</sup>	1.8 <sup>ab</sup>	1.7 <sup>ab</sup>	5.8	80
	0.025	87	1.3 <sup>b</sup>	2.3 <sup>a</sup>	1.0 <sup>b</sup>	5.9	85
	0.05	69	1.1 <sup>b</sup>	1.2 <sup>b</sup>	0.8 <sup>b</sup>	5.3	63
	0.1	75	0.0	—	—	—	—
Thiaproline							
3	Control	82	3.5 <sup>a</sup>	1.4 <sup>a</sup>	2.7 <sup>a</sup>	6.6	76
	0.05	76	3.0 <sup>a</sup>	1.7 <sup>a</sup>	2.7 <sup>a</sup>	6.5	92
	0.1	71	2.4 <sup>a</sup>	1.5 <sup>a</sup>	2.2 <sup>a</sup>	6.7	88
	0.2	71	2.5 <sup>a</sup>	1.4 <sup>a</sup>	2.4 <sup>a</sup>	6.6	82
	0.4	69	1.0 <sup>b</sup>	0.9 <sup>b</sup>	1.5 <sup>b</sup>	6.3	72
4	Control	73	3.2 <sup>a</sup>	1.5 <sup>a</sup>	2.6 <sup>a</sup>	6.3	80
	0.2	70	3.0 <sup>a</sup>	1.4 <sup>a</sup>	2.4 <sup>a</sup>	6.4	90
	0.4	68	1.8 <sup>b</sup>	1.0 <sup>ab</sup>	1.3 <sup>b</sup>	6.3	88
	0.8	67	0.8 <sup>c</sup>	0.7 <sup>b</sup>	0.0	—	—
	1.6	71	0.0	—	—	—	—

<sup>1</sup> Means in the same column followed by the same or no letter do not differ significantly, at the 0.05 level of probability by Duncan's new range test, in each experiment.



ation at the level of 0.4 when compared to the control. The level of 0.8 (exp. 4) further depressed larval survival and weight, did not permit any larvae to pupate, while the level of 1.6 did not permit any larvae to survive. Hatchability, pupal weight and adult emergence was not affected by any of the levels which permitted pupation.

L-canavanine was found to affect other insects when included in the diet. Thus, it inhibited growth and arginase activity of *Tribolium castaneum* (Herbst) at the concentration of 36 mM/Kg diet (Harry et al. 1976), inhibited growth of *Anthonomus grandis* (Boheman) at 100 mg/100ml (Vanderzant and Chremos, 1971). It seems that olive fly larvae are more sensitive to the L-canavanine than other insects studied. In addition L-canavanine suppressed growth and caused various malfunctions when it was administered in various ways into the body of insects (Isogai et al. 1973, Dahlman and Rosenthal 1975, 1976, Rosenthal and Dahlman 1975, Palumbo and Dahlman 1978, Dahlman et. al. 1979 and Dahlman 1980).

L-DOPA inhibited growth and pupation in *Agrotis ipsilon* (Hufnagel) (Reese and Beck, 1976) and it gave abnormal pupation in *Spodoptera eridania* (Craner) (Rehr et al. 1973). At present DL-DOPA is ten times cheaper than L-DOPA. DL-ethionine at the level of 0.07% inhibited reproduction of *Pseudosarcophaga affinis* (Fallen) (Hegdekar 1970), while it did not depress pupal weight and adult emergence of the olive fruit fly even at the highest level used (0.2). The survived larvae gave normal pupal weight and adult emergence, but it took much longer period for the larvae to pupate (15 to 18 days) at the highest DL-ethionine levels used (0.1 to 0.2) when compared to the control (14 days). In the experiments with L-glutamic acid- $\gamma$ -hydrazide and thiaproline the survived larvae gave pupal weight and adult emergence equivalent to the control but time to pupation was longer than the control.

It seems that under the experimental conditions employed larval survival at the 10th day and at pupation was the most reliable criterion for determining the inhibition level of the chemicals tested with the olive fruit fly. On the contrary larval weight in most cases recovered due to longer larval period and in some cases when larvae pupated attained a pupal weight equivalent to the control as in the case of DL-ethionine, L-glutamic acid- $\gamma$ -hydrazide and thiaproline. A study with adult olive fruit flies showed that L-canavanine, DL-allyl glycine, D-cycloserine and

L-glutamic acid- $\gamma$ -hydrazide affected survival and reproduction at higher dietary concentrations (Zografou et al. 1998), than used in the present study. Thus, survival was depressed at the concentration of 1.5 gr/100 ml diet for L-canavanine, 3 for DL-allyl-glycine, 0.25 for D-cycloserine, and 1.5 for L-glutamic acid- $\gamma$ -hydrazide. Thiaproline did not affect survival even at the highest level used (10gr/100 ml diet). Some explanations have been given for the detrimental effects of the amino acid analogues on the olive fruit fly (Zografou et al. 1998). Also it is possible that, specifically for the larvae, these effects are due to creation of amino acid deficiencies, imbalances and toxicities (Manoukas 1981) in relation with the free amino acid pool formed at different stages of this insect (Manoukas 1972). On the other hand the olive fruit mesocarp in which the larva feeds exclusively in nature contains very low amounts of free amino acids (Manoukas et al. 1973) compared to the artificial diet (Manoukas, 1989). Because of the unique relationships of the olive fruit with the free amino acids, further work on amino acid analogues may lead to a new approach for the management of this insect.

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**KEY WORDS:** Olive fruit fly, amino acid analogues, toxic effects, *Bactrocera oleae*

**Επίδραση των αναλόγων αμινοξέων στην ανάπτυξη και επιβίωση των προνυμφών του Δάκου της ελιάς *Bactrocera oleae* (Diptera: Tephritidae)**

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**ΠΕΡΙΛΗΨΗ**

Μελετήθηκε η επίδραση οκτώ μη πρωτεϊνικών αμινοξέων (L-canavanine, D-cycloserine, allyl-glycine, L-glutamic acid- $\gamma$ -hydrazide, DL-ethionine, L- $\beta$ -3,4 dihydroxyphenyl-alanine [L-DOPA], DL- $\beta$ -3,4 dihydroxyphenyl-alanine [DL-DOPA] και thiaproline) που προστέθηκαν σε ένα τεχνητό σιτηρέσιο, πάνω στην εκκολαπτικότητα των αυγών, στην επιβίωση προνυμφών, στο βάρος προνυμφών, στο βάρος νυμφών και στην έξοδο τελείων του Δάκου της ελιάς *Bactrocera oleae*, (Gmelin) (Diptera: Tephritidae). Η επιβίωση και το βάρος των προνυμφών μειώθηκε στατιστικά σημαντικά από όλα τα ανάλογα των αμινοξέων που δοκιμάστηκαν. Το βάρος των νυμφών και η έξοδος των τελείων μειώθηκε από την L-canavanine, D-cycloserine, L-DOPA, DL-DOPA και allyl-glycine. Από όλα τα ανάλογα αμινοξέων μόνο η L-canavanine παρεμπόδισε την εκκόλαψη των αυγών. Η μείωση των παραμέτρων που επηρεάστηκαν μεγάλωσε με την αύξηση της συγκέντρωσης των αναλόγων αμινοξέων που δοκιμάστηκαν. Οι προνύμφες των περισσότερων πειραματικών σιτηρεσίων χρειάστηκαν περισσότερο χρόνο για νύμφωση σε σύγκριση με εκείνες του μάρτυρα.