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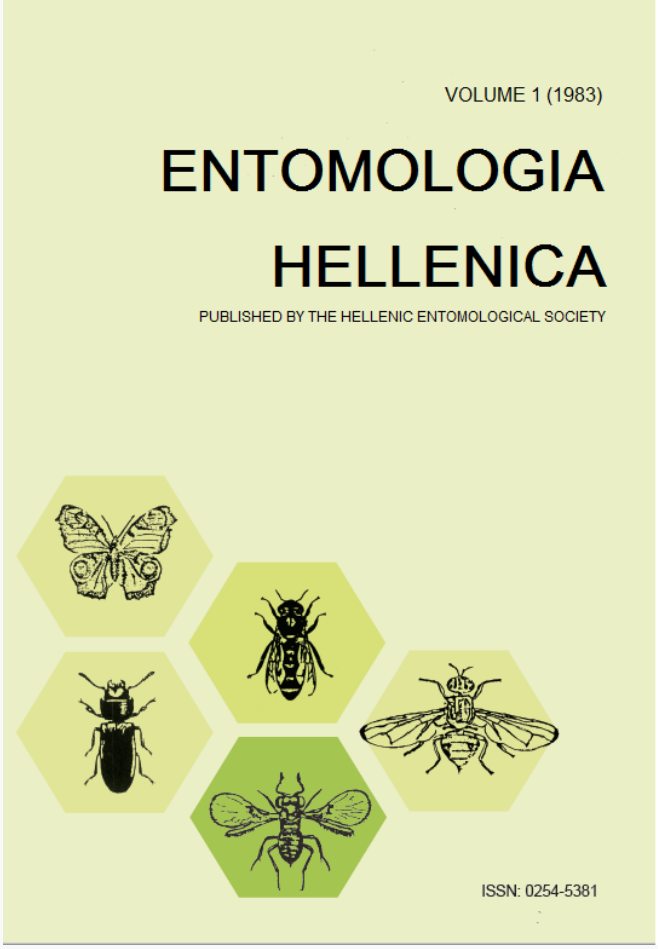

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Improved Solid Adult Diet for the Olive Fruit Fly, *Dacus oleae*[1]

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ABSTRACT

Egg production of the olive fruit fly, *Dacus oleae* (Gmelin) (Diptera: Tephritidae), was higher when insects fed on a liquid diet than on the same diet made solid by enriching it with various amounts of cellite or cellulose. Comparison of a liquid with a solid diet, both composed of water: sucrose: yeast hydrolyzate: chicken egg yolk at ratios 5:4:1:0.7 and 0:4:1:0.7 respectively, given to olive fruit flies kept in pairs, showed that egg production was higher on the liquid diet. With grouped insects, however (30 pairs per cage), egg production, expressed per initial female, was higher with the solid diet. The difference was due to the lower mortality that occurred on the solid diet. Dry egg yolk could substitute fresh egg yolk. Among solid diets differing in the concentration of egg yolk, yeast hydrolyzate, and the presence of vitamins and cholesterol, the diet that gave the highest egg production contained 80 parts sucrose, 30 parts yeast hydrolyzate, and 6.6 parts dry egg yolk.

Introduction

Artificial adult diets for the olive fruit fly, *Dacus oleae* (Gmelin) (Diptera: Tephritidae), containing sucrose and enzymatic yeast hydrolyzate provide good survival and egg production (reviews by Tzanakakis et al. 1967, Tzanakakis 1971). Supplementation of the commonly used diet with egg yolk further improved it (Economopoulos and Tzanakakis 1967).

For the rearing of the olive fruit fly mainly liquid diets have been used. Use of solid diets had been reported as not satisfactory for rearing by Moore (1959), Hagen et al. (1963) but satisfactory by Cavalloro and Girolami (1968). In the rearing practice, however, liquid diets have certain disadvantages as stated by Tzanakakis et al. (1967). These authors working with solid and liquid diets found that olive fruit flies deriving from a dry area could utilize solid diets equally well with liquid diets, but biotypes from humid areas made better use of liquid than solid diets.

The present study aimed at comparing the performance of adult olive fruit flies on liquid to that on solid diet over a number of generations and at further improving the solid diet which was used in our lab. It was found that grouped insects gave a higher egg yield on solid than on liquid diet, and an improved solid diet was developed by increasing its enzymatic yeast hydrolyzate content.

Materials and Methods

The insects used in the experiments on the comparison of liquid with solid diets originated from the area of Attica and had been reared on artificial diets in the lab for about 70 generations. The insects used for the diet improvement experiments came from Crete and had been kept in the lab for 30 generations. In the liquid-solid diet experiments, insects were kept in pairs in small (15 × 15 × 15 cm) or in groups of 30 pairs in large (30 × 30 × 30 cm) cages similar to those used by Hagen et al. (1963). In the diet improvement experiments, large cages, previously mentioned, with groups of 25 pairs of flies were used. All insects were reared on artificial diet according to the method described by Tsitsipis (1975)

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at 25 ± 2° C and 60 ± 5 % RH. Egg production was recorded daily in all experiments. Liquid and solid diets were composed of 40g sucrose, 10g enzymatic yeast hydrolyzate (ICN, Pharmaceuticals, Inc., Life Sciences Group, 26201 Miles Rd., Cleveland, Ohio 44128, USA), 7 g fresh egg yolk and 50 ml water for the former diet (Economopoulos and Tzanakakis 1967). All diets contained 0.05 % streptomycine sulphate (on the basis of solid residue). The solid diet was obtained by evaporating the liquid diet under vacuum at 40° C and pulverizing it in a blender. The solid diets used in the diet improvement experiments were prepared by direct mixing of the solid constituents. Instead of fresh egg yolk and regular sugar, dry egg yolk (spray yolk, Sanovo Food and Engineering Ltd., 5100 Odence, Havnegade 33-35, Denmark) and fine sugar powder were used respectively. The liquid-solid diet comparison experiments lasted 8 weeks, and the diet improvement ones 5 weeks.

The evaluation of data in the liquid-solid diet comparisons was done with the Student's t test, those for the diet improvement analysis of variance was done and for the comparison of the means, the Duncan's multiple range test was used.

Results

Considering the advantages of using solid adult diets (Tzanakakis et al. 1967), in a preliminary experiment, liquid diet was compared with a series of dry diets prepared by supplementing the liquid diet with cellulose powder, or cellite. Each diet was tested on groups of 20 pairs of insects over a period of 6 weeks. The daily egg production per initial female (per living, in parenthesis) was 18 (22.4), 8 (12.6), 6.3 (8.7), 6.1 (9.4), 9.7 (-) and 6.4 (10.1) for the liquid diet and the ones with 20 %, 30 %, 50 % cellulose, 20 %, 30 % cellite, respectively. The results show that the presence of inerts more than halved egg production. The higher the amount of the inert, the lower the egg production.

The results of the comparison of solid with liquid adult diet over a number of generations are shown in Table 1. In insects kept in pairs, the survival of females feeding on either solid or liquid diet did not differ significantly in all generations tested. The egg production of females feeding on liquid diet was consistently higher than the egg production of females feeding on solid diet. In the 1st and 2nd generations, differences were statistically significant. Of all females set up, only a very small number did not lay any eggs. There seem to exist no differences between insects feeding on either diet in respect to the number of non ovipositing females. All non ovipositing females died within the first 2 weeks. Only 1 female, in the 1st generation, feeding on liquid diet did not lay eggs during the 12 weeks she lived. Individual females were very fecund in both diets. Certain females laid more than 1500 eggs in 12 weeks (maximum values obtained: 1910 and 1922 for liquid and solid diet respectively). Fig. 1 shows the frequency distribution of females which laid various numbers of eggs during the first 8 weeks of their life. It is clearly shown that most of the females fed on solid diet laid relatively fewer eggs (less than 500) than the females which fed on liquid diet (most of them laid 250-750 eggs). In grouped insects, survival as well as egg production of

TABLE 1. Egg production and survival (days) of *Dacus oleae* females, feeding on liquid (LD) or solid diet (SD), in the 1st, 2nd, 4th and 10th generation. Insects kept in single pairs (S) or in groups (G) of 30 pais per cage. Egg production of 8 weeks of life, n = no. replicates.

Generation	n		Mean survival (d)		Eggs /initial ♀		Non-ovipositing vs. initial ♀♀	
	LD	SD	LD	SD	LD	SD	LD	SD
1 S	20	20	19.1	19.0	731.0 *	451.1	2:25	3:25
1 G	1	1	16.8	20.3	334.0	372.9	—	—
2 S	26	25	21.6	23.4	557.5*	407.2	0:26	0:26
2 G	4	4	17.2	26.9*	431.2	584.7*	—	—
4 S	28	26	23.1	23.4	532.9	345.4	2:30	1:30
4 G	4	4	19.8	24.6*	498.9	544.0*	—	—
10 S	33	32	26.1	22.9	559.0	402.5	2:40	4:40

* In each data category, differences within same line statistically significant ($P < 0.05$, Student's t test).

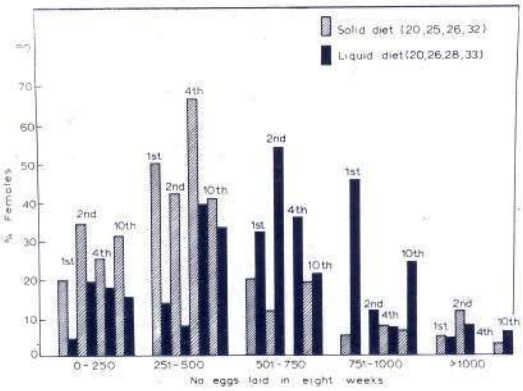

FIG. 1. Frequency distribution of *Dacus oleae* females that laid various numbers of eggs within the first 8 weeks of their life. The insects were kept in pairs and fed on liquid or solid diet for 10 consecutive generations. Checks were made in the 1st, 2nd, 4th, and 10th generation. Numbers in parentheses indicate number of females, the egg production of which was recorded in the 1st, 2nd, 4th, and 10th generation respectively.

females feeding on solid diet were significantly higher than in the liquid diet in the 2nd and 4th generations. The same tendency was shown in the 1st generation although it cannot be statistically evaluated due to the existence of only one replication of the test.

Egg hatchability did not seem to be affected by the kind of adult diet as indicated by sporadic checks.

After it was shown that flies kept in groups could utilize well solid diet, it was of interest to further improve it by: a) attempting to substitute fresh egg yolk, that necessitated drying of a liquid diet to dry form, with dry egg yolk (spray yolk), b) optimizing the egg yolk and yeast hydrolyzate concentrations, c) comparing it with the diet of George and Ruhm (1977), and d) testing the effect of VanderZant, or Hoffmann La Roche vitamin mixtures complemented with cholesterol. The results are shown in Table 2. In the 1st experiment no statistically significant differences were found in female survival, egg production and hatchability between diets containing fresh egg yolk and dry egg yolk at levels ranging from 6 to 12 parts. No differences were also found between these diets and a diet without egg yolk supplemented with vitamins and cholesterol (George and Ruhm 1977). In the 2nd experiment even a higher dry egg yolk level (24 parts) did not improve the diet in regard to any of the parameters tested. In the 3rd experiment, a diet containing 30 parts of yeast hydrolyzate gave a higher egg production than the diets containing lower or higher levels of the constituent (the ratio of egg yolk to the total amount of diet was maintained constant in all diets). In the 4th experiment, diets containing vitamins and cholesterol at various levels did not differ significantly from a diet deficient in these constituents.

Discussion

The adult diet used routinely till now was improved by increasing the level of enzymatic yeast hydrolyzate, but not by increasing egg yolk or enriching the diet with vitamins and cholesterol. Of all the diets tested, the best one that gave the highest egg production was the one that contained sucrose, yeast hydrolyzate and dry egg yolk in the ratio 80:30:6.6.

The lower egg production values attained by the incorporation to the liquid diet of the nutritionally inerts cellite and cellulose can be attributed to reduced, or selective ingestion due to the presence of these particulate water insoluble materials. Egg production of single pairs of 4th and 10th generation insects fed on liquid diets was superior to that of insects fed on solid diets. Although differences were large, they were not statistically significant, in contrast to 1st and 2nd generation egg production where differences were significant (Table 1). This possibly indicates a certain degree of selection of insects utilizing better than in previous generations solid diet. In grouped insects egg production, expressed per initial female, was higher in solid than in liquid diet. This is a consequence of the higher female mortality in the liquid diet, since egg production, expressed per living female, was higher in insects feeding on liquid diet (respective liquid vs. solid diet 8 week egg production ratios: 2nd generation 722.1/634.4, 4th generation 758.3/663.9, the values in the latter case differ statistically). Some flies were accidentally trapped and drowned in the liquid diet. Additionally, this form of diet is more apt to be contaminated by microorganisms.

Substitution of fresh egg yolk with dry facilitated diet preparation. Previously, solid diet was prepared from liquid diet by evaporating the water under vacuum (Tsitsipis 1975), while now it is prepared by direct mixing of the ingredients, all being solid. Fine powdered sugar, used in the diet, makes it homogeneous. Use of granular sugar could lead to unbalanced nutrition due to increased feeding on sugar crystals, as sucrose is more frequently fed upon than yeast hydrolyzate

TABLE 2. Survival, egg production and hatchability of *Dacus oleae* females, fed different solid diets. Five-week egg production with 4 replicates of 25 pairs of flies per cage in each treatment (3 replicates in the 1st experiment). Each diet contained 50 mg streptomycin sulfate.

Diet component[a]							
SU	YH	DEY	V	C	Female survival (days)	Eggs/living ♀ day	Egg hatch %
			1st experiment				
80	20	14[b]	—	—	15.9±8.1	17.9±2.1	90.5±3.0
80	20	6	—	—	17.7±4.9	13.1±4.2	86.1±5.4
80	20	9	—	—	15.7±7.2	15.9±4.9	87.4±3.1
80	20	12	—	—	16.3±5.3	17.5±3.6	86.5±4.3
77[e]	23[e]	—	0.25[ce]	0.05[e]	20.3±3.1	13.8±2.5	91.8±2.0
			2nd experiment				
80	20	6	—	—	22.5±2.2	18.3±5.3	85.7±10.0
80	20	12	—	—	23.6±1.5	20.0±5.0	82.2±13.4
80	20	24	—	—	21.9±2.7	19.0±3.5	81.8±14.6
			3rd experiment				
80	10	5.4	—	—	21.2±3.2	11.8±2.7	70.2±10.9
80	20	6	—	—	22.3±2.4	15.2±2.4	77.4±5.6
80	30	6.6	—	—	20.2±4.0	18.7±2.1*	73.1±4.9
80	40	7.2	—	—	21.1±3.1	15.5±3.2	75.4±11.6
80	50	7.8	—	—	21.0±4.2	12.5±3.2	68.7±8.0
80	60	8.4	—	—	21.9±3.4	14.0±3.2	74.0±6.1
			4th experiment				
80	30	6.6	0.25[c]	0.05	22.7±2.2	21.6±7.1	88.2±4.9
80	30	6.6	0.5[c]	0.1	22.8±1.7	20.7±6.2	87.8±4.4
80	30	6.6	0.125[d]	0.05	22.8±1.9	20.0±5.7	85.1±6.6
80	30	6.6	0.25[d]	0.1	22.1±1.8	20.1±8.0	83.2±6.2
80	30	6.6	0.5[d]	0.2	22.6±2.6	19.6±6.0	87.8±3.2
80	30	6.6	—	—	22.3±2.3	18.0±6.1	87.7±5.0

a SU = sugar, YH = yeast hydrolyzate, DEY = Dry egg yolk, V = Vitamin mix, C = cholesterol.
b The only diet with fresh egg yolk.
c VanderZant vitamin mix.
d Hoffmann La Roche vitamin mix.
e George and Ruhm (1977).
* Value significantly different ($P < 0.05$, Duncan's multiple range test).

(Tzanakakis 1969)[3]. Enrichment of diet with vitamins and cholesterol did not improve it. This indicates that required nutrients are provided by the constituents of the solid reference diet. Although George and Ruhm (1977) found that their best diet, containing sucrose, yeast hydrolyzate, VanderZant vitamin mix and cholesterol in the ratio 77:23:0.25:0.05, was better than our diet containing sucrose, yeast hydrolyzate and fresh egg yolk in the ratio 80:20:14, our results in the present study did not show any differences in the egg production between the two diets (Table 2, 1st experiment). On the contrary, egg production value was lower, in the George's diet (cf. 17.9 with 13.8; Table 2, 1st exp.) though not statistically different. Differences could be due to the different insect biotypes used in the two studies.

The solid diet used in our lab (sucrose to yeast hydrolyzate to dry egg yolk ratio, 80:20:6) has been successfully used to mass rear millions of insects over the last few years.

The mean egg production recorded in the present study is considerably higher than the values reported in the literature with artificial holigidic (Moore 1960, Economopoulos and Tzanakakis 1967, Tzanakakis et al. 1967, Cavalloro and Girolami 1968, George and Ruhm 1977), holidic (Tsiropoulos 1977a), or natural (Tsiropoulos 1977b) diets, whether solid or liquid. Cavalloro and Girolami (1968) in the two solid diets they tested (containing hydrolyzed brewer's yeast, unhydrolyzed brewer's yeast, sucrose in the ratios 10:0:40 and

[3] Tzanakakis, M.E. 1969. Experiments with the olive fruit fly at the "Demokritos" Nucl. Res. Center, Greece, 1967-1968 (a summary of progress). Eighth ad hoc Conference on the Control of Olive Pests and Diseases, Athens, 8-12 May 1969. Mimio 12pp.

10:20:40, respectively) obtained much lower egg production values than in the present study (cf. 3.1 with 11 eggs/initial female/day). Tsiropoulos (1977a) recorded slightly higher egg production values with the solid diet developed by Tsitsipis (1975) than with the liquid diet No. 2 of Economopoulos and Tzanakakis (1967), though differences were not significant. In the present study no substantial difference in the % of egg laying females in laboratory populations kept on solid or liquid diet was observed. Tzanakakis et al. (1967), however, found that larger number of females laid eggs when fed on liquid than on solid diet. Aside from differences in the diet, differences could be due to the different biotypes of insects used, and also to the fact that insects used by the latter authors came from field infested olives.

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KEY WORDS: Olive fruit fly, olive fly, fruit fly, *Dacus oleae*, Diptera, Tephritidae, adult diet, solid adult diet

Βελτιωμένη Στερεή Τροφή για τα Τέλεια του Δάκου της Ελιάς, *Dacus oleae*

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ΠΕΡΙΛΗΨΗ

Θηλυκά του δάκου της ελιάς, *Dacus oleae* (Gmelin), γέννησαν περισσότερα αυγά όταν τράφηκαν με υγρή τροφή παρά όταν τράφηκαν με την ίδια τροφή, που είχε γίνει όμως στερεή μετά από τον εμπλουτισμό της με διάφορες ποσότητες κελλίτη και κυτταρίνης. Σύγκριση υγρής με στερεή τροφή, που δόθηκε σε έντομα διατηρούμενα κατά ζεύγη, όταν οι δύο τροφές αποτελούνταν από νερό, ζάχαρη, ενζυματικά υδρολυμένη μαγιά και φρέσκο κρόκο αυγού κότας σε αναλογία 5:4:1:0.7 και 0:4:1:0.7, αντίστοιχα, έδειξε ότι η ωοπαραγωγή ήταν μεγαλύτερη στην υγρή τροφή. Σε έντομα όμως, που διατηρούνταν σε ομάδες, 30

ζεύγη κατά κλουβί, η ωοπαραγωγή εκφρασμένη κατά αρχικό θηλυκό, ήταν μεγαλύτερη με στερεή τροφή. Η διαφορά οφειλόταν στη μικρότερη θνησιμότητα, που παρατηρήθηκε στη στερεή τροφή. Αποξηραμένος κρόκος αυγού μπόρεσε να αντικαταστήσει τον φρέσκο κρόκο. Όταν έγινε σύγκριση στερεών τροφών, που διέφεραν στην ποσότητα του κρόκου αυγού, της υδρολυμένης μαγιάς, και της παρουσίας και ποσότητας βιταμινών και χολη-στερόλης, αυτή που έδωσε στατιστικά σημαντική μεγαλύτερη ωοπαραγωγή, ήταν αυτή που περιείχε 80 μέρη ζάχαρη, 30 μέρη υδρολυμένη μαγιά και 6.6 μέρη αποξηραμένο κρόκο αυγού.