

# ENTOMOLOGIA HELLENICA

Vol 6 (1988)



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doi: [10.12681/eh.13955](https://doi.org/10.12681/eh.13955)

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## To cite this article:

Stamopoulos, D. C. (1988). Some enzymatic activities registered in eggs and gut tissues of the olive fruit fly, *Dacus oleae* (Gmelin). *ENTOMOLOGIA HELLENICA*, 6, 23–27. <https://doi.org/10.12681/eh.13955>

# Some Enzymatic Activities Registered in Eggs and Gut Tissues of the Olive Fruit Fly, *Dacus oleae* (Gmelin)<sup>1</sup>

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## ABSTRACT

Nineteen enzymatic activities of eggs, gut tissues of larvae and adults of *Dacus oleae* have been determined using the API-ZYM micromethod. The enzymatic activities of eggs were much weaker than those of larval or adult guts. The activities increased progressively to the L<sub>2</sub> and L<sub>3</sub>. The lack of feeding caused a reduction of certain activities in larvae (alkaline phosphatase, esterases, aminopeptidases) but not in adults. The addition of streptomycin to the food of adults, to obtain "aposymbiotic" individuals, did not have a clear effect on the activities of the insect's various stages. Although the enzymes studied were not the only ones that occur in the insect's gut tissues, it seems that there are few similarities between the enzymatic system of larvae and that of adults, most probably because of the different content of the two diets in essential nutrients such as amino acids, proteins, and lipids.

## Introduction

In nature, the adult olive fruit fly, *Dacus oleae* (Gmelin) (Diptera: Tephritidae), feeds on nectar, pollen, juices of ripe or decomposing fruits and honeydew of aphids or scale insects (Tsiropoulos 1977, Katsoyannos 1983). The larva feeds only on ripe olives or olives in the process of maturation. Artificial rearing in the laboratory is quite easy using a mixture of sucrose, hydrolysed protein and water for the adults and freshly picked or cold-stored olives for the larvae. For the rearing of larvae, solid artificial diets can also be used (Tzanakakis et al. 1970, Tsitsipis 1977). The composition of those foods, quite diversified, implies that the insect possesses well adapted enzymatic activities to be able to acquire such essential basic nutrients as amino acids, monohexoses, fatty acids etc. at all developmental stages. The secretion of the enzymes which are essential for such a utilization of the trophic resources takes place principally in the digestive tract, and, ac-

cording to certain authors it is possible that a number of them is of microbial origin (Hagen 1966, Fytizas and Tzanakakis 1966, Tzanakakis and Stavranides 1973). In fact, the microorganisms which abound in the intestine or in the diverticulum of the adult's head capsule are considered as "symbiotes" in the sense that they are involved in the process of food digestion.

The purpose of the present work was to contribute to the knowledge of the gut digestive enzymatic activities that occur in the various stages of *D. oleae*. I do not claim that these are the only ones that occur in the insect's tissues nor that there is always a close correlation between the enzymatic activities observed and the constituents of the insect's food. As Barrington (after Morgan 1976) states, "the ability of certain enzymes to digest a given substrate does not mean that the particular substrate must necessarily be present in their diet". Yet, research on the enzymatic activities of a tissue or an organ may contribute to the understanding of the mechanisms involved in the digestion of foods and to guiding of studies concerning the insect - trophic substrate - symbiotes complex.

<sup>1</sup> Received for publication January 26, 1989.

In this respect, the enzymatic activity of the digestive tract was examined not only of "normal" insects, but also of insects deprived of food and also of insects treated with streptomycin and considered as "aposymbiotic" (Hagen et al. 1963, Hagen 1966, Tzanakakis 1984).

### Materials and Methods

The adults were derived from pupae taken out of field - infested olives of the variety Megaritikiki, that were collected in the region of Halkidiki (northern Greece), in late September. Three "types" of insects were used: 1. Adult females, one-day-old, unfed. 2. Adult females fed for 5 days a mixture of hydrolyzed yeast - sugar - water in the ratio of 1 - 4 - 5 by weight. 3. Adult females to the diet of which 0.25% streptomycin sulphate was added from the 5th day for 3 days. The larvae came from females which laid eggs either in paraffin domes (Hagen et al. 1963) or in green olives of the same variety. The digestive tract was examined of: 1. Larvae soon after their exit from eggs laid in paraffin domes. 2. Larvae of the same type as 1, but from mother flies fed streptomycin. 3. Larvae developed in the mesocarp of green olives (all three larval stadia). The enzymatic activity of eggs from "normal" and "aposymbiotic" females was also determined.

The determination of 19 enzymatic activities was done according to the procedure of the API system S.A. This system is a semiquantitative research micromethod of enzymatic activities applicable to all substrates (tissues, cells, microorganisms etc.), with very small quantities of samples. The following procedure, according to Monget (1978) was followed:

Dissection of adults or larvae in cold distilled water.

*Dissection of the gut, after it has been cleared of tracheae and associated tissues, except of the Malpighian tubules, and emptied of its contents.*

Breaking up of the tissues thus obtained in a Potter tube, in 1.5 ml of cold distilled water. The eggs were broken in toto.

Inoculation, with a Pasteur pipette, of the prepared sample in the API-ZYM tray, at two drops (= 65 µl) per cupule.

Incubation for 4 hours at 37°C.

After the incubation, addition to each cupule of one drop of reagent ZYM-A (permitting the solving of reagent B) and one drop of reagent ZYM-B (Fast Blue BB).

After 5 minutes, exposing of the trays to the rays of a powerful light source (= 1000 watt bulb).

Registering of results on a scale of 0 to 5. Zero corresponds to a negative reaction, while 5 to a reaction of maximum intensity.

We have tried to use approximately the same quantity of digestive tracts in all cases, even in small

larvae. The sensitivity of the method allows the use of very small quantities of tissues. In fact, in a previous work (Stamopoulos 1980), we have found that only 9 digestive tracts of L4 of *Acaethoscelides obtectus* showed the same totals of enzymatic activity with 30 tracts. In general, with *D. oleae* we used 2 mg of digestive tracts and 30 eggs in every series of tests.

### Results and Discussion

From the results seen in Table 1 we can conclude that, in general, the enzymatic activities of eggs are much weaker than those of larvae or adults. Their profile resembles that of starved larvae, except for the activity of acid phosphatase, phosphamidase and  $\alpha$ -glucosidase which are stronger in eggs. The enzymatic activities increase progressively when the larvae reach the stadia L2 and L3. This phenomenon has been observed also in *A. obtectus* (Stamopoulos 1980). The starved condition seems to influence considerably certain enzymatic activities of larvae, as shown by Plantevin and Nardon (1972) for *Galleria mellonella* and *Periplaneta americana*. On the contrary, the addition of streptomycin to the diet of adults seems to have no effect whatsoever on the activities studied. However, for the time being, we cannot reject, even indirectly, the theory of the role the "symbiotes" play in digestion, because other factors may interfere in such a complex system.

Phosphatase activity (2, 11, 12). We find a quite strong alkaline phosphatase activity in larvae and adults but not in eggs or in unfed larvae. This enzyme is involved in various transfer phenomena and its ubiquity in the class of insects is not surprising. Yet, the lack of feeding seems to affect this activity considerably, as also seen in *G. mellonella* and *P. americana* (Plantevin and Nardon 1972). Acid phosphatase was present in all stadia examined, at a generally high level, without a substantial reduction in unfed individuals. It is involved in certain biological processes, such as protein synthesis. The lower activity in eggs from streptomycin-fed mothers should be examined further, same as phosphamidase and  $\alpha$ -glucosidase, because if they are not artifacts due to the experimental procedure, there are two possible explanations: the presence of traces of streptomycin in the eggs partially blocks the substrate of the API-ZYM system, or the secretion of enzymes due to the micro-

TABLE 1. Enzymatic activities of eggs, larvae and adults of *D. oleae* according to the API-ZYM system (N: normal, St: streptomycin-fed, Uf: unfed individuals, t = traces).

Activity looked for	Reaction									
	eggs		larvae					adults		
	N	St	Uf	St	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	Uf	St	N
1. Control	0	0	0	0	0	0	0	0	0	0
2. Phosphatase alkaline	t	t	t	t	3	5	5	5	5	5
3. Esterase (C4)	t	t	0	0	1	2	2	3	4	3
4. Esterase Lipase (C8)	t	t	0	0	1	1	1	2	1	2
5. Lipase (14)	t	t	0	0	1	1	1	1	1	1
6. Leucine arylamidase	t	t	2	2	5	5	5	5	5	5
7. Valine arylamidase	t	t	0	0	1	3	4	2	2	2
8. Cystine arylamidase	t	t	0	0	2	3	4	2	2	2
9. Trypsin	0	0	t	0	0	t	t	t	t	0
10. Chymotrypsin	0	0	0	0	0	0	0	0	0	0
11. Phosphatase acid	4	2	2	2	2	3	4	5	5	5
12. Phosphamidase	4	3	1	2	2	3	3	4	4	4
13. $\alpha$ -galactosidase	0	0	0	0	0	0	0	0	0	0
14. $\beta$ -galactosidase	0	0	0	0	0	t	1	2	1	1
15. $\beta$ -glucuronidase	0	0	0	0	0	t	t	1	1	1
16. $\alpha$ -glucosidase	5	4	0	0	t	1	1	3	4	5
17. $\beta$ -glucosidase	0	0	0	0	0	0	0	0	0	0
18. N-acetyl- $\beta$ -glucosaminidase	0	0	0	0	0	3	3	3	1	1
19. $\alpha$ -mannosidase	0	0	0	0	0	0	0	0	0	0
20. $\alpha$ -fucosidase	0	0	0	0	0	0	0	1	t	t

bial flora of the eggs weakens because of the antibiotic.

Esterases - lipases (3, 4, 5). According to Monget (1978), the line between esterases and lipases remains vague. It is generally accepted that the aliphatic chain of the ester should have at least 14 carbon atoms for the hydrolysis to be caused by a lipase. The esterase and lipase activities observed were of a more or less medium level, except in eggs and in unfed larvae where they were virtually absent. What is unexpected in *D. oleae* is that lipase activities are fairly weak in the larvae. This seems abnormal for an insect which passes its larval stage in a substrate rich in fatty acids. It is possible that this activity is manifested in connection with the level of fatty acids in the mesocarp; in other words, the larvae which develop in green olives, such as those of the present work, which are less rich in lipids, have a weak lipase activity which may increase in larvae developing in ripe olives. However, such a hypothesis remains to be proven.

Proteinase activity (9, 10). It is totally absent

in all the cases examined, while aminopeptidases are present at a high level.

Aminopeptidases (6, 7, 8). They specifically hydrolyse the acid - CO - NH bond and in addition to their digestive function could participate in protein synthesis. In insects there is something a contrast between aminopeptidase and lipase activity (Plantevin and Nardon 1972). In fact, one observes in *D. oleae* as well in *Blaberus cranifer*, in *A. obtectus* and in other insects that the greater the aminopeptidase activity the smaller the lipase activity. Yet, no correlation between these two activities has been demonstrated.

Glucosidases (13, 14, 15, 16, 17, 18, 19, 20). These enzymes hydrolysing glucosides are very specific as to the glucone part of the substrate and to the bond between glucone and aglucone (Monget 1978). They are involved in the digestion of cellular or extracellular oligo and polysaccharides and of mucopolysaccharides contained in tissues. In view of the fact that, in insects, the absorption of carbohydrates takes place in general in the form of

monosaccharides (Treherne 1967), the study of the glucosidase arsenal of an insect may provide interesting information about its diet or about its capacity to be nourished on a certain substrate. According to Morgan (1976), the glucosidases have also transglycosylation functions, the acceptor generally being an alcohol or a sugar. This transferring action could constitute a detoxication mechanism in insects.

The study of the glucosidase system in *D. oleae* shows that there is no similarity between that of larvae and that of adults, same as was observed by Leroi et al. (1984) in *A. obtectus*. The different composition in carbohydrates of the adult diet from the larval diet could explain such a difference in the enzymatic activities. Adult nourishment, already mentioned is very rich in sugars, while the olive mesocarp is very poor, especially during the mid October – early December period (Katakura and Narasaki 1954). According to our results, a strong  $\alpha$ -glucosidase activity is observed in adults and a very weak one in larvae. This allows the imago to attack certain sugars such as sucrose, maltose, melezitose and trehalose. In fact, Tsiropoulos (1980) proved such a capacity in adults of *D. oleae* and suggested the hypothesis that “an  $\alpha$ -glucosidase must be present (in the insect's digestive system)”. A quite clear  $\beta$ -glucosaminidase activity is also observed, except in eggs and in  $L_1$ , which is not surprising because this enzyme is involved in the formation of the integument in insects (Nardon and Plantevin 1972).

The total absence of a-galactosidase implies that the insect has not the capacity to attack melibiose. This seems in contradiction with what Tsiropoulos (1980) has observed. On the contrary, the weak  $\beta$ -galactosidase activity, can explain the non utilization of lactose which that author observed. Yet, as he points out, “some of the larger molecules may be absorbed directly, (and the) presence in the olive fruit fly of these enzymes is only indicative”.

The absence of differences between the enzymatic activities of “normal” insects and those of “aposymbiotic” ones can be explained by admitting that: 1. The procedure used, e.g. continuous washing of the digestive tract in distilled water, has removed the microbial flora of “normal” insects and, consequently, has reduced their total enzymatic activity, or 2. The microbial flora plays a role other than that of

enzyme “producer”. Further studies of the microbial flora of *D. oleae* are indispensable to precisely determine the role of its “symbiotes”.

### Acknowledgment

I thank Prof. M.E. Tzanakakis for comments on the manuscript.

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**KEY WORDS:** *Dacus oleae*, Olive fruit fly, *Dacus oleae* enzymatic activities

## Μερικές Ενζυμικές Δράσεις που Καταγράφηκαν στο Αβγό και στο Πεπτικό Σύστημα του Δάκου της Ελιάς, *Dacus oleae*

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### ΠΕΡΙΛΗΨΗ

Σε αβγά, προνύμφες και τέλεια του *Dacus oleae* εξετάσθηκαν δεκαεννέα ενζυμικές δράσεις, με τη μέθοδο API-ZYM. Τα αποτελέσματα έδειξαν ότι στα αβγά οι τιμές που καταγράφηκαν ήταν πολύ χαμηλότερες από αυτές των τελείων ή των προνυμφών. Στις τελευταίες, οι ενζυμικές δράσεις αυξάνουν προοδευτικά με την αύξηση του σταδίου. Η μη λήψη τροφής από τις νεαρές προνύμφες φαίνεται να προκαλεί μείωση της δράσης ορισμένων ενζύμων (αλκαλική φωσφατάση, εστεράσες, αμινοπεπτιδάσες), ενώ δεν παρατηρείται κάτι ανάλογο στα τέλεια. Η χορήγηση στρεπτομυκίνης σε τέλεια, με σκοπό τη λήψη «αποσυμβιοτικών» ατόμων δε δείχνει να έχει μια ξεκάθαρη επίδραση στις ενζυμικές δράσεις των διαφόρων σταδίων του εντόμου. Παρ' όλο που οι ενζυμικές δράσεις που εξετάσθηκαν δεν είναι και οι μόνες που απαντώνται στο πεπτικό σύστημα του εντόμου, δεν φαίνεται να υπάρχει σε πολλές περιπτώσεις κάποια αντιστοιχία μεταξύ του ενζυμικού «οπλισμού» των προνυμφών και αυτού των τελείων, πιθανώς λόγω της διαφορετικής σύνθεσης της τροφής σε απαραίτητες θρεπτικές ουσίες όπως π.χ. αμινοξέα, πρωτεΐνες και λιπίδια.