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Responses of Tephritid Fruit Flies to Baits

R. Galun, S. Gothilf, D.L. Chambers,
J.L. Sharp

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P.O.B. 6

Bet Dagan, 50250 ISRAEL

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Name and Address of Investigators:

Principal Investigators: R. Galun; S. Gothilf

Cooperating Investigators: D. L. Chambers; J. L. Sharp

Name and Address of Affiliated Institutions:

Principal Institution: Hebrew University of Jerusalem, Israel

Cooperating Institution: Insect Attractants, Behavior and Basic
Biology Research Laboratory, USDA ARS,
Gainesville, FL

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Signature:

Principal Investigator

...R. Galun...

Rachel Galun

Institution's Authorized Official

(signature and official stamp)

....

M. Ottolenghi

TABLE OF CONTENTS

<u>ABSTRACT</u>	1
<u>EVALUATION OF THE RESEARCH ACHIEVEMENT WITH RESPECT TO THE ORIGINAL</u>	
<u>RESEARCH PROPOSAL</u>	2-4
<u>RESEARCH FINDINGS</u>	5-122
-Evaluation of Commercial Preparations	6-12
-Olfactometer and trap for evaluating attractants for the Mediterranean Fruit Fly, <u>Ceratitis capitata</u>	13-18
-Feeding Behavior of <u>Anastrepha suspensa</u> (Diptera: Tephritidae) in the Laboratory	19-48
-Aggregation Response of <u>Anastrepha suspensa</u> (Diptera: Tephritidae) to Proteins and Amino Acids	49-76
-Responses of the Mediterranean Fruitfly <u>Ceratitis capitata</u> to Amino Acids	77-86
-Protein and Sugar Hunger in the Mediterranean Fruit Fly <u>Ceratitis capitata</u> (Wied.)(Diptera:Tephritidae)	88-94
-Olfactory and Gustatory Responses of Normal and of Irradiated Fruit Flies <u>Ceratitis capitata</u> (Wied.) and <u>Anastrepha suspensa</u> (Loew) to Nutrients	95-122
<u>DESCRIPTION AND RESULTS OF COOPERATION</u>	123
<u>THESIS</u>	123
<u>BENEFIT TO AGRICULTURE</u>	123
<u>PUBLISHED REPRINTS AND MANUSCRIPTS</u>	124-125

ABSTRACT

Mediterranean and Caribbean fruit flies pupate in the immediate vicinity of the fruits which adult females have damaged by ovipositing into them. Adult flies normally feed on naturally-occurring protein and sugar-rich substances present on the fruit-bearing plant/s with which each species is associated.

Any attempt at manipulation of normal feeding, mating and oviposition habits, requires a prior understanding of the biological bases for the choice of feeding, mating and oviposition substrates.

This work provides a basis for understanding the feeding preferences of both sexes of both species of flies, with respect to some protein hydrolysates, amino acids--singly and in various combinations--and sugar; and relates these preferences to critical stages in the life history of the flies. Protein consumption increases prior to the time of the first matings and oviposition, and is greater for females than for males of both species; sugar is vital to both sexes, though male medflies consume greater quantities than females.

Manipulation of the normal biology of the flies through irradiation of late-stage pupae, resulted in complete gonadal degeneration. Feeding behavior of irradiated flies was compared to that of normal flies. Total protein consumption by females and sugar consumption by males were depressed, but not to the extent that irradiated flies would fail to seek food and mates at normal breeding sites. Therefore, sterile mating of irradiated males with normal females would still be realized in spite of the depressed feeding behavior occurring as a side-effect of sterilization.

Of several protein hydrolysates and commercial baits tested, casein hydrolysate was by far the most attractive, whether evaluation was based on olfactory or on phagostimulatory responses of the flies. The methods developed for evaluating fly response provide a basis for bioassay of commercial products proposed for use in bait sprays.

EVALUATION OF THE RESEARCH ACHIEVEMENT WITH RESPECT TO THE ORIGINAL RESEARCHPROPOSAL"Responses of Tephritid Fruit Flies to Baits"

The aims of this project as set out in the grant proposal were threefold:

- 1) to identify the chemical nature of the attractant and phagostimulatory components of protein hydrolysates used in fruit fly baits;
- 2) to test experimentally the behavioral responses of the medfly and the caribfly to proteins as related to the age, sex, nutritional history and reproductive state of the flies;
- 3) to evaluate the effect of irradiation on these responses.

Major Results and Conclusions:

1) Protein hydrolysates of different sources constitute the basic ingredient of commercial baits as well as of media used for rearing flies in the laboratory. Some preparations tested here such as hydrolysates of casein, yeast, soy and lactoalbumin as well as such commercial fruitfly baits as Naziman, Buminal, PIB 7, fishmeal and an autolysate of yeast, contain about 50% free amino acids. Therefore, a study of the olfactory, aggregatory and feeding responses of both medfly and caribfly to several commercial compounds, to protein hydrolysates and/or to amino acids were carried out in the course of this study. Among the hydrolysates and commercial baits tested, casein hydrolysate was by far the most effective, from the standpoints of olfactory, aggregatory and feeding response. Responses to tests involving individual amino acids and various combinations of them ranged from negative through indifferent to positive and even to highly positive. However, no single amino acid nor any of the

combinations assayed, even approached the effectiveness of the hydrolysate. The obvious conclusion is that the remaining components should be carefully studied per se, and as synergists to the free amino acids.

2- An age-dependent pattern of protein feeding was observed in both species. For 24 hours after hatching, olfactory attraction to, as well as aggregation and feeding on protein were minimal, thereafter rising to a peak on the 3rd or 4th day in the medfly. This corresponded behaviorally to a peak in initial matings and imminence of first ovipositions. In the caribfly, this same feeding pattern was apparent, but was synchronized with a different time table, first matings and intensive egg maturation occurring on about the 7-8th day of adult life.

In the medfly, an additional, lower peak of protein feeding occurred between the 5th and 10th days. Beyond this age, feeding remained on a plateau at a low level, or declined gradually to a low level. In the normal female, this feeding pattern could be related directly to egg development. Though males did not require protein for egg production, they also showed an initial peak in protein feeding which was lower than that of females; however, there was no second peak.

Exposure of flies to a diet of protein hydrolysate reduced the number of medflies subsequently trapped in an olfactometer baited with casein hydrolysate, by about 50%. Similarly, only about one fourth as many flies aggregated on a casein hydrolysate-containing test substrate if their pre-test maintenance diet had contained yeast hydrolysate with sucrose, rather than sucrose alone. This diminished aggregation on casein hydrolysate was apparent for at least 3-4 days following even a single day's exposure to a protein-containing food substrate. Aggregation on sucrose, on the other hand, remained more or less constant, despite its presence in the maintenance diet.

Marked differences in olfactory attraction to or feeding on protein hydrolysate were not apparent between mated and virgin females tested up to the age of 10 days.

Protein availability had a marked effect on the number of eggs produced by normal medfly females: autogenous egg production was only about one-third that by females fed on a yeast-sugar food substrate.

3- Following irradiation, there was a reduction in:

- olfactory attraction of both sexes of medfly and of female caribflies to protein hydrolysate;

- aggregation on protein hydrolysate by both species (for caribfly, each sex counted separately; for medfly, total number counted, without respect to sex);

- consumption of sucrose by male medflies;

- consumption of protein hydrolysate solution by caribflies of both sexes and by female medflies.

Note is taken of the fact that though reduced, feeding response of irradiated flies is probably adequate to ensure their presence on natural food substrates of the species where mating takes place.

RESEARCH FINDINGS

The scientific findings of this research project are presented in the following pages in the form of separate units which, taken together, represent the experimental procedures and the findings of this project. The status of each unit (published material, manuscripts already submitted, manuscripts ready to be submitted, and the report of a study nearing completion, e.g. "Evaluation of Commercial Preparations") is given on the last page of this report under the heading "PUBLISHED REPRINTS AND MANUSCRIPTS".

Evaluation of Commercial Preparations:

Olfactory response: The response of medflies to commercial baits was compared in the olfactometer and in aggregation experiments in which the phagostimulatory nature of the material also played a part in the fly's response.

The olfactory response of female medflies was tested in the olfactometer described by Gothilf and Galun (1982). 500 females, hatched within 24 hours from the same batch of pupae, were transferred to the 60 x 60 x 60 cm cage of the olfactometer when they were 3-10 days old. They were kept at $25 \pm 2^{\circ}\text{C}$ with solid sugar and water constantly available.

The following commercial products were tested: enzymatic casein hydrolysate, lactoalbumin hydrolysate, soy hydrolysate and yeast hydrolysate, all products of ICN, Cleveland, Ohio; fish solubles and Buminal, products of Bayer Leverkusen, F.D.R.; yeast autolysate, of Mauri Brothers, Homebush N.S.W. Australia; Naziman, Nasid Ltd., Tel Aviv, Israel; and PIB 7, Staley Manufacturing Co., Decatur, Ill. The hydrolysates were in powder form, while the other baits were in the form of pastes, solutions or suspensions.

Olfactory response was tested in two experimental set-ups; in two-choice tests in which two commercial baits were placed alternately, each in three of the six traps suspended from the horizontally rotating wheel of the olfactometer; and in tests in which a single commercial preparation was present in three traps which alternated with three empty traps.

Each test lasted one hour. At the end of that time, the total number of flies was counted for each set of three traps. Since the structure of the trap precluded any actual contact between the flies and the bait, flies were released from the traps back into the cage. Moreover, since preliminary tests had shown that response was not age

dependent between 3-10 days, the same females were tested repeatedly between these ages.

Since test flies were protein-starved, they reacted to introduction of the baited traps with apparent search behavior. Flies landed on the glass traps and walked around until they found one of the two entrance holes. Most of the trapped flies were caught within a half hour of the start of the test.

The result of the two-choice test was expressed as follows: the number of flies in the three traps containing the same bait was summed for each olfactometer and the ratio between the number attracted to each of the two baits in the same olfactometer was calculated, the higher number serving as the numerator. The ratios obtained from replicates of the same choice of baits were averaged and the standard deviation was calculated. If the flies showed a marked and consistent preference for one of the two substances in five tests, this combination of baits was not tested further. If the choice was not clear-cut, or was inconsistent, up to 14 replications of the same choice were carried out.

The order of preference for the nine substances tested was: casein hydrolysate > Buminal = soy hydrolysate > lactoalbumin hydrolysate > fish solubles > Naziman=PIB 7 > yeast autolysate > yeast hydrolysate. The degree of preference is shown in Table I.

In single bait experiments, all substances tested attracted significantly more flies than empty traps. Those caught in unbaited traps were considered to have entered in the course of random flight, rather than as a result of positive attraction. Their number was therefore deducted from the number in the baited traps. As in the two-choice tests, casein hydrolysate attracted by far the largest number of flies (192.0 ± 13.03 , mean $\pm$ S.D.) while that which had ranked lowest in two-choice tests, yeast hydrolysate, attracted by far the smallest number of flies (29.4 ± 13.7) (Table II). Moreover, enzymatic casein hydrolysate, despite

ATTRACTION OF FEMALE MEDFLY TO COMMERCIAL BAITS IN TWO CHOICE OLEATORY EXPERIMENTS

	casein hydrolysate	Buminal	soy hydrolysate	lactalbumin hydrolysate	fish solubles	Naziman	PIB 7	yeast autolysate	yeast hydrolysate
Buminal	1.51 ± 0.44								
Soy hydro- lysate	1.57 ± 0.34	0.99 ± 0.15							
lactalbumin hydrolysate	1.85 ± 0.49		1.30 ± 0.35						
fish sol- ubles	3.50 ± 0.50	2.51 ± 1.71		1.24 ± 0.14					
Naziman		2.0 ± 0.49	2.46 ± 0.92		1.28 ± 0.25				
PIB 7		2.20 ± 0.73			1.11 ± 0.36	1.14 ± 0.21			
yeast auto- lysate			2.41 ± 0.39		1.51 ± 0.61	1.29 ± 0.8	3.20 ± 1.13		
yeast hydro- lysate	13.04 ± 10.36	5.48 ± 2.69	2.29 ± 0.12	3.31 ± 0.61				2.89 ± 1.74	

Numbers represent mean ratio $\pm$ S.D. of flies caught on bait listed in horizontal plane to th
in vertical plane. All differences are statistically significant except Buminal:soy hydrolysate
and fish solubles:PIB 7.

Table IIATTRACTION OF FEMALE MEDFLIES TO COMMERCIAL BAIT

<u>BAIT</u>	<u>MEAN + S.D./OLFACTOMETER</u>	<u>GROUP</u>
casein hydrolysate	192.0 $\pm$ 13.03	a
fish solubles	144.6 $\pm$ 22.94	b
lactoalbumin hydrolysate	141.0 $\pm$ 12.57	b
Buminal	135.6 $\pm$ 13.41	b
yeast autolysate	119.2 $\pm$ 22.95	b,c
soy hydrolysate	109.2 $\pm$ 23.76	b,e,d
Naziman	92.4 $\pm$ 21.66	c,d
PIB 7	83.6 $\pm$ 21.20	d
yeast hydrolysate	29.4 $\pm$ 13.70	e

Numbers represent mean ($\pm$ S.D.) number of flies trapped/cage for all replications of each bait. Letters are based on F-test.

Table III

Preference of medfly for commercial baits *

test bait	number cages	number*flies on bait	number flies, CH standard	total, both dishes	percentage on test
Naziman	5	899	529	1428	62.9
Buminal	3	482	709	1191	40.5
Lactalbumin hydrolysate	3	522	767	1289	40.5
yeast hydroly- ysate	5	546	522	1068	51.1
soy hydroly- ysate	4	383	417	800	47.8

* Tested in aggregation-type, 2-choice experiments.

* Sum of four readings taken at 5, 30, 60 and 120 min. after introduction of petri dishes.

Flies of both sexes, aged 3-8 days, were used.

a shelf life of three years, produced reproducible results and was therefore used as the standard in further tests of commercial products where phagostimulation, as well as olfaction, was involved.

Phagostimulatory response: Samples of the same baits to which the olfactory response of the medfly had been tested, were employed in evaluating phagostimulatory response as expressed in aggregation-type experiments.

In preliminary tests, various formats were assayed: e.g. simultaneous introduction of several baits to each cage; testing of several baits, each in a separate cage vs agar, always including at least one cage with CH vs agar (as standard); and a test bait vs CH, in equivalent concentrations. Insofar as possible, the same batch of flies was employed for testing several different baits, to minimize the variability arising from the use of different fly populations.

Discrimination between the baits could best be evaluated in the format in which the choice was between the test bait in one dish, and CH in the other. Protein hydrolysates were made up in 2.5% concentration, commercial preparations like Naziman and Buminal, in 10% solution (approximating the 2.5% concentration of their protein hydrolysate content) (Table III).

Note is taken of the marked difference in response of the flies when olfactory, as opposed to phagostimulatory response, was tested. In the present phagostimulation experiments, appetite could be stimulated by eating, as well as through olfactory, gustatory and contact tarsal receptors.

In the course of these experiments, it became clear that commercial baits lack uniformity: the same substance from the same company but

from batches of different stock number, may differ greatly in their capacity to attract flies. THIS LACK OF UNIFORMITY MAKES IT ESSENTIAL TO HAVE A BIOLOGICAL ASSAY CARRIED OUT ON ANY COMMERCIAL PREPARATION BEFORE IT IS PURCHASED IN QUANTITY FOR USE IN A LARGE-SCALE CONTROL PROGRAM.

Furthermore, these results emphasized the necessity to distinguish which component parts of the bait are essential for attraction so that manufacturers can be guided towards achieving a uniform and maximally effective product.

Caribfly:

In olfactory tests using only females, and in aggregation tests employing both sexes of caribfly, enzymatic CH was preferred to enzymatic yeast hydrolysate, enzymatic soy hydrolysate and non-hydrolyzed torula yeast. (see attached manuscript: Aggregation Response of Anastrepha suspensa (Diptera: Tephritidae) to Proteins and Amino Acids (Sharp, J. and Chambers, D.L.).

DESCRIPTION AND RESULTS OF COOPERATION:

The work carried out in parallel on two different flies, showed many similar features: responses to hydrolysates, effects of irradiation on feeding behavior, sex differences in feeding responses, etc. The methodology developed for the medfly could be transferred almost directly to the caribfly, thus utilizing the same investment on a larger scale. Furthermore, the similar results obtained with two different genera emphasize the generality of the observations.

THESIS

An MSc thesis in Hebrew on the relationship between protein feeding and egg production in normal medflies and the effect of irradiation on both parameters is being typed up and the English summary will soon be forwarded to BARD, Beit Dagan.

BENEFIT TO AGRICULTURE

Our work demonstrated the variability in effectiveness of commercial baits for the medfly and proposes a reliable way of assessing it before purchasing large batches of baits. Since the standard control of medfly is based on malathion bait sprays, the development of a bioassay for bait evaluation is quite meaningful.

PATENTS:

None.

PUBLISHED REPRINTS AND MANUSCRIPTS

Published:

- ✓ Galun, R., Nitzan, Y., Blondheim, S. and Gothilf, S. 1980.

Responses of the Mediterranean Fruitfly Ceratitidis capitata to amino acids. Proceedings of a Symposium on Fruit Fly Problems. Kyoto and Naha, pp. 55-63.

- Galun, R., Gothilf, S., Blondheim, S. and Lachman, A. 1981.

Protein and sugar hunger in the Mediterranean fruit fly Ceratitidis capitata (Wied.) (Diptera: Tephritidae) in Determination of Behaviour by Chemical Stimuli; ed. J. Steiner: pp. 245-51.

- Gothilf, S. and Galun, R. 1982.

Olfactometer and trap for evaluating attractants for the Mediterranean Fruit Fly, Ceratitidis capitata. Phytoparasitica 10(2):79-84.

- ✓ Sharp, J. L. and Chambers, D.L. 1983.

Aggregation response of Anastrepha suspensa (Diptera: Tephritidae) to proteins and amino acids. Environmental Entomology 12(3):923-8.

Submitted:

- Sharp, J. L. and Chambers, D.L.

Feeding behavior of Anastrepha suspensa (Diptera: Tephritidae) in the laboratory.

Ready for submission:

- Galun, R., Gothilf, S., Blondheim, S., Sharp, J.L., Lachman, A., and Chambers, D.L.

Olfactory and gustatory responses of normal and irradiated fruit flies Ceratitidis capitata (Wied.) and Anastrepha suspensa (Loew) to nutrients.

Final data needed for substantiation of experiments on attraction of flies to commercial baits, are being analyzed. These data will enable

us to complete a paper on attraction to and aggregation of flies on commercial baits. Some results available to date are included in this report (see above, "Evaluation of Commercial Preparations").

Papers in English based on the MSc thesis of A. Lachman will acknowledge support by BARD. As these papers appear, they will be forwarded to BARD, Beit Dagan.