FRUIT FLIES

PROCEEDINGS

OF THE SECOND

INTERNATIONAL SYMPOSIUM

16-21 SEPTEMBER 1986, COLYMBARI, CRETE, GREECE
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Edited by A.P. ECONOMOPOULOS

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SECOND INTERNATIONAL SYMPOSIUM ON FRUIT FLIES

Orthodox Academy of Crete
Colymbari, Hania - Crete, Greece
16 - 21 September 1986

ORGANIZING COMMITTEE

Cirio, U. (ENEA, Italy), Economopoulos, A. (IAEA, Co-ordinator), Enkerlin, D. (Mexico), Gilmore, J. (USDA, USA), Kafatos, F. (Universities of Crete and Harvard), Lindquist, D. (IAEA), Loukas, M. (Agricultural College of Athens), Michelakis, N. and Michelakis, S. (Subtropicals and Olive Institute of Crete), Papaderos, A. (Orthodox Academy of Crete), Prokopy, R. (University of Amherst), Tzanakakis, M. (University of Thessaloniki)

CO-OPERATING ORGANIZATIONS

Food and Agriculture Organization of the United Nations, Rome
International Atomic Energy Agency, Vienna
Ministry of Agriculture of Greece
Ministry of Culture and Science of Greece
Ministry of Industry, Energy and Technology of Greece
Agricultural Bank of Greece
«Eleourgiki» Central Union of Olive Co-operatives of Greece
Hellenic Entomological Society
Orthodox Academy of Crete
Italian National Committee for Nuclear and Alternative Energies (ENEA), Rome

In conjunction with the Symposium, IAEA Fruit Fly Genetic Sexing Research Co-ordination Meeting
The fruit fly scientific community is characterized by an impressive number of active members and intense research effort on diverse subjects in all five continents. High quality research on basic and applied topics and sophisticated applications, often covering vast areas, are characteristics of this vital community.

In 1982, the first large international symposium on fruit flies was jointly organized by the Greek Government, EEC and IOBC. Although it was not named so, in reality it was the first international symposium on the subject. Its success resulted in a unanimous recommendation of participants for the continuation of quadrennial fruit fly symposia.

In 1985, a group of scientists formed the committee which organized the present second fruit fly symposium in Crete, four years after the first one in Athens. Several Greek organizations, FAO, IAEA, and ENEA of Italy supported the symposium. Ninety-six active participants from 21 countries and 2 international organizations presented 75 papers. The symposium was organized in 10 sessions, from very basic information to field control applications. About 40% of papers were of basic research, while 55% dealt exclusively or extensively with the Mediterranean fruit fly indicating the dominant economic importance of this fly all over the world. The aim of involving much basic information had been considered important given its critical role in developing sophisticated control technologies of reduced environmental destruction.

In conjunction with the symposium, the IAEA held its Genetic Sexing Research Co-ordination Meeting. Also, the prospects of a substantial contribution of basic research to control applications and the organization of large field control programmes, were discussed extensively during the symposium.

The closing plenary session of the symposium strongly recommended that the Third International Symposium on Fruit Flies of 1990 be organized in Central America or the U.S.A., given the intensive research activity and large field programmes in the area. It was also recommended that for the third symposium, a steering committee should select the host country and organizing committee. For future symposia, host areas and organizing committees should be elected during the previous symposium.

The approval and support provided by Prof. Zifferero (IAEA), Dr. Sigurbjörnsson (FAO/IAEA), Dr. Brader (FAO) and Dr. Lindquist (FAO/IAEA) made this symposium possible. In particular, the strong and continuous interest of Dr. Lindquist was a key factor for its overall success and strengthened the prospects of its continuation. The enthusiastic support and help of Profs. Loukas (Agricultural College of Athens) and Tzanakakis (University of Thessaloniki) and Dr. Cirio (ENEA, Rome) were decisive for the successful organization of the symposium. The director of the host Orthodox Academy of Crete Dr Papaderos and all the Academy personnel provided excellent cooperation and hospitality.

A.P.E.
EDITORIAL

The papers are arranged according to the presentation sequence of the symposium. An asterisk in the table of contents denotes author who presented the paper. Typing instructions for camera-ready manuscripts were given to authors. The views expressed remain the responsibility of the authors concerned who are also responsible for any reproduction of copyright material. In certain cases only abstracts are presented due to incomplete research, thesis material, submission for publication elsewhere, or where the manuscript is not suitable for reproduction.

Many thanks are due to the IAEA secretaries, M. Cohen and L. Kruzic, for their excellent co-operation in typing all proceedings material (except the full papers) and most of the correspondence related to the proceedings and symposium in general.
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OPENING SESSION
Ladies and Gentlemen,

I feel privileged to be here today. Your scientific meeting will cover recent developments on a subject very important to the agriculture of many countries around the world, among them, my country, with its many olive and fruit trees. Of the insects covered extensively in your Symposium, the Mediterranean fruit fly, the olive fruit fly and the cherry fly are key pests of our agricultural production. They compete with us for some of our most valuable fruit crops. I understand that the same is true of the above and other fruit flies to be covered in this Symposium in many countries in all of the five continents.

Having seen your programme, I was impressed by the large number of countries participating and the apparent interest of large international organizations. I was also impressed by the diversity of subjects which are to be covered, from very basic topics to field control applications. The above gives me confidence that better methodologies will soon be available to deal with fruit flies. Methodologies which will protect fruits from infestation, have little adverse effect on the environment, and reduce fly populations to comfortable densities at a distance from our fruits.

On behalf of the Greek Government, I would like to thank the Organizing Committee, and especially the Food and Agriculture Organization of the United Nations, the International Atomic Energy Agency, and the Italian Nuclear Energy and Alternative Energies Commission, who were co-supporters of the Symposium with the Greek Government. Last but not least, I would like to express my most sincere thanks to all the participants here in Crete who have come to help build a better tomorrow for agriculture and the world.

On this note, I wish you all a successful meeting and declare the Symposium open.
OPENING ADDRESS ON BEHALF OF THE ORGANIZING COMMITTEE

A. P. ECONOMOPOULOS
Organizing Committee Co-ordinator

Ladies and Gentlemen, dear Colleagues,

It gives me great pleasure to welcome you all on behalf of the Organizing Committee to the Second International Symposium on Fruit Flies. Four years after the First Symposium, which was held in Athens in November 1982, we meet again to exchange information on fruit flies. We gather once more to renew and expand friendships. The recommendation and desire expressed in Athens for the establishment of a quadrennial fruit fly symposium appears to be becoming a reality. This, in my opinion, has become possible because of the considerable size and impressive scientific vigour of the fruit fly community and its willingness for progress, among others, through the exchange of information at regular meetings. It appears now that our scientific community can sustain its own regular international meeting on basic and applied study of fruit flies.

This Second Symposium includes papers which deal extensively with 15 different species (and to a smaller degree with several more) from 4 genera. Three of the genera belong to Trypetinae (Anastrepha, Ceratitis and Rhagoletis) and one to Dacinae (Dacus). The above sub-families of Tephritidae include the major economic pests of fruit flies. As was the case in Athens, the cosmopolitan medfly, a multivoltine tropical species, dominates the Symposium once again. It could in itself occupy a whole Symposium. The olive fly is also covered in many of the papers, most probably due to the site of the Symposium which has enabled considerable Greek participation.

Ninety-six fruit fly colleagues from 21 countries and 2 international organizations, altogether presenting 75 papers, are participating. In Athens, about the same number of papers were also presented, but fewer species covered. Of the 75 papers to be presented at this Symposium about half are of a basic nature and the rest of an applied nature. The above partition reflects an important fact: both basic and applied topics are receiving strong emphasis in fruit fly research. The complex problems, created by insects competing for the same resources with man, cannot be easily solved. Species-specific methodologies based on a thorough knowledge of the organism, the population and the ecosystem, are required. The "no insect" goal seems very often impractical or destructive to the ecosystem. Thus, for novel species-specific control strategies, a thorough knowledge of insect biological and ecological characteristics is necessary. This appears to be the latest trend in fruit flies, as suggested by the above dual research emphasis.

Dear Colleagues, we are a long way from the fifth decade of this century when the miraculous results of synthetic insecticides turned insect control into a routine operation. Our optimism today stems from
the prospect of utilizing the extraordinary achievements of basic science,
extensive knowledge at the organism and population levels, and
sophisticated integration of control methodologies.

Before terminating this address, I would like to thank very much, on
behalf of the Organizing Committee, the following Organizations whose
support made our Symposium possible:

1. From the host country — the Ministry of Agriculture;
   the Ministry of Culture and Science;
   the Ministry of Energy and Technology;
   the Agricultural Bank;
   the Eleourgiki Central Olive Union;
   the Hellenic Entomological Society; and
   the Orthodox Academy of Crete.

2. The Food and Agriculture Organization of the United Nations.

3. The International Atomic Energy Agency; and

4. ENEA, Comitato Nazionale per la ricerca e per lo sviluppo
dell'Energia Nucleare e delle Energie Alternative, of Italy.

It is not the practice of the Organizing Committee to thank its
members, and although I have no intention of thanking individual persons,
there is one colleague whose contribution must be mentioned:
that of Dr. D.A. Lindquist of the IAEA, whose consistent and strong
support throughout the 14-month preparatory period played a decisive role
in making this Symposium a success.

Ladies and Gentlemen, dear Colleagues,

Our Organizing Committee welcomes you to the Second International
Symposium on Fruit Flies. We wish everybody a fruitful and pleasant stay
on the island of Crete.
Mr. Chairman, Dear Colleagues,

On behalf of the Hellenic Entomological Society, I would like to express my appreciation for the kind invitation to attend and address this symposium.

I avail myself of this opportunity to extend a cordial welcome to the participants of this symposium, which has already established for itself a world-wide reputation and recognition.

Crop damages incurred by fruit flies in our country constitute one of the most serious problems in the field of crop protection, because Greece's climatic conditions are favourable to the continuous presence of these noxious Diptera on various fruit crops.

The most important fruit flies in Greece are: the olive fruit fly (Dacus oleae) and the Mediterranean fruit fly (Ceratitis capitata). The protection of olives, oranges and other fruits from attack by these two insect pests is a problem of major economic importance for the Greek olive and fruit grower. Annual variations of the extent of damages caused by Dacus oleae and Ceratitis capitata, make the assessment of the resulting economic impact difficult, however, losses are considered to reach significant magnitudes.

It is obvious that international co-operation is necessary in promoting efforts to reach satisfactory solutions for the control of these pests.

The Hellenic Entomological Society, a relatively new scientific institution, strongly supports the spirit of international co-operation within the various disciplines of entomology.

The Hellenic Entomological Society was founded in 1979, and its members are qualified entomologists both from Greece and other foreign countries. Its primary concern is the dissemination of recently acquired knowledge on entomological research through publications, conferences, lectures, meetings, and other activities. It is also interested in the education of future entomologists and encourages young scientists who display a vivid interest in entomology.

Since the establishment of the Hellenic Entomological Society, the necessity of the publication of an Entomological Journal was evident. The first issue of the "Entomologia Hellenica", the official journal of our society, was published in 1983, two issues being published annually thereafter. The journal contains research articles and short notes in English or French, with a summary in Greek.
Articles on various disciplines of entomology and acarology may be presented by society and non-society members from foreign countries as well.

It is of special importance, in my opinion, that the symposium is being held in Greece, where agriculture constitutes a large percent of the national income.

I am positive that the present symposium will assist the participants in promoting mutual friendship, enable discussion on recent developments in fruit fly control, and explore future potential procedures for the benefit of the agricultural economies of our countries.
PLENARY SESSION
(Invited Papers)
INSECTICIDE RESISTANCE: THE TEPHRITIDAE NEXT?

G. P. GEORGHIOU
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University of California, Riverside, California 92521, U.S.A.

Abstract

Selection of colonies of Ceratitis capitata (Wied.) by insecticides in various laboratories has provided evidence for resistance potential toward organophosphate, DDT, and dieldrin insecticides. Biochemical investigations have also shown the presence of organophosphate-metabolizing esterases and of acetylcholinesterase with lower sensitivity toward inhibitors. Yet, no documented cases of resistance to insecticides in natural populations of this or other species of fruit flies (Tephritidae) have so far been reported. While encouraging, the present situation requires continuous monitoring, in view of the long standing, heavy exposure of fruit flies to insecticides.

Introduction

There is no doubt that the application of insecticide against an insect population is a catastrophic event: the concentration of toxicant applied is usually intended to provide near 100% mortality among the insects that are reached by the spray. Many populations have responded to this challenge by evolving resistance, and by so doing have provided clear evidence of the evolutionary process.

However, resistance does not evolve at the same rate in all arthropod species or populations that come under selection pressure. It may develop rapidly in one species, more slowly in another, and not at all in a third. For example, despite enormous selection pressure during many years of intensive DDT treatment the European corn borer [(Ostrinia nubilalis (Hubner)] in the U.S. has shown no evidence of resistance. Nor has the codling moth [Cydia pomonella (L.)] toward azinphosmethyl, the mainstay of chemical control on apple for over 30 years, although the species had previously developed high resistance to DDT in many countries (Croft, 1982). Some populations obviously do not have the capacity to come up with resistance alleles to certain chemicals, despite what would seem to be an obvious advantage for doing so. In contrast to these cases of recalcitrance for resistance, house flies (Musca domestica L.) in many areas have developed resistance to nearly every insecticide within two to three years under selection pressure. Species of mosquitoes, aphids, mites, leafhoppers, and many Lepidoptera and Coleoptera, have likewise responded rapidly to selection pressure (see Georgiou, 1981).

Fortunately for those whose livelihood is challenged by fruit flies, this group of insects has not so far shown clear evidence of resistance under practical field conditions. The question may, therefore, be asked whether this is due to inadequate selection pressure, or to lack of genetic potential for resistance to chemicals. Or, could it be that a sufficiently strong selection has already been exerted on some populations,
as for instance against the olive fly in Greece, or the medfly in Guatemala, such that the resistance gene frequency in these is now approaching levels of detectability? It is known from simulations as well as from field experience, that resistance remains undetectable while the frequency of resistance alleles increases progressively from rare (perhaps 0.00001-0.0001) to not so rare (U. UU 0.1). Beyond that point, continued strong selection could have dramatic effects, as control failures become rapidly evident. In view of the extensive use of insecticides in fruit fly control for many years, the consideration of this question is timely and urgent.

Status of resistance in agricultural insects

A data-base on cases of resistance that we are maintaining at the University of California, Riverside, indicates that by the end of 1984 resistance to one or more insecticides had been found in at least 447 species of insects and mites (Georghiou 1986). Excluding 171 species that are of medical or veterinary importance and 12 species that are beneficial, the agriculturally relevant cases are distributed in the following Orders:

<table>
<thead>
<tr>
<th>Order</th>
<th>Number of Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidoptera</td>
<td>67</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>64</td>
</tr>
<tr>
<td>Homoptera</td>
<td>46</td>
</tr>
<tr>
<td>Acarina</td>
<td>36</td>
</tr>
<tr>
<td>Diptera</td>
<td>21</td>
</tr>
<tr>
<td>Heteroptera</td>
<td>16</td>
</tr>
<tr>
<td>Other</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>264</td>
</tr>
</tbody>
</table>

The 21 species of Diptera represent 8 families of phytophagous pests, including root maggots, leaf miners, fruit feeders and other. Thus,

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agromyzidae</td>
<td>6</td>
</tr>
<tr>
<td>Anthomyiidae</td>
<td>5</td>
</tr>
<tr>
<td>Cecidomyiidae</td>
<td>2</td>
</tr>
<tr>
<td>Drosophilidae</td>
<td>2</td>
</tr>
<tr>
<td>Otitidae</td>
<td>2</td>
</tr>
<tr>
<td>Phoridae</td>
<td>1</td>
</tr>
<tr>
<td>Psilidae</td>
<td>1</td>
</tr>
<tr>
<td>Syrphidae</td>
<td>2</td>
</tr>
</tbody>
</table>

It is apparent that agriculturally important diptera have demonstrated ample capacity for resistance as have pests in other taxonomic Orders.

*While this paper was being completed, it was discovered that this subject was discussed also by Professor R. J. Wood at a NATO Systems Analysis Workshop on Pest Control in August, 1985, and published recently (Wood, 1986). I have made an effort to avoid excessive overlapping by extending the scope and coverage of my presentation, so that our two papers would supplement each other.
Mechanisms of resistance in insects

Before proceeding to a discussion of resistance in fruit flies, it would be useful to highlight the principle mechanisms of resistance known to-date (Table I). Of special significance to the subject at hand are metabolic mechanisms mediated by mixed function oxidases (MFO) and esterases, and the mechanisms that involve reduced sensitivity at the site of action of the insecticide.

TABLE I. Principal mechanisms of insecticide resistance in insects

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Insecticides affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Increased detoxication:</td>
<td>DDT</td>
</tr>
<tr>
<td>dehydrochlorinase</td>
<td>carbamates</td>
</tr>
<tr>
<td>microsomal oxidases</td>
<td>pyrethroids</td>
</tr>
<tr>
<td>glutathione transferases</td>
<td>IGRs (diflubenzuron)</td>
</tr>
<tr>
<td>esterases (broad spectrum)</td>
<td>organophosphates (O-ethyl)</td>
</tr>
<tr>
<td>carboxylesterase</td>
<td>organophosphates (O-methyl)</td>
</tr>
<tr>
<td></td>
<td>organophosphates</td>
</tr>
<tr>
<td>2. Decreased sensitivity of target site:</td>
<td>malathion</td>
</tr>
<tr>
<td>decreased sensitivity of acetylcholinesterase (AChE)</td>
<td>carbamates, organophosphates</td>
</tr>
<tr>
<td>decreased sensitivity of nerve (kdr mechanism)</td>
<td>pyrethroids, DDT</td>
</tr>
<tr>
<td>cyclodienes resistance genes</td>
<td>dieldrin, BHC</td>
</tr>
<tr>
<td>3. Decreased cuticular penetration</td>
<td>most insecticides</td>
</tr>
<tr>
<td>4. Behavioral</td>
<td>many</td>
</tr>
<tr>
<td>Avoidance of treated microhabitats</td>
<td></td>
</tr>
</tbody>
</table>

Mixed function oxidases are relatively non-specific, they are associated with the microsomal fraction of cells, and involve multiple forms of cytochrome P-450 (Wilkinson, 1982). MFO's are known to metabolize broad classes of chemicals including carbamates, organophosphates, pyrethroids and organochlorines; they are located in strategic tissues such as the midgut/fatbody; and they are synchronized to a time of need, showing highest activity during the larval feeding stage and lowest during the adult stage.

Carboxylesterases, broad spectrum esterases, phosphotriester hydrolases, and glutathione transferases are widely known as mechanisms of resistance, especially toward organophosphates. Unlike the relatively broad spectrum of resistance that is conferred by esterases, carboxylesterases confer resistance only to malathion, a fact that is especially important in view of the wide use of malathion against fruit flies. It has been observed that non-specific esterase activity against α- or β-naphthyl acetate often parallels closely the degree of resistance, and in some cases these substrates have been employed in diagnostic tests for the detection of organophosphate resistance in individual insects (Pasteur &
Georghiou 1981, Rees et al. 1985). Interest in the role of esterases in resistance has been strongly enhanced by the recent demonstration that gene amplification, previously known only in mammalian cells in culture (Schimke, 1980, 1982), occurs also in mosquitoes (Mouchès et al. 1986) and possibly other insects. The presence of multiple copies of specific genes (more than 250 copies in the case of the mosquito Culex quinquefasciatus Say) results not only in the production of large quantities of the resistance enzyme, and hence substantially higher resistance, but may also provide greater opportunities for mutation of alleles coding enzymes that are specific toward newly introduced chemicals.

In addition to increased detoxification, resistance may be produced through the selection of alleles that confer insensitivity at the site of action. Three categories of such mechanism are known, i.e. reduced sensitivity of acetylcholinesterase (AChE) (toward organophosphates and carbamates), reduced sensitivity of nerve tissue (toward pyrethroids and DDT), and reduced sensitivity of the target of dieldrin and other cyclo-diene insecticides. These mechanisms may confer extremely high levels of resistance, frequently amounting to several hundred fold, and they impart a broad spectrum of cross-resistance within the chemical family they affect.

Absence of resistance in natural populations of fruit flies

Although there are no documented cases of resistance to insecticides in natural populations of fruit flies, "incipient" resistance has been suspected in a small number of cases.

The walnut husk fly in California (Rhagoletis completa Cresson), was suspected by Quale (1943) to be developing resistance to the inorganic insecticide cryolite (sodium fluoaluminate). Quayle wrote: "...cryolite for the control of R. completa... was formerly satisfactory (Boyce, 1934) but in recent years the results have been much less so". In the absence of confirmatory evidence, and the subsequent abandonment of cryolite for DDT, this case remains of historic interest only. Two other reports concern the medfly: In 1968, Maher Ali advised FAO that the medfly in Egypt was showing resistance to lindane (FAO, 1969). There was no confirmation or follow-up of this case. Finally, the most recent report is by Koren et al (1984) in Israel, who wrote: "In spite of the highly efficient control system, there are sporadic increases of the (med) fly population which may very likely be interpreted as the result of increased tolerance to malathion".

Resistance potential of fruit flies as revealed by laboratory selection

In view of the absence of documented field cases of resistance, the results of studies involving selection of fruit flies in the laboratory are of special interest (Table II). While these results do not indicate the full potential of field populations for resistance, they serve as indicators of the resistance mechanisms that are present in the colonized sample. Unfortunately, some of the studies cited below are limited by the fact that the selected populations had been under laboratory rearing for a number of years, during which their genetic heterogeneity must have been reduced.
### TABLE II. Cases of selection for insecticide resistance in Tephritidae

<table>
<thead>
<tr>
<th>Species</th>
<th>Selecting insecticide</th>
<th>Stage treated</th>
<th>Generations selected</th>
<th>Resistance ratio at LC₅₀</th>
<th>LC₉₅</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ceratitis capitata</em></td>
<td>DDT</td>
<td>Adult</td>
<td>55+84</td>
<td>15.17</td>
<td>-230</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>dimethoate</td>
<td>Adult</td>
<td></td>
<td>1.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dieldrin</td>
<td>Larva</td>
<td>21</td>
<td>45</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>Larva</td>
<td>26</td>
<td>24</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>Larva (sib)</td>
<td>49 to 67</td>
<td>2.8</td>
<td>-4.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>malathion</td>
<td>Adult</td>
<td>18</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dacus cucurbitae</em></td>
<td>BHC</td>
<td>Adult</td>
<td>11</td>
<td>16.36</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>carbaryl</td>
<td>Adult</td>
<td>13</td>
<td>1.51</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

1. Orphanidis et al., 1980.

In Greece, Orphanidis et al. (1980) subjected adult medflies to intermittent selection pressure by DDT and dimethoate for 55 generations. Following outcrossing of the population to newly-colonized adults, selection was resumed for 84 additional generations. The results (Fig. 1) evaluated by bioassay of adults in petri dishes, indicate that resistance was developed only toward DDT in the second series of selection. Although the

![Figure 1](image_url)

**FIG. 1.** Changes in susceptibility to DDT and dimethoate in adult *C. capitata* selected by these insecticides in the laboratory. (Adapted from Orphanidis et al. 1980)
degree of resistance calculated at the LC$_{50}$ (15.17-fold) was rather modest, there was a significant decline in the slope of the regression line, so that at the LC$_{95}$ the resistance amounted to 230-fold the normal (Fig. 2). This work thus provides evidence of the ability of the medfly to develop resistance to DDT. No resistance to dimethoate was found, the LC$_{50}$ having increased only 1.8-fold.

Some evidence for organophosphate-resistance potential has been obtained by Koren et al. (1984). A medfly colony that had been established in Israel some 10 years earlier, was subjected to selection in the adult stage by malathion in petri dishes. After 18 successive generations of selection, some resistance appeared, but only in females, and amounted to 2.8-fold at the LC$_{50}$ (Fig. 3). Extrapolation of the data shows that
resistance at the LC\textsubscript{95} was 4.3-fold. Although the quantitative significance of this work is obscured by the long age of the colony, the study provided qualitative information on potential mechanisms of resistance in this insect, as will be described later.

The possibility of resistance by the medfly to the cyclodiene group of insecticides was investigated by Wood and Busch-Petersen (1982) and Busch-Petersen and Wood (1983) in a feasibility study on the use of dieldrin in genetic sexing. Successive generations were selected by dipping larvae in dieldrin suspensions in water. Two lines selected for 21 or 26 generations showed increases in EC\textsubscript{50} values of 45-fold and 24-fold, respectively. Further sib selection of progenies from single pairs increased the EC\textsubscript{50} value to a resistance level of 49 to 67-fold (Fig. 4).

![Image](417x100 to 420x103)

**FIG. 4.** Inheritance of dieldrin resistance in larvae of *Ceratitis capitata*. (after Busch-Petersen & Wood, 1983)

The only other fruit fly species examined for potential resistance is *Dacus cucurbitae* Coq. Selection of a laboratory colony by topical application of lindane or carbaryl to adults for 11 and 13 generations, respectively, produced 16.36-fold resistance to lindane but none to carbaryl (Khan and Khan, 1976).

These studies thus provide evidence for the presence in members of the Trypetidae of resistance potential toward three major classes of insecticides, e.g. DDT, dieldrin/BHC and organophosphate. It is of interest to examine the mechanisms of resistance in fruit flies as revealed by this limited number of studies.

The known mechanisms of insecticide resistance in fruit flies

That detoxification by esterases is of potential significance in fruit flies is evident from the work of Koren et al (1984) on their malathion-selected strain. Carboxylesterase determinations revealed a somewhat higher activity (1.29-fold) in the selected than in the parental
strain (Table III). It would be desirable to investigate the carboxylesterase level of flies collected soon after application of malathion. These studies showed no evidence of involvement of glutathione transferases in the malathion resistance.

TABLE III. Biochemical characteristics of "resistant" (R) and "susceptible" (S) Ceratitis capitata adult females

<table>
<thead>
<tr>
<th>Enzyme measured</th>
<th>Substrate</th>
<th>R</th>
<th>S</th>
<th>R/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxylesterase+/−</td>
<td>p-nitrophenyl acetate</td>
<td>4.28</td>
<td>3.33</td>
<td>1.29</td>
</tr>
<tr>
<td>Glutathione S-transferase+/−</td>
<td>CDNB+/−</td>
<td>7.31</td>
<td>7.21</td>
<td>1.01</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>acetylthiocholine iodide</td>
<td>0.83+/−</td>
<td>1.80+/−</td>
<td>0.46</td>
</tr>
</tbody>
</table>

+/−In nM p-nitrophenol/min/25 μl of 5% whole fly homogenate.
+/−In nM CDNB (1-chloro-2,4-dinitrobenzene) conjugated/min/25 μl of 5% whole fly homogenate.
+/−X10−6; Kᵢ for AChE inhibition by malaoxon.
(Data from Koren et al., 1984)

Equally significant are published data on the presence of medflies displaying lower sensitivity of AChE, the target enzyme of organophosphates and carbamates. Early work by Zahavi and Tahori (1970) revealed that the AChE of medflies derived from a collection from Mikve, Israel, was 20% less sensitive to inhibition by phosphamidon than was found in other field or laboratory strains. The importance of this observation was ignored since phosphamidon is a relatively poor inhibitor of medfly acetylcholinesterase, thus differences between strains would tend to be magnified. However, clear differences were also observed with malaoxon between the malathion-selected and parental strains studied by Koren et al (1984). The AChE of the "resistant" strain was only 0.46 as sensitive as that of the susceptible strain.

Pertinent research on AChE was also conducted on Dacus oleae (Gmel). The main amount of esterase in this insect was found to be controlled by two autosomal, independently segregating loci, A and B. Locus A was shown to control the synthesis of an AChE and locus B of a pseudocholinesterase or lipase (Zouros et al. 1968). Laboratory experiments revealed that dimethoate preferentially kills flies homozygous or heterozygous for the silent allele of locus A (Tsakas and Krimbas, 1970). A subsequent survey of several field populations indicated that those exposed to organophosphates had a lower frequency of silent variants and a higher frequency of duplications than the non-treated ones (Tsakas, 1977). The significance of these observations to potential field resistance must await comparative kinetic studies on the enzyme.

The mechanism of resistance to dieldrin investigated by Wood and Busch-Petersen (1982) and Busch-Petersen and Wood (1983) presents unusual
features. In all cases of cyclodiene resistance studied to-date, resistance is monofactorial, and of the "site-insensitivity" type. Hence it extends at various degrees to all members of the cyclodiene group. There is no cross-resistance to other classes of insecticides. In contrast, Busch-Petersen and Wood (1983) observed that the 67.6-fold resistance to dieldrin was accompanied by only limited resistance to aldrin and endrin (4.1 and 4.4-fold, respectively), and by high resistance to malathion (97.7-fold) and permethrin (19.6-fold). It was suggested (Wood, 1983) that this case may be similar to that of gene RI of Drosophila melanogaster which also involves multiple resistance to compounds in various chemical groups. Whether this is the result of microsomal mixed function oxidases (Wood, 1983) must be demonstrated by appropriate biochemical tests.

Dynamics of resistance in fruit flies

The proliferation of cases of resistance in species that represent a wide range of genetic, biological and ethological characteristics makes possible the examination of the dynamics of resistance in greater perspective. The advent of computer technology and modelling has been extremely valuable in such studies. There is concurrence of opinion that the evolution of resistance is influenced by a variety of genetic, biological and operational factors, which in concert determine the degree of selection pressure that is exerted in a given ecological situation (Georghiou and Taylor, 1976, 1977a,b) (Table IV). If it were possible to assign individual ratings to each factor, we could conceivably arrive at a quantitative expression of the risk for resistance in a given population for a certain control program.

<table>
<thead>
<tr>
<th>TABLE IV. Known or suggested factors influencing the selection of resistance to insecticides in field populations</th>
</tr>
</thead>
</table>

A. Genetic

a. Frequency of R alleles
b. Number of R alleles
c. Dominance of R alleles
d. Penetrance, expressively, interactions of R alleles
e. Past selection by other chemicals
f. Extent of integration of R genome with fitness factors

B. Biological/Ecological

1. Biotic

   a. Generation turnover
   b. Offspring per generation
   c. Monogamy/polygamy, parthenogenesis

2. Behavioral/Ecological

   a. Isolation, mobility, migration
   b. Monophagy/polyphagy
   c. Fortuitous survival, refugia
TABLE IV. (con't)

C. Operational

1. The Chemical
   a. Chemical nature of pesticide
   b. Relationship to earlier-used chemicals
   c. Persistence of residues, formulation

2. The application
   a. Application threshold
   b. Selection threshold
   c. Life stage(s) selected
   d. Mode of application
   e. Space-limited selection
   f. Alternating selection

(Adapted from Georghiou and Taylor, 1976)

The record of fruit fly control to-date reveals a low potential for resistance, if one considers the frequency, duration, and intensity of the chemical control interventions against a number of species, particularly C. capitata, D. oleae, S. dorsalis, and U. cucurbitae.

The medfly control program in Israel, now in effect for some 29 years, consists of ULV applications of malathion bait with hydrolyzed proteins, at the rate of 227.5 gr. malathion a.i. per hectare, and 4-6 treatments per year (Wood, 1986). Rossler, quoted by Wood (1986) considers the degree of selection pressure to exceed 99%. Since 4-6 generations of the fly are completed per year, such selection must have involved 116-174 generations.

The selection pressure exerted against the olive fly in Greece has also been considerable. Control since the mid 1960's has been based on the use of bait sprays consisting of 2% protein hydrolysate and 0.3% dimethoate or fenthion. The treatments are made by ground equipment or by air, their frequency varying from 1 or 2 per year in the drier areas (E. Crete and islands of eastern Aegean) to as many as 6-8 in the more wet regions (Volos, Lamia, Pelion). Until recently, control in NW Peloponesos, Corfu and Zante involved total coverage sprays of dimethoate or fenthion.

In addition to these regular programs of control, fruit fly populations have been subjected to massive, highly organized eradication programs, in which insecticides are a major component. Examples are the medfly barrier zone treatments on the Mexico-Guatemala border (Hendrichs et al. 1983), and the medfly eradication program in California during 1980-82. The latter involved the aerial spraying of about 900,000 acres in 44 cities and 8 counties with malathion bait. Counting multiple applications, more than 10 million acres were sprayed with 190,000 gallons of 91% malathion (Scribner, 1983).

The list of genetic, biological/ecological, and operational factors that influence the evolution of resistance in field populations may serve as a guide in assaying the dynamics of resistance in fruit flies (see Table III). Despite many similarities among the various species of fruit flies, there are also differences, especially in the biological/ecological factors, which make generalizations unattainable. Additionally, as pointed out by Carey (1982), surprisingly little work has been done on the
demography of insects such as the medfly, and there are far too many "unit" questions (sensu Pielou, 1981) that must first be answered. At present it is only possible to explore the potential impact of each of the factors listed on the evolution of resistance.

Among the genetic factors, many obviously cannot be evaluated until after resistance has developed. Wood (1986), on the basis of the instability of the dieldrin resistance in the medfly strain studied, suggested poor penetrance or expressivity of the resistance gene, or lack of fitness of the resistant phenotype in the absence of the insecticide. These problems, however, are more pronounced in long-established laboratory colonies than in field populations.

The biological/ecological factors may affect dramatically the responsiveness to the selection. Most obviously, the larger the number of generations per year, the faster the evolution of resistance. Fruit flies with a large number of generations in warm climates should present a greater risk for developing resistance. High fecundity and fertility would be another positive factor for resistance, since populations with a higher reproductive potential are able to withstand a higher substitutional load. The medfly with a net fertility of some 925 eggs per female (Carey, 1984) would be an outstanding candidate. Resistance may also be favored by polygamy, as is found in many fruit flies including the medfly, since polygamy offers increased opportunities for a resistant male to distribute his genes in the population.

Among the factors that may have a delaying effect on the evolution of resistance in fruit flies are high mobility and the tendency for spatial dispersal, both characters enabling the population to move to other or alternative hosts, thus escaping continuous exposure to the chemical.

Polyphagous species tend to develop resistance more slowly than monophagous ones. Two factors may contribute to this: firstly, a smaller proportion of a polyphagous species is likely to be exposed to the insecticide, hence the selection is less intense; secondly, because some of the insects would be in untreated refugia, they could constitute a reservoir from which untreated, susceptible migrants could come. All other conditions being equal, it would appear that the monophagous olive fly would be more severely selected than the polyphagous medfly. It is interesting that on strictly biochemical criteria, polyphagy may enhance the potential of a species to develop resistance. Krieger et al. (1971) have provided evidence that in lepidopteran larvae the insecticide metabolizing microsomal oxidases show higher activity in polyphagous than in monophagous species. On this basis the medfly might be expected to display greater biochemical polymorphism than the olive fly.

As indicated above, a factor that may be expected to delay the evolution of resistance is refugia. In normal pest control, not all individuals are reached by chemical treatment. Depending on the biological and behavioral characteristics of a species, a proportion of the population may be present in refugia at the time of the treatment, thus escaping selection (Georgiou and Taylor, 1976). Refugia may consist of fruit tissues, alternative hosts, non-treated hosts, or other shelters. Whatever the reason, such refugia may be very important in providing a source of susceptible immigrants, thus retarding the evolution of resistance. It is obvious that in the case of fruit flies, the role of refugia may be drastically reduced by the type of treatment being applied.
Thus, use of a systemic larvicide as dimethoate and fenthion must severely reduce the percentage of larvae escaping selection. Likewise, baits and lures, by attracting flies to the chemical, decrease their chances of fortuitously avoiding exposure to the chemical.

Conclusions

It is apparent that populations of fruit flies have shown limited potential for development of resistance to the chemical control programs mounted against them. With the exception of the observation of sporadic increases in medfly populations in Israel despite a strict control program involving ULV application of malathion and hydrolyzed proteins, no documented evidence of resistance in fruit flies is available.

While encouraging, this situation must not lead to complacency. Ample evidence is available from selection of medflies in the laboratory that the genetic capability for biochemical resistance is available. Esterases that metabolize organophosphate insecticides, and AChE that displays lower sensitivity to inhibition by these compounds, both potentially important mechanisms of resistance, have been demonstrated in medflies. Other sources for biochemical resistance may also exist, judging from the successful development of resistance to DDT and dieldrin.

The development of resistance based on the selection of behavioral traits that reduce fruit fly contact with the chemical must also be considered. Since the prevailing attractant in use is also a source of nourishment, the likelihood of resistance to it may be remote. However, the existence of biotypes that do not respond to certain attractant chemicals or pheromones cannot be ruled out.

Despite such biological characteristics of medflies as high fecundity, polyphagy and polygamy that tend to favor the evolution of resistance under persistent chemical stress, resistance has not yet evolved. These qualities are apparently countered by others that delay resistance, such as high mobility, abundant refugia, untreated reservoirs, and the exposure to substantial doses of insecticide in the bait. Only time will show whether these opposing forces will continue to hold resistance at bay.

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MODERN METHODS OF INSECT CONTROL

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Abstract

The major advances during this century in insect control have been host-plant resistance, the development of synthetic organic insecticides, the sterile insect technique, and integrated pest management as a systems approach to insect control.

There are exciting research developments taking place which will lead to improved methods of insect control in the future; it is anticipated that some of these methods will be more environmentally acceptable than current methods. Totally new methods of insect control are not likely in the immediate future. Host-plant resistance, the sterile insect technique, progress in developing further genetic methods of insect control, more effective use of biocontrol agents, etc. will be improved. Genetic engineering will play a major role in many of these improvements.

There probably will be greater changes in the strategy of using insect control technologies than in the insect control technologies themselves. Specifically, the development of area-wide insect control for certain key insect pests will be implemented. One of the primary reasons for this will be the availability of technologies which are applicable primarily, or only, on an area-wide basis. Furthermore, preventative insect control using biological methods, within area-wide control, will likely be looked upon with favour and utilized to a far greater extent than at present. Area-wide control requires somewhat different types of knowledge and thus research scientists must design their research projects to obtain the necessary data.

Fruit fly control will be no exception to the above and area-wide control and eradication can be expected to play an increasing role in managing fruit fly problems.

Introduction

During this century the major advances in insect control have included host-plant resistance, the development of powerful synthetic organic insecticides, the development of genetic methods, notably the sterile insect technique (SIT), and the development of integrated pest management (IPM) as a systems approach to insect control. With the exception of host-plant resistance, all of the above have caused controversy, yet all have been successfully implemented and have benefited
agricultural production. The use of synthetic organic insecticides has been the major method of insect control, and will continue to be so.

Apart from specific insect control technologies, the strategic use of insect control technologies is receiving increased attention. Specifically, the control, or sometimes eradication, of an insect species over a large area which includes land farmed by many individuals, is receiving increased attention.

Research is being conducted on a number of new approaches to insect control or eradication. Included are biological control, the use of insect genetics, and attractants.

Current Methods of Fruit Fly Control/Eradication

The use of insecticide bait sprays is the predominant method of controlling fruit flies. This method combines a feeding attractant, usually a protein hydrolysate, with an insecticide such as malathion. This material is sprayed in relatively large droplets onto the crop to be protected. The fruit flies are attracted to, and feed on, the protein hydrolysate bait, and at the same time ingest the insecticide which kills them. The major alternative to bait sprays is a cover spray without the protein hydrolysate bait. The use of physical attractants, such as shape and colour, have also proven effective in controlling certain species of fruit flies. However, this method has not been widely accepted, primarily because it has not been successfully commercialized and partly because it is labour intensive. Parasites, predators and insect pathogens have not proven effective in managing fruit fly infestations.

Where eradication of a fruit fly species is an objective, three technologies have been successfully used: bait sprays, the sterile insect technique (SIT), and male annihilation.

Research and Development

All of the control and eradication technologies mentioned above are receiving attention from research scientists. In addition, there is considerable research being conducted on the behaviour of fruit flies, frequently in relation to various control technologies. Eradication campaigns require research support to solve immediate problems; this crash research has frequently led to more in-depth research, particularly in mass-rearing, behaviour and ecology.

Recent research at the IAEA laboratory has shown that certain strains of *Bacillus thuringiensis* are active against adult medflies. This programme is continuing and probably will be expanded by other scientists to include other species of fruit flies. *Bacillus thuringiensis* has rarely, if ever, been evaluated against adult insects. However, with the powerful attractants available for fruit flies, including feeding
attractants, synthetic chemicals which attract only one sex (usually the male), and physical attractants, there is an excellent opportunity to deliver an insect pathogen to adult fruit flies.

In general, attractants have been discovered and, at least, partially developed for a number of species of day-flying Diptera. This phenomena has not been sufficiently studied. It would appear that the combination of attractants with insect pathogens offers a real opportunity for fruit fly control. Unfortunately, virtually no research along these lines has been conducted to take advantage of the powerful attractants available for fruit flies. A search for insect pathogens effective against adult fruit flies should pay enormous dividends.

Parasites have not proven very effective in controlling fruit flies. However, it is well known that there are parasites which are somewhat effective in reducing the fruit fly populations after the fruit fly populations reach very high levels. In all probability it will require the inundative release of parasites to achieve satisfactory fruit fly control. Experiments designed to measure accurately the efficacy of inundative releases of parasites have rarely, if ever, been conducted. These experiments must be done on a fairly large area and the numbers of released parasites must be sufficiently high to have a reasonably rapid impact on the fruit fly population. The relatively high cost of this type of experiment has undoubtedly deterred this type of research. However, with the increasing concern about the environmental effects of insecticides, the time is rapidly approaching when inundative releases of parasites should be seriously investigated.

Strategy

Application of fruit fly control technology by the individual producer is the predominant way in which fruit flies are controlled. Bait sprays or cover sprays are most common. When the producer has a large plantation, bait sprays are very effective in protecting the crop. However, when the producer has very small plantations it is more difficult to control fruit flies with bait sprays, particularly when neighbouring producers do not apply effective control methods. In addition, many individual fruit trees in yards and gardens are not treated for fruit fly control and thus become sources of a large number of pests which will infest not only other yard and garden fruit trees but commercial plantings in the same area.

The problems of controlling fruit flies by individual producers can be overcome by the strategy of area-wide control. The application of this strategy involves a relatively large area, and includes all host-plants within the area, commercial, ornamental, and individual trees in gardens. Area-wide control of fruit flies can be achieved with bait sprays. The strategy can be particularly effective when bait sprays are applied to the earliest fruiting host in the area, i.e. before the fruit fly population becomes very high. By selective bait sprays, the fruit fly population in the entire area can be greatly reduced with minimal insecticide treatment and, if effectively done, very little treatment will be required when the major crop starts to mature. Area-wide control strategy requires good
knowledge of the ecology of the fruit fly, the fruit fly hosts, their fruiting sequence, movements of the fruit fly population, and particularly wild alternate hosts.

There are relatively few examples of fruit fly control using the area-wide strategy. The olive fruit fly in Greece and the onion fly in the Netherlands are the best known. The latter is paid for by the onion producers in the Netherlands. The increase in costs of fruit fly control should lead producers to the conclusion that an effective area-wide control strategy offers the opportunity to reduce costs and reduce the losses caused by fruit flies.

Fruit fly eradication campaigns are of necessity conducted on an area-wide basis. Bait sprays, the SIT, or most frequently a combination of the two, have been used successfully for fruit fly eradication. Under some conditions, eradication is the only realistic approach to solving a fruit fly problem. The Mediterranean fruit fly has been eradicated a number of times from different areas in the U.S. The medfly has also been recently eradicated from Mexico and the programme is continuing into Guatemala. The medfly does not exist in Mexico and the mainland U.S.A. It does occur in Hawaii. The eradication campaigns undertaken were to prevent the establishment of this pest. It was clearly demonstrated that the economic and environmental cost of allowing the medfly to become established in Mexico and the U.S. was totally unacceptable. Thus eradication campaigns were initiated.

Eradication of an insect species from a given area is a controversial concept. However, the eradication of newly introduced pests is less controversial. The introduction of the cassava mealy bug into Africa has caused serious losses of cassava production. Likewise, the introduction of the boll weevil into Brazil has cost staggering sums of money in cotton production and is causing serious social problems among small cotton producers. In the case of the cassava mealy bug, an active area-wide biological control programme has been successfully initiated. This will eventually reduce the losses caused by the cassava mealy bug to levels which can be tolerated. Although the technology was available, a decision was made not to attempt boll weevil eradication in Brazil when it was first discovered. Thus, not only Brazil, but adjacent cotton producing countries will suffer enormous losses because of this introduced pest. Eradication of an insect pest from geographically isolated or partially isolated areas, particularly when it can be achieved primarily by biological technologies, must be considered as an option. Entomologists have a responsibility to develop technologies which can be used for eradication.

Quarantine and Quarantine Treatments

Fruit flies attack a large variety of fruits which, in many countries, constitute high-priced commodities. Control (as compared to eradication) is satisfactory for consumption of the fruit within the country and to a certain extent when the fruit is processed into juice, preserves, or canned fruit. However, when export of fresh fruit is the objective of production, quarantine and quarantine treatments become an
overriding factor in insect control. The loss of ethylenedibromide (EDB) as a fumigation treatment is having a devastating effect on countries which export fresh fruit and vegetables. EDB treatments have been the primary quarantine method used for killing fruit flies which might become introduced into the importing countries. The loss of EDB, coupled with the increasing demand for fresh fruit, is resulting in a number of changes in approaches to fruit fly control. Thus, fruit fly eradication (rather than control) is now considered and practised where eradication appears feasible, and where the technology has been developed. However, in most situations this is not possible at the present time.

While alternative methods of quarantine treatments, such as hot water dips, lengthy cold storage, irradiation treatments, etc. are all the subject of research, they have not yet been widely implemented. The changes which are being considered in quarantine and quarantine treatment, at least for the U.S.A., include a very high degree of fruit fly control, approaching eradication, in the fields from which the fruit will be exported. This is combined with on-site inspection by representatives of the importing countries so that statistically there is little or no chance that the pest species will be imported into the country. If this approach to quarantine continues, then area-wide control will certainly become of major importance, particularly when the crop is being produced in small fields.

Conclusions

New control technologies undoubtedly will be developed for managing insect populations. However, the greatest change likely to take place involves a shifting emphasis from field-by-field control to an area-wide control strategy. The SIT, the use of inundative releases of parasites; and the use of insect pathogens, all require an area-wide approach because they act slowly on the population. Additional emphasis will be placed on eradication where it is technically and economically feasible.

Research scientists who are responsible for new and improved methods of fruit fly control should seriously consider whether the technologies they are developing are applicable on a field-by-field basis or amenable to area-wide strategy. Some control technologies are applicable to both strategies, however, most of the biological, genetic, and physical methods of fruit fly control under investigation are effective only when applied on an area-wide basis.
POSSIBILITIES FOR GENETIC ENGINEERING IN INSECTS OF ECONOMIC INTEREST

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ABSTRACT

The use of genetic engineering techniques in applied entomology is fast approaching: both for control or eradication of harmful insects, and for protection or improvement of beneficial species. Some areas of possible use are discussed, and specific strategies are proposed. This field will be greatly aided by the rapid advances in Drosophila molecular genetics.

INTRODUCTION

In barely 15 years, recombinant DNA (rDNA) technology has become a most powerful tool for the life sciences, and has profoundly affected studies of basic biological problems in a variety of fields. This technology, also known as gene splicing or genetic engineering, has revolutionized the fields of genetics, molecular biology, developmental biology and cell biology, but also more distantly related fields such as neurobiology and evolutionary biology. For example, although that would have seemed science fiction only a few years ago, we now understand in considerable detail the structure of genes in higher organisms and the mechanisms that regulate their expression, thanks to the structural analysis of cloned genes from a variety of organisms, coupled with the ability to modify these DNA sequences in vitro and to subsequently reintroduce them into living cells, or even the germ line of related organisms.

These advances, in turn, have been immediately utilized in applied biology. Already several proteins (ranging from enzymes to therapeutically used hormones) are commercially produced via cloning. Moreover important diagnostic reagents and vaccines for human and animal use are at various stages of development, using rDNA technology. The field of Medical genetics has advanced rapidly through the use of these methods. Finally, the techniques have also yielded many bacterial strains of commercial interest, and have been incorporated into active commercial programs for plant improvement and plant protection in agriculture. The wide applicability of genetic engineering raises the question of whether this technology might not also be used in the field of applied entomology, namely for the biological control or eradication of
harmful insects and for the improvement of valuable species, including their protection from insecticides, parasites or competitors. It is our belief that such applications will be forthcoming soon, and that their rapid development will be based on the impressive recent advances in the molecular genetics of \textit{Drosophila melanogaster}.

In this paper we will discuss the current status of \textit{Drosophila} molecular genetics in respect to potential use in applied entomology, and propose three ways in which know-how from \textit{Drosophila} to insects of economic importance might be accomplished.

**THE STATUS OF DROSOPHILA MOLECULAR GENETICS: HOW TO CLONE ANY FRUIT FLY GENE**

The purpose of this section is not to review all of the molecular genetics of \textit{Drosophila melanogaster}, but to identify key techniques that could be of use in applied entomology. In particular we wish to briefly describe cloning strategies which now make it possible to isolate any fly gene of interest and also the gene transfer (transformation) technique which is essential if gene isolation is to lead to genetic manipulation for insect populations.

There are three reasons that make \textit{Drosophila melanogaster} an ideal system for the study of molecular genetics. The first one is historical, namely the more than 75 years of genetic analysis that started with the first description of the \textit{white} locus of \textit{Drosophila} by Morgan (1910). Since then, hundreds of genetic loci have been identified, ranging from relatively simple biochemical loci like \textit{Adh} to biologically intriguing and highly complex homeotic genes. This steady accumulation of knowledge has made \textit{Drosophila melanogaster} the best genetically understood higher organism. The other two features have to do with the biology of this insect: First, the relatively small genome size (170 000 000 base pairs, approximately one twentieth of a mammalian genome (Rasch et al., 1971)) greatly facilitated the recovery of specific DNA sequences. The second important feature is the existence of polytene chromosomes. These have been mapped cytotogenetically in great detail (Lefevre, 1976), and they provide a means to localize a gene on the chromosome, both by classical cytological analysis of rearrangement mutations, and by the technique of \textit{in situ} hybridization (Pardue and Gall, 1975). This technique enables the localization of any isolated nucleic acid sequence to the corresponding DNA segment on the polytene chromosomes, providing a powerful tool for linking genetic and molecular biological analysis.

Because of its uniquely favorable features, \textit{Drosophila} permits cloning of virtually any gene, by methods which complement standard procedures, such as those based on differential expression in various tissues or stages (mRNA enrichment), sequence information, or availability of antibodies.
directed against the gene product. Thus, a substantial part of the D. melanogaster genome has already been cloned and characterized at some level. Furthermore, D. melanogaster is a favorable organism for constructing a complete physical map of the genome by the characterization of overlapping clones, along the same lines as used for Caenorhabditis elegans (Coulson et al., 1986). Such an effort is likely to be undertaken soon by Drosophila biologists, and would be considerably facilitated by the extensive information already available on fly DNA clones, and by the cytogenetics possible for that species. Once the entire genome of D. melanogaster is cloned and mapped, the ease of recovery of any desired gene would be further increased by as much as two orders of magnitude, relative to the present.

The cloning techniques which are more or less specific for Drosophila, and which have helped the rapid progress to date, include chromosomal walking and jumping, chromosomal microdissection, and transposon tagging. These techniques will be briefly described below.

a) chromosomal walking and jumping. This method was first used in the cloning of the Bithorax Complex of Drosophila melanogaster in the laboratory of D.S. Hogness (Bender et al., 1983). The basic principle of the walking technique is that, starting from any cloned DNA sequence which has been previously mapped cytogenetically by in situ hybridization and is close to the desired chromosomal DNA segment, one can advance toward the desired segment in steps: by repeatedly isolating overlapping recombinants from a genomic library, using for each step of screening a DNA fragment located at the physical end of the recombinant isolated at the previous step. This method allows the isolation of contiguous segments of DNA with a speed which, under ideal conditions, is about 10-20 kilobases/week (20-40 if cosmid rather than phage libraries are used). This speed is only acceptable if the DNA segment of interest is close to the starting rDNA sequence, but Drosophila genetics can considerably shorten the distance that must be walked. This is achieved by chromosomal jumping, which simply means the use of chromosomal rearrangements, such as inversions, deletions and translocations to skip over chromosomal segments, or even to enter a desired location in a different chromosome (in the case of translocations). Jumping is achieved by isolating in one of the walking steps in a wild-type library, a DNA segment that spans a breakpoint of a favorable rearrangement, which is evidenced by in situ hybridization to two expected chromosomal bands in the rearrangement mutant. The next step of walking is then taken in a genomic library constructed from DNA isolated from the mutant. As a result, walking proceeds on the other side of the breakpoint, which may be several hundreds of kilobases closer to the segment desired. Given the large number of chromosomal rearrangements isolated over the last decades, and the free exchange of stocks among the various laboratories of the Drosophila melanogaster research community, the walking/jumping method has become routine for isolating genes of known cytogenetic location. At the end of the walk, breakpoints are again very useful for locating the gene on the molecular map:
breakpoints which disrupt the DNA (relative to wild-type) but do not result in mutant phenotype are presumed to be outside the limits of the pertinent genetic unit, while those that do result in mutant phenotype are presumed to be within or near the gene.

b) chromosomal microdissection. Should no cloned DNA be found to map close to the desired segment, or should no favorable breakpoint mutations be available, the chromosomal walk can be initiated by the actual microdissection and microcloning of the region of interest from the polychene salivary chromosomes of third instar larvae. In this technique, pioneered by Edstrom and Pirotta (Scalenghe et al., 1981), the "cutting" of the chromosomal area and its subsequent cloning produces a few recombinants, which will eventually have to be connected by walking in a normal (total genomic) library. Although very successful, this technique has the drawback of requiring specialized equipment, but this disadvantage is not so prohibitive, since it is usually easy to arrange a visit for that purpose in one of the laboratories that are set up for such work.

c) transposon tagging. This technique has also been very successful (e.g., Searles et al., 1982). It relies on the principle that transposable elements can cause mutations by their insertion in, or very near genes. If such an insertional mutation is available for a locus that one wants to clone, and the transposable element that is the cause for the mutation has been identified, one undertakes screening of a genomic library constructed from the mutant line, using the labelled transposon DNA as probe. This yields clones of all DNA segments that harbor the element. Subsequent screening of these clones by in situ hybridization to wild-type chromosomes will determine which of the isolated recombinants originated from the locus of interest. In some cases, an appropriate insertion may be identified by chance, in the course of analyzing the distribution of repetitive, putative transposable elements in different strains. More frequently, one has to generate such insertions by mobilizing transposable elements at will. This task became feasible when the phenomenon known as hybrid dysgenesis was elucidated.

HYBRID DYSGENESIS AND TRANSFORMATION IN DROSOPHILA

Hybrid dysgenesis is the name given to a genetic syndrome, in which sterility, insertional mutations, male recombination and chromosomal rearrangements are found among the progeny of specific inter-strain crosses (Kidwell, 1977). In the type known as P-M dysgenesis, these crosses always involve fathers that belong to the so called P strains and mothers that belong to the M class; the resulting dysgenesis is due to the presence of complete, functional "P" transposable elements in the paternal genome, combined with their absence in the maternal genome. By carefully choosing the strains one wishes to mutagenize, and under certain conditions such as low temperature,
a cross between P fathers and M mothers will yield transposition of P elements and a very high incidence of mutations, for a wide variety of loci. In general, transposition requires the presence of the sequences found at the two ends of the element as well as a "transposase" enzyme internally encoded by the element (Engels, 1983). In most cases, the mutations are caused, as indicated above, by the insertion of P elements, although HD may also be associated with non-specific "transposition explosions" that may yield mutations caused by the insertion of a different transposon (Rubin et al., 1982). Some loci are particularly susceptible to P-M dysgenesis, while others are more refractory. Two additional systems of hybrid dysgenesis are known today, namely I-R and Hobo (Bucheton et al., 1976; Yannopoulos et al., 1986, B. Gelbart, pers. comm.); if these are used to complement the P-M system, every Drosophila melanogaster gene might be clonable by transposon tagging.

The understanding of P-M hybrid dysgenesis had a consequence much more profound than the development of an additional tool for cloning. It led directly to the development of a practicable and convenient method for germ line transformation, i.e. for the integration of cloned DNA fragments into the Drosophila genome, in a heritable manner. This technique was one of the major breakthroughs for Drosophila research, as well as a major stimulus for attempts to apply genetic engineering to insects of economic importance. The technique was established by Rubin and Spradling (1982). In brief, it was found that when a DNA segment is cloned in such a way, that it is flanked on both sides with the two ends of the P-element, and is subsequently injected into the posterior cytoplasm of a Drosophila embryo at a preblastoderm stage, it becomes stably integrated in the genome of some germ cells of the recipient fly. For this to occur however, one also has to provide the P-element-encoded transposase activity, usually by co-injection of an integration-defective but otherwise intact, functional P-element; an example of such a "helper" construct is the one named "wings clipped", which carries a small deletion in one of the two ends of P (Rubin & Spradling, 1983). A high proportion of the eggs usually survive the injection of P-DNA constructs, and typically 5-20% of the survivors (G0) give progeny (G1) which include at least some individual transformants, from which in turn, transformed lines (G2) can be established. Thus, with some experience, by injecting approximately 100 eggs (one day's work), one can confidently expect to have several transformed lines, a few weeks later. Only one, or a few inserts per genome are usually obtained and each insert consists of the original construct terminating with the P ends, without any internal rearrangements (Hazelrigg et al., 1984).

This technique has now been widely used to study fundamental biological problems in the fruit fly, such as regulation of gene expression. Interestingly, this method has been successful in transforming not only D. melanogaster, but also the very distantly related species D. hawaiiensis (Brennan et al., 1984). This, raises the possibility of using the P transformation system to transfer genes into non-Drosophila
insects as well. While this is presently under investigation in several laboratories, no positive results have been reported as yet. Interestingly, it has been discovered recently that the normal restriction of transposition to the germ line can be lifted (i.e. somatic transposition can be achieved) by deleting the third intron of the P element (Laski et al., 1986). It is possible that this, or other modifications of the P element may facilitate the expression of transposase in other species as well. Another possibility is that additional hybrid dysgenesis systems (e.g. Hobo) might also provide tools for transformation. In addition, the study of transposable elements and potential hybrid dysgenesis systems in other insects, might help to develop suitable species specific gene transfer techniques.

TRANSFERABILITY OF INFORMATION FROM DROSOPHILA TO OTHER INSECTS

How much can we use the basic information accumulated in Drosophila for designing useful genetic constructs in other systems? The answer seems to be: a great deal, because of conservation of structural genes as well as regulatory mechanisms across broad evolutionary periods.

The rate of sequence divergence differs widely for different types of genes. For example, actins, as well as the proteins involved in the heat shock response are so well conserved from yeast to humans, that the respective genes can be selected out of "libraries" of clones from any eucaryotic organism, by using as hybridization probe the genes from one species, such as Drosophila. At the other extreme, genes for some structural proteins evolve so rapidly that they cannot possibly be recovered by cross-hybridization across wide phylogenetic distances. Most genes of interest, such as those encoding selectable enzymes, are likely to fall in between. Thus, with due attention to the necessary "permissive" conditions for cross-hybridization, recovery of a gene in Drosophila will frequently be a convenient starting point for its isolation in a species of interest. As an example, it has been possible to clone the genes for yolk proteins (vitellogenins) in the medfly Ceratitis capitata, using as probes the corresponding genes from Drosophila grimshawi (Rina, Savakis & Mintzas, this Volume). In many cases one need not even bother to isolate genes from species of interest, since many genes work in widely different species. For example, the bacterial neomycin-resistance gene can confer resistance to the antibiotic G418 when transferred into virtually any eucaryote.

It is now beginning to be appreciated that many gene regulatory mechanisms are also widely conserved in evolution. For example, regulatory sequences of the D. melanogaster major heat shock genes (hsp 70) are recognized by the pertinent mammalian regulatory factors, thus permitting heat-inducibility of hsp 70 genes transferred into mouse cells (Corces et al., 1981). Reciprocally, the regulatory sequences and diffu-
Possible factors involved in the developmental regulation of chorion (eggshell) gene expression, are so well conserved, that chorion genes from the silkmoth Bombyx mori function with normal tissue and temporal specificity when transferred into the Drosophila genome (Mitsialis and Kafatos, 1985).

It is not certain how universal such structural and functional genetic conservation will prove to be. It is a matter of interest, for example for both basic and applied biology, to learn the extent of conservation of the genetic circuitry involved in sex determination or dosage compensation for sex-linked genes. In any case, the evidence to date strongly indicates that the wealth of molecular genetic information in Drosophila is a good starting point for economically relevant insect genetic engineering. In some cases, constructs can be developed in Drosophila and used directly in any insect species where transformation has been achieved. In other cases, for optimal functioning, the Drosophila constructs will need to be modified, or rebuilt from components isolated from species of interest, frequently using cross-hybridization as a tool for quick cloning.

**Possible Areas of Use for rDNA Technology in Applied Entomology**

In the previous sections we reviewed some recent advances in the molecular genetics of Drosophila which are potentially useful in applied entomology. Here we will outline some strategies for using these findings, and the areas in which they may be applied. As mentioned in the introduction, the two broad fields of application are the control or eradication of harmful insects and the protection or improvement of economically valuable species. The economic importance of such projects could be enormous, and will be easily appreciated; it is not the purpose of this section to demonstrate it. In both areas of application, an essential prerequisite is the technology of transformation, i.e. the ability to introduce exogenous genes into the genome of the insect of interest. As already discussed, the Drosophila transformation system will be the ideal starting point for the development of similar techniques in other insects. The kinds of constructs that might be used, once transformation is accomplished, are summarized below.

a) **Eradication of harmful insects: Genetic sexing.** There are two possibilities to use gene transfer with the ultimate goal of eradicating insects. The first one, which we believe is also the most promising, is to use rDNA technology for genetic sexing in conjunction with sterile male release programs. There is of course no need to analyse here, the enormous potential, as well as the limitations of the sterile male technique. Genetic sexing, i.e. selective elimination of females, would be extremely important in this context, for several reasons: halving the cost when only males are mass-reared, enhancing the effectiveness of the released males,
which are often diverted to preferential mating with co-released sisters, and preventing crop damage by released females, which are active albeit sterile (e.g. sterile medfly females still try to oviposit, and hence damage the current crop). We can imagine three main strategies for achieving genetic sexing:

i) Y-linked genes. This is analogous to currently used schemes which are based on classical genetic methods. Sexing can be achieved by using for transformation a positively selectable gene, which should be integrated into the Y chromosome, and thereby gain sex specificity because of the site of integration rather than because of its intrinsic regulatory elements (promoters in the loose sense). The selectable gene could be generalized (e.g. G418 resistance), or used only with specialized host strains (e.g. gene for alcohol dehydrogenase, to be used in Adh- strains). A refinement of the approach would be to make the promoter inducible, e.g. by heat shock or heavy metal treatment, so as to reduce general expression and a possible competitive disadvantage of the strain in the field. Unfortunately, so far in Drosophila, an integration of a transduced DNA on the Y chromosome has not yet been achieved. The reason for that may be the actual failure of integration, or failure of detection, as a result of the special nature of the Y chromosome, which is thought to be devoid of genes other than fertility "factors".

ii) Genes with sex-specific expression. This method would rely on the engineering of a selectable marker gene, so as to bring it under the control of regulatory DNA segments that would render it expressible in only one sex. Thus, a negatively selectable gene could be controlled by a female promoter, or a positively selectable one, by male regulatory sequences. In this case the sex specificity is intrinsic to the regulatory element, rather than the site of insertion. A desirable refinement would be to make the construct inducible, e.g. by heat shock or metals. Whether that "additive regulation" could be achieved remains to be determined even in Drosophila.

iii) Sex-transforming genes. This method for sexing is in principle completely different from the previous ones, as one would not need to put any selective pressure onto the transgenic insect line; the gene used would be coupled to an inducible promoter, and when induced, would change the sex of the insects 'from female to male. In this case specificity is inherent to the gene itself, rather than the regulatory element or the site of integration. Genes have been cloned in Drosophila, which would make such constructs possible. Whether they would work on other insects is unknown.

What is needed for the three sexing methods outlined above? Other than a transformation system, one would need a toolbox of good selectable genes, sex-specific promoters inducible promoters, and sex-transforming genes. Many of these elements are already available or obtainable in Drosophila. Ideally, these genes and their regulatory sequences should be active in early phases of development, and the selection
procedure should be inexpensive, if the costs of mass rearing are to be substantially reduced. In Table 1, we try to summarize the availability of elements and the advantages and disadvantages of the three strategies outlined.

**Table 1: Genetic Sexing Strategies**

<table>
<thead>
<tr>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
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<tr>
<td><strong>STRATEGY 1 (Y-LINKAGE)</strong></td>
<td></td>
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<tr>
<td>- Inducible promoters available (hsp, metallothionein)</td>
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<tr>
<td>- Generalized selectable genes available (neo resistance)</td>
<td></td>
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<tr>
<td>- Specific dominantly selectable genes available (Adh, ry)</td>
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<tr>
<td>- Dominant insecticide resistance mutants clonable</td>
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<tr>
<td>Expression in Y uncertain</td>
<td></td>
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<tr>
<td>G418 is expensive</td>
<td></td>
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<tr>
<td>Necessity to construct special host strains (Adh, ry)</td>
<td></td>
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<tr>
<td>Inadvisability of releasing insecticide-resistant males</td>
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</table>

| **STRATEGY 2 (SEX SPECIFIC EXPRESSION)** |
| - Many sex-specific promoters probably exist |
| - Sex-specific adult-active promoters cloned (vitello-genins, chorion etc.) |
| - Female-specific, larval-active promoter cloned (tra) |
| - Constructs probably of general use |
| Inducibility may not be feasible |
| Selection only in adults |
| Cloned, but not yet characterized |
| Disadvantages of selectable genes (see strategy 1) |

| **STRATEGY 3 (SEX TRANSFORMATION)** |
| - Avoids elimination of half the progeny |
| - Genes exist in *Drosophila* |
| - Several genes have been cloned |
| Generality for other insects unknown |
| Cloned genes must be further characterized |

The list is by no means complete. Additional potentially useful genes are constantly being cloned. For example the prospects of obtaining for example promoters acting in sex specific manner in early stages are excellent. Also many more selectable genes are becoming obtainable, e.g. the *Pseudomonas* genes that increase the freezing temperature of water, insecticide resistance genes and toxin genes.

b) *Eradication of harmful insects: Autocidal strains*

The second potential way of fighting harmful insects with methods based on rDNA technology, is the development of strains that are autocidal under field conditions. Releasing these strains would introduce deleterious genes into wild populations. The molecular part of this approach is based on the same parameters as genetic sexing. First, there should be a gene transfer method available, and second a gene construct
capable of the desired expression within the host should be devised. One possible example would be female sterilizing constructs consisting of a gene that encodes an intracellular toxin, coupled to the regulatory sequences that both confer inducibility (e.g. metal treatment or mild heat shock) and specify expression in developing oocytes. Of course this approach will have to await the development of "hybrid promoters", in which inducible and sex-specific elements are combined and additive. In this case, one would raise large numbers of insects under conditions that permit propagation of the strain, and release, the insects into the wild. Sterilization of the population might be achieved by normal environmental conditions such as mid-day heat, or by treatment of the field with a copper spray similar to those already used in agriculture. A second alternative would be to use insecticide-hypersensitivity genes for autocidal purposes.

The effectiveness of any autocidal strategy, whether direct (e.g. insecticide hypersensitivity) or indirect (e.g. female sterilization) needs to be evaluated by quantitative modelling. The relative frequency of any selectively disadvantageous gene within a population might be kept at a sufficiently high level while the population is being decimated by the very existence of that same gene, by linking the gene in question to positively selectable one, such an insecticide-resistance gene. Of course, such a method is only advisable in insect pests which have already developed insecticide resistance genes, in populations other than the one under treatment.

c) Protection or improvement of beneficial insects. We will not discuss this topic in detail, other than to point out that Drosophila should again offer a good model for developing and testing the necessary constructs. For example, it should be relatively easy to clone resistance genes from Drosophila, given the reasons stated in the previous sections. Then, as is the case of the eradication schemes, the Drosophila constructs could be transferred to commercially beneficial insects, once transformation has been accomplished in that species. Such a strategy might be appropriate for valuable pollinators, such as bees, exposed to agricultural pesticides. The environmental dangers of releasing any organisms that bear resistance genes to important insecticides must be evaluated, of course, before such an approach is used. Other schemes that improve beneficial species, e.g. increasing their resistance to parasites or improving their field competitiveness, can also be imagined.

CONCLUSIONS

The time is fast approaching for agriculture related uses of rDNA technology. Its application in the field of applied entomology could be relatively restricted by the rather limited knowledge on the genetics of insects of economic importance. On the other hand, as stated before, the biology and
genetics of *Drosophila melanogaster*, offer an excellent source from which to transfer the necessary technology. The tool box that this fruit fly makes available, already includes inducible promoters, general and special dominant selectable genes, sex specific promoters, sex-transforming genes, well characterized transposable elements with defined transforming abilities, and the large reservoir of genetic information and molecular genetic techniques. It is our firm belief that, beginning with genetic sexing, genetic engineering strategies developed and tested in *Drosophila* will soon be transferred to insects of economic importance. That process will be facilitated by devoting some significant effort to genetic and molecular biological studies of the insects of interest

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FURTHER DATA ON THE ULTRASTRUCTURE AND THE SECRETORY PRODUCTS OF THE FEMALE ACCESSORY GLANDS OF CERATITIS CAPITATA (DIPTERA, TEPHRITIDAE)

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Abstract

The female reproductive accessory glands of Ceratitis capitata are formed by two structures opening into the vagina. Each of them comprises an apical reservoir and a glandular duct which in the distal part has a typical sphincter. The reservoir shows two kinds of cells, as in class III insect epidermal glands (Noirot and Quennedey, 1974): secretory and duct forming cells. After emerging, the former produce a highly proteinaceous secretion; the latter organize an efferent duct for the transport of the secretion into the glandular lumen. Gel electrophoresis indicates that the main components are comprised between 13 Kd and 38 Kd. Aminoacid composition showed a large amount of aspartic acid, arginine and histidine in the secretions. Preliminary observations reveal that secretions from the accessory glands do not remove the egg micropylar cap, which is still evident after egg laying. Such secretions seem to modify the DBA lectin-binding sites. An endochorion formed by branched protrusions is evident at the micropylar region.

Introduction

The results obtained by Leopold (1980) on the removal of the female reproductive accessory glands in Musca clearly indicate that these structures are strongly involved in the efficiency of fertilization. Whether such structures have a similar important function in Ceratitis capitata is not yet known. In this paper we report the fine structure of the medfly accessory glands and the chemical characterization of the secretions produced by these glands. The results represent general information for the understanding of the reproductive process in this organism.

Observations

The female reproductive system of Ceratitis capitata has a pair of accessory glands constituted by an apical reservoir and a long duct. The former is heart-shaped and contains an abundant proteinaceous secretion. The fine structure of these glands have recently been described by Dallai et al. (1985). The epithelial wall is formed by a flattened layer of cells lined by a thin cuticle. The cell organization is that...
Fig. 1. Female reproductive glands of Ceratitis capitata.

a) Cross section of secretory cell in a newly emerged female. The structure of the apical invagination (in) of the cell is bordered by microvilli (mv). N, nucleus; ed, efferent duct; cl, cuticular layer. x 15500.
b) Cross section of a secretory cell in a 1-2 day old female. Note the microvilli (mv) delimiting the cell invagination (in) and the filamentous material (fm) over them. The intercellular space, in some regions (arrows), appears dilated. x 8500.

c) Cross section of accessory gland in a 5 day old female. The apical invagination (in) is full of homogeneous dense material produced in the Golgi systems (G). The efferent duct (ed) opens into the apical invagination (in) through a spheroidal structure (sph). L, lumen of the apical gland reservoir; cu, cuticular intima. x 8500.

d) Cross section of accessory gland in a 5 day old female. The continuity of the efferent duct (ed) from the apical invagination (in) to the gland lumen (L) is shown. The extremity of the efferent duct opens into the apical invagination through a spheroidal structure (sph). x 19000.

Paraformaldehyde 4%-glutaraldehyde 3%; osmium tetroxide 1%; Epon.

of many other insect ectodermic glands showing secretory cells and duct-forming cells arranged as in the class III glands of Noirot and Quennedey (1974).

In the pupae or the newly emerged females the secretion does not occur (Fig. 1a). The first signs of secretion become evident in 1-2 day old females and take the form of small dense droplets which accumulate in the lumen of the apical reservoir (Fig. 1b). The secretion increases greatly in the older females, independently of mating but in relation to ovarian maturity, and concentrates first in the cystern of the secretory cells (Fig. 1c) and then flows through an efferent duct (Fig. 1d) into the gland lumen.

The nature of the secretion produced by the secretory cells, according to histochemical procedures, is highly proteinaceous with a weak polysaccharide content.

We recently investigated chemical composition of the secretion. By Bradford's method (1976) each gland was found to contain an average of 0.54 μg of total proteins.

SDS-polyacrylamide gel electrophoresis of the secretion obtained from dissected accessory glands of adult females revealed several electrophoretic bands. Some of these are extraneous to the secretion and comparison with a similar electrophoresis obtained from whole glands and/or epithelial wall, indicated that the electrophoretic bands typical of the secretion are only a few, comprised between 13 and 38 Kd. Of these bands the most relevant were those (1, 2, 3 5, 7 and 8) with molecular weight of 13, 26, 28, 32, 36 and 38 Kd respectively (Fig. 2a). The PAS staining of the same gel electrophoresis gave positive results at the 3rd, 5th and 8th bands (Fig. 2b).

The aminoacid composition of the secretion is reported in Table I. From this it appears that the most common aminoacids are aspartic acid, arginine and histidine. The acidic aminoacids constitute 20.28% and the basic ones
Fig. 2. SDS-polyacrylamide gel electrophoresis of accessory gland secretion from adult females of Ceratitis capitata.
a) Coomassie blue staining; b) PAS staining.
Lanes A-D, molecular weight standards: A, actin; B, bovine serum albumin; C, trypsin inhibitor; D, calf neurofilaments.
Lane E, accessory gland secretion: band 1, MW = 13 Kd; band 2, MW = 26 Kd; band 3, MW = 28 Kd; band 4, MW = 30 Kd; band 5, MW = 32 Kd; band 6, MW = 34 Kd; band 7, MW = 36 Kd; band 8, MW = 38 Kd.
The secretion was obtained from about 350 accessory glands opened in cacodylate buffer 0.1 M pH 7.2 at 4°C. The sample, constituted by 40 µl (secretion + buffer) was denatured with Laemmli sample buffer (Laemmli, 1970). The SDS slab gel of 12% polyacrylamide was prepared according to Laemmli (1970).

34.81%. It will be interesting to establish the enzymatic properties of the secretion but this will be the subject of a future study. In order to know the possible action of the secretion on the female gametes we examined the organization of the egg micropylar region in eggs removed from the ovary and in recently laid eggs. In both cases there is a similar structure. The general structure of the egg chorion of Ceratitis capitata has already been investigated by Margaritis (1983, 1985). According to this author the micropyle region has a chorionic protrusion but does not present an anterior cap as does Dacus oleae. We showed, on the contrary, that a cap-like structure is present even in Ceratitis capitata (Fig. 3a,b,c,d). A cross section through the micropyle egg region shows an innermost vitelline envelope, a trabecular endochorion from which a branched structure radiates, and an outer fibrous exochorion (Fig. 4a,b,c). The most interesting is the branched
Tab. I. Aminoacid composition of the accessory gland secretion of *Ceratitis capitata*. The secretion was obtained from the dissection of about 500 adult females. It was recovered by opening the glands in a drop of cacodylate buffer; then it was lyophilized and used for three samples analyzed by Waters fluorimeter (results kindly sent us by Dr. J. François from Dijon, France).

<table>
<thead>
<tr>
<th>AMINOACID</th>
<th>Average amount in ng/µg of secretion</th>
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<tr>
<td>Gly</td>
<td>6.51</td>
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<tr>
<td>Ala</td>
<td>0.47</td>
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<tr>
<td>Val</td>
<td>4.26</td>
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<tr>
<td>Leu</td>
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<tr>
<td>Ile</td>
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<td>Pro</td>
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<td>Ser</td>
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<td>Thr</td>
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<tr>
<td>Phe</td>
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<td>Trp</td>
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* Cys = Cystine

endochorion: it is denser than the underlying endochorion and consists of protrusions of 0.12 µm regularly emerging from the outermost endochorion (Fig. 4b, c,d). The pillars after a short distance from the outer endochorion begin to branch and at the peripheral micropylar region form an elaborate network 1 µm high which progressively increases to 7.3 µm toward the central part of the micropylar area (Fig. 4a,b). The micropylar area has, as consequence, a crater-like appearance (Fig. 4a). The central part, however, is devoid of this dense network and instead is filled with a large amount of fibrous material of the exochorion (Fig. 4a,b). This material also forms a thin layer over the dense branched endochorion and the whole egg surface. In the posterior pole of the egg the exochorion expands to form a semilunar structure 8 µm high. The above described organization of the micropylar region apparently remains unchanged in the ovary and laid eggs. Even "in vitro" treatments with solutions containing increased concentrations of the female accessory gland secretion did not remove the micropylar structure. We cannot exclude, however, that some minor differences occurred. To verify possible modifications in the topographic distribution of some chorionic bound carbohydrates we analyzed these compounds using several FITC-coupled lectins. First of all it is interesting to note that the chorion is usually weakly autofluorescent, but that the two opposite apical regions, the micropyle and the semilune at the posterior pole did not show any fluorescence. As we can see from the Table, none of the lectins, apart from DBA, revealed any distinguishable change in binding-site distribution between ovary and laid eggs (Figs. 5,6). The specificity of the reaction was indicated by the complete inhibition of the fluorescence when appropriate sugars were added to the different lectin solutions. DBA lectin binding-sites were differently located in ovary and laid eggs. In the former a positive reaction was evident at
Fig. 3. a) *Ceratitis capitata* laid egg by interference contrast microscopy. The arrow indicates the micropylar cap. The micropyle is visible in the center of the cap structure. x 700.
b) SEM low magnification of the micropylar cap showing the micropyle in the center of the structure. x 700.
c) SEM lateral view of the micropyle. x 1250.
d) SEM frontal view of the micropyle. x 2500.

the base of the micropylar cap (Fig. 6a); in the laid eggs this region appeared negative. A very weak reaction was visible along the marginal site of the micropylar cap in a long photographic exposure (Fig. 6b).

The use of FITC-coupled lectins also allowed us to detect the presence of binding-sites on the posterior pole of the egg. In particular Con A gave a positive diffuse staining both in ovary and laid eggs (Fig. 5a,b). WGA, on the contrary, gave a positive reaction on the marginal sites of the semilunar posterior feature (Fig. 5c,e).

Discussion

As mentioned in a previous paper (*Ballai et al., 1985*) the ultrastructure of the apical reservoir of the accessory glands of *Ceratitis capitata* is similar to that of many other insect ectodermic glands (*Noirot and Quennedey, 1974*)

The secretory cells begin secretory activity in 1-2 day old females; the secretion greatly increases in the mature female (5 days old). The secretion contained in the apical reservoir is mainly proteinaceous.
Fig. 4. a) Sagittal section of the micropylar cap. Note the branched structure of the outermost endochorion (be), the trabecular endochorion (en), the exochorion (ex) in the center of which the micropyle (m) opens and the vitelline envelope (ve). x 4800.
b) Detail of the previous figure showing the branched endochorion (be), the trabecular endochorion (en) and the exochorion (ex). Arrows indicate the starting points of the protrusions forming the branched endochorion. x 8400.
c) Cross section of the micropylar cap. The peripheral area shows the branched endochorion (be), the exochorion (ex)
and the micropyle (m). x 6800.
d) Detail of the previous figure to show the structure of the branched endochorion (be) showing ramifications of the protrusions (arrows) and the exochorion (ex). x 18800.
Paraformaldehyde 4%-glutaraldehyde 3%; osmium tetroxide 1%; Epon.

Fig. 5. Fluorescein-coupled lectins.
  a) Con A laid eggs, anterior and posterior poles. x 200.
  b) Con A laid egg, posterior pole. x 400. c) WGA laid egg, posterior pole. x 200. d) Specificity of FITC-Con A binding was demonstrated with 50 mM L-methyl-D-mannoside. x 400. e) WGA laid egg, posterior pole. x 400. f) WGA laid egg, anterior pole. x 400.
The electrophoretic results indicate that this secretion produces bands mainly between molecular weight 13 and 38 Kd. The aminoacid pattern reveals a major proportion of aspartic acid, arginine and histidine. A relation between the electrophoretic results and those from the aminoacid analysis has not yet been established.

The enzymatic properties of the accessory gland secretion of Ceratitis have not yet been investigated so comparative analysis with other data (Leopold, 1980) is not yet possible. Nevertheless the negative results of experiments on the removal of the micropylar material led us to affirm that in medfly Ceratitis the micropylar region is differently organized compared with that of Musca or other Diptera (Leopold, 1980; Margaritis, 1980, 1985). We were able to demonstrate that a micropylar cap, similar in shape to that of Musca, is really present in Ceratitis. The micropylar
cap, visible with light and scanning electron microscopy, is a delicate structure, easily mechanically removed. This may be the reason for its absence in the photographs of Margaritis (1983, 1985). It mainly consists of a network emerging from the outermost endochorion so it constitutes an integral part of the egg and its removal, as consequence, is not possible by accessory gland secretions.

These secretions, however, seem to be involved in the modification of the topographic distribution of chorionic-bound carbohydrates. Though preliminary, our results give evidence of such modification at least in the DBA-binding sites. The meaning of these results is still matter to investigate.

As a general conclusion we can assert that medfly fertilization occurs without removal of the micropylar cap. Studies are in progress to investigate the possible action of the female accessory gland secretions on spermatozoa.

References


MORPHOLOGY OF SEX-PHEROMONE GLAND IN MALE ORIENTAL FRUIT FLY AND ITS SUSPECTED MECHANISM OF PHEROMONE RELEASE

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Abstract

The morphology of the sex pheromone producing gland, located in the rectum of the male Dacus dorsalis was studied by the use of the scanning electron microscopy. Sexual differentiation of the rectum morphology was found in this fly. In females, a cucumber-like rectum with four round rectal papillae located at the anterior end and surrounded with the well developed circular muscle were observed. In males, except the four rectal papillae and the circular muscle, an enlarged evagination is developed on the dorso-posterior portion of the rectum. This evagination structure is the reservoir, a storage of pheromone. At the near end of the reservoir, a small cone like sac, the secretory sac, is attached and which is suspected to produce the pheromone. While the male fly is in sexual maturation, the reservoir enlarged gradually. It continuously enlarges and finally becomes a balloon like structure.

The release mechanism of the pheromone was observed by the wild M8 type of stereomicroscopy. The rectum of the mature male fly mostly starts to enlarge gradually at about 14:00-15:00 and more frequently at 16:00-19:00 in the summer season. One or more transparent odor oily droplets released from the anal tube was suspected as the pheromone. In the female, the enlargement and the oily droplet secretion are never occurred in the rectum.

Introduction

The use of sex-pheromone in pest control of cabbage fields in Taiwan has been establishing in two species, the armyworm moth, Spodoptera litura (F), and the diamondback moth, Plutella xylostella (L.) (Chow et al. 1984). Moreover, the synthetic sex pheromone of several more Lepidopterans was studied in the recent time, such as the smaller tea tortrix, Adoxophyes privata re, the oriental tea tortrix, Homona magnantima (Shry et al. 1986) and the Asian corn borer (Chow et al. 1984).

The oriental fruit fly, Dacus dorsalis Hendel is one of the most economic important fruit pest. The poisoned protein hydrolysis, insecticide of Fenthion, the trapping with the poisoned methyl eugenol and the sterile fly release technique had been used for control and tried to eradicate this insect in Taiwan (Hsu et al. 1977; Lee 1980). The annihilation method is applied for the current control program. Entomologists of the fruit flies in the interested countries have been working intensively toward developing integrated control methods. The sex pheromone produced has been discovered by the male flies (Economopoulos et al. 1971; Fletcher, 1968, 1969; Kobayashi et al. 1978; Lee et al. 1986; Nation, 1972, 1974 and Landolt et al. 1985). Also its chemical identification and synthesis (Baker et al. 1980; Mazomenos et al. 1985, Nation, 1975) have stimulated research among it's use for monitoring and control of the different species of fruit flies.

1 This research was supported under the grant No. NSC 75-0201-B001-43 of the National Science Council of the Republic of China.
The laboratory bioassay studies have shown that the sex pheromone produced by the male oriental fruit fly (Kobayashi et al. 1978; Lee et al. unpublished). This study attempts to investigate the morphological changes and the mechanism while the sex-pheromone gland secretes, stores and releases the pheromone of this fly.

Materials and Methods

Procedures of colony maintenance of the oriental fruit flies in laboratory have been published (Lee et al. 1985). The pupal and the adult stage were kept in a growth chamber with temperature at 27±1°C and R.H. at 65±5%. The 1, 3, 5, 7 and 9 day old adult, then once for each 5 days since the 15th day adult males and females were dissected at 10:00-12:00 and 14:00-19:00 o'clock. The rectum were pulled out into the ringer solution. The morphology of the normal and the enlarged recta were studied by the scanning electron microscope. The normal and the different degree of enlargement of recta were fixed in 2.5% glutaraldehyde with 0.1M cacodylate buffer at 4°C for 2 hours, then washed in distilled water, dehydrated through a series of acetone to absolute acetone. After critical-point dehydration treated in liquid CO2, the recta were mounted on a metal stub, coated with gold in the ion-coater IB-2 and observed with the Hitachi S-450 scanning electron microscope.

The morphology changes and the mechanism of the suspected sex pheromone release were observed under the Wild M8 type of stereomicroscope and the photographs were taken right away with the Wild MPS 20 Semiphotomat.

Results and Discussions

The oriental fruit flies usually mate at dusk from about 16:00-19:00 (Lee et al. 1983) few courtships occur earlier the natural light intensity at about 2300 lux, abundant at about 175-460 lux. While the flies are going to mate, they are flying and dancing around in the insect cage. The male stridulates by drawing their wing across a pair of comb-like large bristle on two sides of their abdominal tergite, a strong olfactory odor is produced at the same time. This structure and mating behavior are similar to those of Dacus tryoni (Fletcher 1968, 1969) and D. oleae (Economopoulos et al. 1971) considered stridulation sang to be a mating call. The olfactory odor is suspected a sex pheromone which stimulates the female to start the mating behavior (Economopoulos et al. 1971). This behavior also occurs on the papaya fruit flies, Toxotrypana curvicauda (Landolt et al. 1985).

The sex-pheromone producing gland of the oriental fruit flies and other Dacus flies, located in the rectum of the male fly has been demonstrated by several entomologists (Fletcher 1969; Schultz et al. 1971; Koboyashi et al. 1977; Lee et al. unpublished), the external morphology of the rectum of the oriental fly was reported by Lee et al. (1986) with the scanning electron microscopic study. It is different between the female and the male. The rectum of the female appears as a cucumber (Fig. O-D and Fig. 1). It anterior portion has four rectal papillae (Rp) and a anal tube (A) is at the posterior part. In the male, except 4 rectal papillae (Fig. 2 and 7, Rp) it is also a ball like structure of reservoir (R) and a small cone of secretory sac (S) at the posterior portion near the anal tube (A).

From the scanning electron microscopic studies, the circular muscle is well developed surrounding the female rectum (Fig. 1) and the male
Fig. 1. A scanning electron micrograph of the female rectum which is surrounded by well developed circular muscle.

Fig. 2. A scanning electron micrograph of the normal male rectum.

Fig. 3-5. The reservoir (R) of the male rectum is being enlarged.

Fig. 6. A scanning electron micrograph of the balloon-like-rectum the striation circular muscle is completely disappeared by the rectal enlargement.

Fig. 7. The normal male rectum was taken under stereomicroscopy.

Fig. 8-11. Photographs of stereomicroscopy show the way of enlargement of the male rectum.

Fig. 12. The balloon like rectum release a drop of the oily droplet from the anal tube.

Fig. 0. The photographs show the male rectum approaching its enlargement and the comparision of the shape between the rectum of the male and the female.
A. Normal male rectum.
B. The male rectum is enlarging.
C. The male rectum enlarges as a balloon.
D. The female rectum never changes.

Abbreviations:

A- Anal Tube, C-Colon, R- Reservoir, Rp- Rectal papillae, S- Secretory sac.

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rectum is in the normal condition (Fig. 2). When the pheromone were secreted, the reservoir is enlarged, the muscular striation on the reservoir area is first stretched and disappear (Fig. 3). Until the whole rectum enlarges as a balloon, the muscular striation completely loss as shown in the figure 4-6.

The mechanism of the pheromone release were observed with the wild M8 Stereomicroscope and the microphotographs were taken as quick as possible while the morphology of the male rectum was changing. The recta are pulled out in the ringer solution about after 15:00 'o'clock. One can see that the rectum is moving, the secretory sac (S) is shaking; the rectum is enlarged from the reservoir (R) toward the anterior portion until the rectum becomes a balloon shape. This action and changes of the rectal enlargement are shown from the figure 7 to the figure 11. During or after the rectum enlarged, a transparent oily droplet is released one after one from the anal tube (Fig. 12, 0). These behavior of moving, shaking and enlargement of the rectum differ at the different time controlled by a circadian rhythm. The rectum is pulled out before the dusk about 16:00, the rectum takes more 20 minutes to enlarge as a balloon. Meanwhile, the oily droplet release out slow. After 16:30, up to 19:00, the enlargement of the rectum occurs right away. While the rectum is being pulled out it takes 2 to 3 minutes even shorter time, it become as a balloon shape and releases the oily droplets which will get out to the ringer solution. A strong odor one can be smelled. After those oily droplets release completely, the rectum recovers to the origin shape (Fig. 0-A and Fig. 7). The recovery rate is in proportion to the rate of enlargement or amount of the ringer solution. If the solution is not enough, the rectum is immovable and died. The rectum of female is also moving and shows a few degree of enlargement. It also release the droplets. However, these droplets are feces sinking into the bottom of the solution and without the odor smell. Economopoulos et al. (1971) reported both the male and the female of the olive fruit flies apparently release the yellow oil-like droplets when the flies are sexually active. Droplets with characteristic odor easily detectable by humans, is released by the sexually mature males, and the weaker odor is release by females. These characteristic droplets, we may suspect as the sex-pheromone which should be studied in the near future.

References


COMPARATIVE STRUCTURAL STUDY OF THE EGG-SHELL (CHORION) IN Dacus oleae, Rhagoletis cerasi, Ceratitis capitata and Eurytoma amygdali

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Summary

The eggs of the fruit flies of economic importance Dacus oleae, Ceratitis capitata and Rhagoletis cerasi (Diptera, Trypetidae) exhibit among them similarities but differences as well. Externally the follicles are longitudinal with characteristic anterior-posterior polarity. Their size is nearly the same whereas the egg-shell layers exhibit great differences among the three species: a thickened vitelline membrane from D. oleae to C. capitata respectively, b. a single trabecular layer in D. oleae, while in C. capitata it is double and perforated and in R. cerasi it is double or triple and perforated, too, helping in embryo's respiration. On the contrary, in D. oleae egg-shell, which has only small dispersed holes, respiration might take place through the anterior pole cup as well.

Extending our study to the almond wasp Eurytoma amygdali we found out that the egg morphology is completely different having a small oocyte and a long appendage. In thin sections it reveals two different chorionic layers, a granular and a pillar one, but with no cavities.

Results following o-dianicidine and diaminobenzidine assays have lead us to believe that the participation of peroxidase is involved in chorionic crosslinking of the proteins in all above species, a fact that reinforces the aspect that chorionic proteins are crosslinked with rubber like bonds to insure elastic properties to the egg.

Introduction

The egg-shell of Insects is composed of the chorion and the vitelline membrane. It is produced by the follicular cells (Margaritis et al 1980) and has several functions: to allow the spermatozoon entry, to help embryo's respiration, to avoid the loss of water in dry environmental conditions and flooding in wet environmental conditions.

One of the most thoroughly studied dipteran species Drosophila melanogaster which has been shown to exhibit regional and radial complexity in its egg-shell (Margaritis et al 1976, Margaritis et al 1980) has differences and some similarities to D. oleae and C. capitata (Margaritis 1985b).

Chorion proteins have proved to crosslink, forming di-tyrosine and tri-tyrosine covalent bonds at least in D. melanogaster (Mindrinos et al 1980) and in the case of D. oleae and C. capitata (Margaritis 1985b).

In this study we tried to compare these two species with the cherry fly Rhagoletis cerasi belonging to the same family,
indicating the existence of peroxidase in the third species as well.

We also preliminary studied the almond wasp *Eurytoma amygdali* (Hymenoptera, Eurytomidae) egg-shell. This species has a similar way of oviposition into immature almond seeds through a very narrow ovipositor (Plaut 1971). It is interesting therefore to reveal the similarities in egg-shell features between various species of economic importance.

Materials and Methods

*D. oleae*, *C. capitata* and *R. cerasi* adult insects were kept in a 22-23°C culture room. *Eurytoma amygdali* insects were brought into the laboratory from infested almonds. 3-5 days old insects were dissected in cold Ringer's solution. Mature eggs were removed from the ovaries and processed for transmission and scanning electronmicroscopy as described elsewhere (Margaritis et al. 1980). Stage 13 and 14 follicles were examined for peroxidase activity according to Fahimi (Fahimi 1970) following the diaminobenzidine (DAB) assay for identification of peroxidase in the electron microscope and the o-dianisidine procedure of Worthington Biochemical Corporation (Anonymous 1972). The o-dianisidine assay was also used in the case of *E. amygdali* mature follicles.

Results

The mature eggs of the fruit flies *D. oleae*, *C. capitata* and *R. cerasi* are oblong in shape (fig.1,2,3) with a characteristic anterior pole which includes the micropyle. In *D. oleae* the anterior pole has formed a "cup" (fig.4) where numerous protrusions and holes can be observed. In *C. capitata* it is more like a cone (fig.5) and in *R. cerasi* it has the shape of a disc (fig.6), however, unlike *D. oleae*, it is smooth having no protrusions.

In thin sections the egg-shell of *D. oleae* has a thin vitelline membrane (VM) 0.15-0.2µ thick (fig.7) whereas in *C. capitata* it is 0.5µ thick (fig.8) and in *R. cerasi* it is 0.2-0.25µ (fig.9). The wax layer (fig.8) and the innermost chorionic layer (fig.9) are very similar among the three species.

The endochorion is a very complicated configuration. In *D. oleae* it is constructed of a single trabecular layer 0.6µ thick with a thick and mostly compact inner endochorion (ie) apart from the column (P) bases where holes can be observed (fig 7,10) and a thick outer endochorion (oe) showing small dispersed holes (fig.7). In *C. capitata* it is 1µ thick with a double perforated trabecular layer and a very thin discontinuous inner endochorion (fig.8). In *R. cerasi* it is 1.3µ thick, however, the irregular formation of the trabecular layer does not enable us to distinguish the number of cavity rows. They are either two or three with transversed pillars (fig.9). It is also perforated (fig.9) and has a discontinuous inner endochorion. The last chorionic layer, the exochorion (ex) exists in all three species (fig.7,8,9).

During chorionic formation the microvilli of the follicle
FIG. 1, 2, 3: Whole mount views of mature eggs of
D. oleae (1) bar 0.12 mm, C. capitata (2) bar 0.13 mm, and R. cerasi (3) bar 0.12 mm.
FIG. 4, 5, 6: Scanning micrographs of the anterior "cup" of D. oleae (4), the cone of C. capitata (5) bars 10 μm and the "disc" of R. cerasi (6) bar 20 μm.
Notice the protrusions of the "cup" in opposition to the smooth "disc" of R. cerasi. The micropyle opening is pointed, in each case, with arrows.
Cells play a major role in the construction of the endochorionic cavities creating the perforated endochorion of C. capitata (fig. 11) and R. cerasi (fig. 12) and the holes in the endochorion of D. oleae (fig. 10).

Histochemical approach in R. cerasi for the existence of peroxidase in the chorion showed positive reaction with o-dianisidine in whole mount follicle views (red colour) and endochorion was stained (electron dense) in thin sections with diaminobenzidine (DAB) (fig. 13).

FIG. 7: Transmission micrograph of thin section of the chorion of D. oleae. The holes are apparent in the bases of the pillars and in the outer endochorion. Bar 0.5 μ

FIG. 8: Thin section of the chorion of C. capitata Bar 0.6 μ

FIG. 9: Thin section of the chorion of R. cerasi. Bar 0.5 μ

(VM) vitelline membrane (FC) follicle cells, (wl) wax layer, (ICL) innermost chorionic layer, (oe) outer endochorion (ie) inner endochorion, (P) pillars, (OC) oocyte.
FIG. 10: Thin section of immature egg of *D. oleae* (a) The microvilli of the follicle cells extend into the endochorial cavities (arrows) Bar 0.21µ (b) Notice one of the microvilli (arrow) withdrawing of the cavity. A hole will remain in this place. Bar 0.20µ

FIG. 11: Transmission micrograph of an immature egg of *R. cerasi* The microvilli of the follicle cells are noticable here too (arrow) Bar 0.12µ

FIG. 12: Similar aspect of chorion of *C. capitata*. The microvilli are extending into the endochorion cavities (arrows)
FIG. 14: Whole mount view of mature egg of the almond wasp *Eurytoma amygdali*. Notice the oocyte (OC) and the long and short appendages from both sides of the oocyte. Bar 0.5 mm.

FIG. 15: Thin section of the chorion of *Eurytoma amygdali* egg. The loose vitelline membrane (VM) is barely noticeable. Observe the granular layer (gl) and the pillar (P) layer. No exochorion is observed. (wl) wax layer. Bar 0.5 μ
FIG. 13: Peroxidase assay with DAB in *R. cerasi* (a) the endochorion is positively stained. Bar 0.3μ
Notice the stained secretory vesicles in the follicular cells and (b) detailed same aspect of secretory vesicles

Trying to extend our study to other insects of economic importance we chose *Eurytoma amygdali* which has a similar way of oviposition with the above studied Diptera. The outer egg morphology however, is completely different; it consists of a small elongated oocyte and two thread-like extensions from both sides of the oocyte(fig.14). The long one is a 1500μ long appendage and the short one is 150μ long where possibly the micro-pyle opening is located.

The chorion consists of a loose vitelline membrane(fig.15) a layer that is easily separated from the rest of the chorion and may be of the same material as the wax layer(fig.15) and the main chorionic layer consisting of an inner granular one and an outer pillar one 5μ thick(fig.15) with no cavities. No exochorion was observed. The o-dianicidine assay showed positive reaction(red-coloured oocyte and tails).

Discussion

The eggs of the three fruit fly species show several similarities due to the same oviposition from the insects but differences as well as a result to their adaptation in different
environmental conditions.

A thick vitelline membrane may not be needed for the protection of the embryo as it is in *D. oleae* and *R. cerasi* which have a more or less compact chorion. On the contrary a perforated like the *C. capitata* one might need a thick vitelline membrane for the better protection of the embryo.

The endochorion is the most complex layer of the egg-shell. In *D. oleae* it is the most compact of all, having very small holes in the inner and outer parts; that might be a reason of its less thickness. It doesn't need to be as thick as the others because its compactness can play a protective role. *C. capitata* main body of endochorion may be used for plastron respiration (Margaritis 1985b)(Hinton 1980) and in *R. cerasi* a similar thing might happens only through the anterior pole quite similar to the respiratory appendages of *D. melanogaster* (Galanopoulos 1982)

Their phylogenetic relationships (Kitto 1982) justifies this aspect. Since *R. cerasi* is the oldest of the three Trypetidae and closer to *D. melanogaster* and Drosophilidae in general we can presume that the respiratory appendages have been replaced in the Trypetidae. a) by an area for respiration in the main body of the egg, b) later on by the whole body of the egg (in *C. capitata* where its eggs live under "flood"conditions inside the fruit) and c) in no plastron respiration or very limited through the main body and the anterior cup (in *D. oleae*).

*E. amygdali* on the other hand, needs a thorough examination on several problems: a) What is the use of such a long appendage since it doesn't seem to play the role of respiratory apparatus; it is smooth and it is completely immersed inside the seed (Plaut 1971) unlike the long respiratory appendages of Drosophila grimshawii which are being directed outside the leaf (Kambysellis et al 1971) for that purpose. b) What is the role of the granular and the pillar layers of the chorion?

Peroxidase exists in *R. cerasi* chorion and *E. amygdali* as happens in *D. melanogaster* (Mindrinos et al 1980) in *D. oleae* and *C. capitata* (Margaritis 1985b) and in many other species from different orders (Review, Margaritis 1985a) assuming the chorion's need to harden with resilin type bonds (Andersen 1964) to avoid splitting during oviposition through the narrow ovipositor.

References


VITELLOGENINS AND THEIR GENES IN THE MEDFLY, CERATITIS CAPITATA

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Abstract

Two vitellins have been identified in the medfly. These proteins and their precursors in the hemolymph, vitellogenins 1 and 2 (Vg-1 and Vg-2), have been characterized.

Vitellogenin synthesis during medfly development was studied by radiolabelling in vivo and in organ culture, and by in vitro translation of RNA in a heterologous cell-free system. Vg's are synthesized in the fat body and ovaries. No Vg synthesis can be detected in larvae and in adult males. In females, Vg-1 and Vg-2 synthesis is detectable within a few hours after eclosion, remains relatively low for the first 24 hours, and then increases dramatically during days 2 and 3, to reach approximately 5% of the total protein synthesis.

Although both Vg-1 and Vg-2 are synthesized in fat body and ovaries, their relative synthetic rates differ in the two tissues. In fat body the ratio of Vg-1 to Vg-2 synthesis is approximately 4; in ovaries this ratio is close to 2.

Injection of \(\beta\)-ecdysone into adult males induces synthesis of Vg-1 and Vg-2 in fat body.

A medfly genomic library was constructed using lambda vector EMBL4 and embryonic DNA partially digested with restriction endonuclease Mbol. Several clones containing sequences homologous to a Drosophila vitellogenin gene have been isolated and are currently being characterized.
THE MAJOR LARVAL SERUM PROTEINS (MLSPs) OF CERATITIS CAPITATA: STRUCTURE-BIOSYNTHESIS-REGULATION

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Abstract

During the last larval stage of C. capitata, the fat body synthesizes four MLSPs which accumulate in the hemolymph in large amounts. Three of them (MLSP-1, 2 and 3) have similar structure, amino acid composition, peptide maps and immunological properties as well as the same biosynthetic and accumulation pattern during development, remain in high concentrations during the main part of pupal life (8 days) and disappear gradually within 2 days before adult emergence. MLSP-4 is biochemically and immunologically different from the other three MLSPs and shows two peaks of synthesis: a first one during the last larval stage, about one day after the MLSP-1, 2, 3 biosynthetic peak, and a second one during the first two days of the adult stage. The concentration of MLSP-4 remains at a high level during the entire pupal stage and the first days of the adult life. The synthesis of this protein in the adult flies can be stimulated by 20-OH-ecdysone. The data we have obtained so far suggest that C. capitata has three similar co-regulated MLSP genes which are expressed once during the last larval stage and a fourth MLSP gene which is expressed in both larvae and adults and is probably under ecdysone control.
A STUDY OF FACTORS THAT CONTROL PHEROMONE BIOSYNTHESIS OF THE OLIVE FRUIT FLY, Dacus oleae

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Abstract

The biosynthesis of the female olive fruit fly, Dacus oleae (Gmelin) sex pheromone was depended by physiological and environmental factors. Females began synthesized pheromone from the 3rd day postemergence. Pheromone biosynthesis was cyclical with peaks recurring at about ten days intervals. Females which ovarian development was inhibited by γ-ray synthesized more pheromone and its synthesis was not cyclical. Adult female diet and the temperature they were maintained affected pheromone synthesis. The release rate was regulated by an endogenous rhythm, the maximum release occurred during the last two h of a 12:12 light-dark regime.

Introduction

The olive fruit fly, Dacus oleae (Gmelin), females produce and release a pheromone blend which attracts males (Haniotakis, 1974, Mazomenos and Haniotakis, 1981). The major compound of the pheromone blend has been shown to be a spiroketal (Baker et al., 1980) Mazomenos and Haniotakis (1985) have shown increased attraction of the three secondary compounds which were also isolated from females. These compounds were: α-pinene, n-nonanal and ethyl-dodecanoate. The site of pheromone production and storage was found to be the female rectum (Vita et al., 1979, Mazomenos and Haniotakis, 1981).

Various factors have been shown to effect the amount of the sex pheromone in the pheromone gland of many insect species studied. The quantity of the sex pheromone produced by females of: Plodia interpunctella (Coffelt et al., 1978), Platynota stultana (Webster and Carde, 1982, 1984), Trichoplusia ni (Bjostad et al., 1980), Adoxophyes fasciata (Nagata et al., 1972) Choristoneura fumiferana (Sanders and Lucuik, 1972), Drosophila melanogaster (Tompkins, and Hall, 1981), has been found to increase rapidly after emergence, reach a maximum level and remain high for several days, while mated females diminish pheromone production rapidly.

In previous studies (Mazomenos, 1984) has been shown that female olive fruit fly produces and releases sex pheromone from the third day postemergence. The production of the sex pheromone was cyclical with peaks of production recurring at ten-day intervals, each peak was lasted 2-3 days. Mated females produced less pheromone the first ten days after mating which then increased to near the same level of that in virgin females.

In this paper we will report the results of studies showing the effect of a) ovarian maturation, b) female adult diet, c) temperature in which females were maintained, on the biosynthesis of the pheromone and that pheromone release by females is regulated by an endogenous rhythm.
Methods and Materials

Insects

The insect used were obtained from a colony maintained at the "Democritos" N.R.C. Athens, Greece on an artificial diet for many generations. The flies were reared at the larval stage under the conditions and methods described by Tsitsipis (1977). The pupae obtained were separated in two batches. One batch was treated as late pupae with 11-Krad γ-ray in nitrogen (Economopoulos, 1977). Flies 24 h after emergence were immobilized by chilling at 2-4°C for 1-2 h and then separated by sex. They were maintained in screen cages 270 cm³ in artificial light (3000 lux intensity) and 12:12 light-dark regime. Temperature was 25°C ± 2 and RH 65%/±5%. Irradiated female flies and non-irradiated flies used as control were maintained on a diet described by Tsitsipis (1975), while another group of female flies were maintained on sucrose. Water was provided in both cases. To examine the effects of various temperatures on pheromone biosynthesis, females from the control stock were transferred 24 h prior to analysis into incubators with the desire temperature.

Pheromone collection

Volatile substances were extracted from the rectal glands of the female flies as previously described (Vita et al., 1979, Mazomenos and Hanniotakis, 1981). Glands were extracted with 2 ml of ether for 24 h. An internal standard 2,4-nonadienal (50ng/µl) was added to all samples which were stored at 4°C until analysis. The ether extracts were concentrated and then analyzed by gas chromatography (GC).

Gas Chromatographic Analysis

The ether extracts were of sufficient purity to be gas chromatographed directly after concentration. For separation of the various compounds a Varian model 1400 gas liquid chromatograph (GLC or GC) equipped with a flame ionization detector was used. A 5% OV-101 ²nm 1.8mm (i.d) on 80/100 mesh chromosorb G-HP stainless steel column was used. Temperature was held at 80°C for ten min. and then programmed to 240°C at 60°C/min. Carrier gas was nitrogen at 20 ml/min. Injector temperature was 200°C and the detector temperature was 250°C. Gas-chromatographic conditions were the same for all the analyses. A varian model CDS 401/402 computer integrator provided quantitative determination of the major sex pheromone component, 1,7-dioxaspiro (5,5) undecane, by comparing the total peak area of the pheromone peak with that of the internal standard.

Results and Discussion

Pheromone gland content of virgin females treated with γ-ray and non-treated, fed with normal adult diet or sucrose were examined during the first twenty days after emergence (Fig. 1). Pheromone titre was determined every day starting from the third day postemergence. Five randomly selected females were used for each determination. Five replications were done for each day and each treatment. The pheromone glands were excised from the females and extracted in ether at 08:00 h.
Pheromone titre in non-irradiated females fed with normal adult diet was not different from that reported previously (Mazomenos, 1984). Pheromone synthesis was cyclical with peaks of pheromone production occurring at days 7th and 17th after emergence.

Effect of Ovarian Development on Pheromone Biosynthesis

Female olive fruit flies irradiated as late pupae with 11 Krad of γ-ray synthesized more pheromone than the control females and pheromone synthesis was not cyclical. Pheromone titre increased as the females aged and reached the maximum level on the 7th day after emergence. The pheromone titre in the rectal gland remained high through the 16th day postemergence and then decreased gradually.

The dose of 11-Krad of γ-ray has been found to inhibit ovarian development in female olive fruit flies (Thomou, 1963), although mating behaviour and competitiveness is much the same as that of non-irradiated females (Economopoulos, 1972). In laboratory bioassays with live irradiated and non-irradiated same age females as bait, irradiated females were attracted more males than the non-irradiated ones (Haniotakis, 1979).
The ovaries are known to produce and receive hormones (Adams, 1980, Fuchs and Kang, 1981). In many insect species ovaries have been shown to play important role for the sexual behaviour and pheromone release. Sexual receptivity of the house fly *Musca domestica* (Adams and Hinz, 1969) is correlated with the stage of ovarian development. *Dacus tryoni* females do not become responsive to the male sex pheromone until their ovaries are in the final stage of maturation (Fletcher and Giannakakis, 1973). In, *Dacus oleae*, ovarian maturation does not appear to control directly female receptivity and pheromone synthesis, irradiated females which ovarian development was inhibited synthesize more pheromone than the normal females. However pheromone synthesis appear to be affected by ovarian maturation.

Olive fruit fly females develop eggs by the process of synchronous vitellogenesis with interovariole synchrony (Fytizas and Bacoynniss, 1970), only the first cycle within the ovarioles develop to maturity and the other cycles remain previtellogenic. Seems possible that as the first cycle of eggs mature a feedback inhibitory humoral factor is released from the ovaries, which block pheromone synthesis, for a period of 10 days. This may explain the cyclicity of pheromone synthesis in normal females.

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![Graph](image)

**Fig. 2.** Titre of 1,7-dioxaspiro (5,5) undecane from 24 h rectal gland washes of female olive fruit fly maintained at various temperatures. The quantity is the mean of five samples of five females each.
Humoral factors secreted by ovaries regulate various developmental process in many insect species. Ovaries of cockroaches and mosquitoes produce humoral factors which control the corpora allata (Stay, et al., 1980, Rossignol et al., 1981). Where this humoral factor release by the olive fruit fly female ovaries control corpora allata or brain activity need to be investigated.

Sucrose fed females produce generally the same quantity of pheromone as the females fed with normal adult diet. However pheromone synthesis by females fed with sucrose is higher during the period where pheromone synthesis by normal females is low. The pheromone production is not cyclical. Sucrose fed females develope few eggs during their life. Mature eggs seem to stimulate the ovaries to release the humoral factor which block the process of pheromone synthesis.

![Graph showing the content of 1,7-dioxaspiro (5,5) undecane in the female olive fruit fly rectal gland at different times of the day. Each point represents the mean of five samples from five females.](image)

Fig. 3. Sex pheromone 1,7-dioxaspiro (5,5) undecane content of the female olive fruit fly rectal gland at different h of the day. Each point is the mean of five samples of five females each.
Effect of Temperature on Pheromone Synthesis

Temperature affected the rate of pheromone synthesis by the olive fruit fly females. Group of females which were maintained at 25°C, 12:12 h light-dark regime, when transferred at 17.5°C, 20°C, 22.5°C, and 35°C with the same light-dark regime for 18 h, prior to analysis, synthesized different quantities of pheromone depending on the temperature placed (Fig. 2). At 17.5°C and 35°C, pheromone synthesis proceeds very slow or ceases since the pheromone gland content measured did not varied much from that found at the end of the light period at 25°C. At the range of 20-25°C females synthesized pheromone. The optimum temperature was proved to be the 25°C where females synthesized the higher quantity of pheromone. These results are consistent with those reported by Pomonis and Mazomenos (1985), where the rate of C14-malonate incorporation, a pheromone precursor into pheromone molecule by explanted female gland, incubated in 0.05 M phosphate buffer pH 7.2, was higher at 25°C than any other temperature tested.


Pheromone release by the female olive fruit flies is regulated by an endogenous rhythm induced by light-dark regime. Pheromone gland content measured during the light period at one h intervals indicated that females release pheromone four h prior to the onset of the scotophase (Fig. 3). Pheromone release rate was gradually accelerated and the peak of pheromone release occurred during the last two h of the photophase. 80% of the female matings has been found by many investigators to occur during these two h. At the end of the photophase pheromone content was very low, pheromone biosynthesis seem to proceed during the scotophase. Glands incubated with C14 malonate during the scotophase converted malonate rapidly to pheromone (Pomonis and Mazomenos, 1985). The maximum conversion of malonate to pheromone occurred within the first four h of the scotophase.

References


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RESPONSES TO ANTIMETABOLITES IN THE MEDITERRANEAN FRUIT FLY LARVAE

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Abstract

Laboratory experiments at fixed environmental conditions (26 ± 1°C, 65 ± 5% RH and 12:12 hrs. L:D regime, 1.900 lux.) were carried out in order to study the effects of Cytarabine (1-(O-D-Arabinofuranosylcytosine) and Ftorafur [5-Fluoro-1-(tetrahydro-2-furyl)-uracil] on the larval and pupal stages of Ceratitis capitata Wied., when these antimitabolites were included in the larval diet: concentrations varied from 4 to 20 ppm (Cytarabine) and from 10 to 50 ppm (Ftorafur).

Preliminary results showed that, in general, the toxic effect with respect to the standard diet, was higher with Cytarabine than with Ftorafur. An increase of 4 days in the pupation time was observed when Cytarabine was included in the larval diet at the rate of 20 ppm, but the same effect was found with Ftorafur at 50 ppm.

On the other hand, a decrease in the weights of 7 day old pupae was obtained with Cytarabine at 4 ppm. However, only a tendency to this decrease was found with Ftorafur at 15 ppm. At the rate of 15 ppm in both cases, the yield of pupae was less than the one obtained with the laboratory standard diet.
ACTION DES GIBBERELLINES, HORMONES DE CROISSANCE VEGETALES, SUR LA PHYSIOLOGIE DE LA REPRODUCTION DE CERATITIS CAPITATA (DIPTERA, TRYPETIDAE)

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Abstract

Effect of Gibberellins, Growth phyto regulators on reproduction physiology of Ceratitis capitata (Diptera, Trypetidae):
The effect of high doses of Gibberellic acid (G.A) on C. capitata results in a great decrease of the fecundity of this pest.
This effect can be explained by a retardation of vitellogenesis observed in treated females (reduction of mature oocytes in comparison with the control).
Dealing with the physiologic mecanism shown, it seems that the G.A, keeping without effect on the vitellogenin and vitellin quality, greatly modify the synthesis rythm of take in of the oocyte.
This may be explained by the interference of G.A with the endocrine process involved in the vitellogenin and vitellin mouvement during the vitellogenesis.

Introduction

Les phyto hormones en général et l'acide gibberellique en particulier, sont de plus en plus utilisés sur différentes cultures légumières, fruitières et même industrielles.
Mais si l'impact de ces phyto hormones a été largement étudié et leur mécanisme d'action élucidé au niveau de la plante, il n'en est rien quant à leur influence sur la faune des arthropodes inféodés aux cultures. Les seuls résultats dont on dispose actuellement découlent de simples observations qui se résument en des constatations de réduction des populations de ravageurs sur les plantes traitées (TURNER & AL. 1970, HENNEBERRY & AL. 1982).
Nous avons constaté que les fortes concentrations d'acide gibberellique provoquent une réduction de la fécondité de la cératite. En utilisant les techniques histologiques et immunoélectrophorétiques nous avons tenté d'élucider les modalités d'action de ces gibberellines chez la femelle de Ceratitisis capitata.

Matériels & Méthodes

Nous avons utilisé des femelles de Ceratitisis qui provenaient d'un élevage permanent maintenu au laboratoire d'entomologie de l'INRAT (CHEIKH & COLL. 1977)
Dès l'émergence les mouches reçoivent une alimentation solide formée de Protéolysat de levure et de sucre et une alimentation liquide formée d'eau pour les témoins et d'une so-
lution d'acide gibberelliqne à 2500 ppm pour les traitées.

Techniques histologiques :
Les abdomens de 10 mouches femelles témoins et 10 femelles traitées sont fixés au Bouin alcoolique pendant 48 heures. Après inclusion à la paraffine, les coupes sériees sont colorées à l'hémalun de MASSON (coloration de Patay) selon GABE (1969). Toutes les coupes sont observées sous microscope pour le comptage des ovocytes.

Préparation des homogenats :
Pour l'analyse immunoélectrophorétique nous avons préparé d'une part des broyats de têtes et de thorax de femelles témoins et de femelles traitées à raison de 50 insectes dans 1 ml de Tampon phosphate de sodium pH7 (FINE 1982,ERIC.C & COL.1983) et d'autre part des broyats d'abdomen selon le même protocole à raison de 50 abdomens dans 1 ml de la solution tampon phosphate de sodium. Les broyats sont alors centrifugés à 20.000 g à 4° pendant 20 minutes et conservés à -26°.

Préparation de l'immunserum :
A un lapin mâle nous injectons un homogenat d'oeufs de Ceratitis dans une solution de Nacl à 9%/oo. Après avoir reçu 3 injections intraveineuses quotidiennes de 0,2 ml, 3 injections intraveineuses hebdomadaires de 0,2 ml, 3 injections sous-cutanées de 0,5 ml d'homogénat d'oeufs mélangées à 0,5 ml d'adjuvant complet de Freund et 1 injection intramusculaire de 0,5 ml d'homogénat d'oeuf mélangée à 0,5 ml d'adjuvant de Freund, le lapin est sacrifié pour récupérer le sang. Après rétraction du caillot, le sérum est centrifugé à 3000 tours par minute pendant 20 mn.

Pour rendre ce sérum spécifique aux femelles nous avons précipité les protéines communes aux oeufs, aux larves et aux mâles et ceci en ajoutant à un volume de serum anti-vitellus, 1 volume d'homogénat de mâle dilué au 1/5 et un volume d'homogénat de larves de 3ème stade dilué au 1/5. Le mélange est incubé pendant une heure à 37° et pendant 12 heures à 4°, l'opération est complétée par une centrifugation à 4000 tours/minute pendant 20 mn. La spécificité du serum est contrôlée par les tests d'OUCHTERLO - NY.

Immunodiffusion radiale (MANCINI 1965) :
On laisse diffuser en chambre humide pendant 48 heures 38 µl d'homogénat de tête et de thorax de femelles témoins et de femelles traitées âgées de 1 à 15 jours sur des plaques en verre (80x 80 mm) couvertes de 14 ml du mélange volume à volume d'agarose à 3% dans un tampon phosphate 0,03 M, NacI 0,1 M, pH8 et d'Immun-serum spécifique aux femelles dilué au 1/3 dans le même tampon. La même opération est réalisée pour 20 µl d'homogénat d'abdomen de femelles témoins et de femelles traitées âgées de 1 à 15 jours. Le diamètre des cercles de précipitation (antigènes-anticorps) sont mesurés sous loupe binoculaire.

Immunoelectrophorèse en ligne :
Sur des lames de 30 x 20 mm sont coulés les différents échantillons d'antigènes (homogénat de tête et de thorax de femelles témoins et de femelles traitées et homogénat d'abdomen de femelles témoins et de femelles traitées) mélangés à l'agarose à 2% (1,5 ml d'agarose + 150 µl d'antigène). Ces rectangles de gel sont portés sur le bord d'une plaque en polyéthylène (94x84mm). Sur le reste de la plaque est coulé 10 ml d'agarose à 2% conte-
nant 1,5 ml d'anti-serum spécifique aux femelles. La migration protéique et la formation des lignes de précipitation se fait sous une tension de 2 volts/cm pendant 20 heures dans un appareil de type Pharmacia. Toutes les lames séchées sont colorées par le Noir-amido et décolorées à l'acide acétique à 7 %.

Résultats

1/ Action de l'acide gibberellique (A.G) sur la fécondité des femelles :

Les fortes doses d'A.G entraînent une diminution nette de la fécondité qui peut être réduite jusqu'à 50 % (Fig.1)

Fig.1 : Courbe de ponte des femelles normales et des femelles traitées aux gibberellines.

Cette action inhibitrice ne se manifeste qu'après au moins 3 jours de traitement à partir de l'émergence, elle est réversible dans le temps, mais la reprise n'est pas complète, la fécondité des femelles traitées reste toujours inférieure à celle des témoins (Fig.2).

105
Moyennes cumulées des œufs par femelles et par jour.

Fig. 2 : Courbe de ponte cumulée des mouches traitées pendant 3, 4 et 5 jours et pendant toute la période de ponte.

L'apport intensif en protéines a tendance à diminuer l'effet du traitement mais n'arrive pas à l'empêcher (Fig.3), ce qui semble tout à fait normal puisque l'on sait qu'un régime pauvre en protéine ralentit la vitellogenèse qui se fait au dépens des réserves protéiques absorbées par la femelle au cours de la vie imaginale (THOMSEN 1952, RAMADE & COL 1973 - RACCAUD 1980).
2/ Action sur l'ovaire :

Le nombre d'ovarioles est approximativement le même pour le lot des femelles traitées et de femelles témoins. La différence se manifeste essentiellement au niveau du nombre d'ovocytes chorionnés, ces ovocytes se reconnaissent sur les coupes transversales par la disparition totale des cellules folliculaires qui les entouraient ainsi que celle des cellules nourricières (Fig. 4).

![Image](image-url)
On note en particulier que 75 à 80 % des ovocytes achèvent leur vitellogenèse chez les mouches témoins alors que 50 à 62 % seulement des ovocytes jeunes arrivent à maturité dans les ovaires des femelles traitées (tableau n° I).

Par ailleurs nous n'avons noté aucune différence morphologique entre les ovaires des femelles traitées et ceux des témoins. En effet tous les stades de développement de l'ovocyte se rencontrent et les cellules ont un aspect tout à fait normal.

3/ Action de l'acide gibberellique sur les vitellogénines (Vg) et les vitellines (Vn) :

Dans l'homogénat de tête et thorax de femelles nous avons mis en évidence 3 vitellogénines immunologiquement différentes entre elles. Ces protéines subissent des remaniements au cours de leur absorption dans les ovaires pour former la majeure partie des vitellines qui ne présentent plus la même mobilité électrophorétiques que leurs précursors, bien qu'elles gardent les mêmes sites antigéniques que ces derniers (BARBOUCHE N. BEN HAMOUDA M.H. 1986). L'action des gibberellines sur le mouvement de ces protéines vitellines a été envisagée suivant deux aspects :

-Aspect qualitatif
La comparaison immunologique des Vg et des Vn de femelles traitées et de femelles témoins ne nous a pas révélé de différences notables. Toutes les fractions protéiques observées chez les mouches normales sont présentes chez les femelles ayant reçu
les giberellines (Fig.5)

Fig.5 : Immunoélectrophorèse en ligne comparative de femelles de Ceratites traitées aux giberellines et des femelles témoins : H.t.th.te : Homogénat de tête et thorax de femelles témoins. H.t.th.tr : Homogénat de tête et thorax de femelles traitées. H.Ab.te : Homogénat d'abdomens de femelles témoins. H.Ab.tr = Homogénat d'abdomens de femelles traitées. Vg = vitellogénine. Vn=vitelline, Av.sp.♂ : Immunserum anti-vitellin spécifique aux femelles.

- Aspect quantitatif

Nous avons examiné d'une part le mouvement des Vg et d'autre part celui des Vn.

* Mouvement des Vg (Fig.6) : 24h. après l'émergence, le taux des Vg dans l'hématolymphe et le corps gras (homogénat de tête et thorax de femelles) est plus faible que chez les témoins. Avec le déclenchement de la ponte (3ème jour après l'émergence), on assiste à une augmentation du titre des Vg qui dépasse même légèrement celui des témoins.

À partir du sixième jour, le taux des vitellogénines chez les insectes traités baisse petit à petit jusqu'à la fin de l'essai alors qu'on le voit oscillier chez les insectes témoins tout en gardant un niveau élevé.

* Mouvement des Vn (Fig.7)

Le taux des Vn dans l'hémogénat d'ovaire de femelles traitées suit le même rythme que celui des témoins, mais il reste plus faible dès le premier jour et demeure ainsi jusqu'à la fin de l'essai.
On remarque en particulier que le taux des Vn reste faible entre le 4ème et le 6ème jour malgré la présence dans l'hémolymphhe de grandes quantités de Vg au cours de cette période (Fig. 6)

Discussion

Les fortes doses de gibberellines inhibent partiellement la fécondité des cératites femelles. Bien que l'inhibition est réver-
sible dans le temps, la fécondité ne reprend pas normalement et reste perturbée tout au long du cycle de ponte. Avec un traitement continu, la fécondité est diminuée d'environ 50%.

Cette inhibition trouve son explication si l'on examine le déroulement de la vitellogenèse chez les femelles traitées :

1/ Au cours de la phase de démarrage de la vitellogenèse, la synthèse des vitelloglobines est plus lente que la normale.

2/ Au déclenchement de la ponte, qui s'accompagne chez la cératite d'une accélération de la vitellogenèse (CAUSSE 1972), on remarque une augmentation du titre des Vg dans l'homogénat de tête et thorax des femelles qui serait probablement dûe à une mauvaise utilisation de ces protéines par l'ovaire. La séquestration dans les ovocytes étant partiellement inhibée, ces vitelloglobines restent alors dans l'hémolymphe où elles s'accumulent.

3/ Au cours du ralentissement normal de la vitellogenèse, on voit qu'il est beaucoup plus accentué chez les femelles traitées aux giberellines.

Quant au mouvement des Vn dans l'ovaire il est en rapport avec son fonctionnement. En effet l'accumulation des Vn dans l'ovocyte ne peut être séparée de la synthèse des Vg au niveau du corps gras. Cependant nos résultats montrent que ces protéines demeurent à un niveau très faible par rapport à celui des témoins bien que le taux des Vg soit élevé en particulier entre le 4ème et le 5ème jour. Ce qui est à mettre en rapport avec une éventuelle action de l'acide gibberellique directement sur l'ovaire.

Les perturbations de la vitellogenèse constatées sur coupes histologiques confirment ces observations.


Cependant ces auteurs n'ont pas indiqué l'impact de ces hormones sur les protéines vitellines.

Nous pensons que les giberellines provoquent des perturbations sur le métabolisme général de l'insecte qui se manifeste au niveau du fonctionnement des organes reproducteurs, or tous les chercheurs pensent qu'il existe une corrélation complexe entre le métabolisme général et le développement ovarien chez les insectes, le tout étant placé sous contrôle hormonal (CASSIER 1967, JOLY 1968).

Nous avons montré que les giberellines provoquent des perturbations au niveau de la synthèse des précurseurs vitellins et entraînent une inhibition partielle de l'activité ovarienne. Ceci est probablement dû à une action au niveau du mécanisme en-

Dans le 2ème cas on peut penser à un phénomène de saturation hormonale puisque l'AG peut avoir le même effet que l'ecdyséron sur la mue tel qu'il a été démontré par CARLISLE & AL (1963) chez le criquet. Ceci serait dû au fait que l'ecdyséron et l'acide gibberellique ont un précurseur commun qui est l'acide mevalonique.

Ces résultats tout en confirmant les travaux de DE MANN & AL (1981) qui ont obtenu un arrêt de la vitellogenèse chez Sarcophaga bullata sous l'action de l'acide abscissique, semblent résulter de conditions physiologiques différentes en ce sens que l'acide abscissique est du point de vue structure plutôt voisin de la J.H alors que l'acide gibberellique se rapproche des ecdystéroïdes.

Ces perturbations de la vitellogenèse sous l'action de l'acide gibberellique pourrait alors résulter soit d'un déficit hormonal (inhibition de la J.H) soit d'une saturation des ecdystéroïdes. Quoiqu'il en soit d'autres travaux sont nécessaires pour éclairer l'une ou l'autre de ces hypothèses.

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BIOLOGICAL EFFECTS OF MICROWAVE TREATMENTS ON PUPAE AND ADULTS OF *CERATITIS CAPITATA* WIED.

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Abstract

Experiments were set up to investigate effects of microwave irradiation on pupae and adults of several ages of *Ceratitis capitata* Wied. Flies were irradiated in a rectangular waveguide applicator (WR 90) with power densities ranging from 8.6 W/cm² to 0.21 W/cm² and different exposure times (from 7.5 s to 20 min). The frequency was 9 GHz. A colony-reared strain of this fly, with no history of insecticide exposure, was used in all tests. The influence of treatments on fecundity, fertility, longevity, sex ratio of progeny, female and male reproductive systems and adult behavior was studied. Also, lethal effects of microwaves were recorded using adults of 2 and 9 days-old and a power density of 8.6 W/cm². The results of our experiments suggested, that biological effects of exposure to microwaves in our conditions, were primarily thermal effects. Not any significant difference (at the 5% level) between both treated and control insects was observed for all studied factors. Neither teratogenic effects of irradiation were recorded for pupae or adults of this fly. Influence of adult age on its sensibility to microwaves was observed. Data could be represented by parallel probit-log dose regression lines of common slope \( b = 5.600 \pm 0.711 \), and 2 days-old adults of *C. capitata* were less sensible to irradiation that the older ones (9 days-old).

Introduction

It has been reported that the application of radio-frequency energy (RF) to insects, may give a great variety of effects on different insect species: **lethal effects** (in *Musca domestica* Ondracek et al., 1976; in *Tribolium confusum* Watters, 1976; in *T. castaneum*, *Oryzaephilus surinamensis*, *Sitophilus granarius* and *Euphestia cautella* Hurlock et al., 1979); **theratogenic effects** (in *Tenebrio molitor* Kadoum et al., 1967 and Liu et al., 1975); **changes on reproduction** (in *T. molitor* Rai et al., 1977; in *T. molitor* and *Pieris brassicae* Fleurant Lessard et al., 1979); **Behavioral changes** (in *Caryedon gonagra* Ondracek et al., 1976), etc.

In this work we have examined effects of "closed space" microwave treatment at 9 GHz in several biological parameters of *Ceratitis capitata* when pupae and adults were irradiated.
Materials and methods

Flies used in all tests, were a colony-reared strain according to Vifuela, 1981 with no history of insecticide exposure. Cultures of C. capitata were maintained in a cabinet with temperature and relative humidity maintained at 25±2°C and 75±5 % respectively and a day/night cycle of 16/8. This cabinet was also used for the posttreatment period.

Irradiation technique and test method

"Closed space" experiments were performed at 9 GHz using a rectangular waveguide applicator WR 90 (2.3x 1.0 cm). The experimental arrangement of the microwave apparatus was the same to that used by Vifuela et al., 1983. A Hewlett-Packard HOI 686 C Oscillator and a Hughes 1277 H Amplifier were used to generate the microwave power.

Polystyrene holders supporting 48 pupae in the center line of waveguide were used. For adults, plastic bases (1x1 cm cross section by 15 cm length) were employed to individualize 8 males and 8 females each time.

Each experiment consisted of 4 replicates/dose level and control insects were kept under similar conditions but shielded against microwaves.

During irradiations, ambient temperature and humidity were not strictly controlled, but the conditions were the same for treated and control insects.

Before and after treatments, the adult diet consisted of a dried mixture of sugar and protein (yeast hydrolyzate enzymatic ICN) in a ratio (w/w) of 4/1 and water (supplied by means of 10% agar).

EXPERIMENT 1: Two sorts of experiments were performed to investigate effects of microwave treatment on several biological parameters of C. capitata: mortality, fecundity, fertility, longevity, sex ratio of progeny, female and male reproductive system and adult behavior:

* 4 days-old pupae were irradiated with incident power levels of 1-0.75-0.50 and 0.25 W (power densities extreme values were 0.86 and 0.21 W/cm² respectively) and exposure times of 10 and 20 minutes; and with an incident power level of 10 W (= 8.6 W/cm²) and exposure times ranging from 60 to 7.5 seconds.

Before treatments, pupae were maintained in groups of 48 in ventilated plastic boxes (9.5 cm in diameter by 1.5 cm high) in the same conditions as previously described.

* 2 days-old adults were treated with incident power levels ranging from 10 to 1.25 W and exposure times from 16 mi to 7.5 s (power density varied between 8.6 and 1.07 W/cm²).

EXPERIMENT 2: Lethal doses of microwaves were recorded using adults of 2 and 9 days-old and an incident power level of 10 W (= 8.6 W/cm²) because it was determined to be the most suitable one from the above mentioned trials. Several doses of irradiation were given to the flies by modifying the exposure times from 7.5 to 60 seconds.
During the pretreatment adult periods, 50 pairs of newly emerged adults from culture boxes, were introduced into cylindrical plastic cages (12 cm in diameter by 5.5 cm high) with a ventilation opening and a lateral egg collector.

For facilitate adult handling, pure CO2 was used as anaesthetic because it is not harmful to this fly according to Viñuela, 1982.

Posttreatment period

After microwave exposure, pupae were maintained in groups of 48 in plastic cages similar to those used in the pretreatment adult period, until adult emergency was reached. Then, adults were handled in the same way as the treated ones.

4 replicates were performed in all cases for the below mentioned trials:

*To study mortality, the number of adults emerging from pupae treatments and the percentage of living adults 24 h. after adult treatments, were recorded. In the last irradiations, when mortality oscillated between 75 and 25%, probit-log dose regression lines were calculated.

*To study adult longevity, fecundity and fertility, 5 pairs of adults/dose level were placed at the rearing conditions until they were 26 days-old and in this moment, the percentage of living adults was recorded.

eggs from these couples were collected and counted daily during 18 days after the first oviposition day (flies of 5 days-old) and adult mortality was recorded at the same time, to obtain the number of eggs per female per day.

At 5, 10, 15 and 20 days from oviposition, 200 eggs were sown on a wet filter paper placed on the bottom of an hermetic plastic cage (9 cm in diameter by 2.5 cm high) and the percentage of egg hatching was recorded at 48 h. after the sowing.

*To study sex ratio of progeny, at 6, 11, 16 and 21 days from oviposition, 2 replicates/treatment of 200 eggs were sown on the rearing larval medium in hermetic plastic boxes (9 cm diam., 5.5 cm high). After adult emergence number of males and females was recorded and the sex ratio was expressed as M/F.

*To study reproductive system, 5 males and 5 females were dissected for each treatment according to the method of Salom, 1974 to observe the development of the testicles and the ovaries and the egg number /ovarie.

Analysis of data

Analysis of variance and Newmann-Keuls multiple range tests were performed to determine significant differences between biological parameters of control and treated insects. Square root transformation was applied before percent data were studied.

Probit regression equations were calculated according to the method of Finney, 1971 to study lethal effects of microwaves on adult flies. Lethal doses (TL) were recorded in seconds of irradiation and the 95% fiducial limits were determined for TL50.

A test for parallelism (Finney, 1971) and a studentized range test (Snedecor and Cohram, 1971) were performed to com-
pare probit lines.

Results and discussion

TABLE I summarizes effects of microwaves on C. capitata when 4 days-old pupae were irradiated at 9 GHz. Data are mean of 4 replicates/dosage ± S.E.

Adult emergence was high in all irradiations except when the highest potency level (10 W) was applied during 60 s. In this case, a higher variability was observed, but a significant reduction (P< 0.05) in the number of developing adults was recorded. Nevertheless, not any significant difference at the above mentioned level was obtained for the others biological parameters studied in these series of irradiations:

* Fecundity, expressed by cumulative number of eggs/female between 5 and 22 days-old from 5 females, was near 800 eggs in all cases.

* The percentage of egg hatching (from 200 eggs) was near 80%. As the were not significant differences between treatment efficacies due to the sowing day (females of 5-10-15 and 20 days old) data were pooled in TABLE I.

* 26 days after adult emergence, the percentage of living adults oscillated between 82.5 and 92.5%.

The results of the microwave exposure of 2 days-old adults at 9 GHz with different potency levels and exposure times are given on TABLE II.

The number of living adults 24 h. after irradiations was slightly reduced when the potency applied was 2.5 W and the exposure times were 4 and 2 mi. A higher reduction was recorded for the previous potency when the irradiation times were 8 and 16 mi or when the potency applied was 10 W. Nevertheless not any difference among treatments was detected when 1.25 and 5 W were given to the flies, for all exposure times used. Then, survival of adults after microwave treatment, depends on the product of potency x exposure time, although as we can see on TABLE II, high potency levels are more effective for C. capitata when the previous product has the same value (5 W·15 s=10 W·7.5 s), because the mortality recorded was higher in the last case. In this fly, this seems to be true although a different irradiation technique was applied, because the results agree with our previously reported findings when effects of an "open space" treatment were investigated using adults of C. capitata (Viñuela et al., 1983).

As in the case of microwave treated pupae, statistical analysis of the others biological parameters reported in TABLE II, when adults were irradiated, brought about no significantly differences (P< 0.05) among treatments.

Aichmann et al., 1984 irradiating Drosophila melanogaster adults at 40 GHz and low power levels, also found that the fertility of irradiated flies and of their 1st and 2nd filial generation was no affected by RF treatment. In contrast with these results, Dardalhon et al., 1979 working with the same fly and
TABLE I: Effects of microwaves on several biological parameters of *C. capitata* when 4 days-old pupae were irradiated (means of 4 replicates ± S.E.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% adult emergence (1)</th>
<th>Fecundity (2)</th>
<th>Fertility (3)</th>
<th>Longevity (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>91.7±2.97 a</td>
<td>784.4±27.07</td>
<td>80.8±1.15</td>
<td>88.3±5.20</td>
</tr>
<tr>
<td>10W-7.5 s</td>
<td>90.7±4.45 a</td>
<td>767.4±27.61</td>
<td>80.6±1.77</td>
<td>82.5±9.57</td>
</tr>
<tr>
<td>10W-15 s</td>
<td>91.7±2.97 a</td>
<td>795.4±26.45</td>
<td>81.2±2.47</td>
<td>85.0±10.0</td>
</tr>
<tr>
<td>10W-30 s</td>
<td>90.7±4.45 a</td>
<td>784.3±19.78</td>
<td>81.5±1.55</td>
<td>85.0±5.77</td>
</tr>
<tr>
<td>10W-60 s</td>
<td>55.2±9.32 b</td>
<td>782.0±34.19</td>
<td>81.6±1.35</td>
<td>87.5±9.57</td>
</tr>
<tr>
<td>10 mi-0.25W</td>
<td>90.6±1.48 a</td>
<td>769.3±13.28</td>
<td>80.4±1.19</td>
<td>87.5±5.00</td>
</tr>
<tr>
<td>10 mi-0.50W</td>
<td>90.6±1.41 a</td>
<td>749.5±56.52</td>
<td>80.1±1.75</td>
<td>87.5±9.57</td>
</tr>
<tr>
<td>10 mi-0.75W</td>
<td>93.8±5.87 a</td>
<td>741.3±45.10</td>
<td>80.6±1.18</td>
<td>90.0±8.16</td>
</tr>
<tr>
<td>10 mi-1.00W</td>
<td>91.7±2.90 a</td>
<td>752.5±30.51</td>
<td>79.8±1.04</td>
<td>90.0±8.16</td>
</tr>
<tr>
<td>20 mi-0.25W</td>
<td>91.7±2.90 a</td>
<td>803.0±34.04</td>
<td>80.9±1.38</td>
<td>85.0±5.77</td>
</tr>
<tr>
<td>20 mi-0.50W</td>
<td>93.7±2.97 a</td>
<td>783.4±44.45</td>
<td>81.1±1.72</td>
<td>87.5±5.00</td>
</tr>
<tr>
<td>20 mi-0.75W</td>
<td>90.6±1.41 a</td>
<td>804.7±23.22</td>
<td>79.6±0.89</td>
<td>92.5±5.00</td>
</tr>
<tr>
<td>20 mi-1.00W</td>
<td>93.8±5.87 a</td>
<td>816.9±29.55</td>
<td>80.6±1.78</td>
<td>87.5±9.57</td>
</tr>
</tbody>
</table>

(1) Means followed same letter not significantly different (P<0.05) compared by Newman-Keuls test.

(2) Fecundity was expressed by cumulative number of eggs/♀ between 5 and 22 days-old from 5 ♀♀.

(3) Fertility was recorded as percentage of egg eclosion from 200 eggs.

(4) Adult longevity was recorded as percentage of living adults of 26 days-old.
TABLE II: Effects of microwaves on several biological parameters of *C. capitata* when 2 days-old adults were irradiated (means of 4 replicates ± S.E.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% living adults at 24 h. (1)</th>
<th>Fecundity (2)</th>
<th>Fertility (3)</th>
<th>Longevity (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.0±0.86 a</td>
<td>792.7±63.66</td>
<td>82.2±1.51</td>
<td>93.3±1.44</td>
</tr>
<tr>
<td>1.25W-30 s</td>
<td>100.0±0.00 a</td>
<td>808.3±17.33</td>
<td>81.1±2.23</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td>1.25W-1 mi</td>
<td>100.0±0.00 a</td>
<td>817.6±34.69</td>
<td>81.3±1.82</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td>1.25W-2 mi</td>
<td>98.5±3.10 a</td>
<td>850.2±35.17</td>
<td>81.9±3.51</td>
<td>95.5±5.77</td>
</tr>
<tr>
<td>1.25W-4 mi</td>
<td>98.5±3.10 a</td>
<td>829.7±11.44</td>
<td>82.3±2.30</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td>1.25W-8 mi</td>
<td>98.5±3.10 a</td>
<td>838.9±12.86</td>
<td>80.0±1.05</td>
<td>100.0±0.00</td>
</tr>
<tr>
<td>2.50W-30 s</td>
<td>96.8±6.25 a</td>
<td>815.19±18.35</td>
<td>81.9±1.44</td>
<td>95.5±5.77</td>
</tr>
<tr>
<td>2.50W-1 mi</td>
<td>98.5±3.10 a</td>
<td>791.4±21.96</td>
<td>81.0±0.71</td>
<td>92.5±9.57</td>
</tr>
<tr>
<td>2.50W-2 mi</td>
<td>82.8±9.39 b</td>
<td>832.3±19.76</td>
<td>82.1±2.03</td>
<td>90.0±8.16</td>
</tr>
<tr>
<td>2.50W-4 mi</td>
<td>81.3±11.40 b</td>
<td>814.9±18.19</td>
<td>83.3±3.06</td>
<td>95.0±5.77</td>
</tr>
<tr>
<td>2.50W-8 mi</td>
<td>75.0±8.81 c</td>
<td>815.2±44.09</td>
<td>83.3±1.39</td>
<td>85.0±5.77</td>
</tr>
<tr>
<td>2.50W-16 mi</td>
<td>71.9±3.58 c</td>
<td>809.8±39.53</td>
<td>82.6±2.29</td>
<td>85.0±5.77</td>
</tr>
<tr>
<td>5.00W-7.5 s</td>
<td>96.9±3.58 a</td>
<td>748.4±27.54</td>
<td>82.3±3.18</td>
<td>95.0±5.77</td>
</tr>
<tr>
<td>5.00W-15 s</td>
<td>96.9±3.58 a</td>
<td>751.5±21.22</td>
<td>80.8±1.98</td>
<td>95.0±5.77</td>
</tr>
<tr>
<td>10.00W-7.5 s</td>
<td>75.1±7.22 c</td>
<td>734.8±12.48</td>
<td>82.4±2.43</td>
<td>87.5±9.57</td>
</tr>
</tbody>
</table>

(1) Means followed same letter not significantly different (P < 0.05) compared by Newman-Keuls
(2) Fecundity was expressed by cumulative number of eggs / ♀ between 5 and 22 days-old from ♀
(3) Fertility was recorded as percentage of egg hatching from 200 eggs
(4) Adult longevity was recorded as percentage of living adults of 26 days-old
a frequency of 17 GHz reported an increase in fertility when females were exposed to microwaves and according to these authors it may be explained by the assumption that microwaves can interfere with the metabolism of hormones. A metabolic modification was also observed by Fleurat Lessard et al., 1979 in T. molitor and P. brassicae following RF treatments.

In their studies, Dardalhon et al., 1979 also reported that RF treatments did not induce teratological changes in the adults and this agree with our observations on irradiated pupae and adults of C. capitata, but morphological abnormalities have been reported in T. molitor by several authors (Kadoum et al., 1967; D' Ambrosio and La Manna, 1977).

A reduction on adult fecundity of T. molitor following microwavetreatment and a lower percent egg hatch, were observed by Rai et al., 1977. They pointed out that it could have resulted from the spermatocrit inactivity observed or from the inability of males to transfer spermatocrit successfully because an alteration of adult behavior was observed. When the reproductive system was studied, these authors did not find morphological damage to spermatocrit from adults exposed to RF. In a series of experiments previously described by Rai et al., 1975, it could also be observed a reduction in both the size of ovaries and the number of eggs which developed in females that emerged from treated larvae of T. molitor. Nevertheless in C. capitata not any malformation on both male and female reproductive system or adult behavior alteration could be observed following pupae or adult irradiations. This agree with the results reported by Whitney and Zharadly, 1984 working with T. molitor pupae and several developmental stages of Dendroctonus ponderosae when they were irradiated at frequencies of 10-35 and 74 GHz. They attribute the lack of response to RF to the physiological condition of the cells and tissues being irradiated, because the response to low level RF is a strong function of them.

With regards to the number of developing adult females and males, no differences in mortality of sexes were detected as compared to the controls among treatments or among egg sowing days/treatment (females of 6-11-16 and 21 days-old), after pupae or adult irradiations. The sex ratio value was always near 0.5 (♂♂/♀♀ + ♀♀). Contrary to the present results, Dardalhon et al., 1979 found a slight alteration of the sex ratio after treatment of D. melanogaster eggs, because a higher male mortality was recorded. The differences could be asigned to variations in irradiation technique (open space-17 GHz) and in developmental stage and specie used.

A reduction on adult longevity of T. molitor has been observed by Carpenter and Livistone, 1971 when pupae of this insect were irradiated at 10 GHz and 20 mW. In C. capitata not any alteration of adult longevity has been found in our experiences and this agree with Gary and Westerdahl, 1981 works with Apis mellifera.

It can be concluded that there is contradictory results with regards to biological effects of microwaves depending
mainly on the specie used and that in C. capitata these effects are primarily thermal ones, because not any effect was observed in this fly when the low potency levels were applied.

Lethal doses: On FIG. 1 we can see the probit-log dose regression lines for the experiments performed with 2 and 9 days-old adults. The replicates of each dosage were pooled and doses were expressed as exposure time in seconds.

A test for parallelism showed that data could be represented by parallel lines of common slope $b=5.600 \pm 0.711$ ($\chi^2$ value for parallelism $=0.186$ with 1 d.f.). According to a studentized range test, young adults were significantly slightly more tolerant to RF treatment than the old ones ($F=22.37$ with 1 and 3 d.f.) ($P<0.05$) and the same results were obtained by Frings, 1952 with Oncopeltus fasciatus and by Tateya and Takano, 1977 with T. confusum. The TL50 and their fiducial limits were: 10.443 (11.904; 9.147) to 2 days-old adults and 9.274 (10.014; 8.453) to 9 days old ones, then adult susceptibility to RF is the same based on the criterion of overlapping fiducial limits of the TL50 values, and this agree with Nelson and Kantack, 1966 works with S. grinarius, because these authors do not report mortality differences among ages.

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STUDIES ON THE MEDITERRANEAN FRUIT FLY DARK PUPAE, INHERITANCE, BIOLOGY AND ECOLOGICAL FACTORS

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Abstract

Dark pupae were brought from upper Egypt and a strain has been established from them for two years. The first generation from these pupae have five degrees of colour, yellow, dark yellow, brown, dark brown and black. The adult emerged from black pupa was darker and thorax was black.

In crosses black pupae and wild type pupae (dark yellow) the majority of the first generation consisted of dark yellow brown and dark brown and few numbers of yellow and black pupae.

Insects from dark yellow, brown and black pupae were tested for fecundity, fertility and longevity. The results indicated that all the groups laid equal number of eggs. The generation time of dark yellow, brown and black pupae was (egg to larva 3-4 days, larva to pupae 7-8 days, pupa to adult 7-9 days and oviposition period 4-5 days). Sex ratio did not deviate from 1:1 ratio throughout the test.

The effect of some ecological factors as components of the diet and temperature during the larval development have some effect on the degree of colour of pupae. It was found that the increase of acidity and decrease of temperature increased the number of yellow pupae from wild type. Black pupae were sensitive to low temperature and percentage emergence was 16% at 18°C.

Introduction

Some morphological mutants of the mediterranean fruit-fly have been described since 1973 as: "narrow orbital bristle" (Cavicchi 1973) "apricot eye", "dark pupae" and "double chaetae" (Rossler and Koltnin 1976), "white eye" (sharp and chambers 1973), "white pupae" (Rossler 1979). Recently pupal colour can be linked to sex chromosome through its segregation of sexes is developed.

Materials & Methods

1- Breeding and selection:
A strain from upper Egypt was brought during 1984 and few number of dark pupae were segregated and reared on the standard artificial medium in the laboratory of Plant Protection Research Institute, Agriculture Research centre of Egypt in Cairo. The resulting pupae were selected for dark colour and reared. Selection for the darkest colour was continued for six generations.
2- Inheritance of the mutation:
Crossing between 15 black females with 15 dark yellow males (wild type) and 15 dark yellow females with 15 black males was carried out to study the mode of inheritance of dark pupae as follow. Fifteen pairs of flies were put in a cage and supplied with sugar and enzymatic yeast hydrolyzate. The fourth day of emergence, eggs of each cage were daily collected and placed on the standard artificial medium of our laboratory. The recovered pupae were collected, segregated and counted according to their colours (yellow, dark yellow, brown, dark brown, and black). The experiment was replicated three times. The F2 testcross was carried out by mass reared of 100 of the brown (medium) colour pupae, eggs were placed on the diets and pupae segregated as before.

3- Recovery
Survival of immature stages was compared by placing 100 eggs of each phenotype (dark yellow brown and black) on 20 gm larval diet at 25+1°C. The test was carried out in ten replicates (1000 eggs), egg hatch, pupation and adult emergence was recorded. Survival rate from egg to adult was calculated.

4- Test for viability:
Incubation period of eggs, larval duration, pupal duration, sex ratio, percentage emergence of adults, total number of eggs per female, hatchability and longevity of adults for 100 individuals of each phenotype (dark yellow, brown and black) was carried out at 25°C.

5- Ecological factors (nutrition & Temperature):
Diets of different components were used to study the effect of carbohydrate (molasses), protein (brewer's yeast) contents and PH on pupal phenotype. In this experiment five different larval diets were used, the diets were as follow.
1- 500 gm molasses + 300 gm brewer's yeast
2- 250 gm molasses + 300 gm brewer's yeast +1000 gm wheat bran +2000cm
3- 500 gm molasses + 100 gm brewer's yeast +1000 gm wheat bran
4- 250 gm molasses + 100 gm brewer's yeast +1000 gm wheat bran

5- 250 gm molasses + 100 gm brewer's yeast +1000 gm wheat bran +2000cm water + sodium benzoate 10 gm

On each diet 100 eggs from each phenotype (dark yellow, brown and black pupae) were allowed to hatch and develop at 25 ± 1°C. The pupae recovered were classified to the different phenotypes and counted. The experiments were replicated ten times.

The same series of experiments were carried out at 18°C and pupae recovered were segregated as before.

Results & Discussion

1- Description:
Dark pupae are individuals different from the wild type pupae in colour. Adults emerging from it are darker in appearance. The thorax and the ovipositor of the fly is black.

Normal diet: 250 gm molasses + 250 gm brewer's yeast + 1000 gm wheat bran + 2000 water + 10 gm sodium benzoate
and the coloured spots on the wings are more visible (Fig 1&2)

Figure 1: Phenotypes of the mediterraenan fruit-fly pupae C. capitata

2- Inheritance of the mutation:

The results of crosses between the black pupae mutation and the wild type deviated from the expected F₁ progeny (Table 1). If the mutant was recessive the F₁ progeny would be of the wild type dark yellow and if it was incomplete dominance with a single gene the F₁ progeny would be the medium colour brown. The F₂ testcross clearly indicates five phenotypes yellow, dark yellow, brown, dark brown and black, in a population of 1634 pupae, there were 91 yellow 369 dark yellow (wild type), 654 brown, 410 dark brown and 110 black. This ratio does not significantly deviate from the ratio 1:4:6:4:1 (x² 0.05 = 8.3). It is thus assumed that the black pupae colour is caused by the operation of one, two or more dominant independently inherited genes. The presence of a single gene is sufficient to cause the development of black colour. The pupae that have two genes are darker than those with one gene, and pupae with three are darker than those with two and so on.
These multiple genes have cumulative effects. The results in the table also suggest that the wild type parents are not homozygous (Table I).

3. Recovery

The recovered pupae are equal in weight as mean weight of the wild type is 9.1 mg, compared to 9.0 and 9.3 mg for the brown and black phenotypes. Percentage recovery from egg to adult in a diet composed of 250 gm brewer's yeast (250 gm molasses, 1000 wheat bran, 2000 cm. water 10 HCl and 10 gm
Table I: Phenotype ratios of the $F_2$ offspring in the crosses between wild type pupae and black pupae strain of the Mediterranean fruit-fly

<table>
<thead>
<tr>
<th>Parental phenotypes</th>
<th>Cross No. of pairs</th>
<th>$F_1$ Phenotypes</th>
<th>$X^2_{tab.}(0.05)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Y</td>
<td>D.Y</td>
</tr>
<tr>
<td>Wild type Black oo oo</td>
<td>1</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9</td>
<td>145</td>
</tr>
<tr>
<td>Black wild type oo oo</td>
<td>1</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6</td>
<td>130</td>
</tr>
</tbody>
</table>

$F_2$.obs. | $X^2$ |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>369</td>
</tr>
<tr>
<td>exp.</td>
<td>102.1</td>
</tr>
</tbody>
</table>

Sodium benzoate was 30.5, 29.1 and 32.2 of the dark yellow (wild type), brown and black respectively.

4. Viability:

Adults from black, brown and dark yellow pupae were studied for viability. Females from black and brown pupae laid as many eggs as the wild type (dark yellow) females. Incubation period of eggs, larval and pupal periods are equal to the wild type. The sex ratio does not deviate from the normal 1:1. Percentage hatching of eggs is the same. It is concluded that colour of pupae does not decrease viability of insects (Table II).

Table II: Biological characters of different colour strains of the Mediterranean fruit-fly C. capitata

<table>
<thead>
<tr>
<th>Biological characters</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark yellow</td>
</tr>
<tr>
<td>Incubation period of eggs/days</td>
<td>3.0</td>
</tr>
<tr>
<td>Larval period/days</td>
<td>8.4</td>
</tr>
<tr>
<td>Pupal period/days</td>
<td>9.2</td>
</tr>
<tr>
<td>Percentage emergence</td>
<td>95.0</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>1:1.1</td>
</tr>
<tr>
<td>Total number of eggs per female</td>
<td>404</td>
</tr>
<tr>
<td>Hatchability of eggs</td>
<td>88.9</td>
</tr>
</tbody>
</table>
5/ Ecological factors (nutrition and temperature)

1- Carbohydrate, protein contents and PH

When eggs of dark yellow phenotype were placed on two diets varied in carbohydrate contents (500 gm molasses : 300 gm brewer's yeast & 250 gm molasses : 300 gm brewer's yeast). The recovered pupae clearly indicate that the increase of carbohydrate increases the dark yellow from 60.9% to 92.0%. The same result was also obtained when little amount of brewer's yeast (100 gm) was used with the two amounts of molasses (500 & 250 gm). These results clearly show that the increase of carbohydrate increases the darker colours (Table III).

Table III: Effect of carbohydrate, protein contents and PH of the diet on pupal colour of the mediterranean fruit-fly (dark yellow parents).

<table>
<thead>
<tr>
<th>No.</th>
<th>C&amp;P per 1000 w.b.gm</th>
<th>Progeny %</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>y</td>
<td>DY</td>
</tr>
<tr>
<td>1</td>
<td>500:300</td>
<td>3.3</td>
<td>92.0</td>
</tr>
<tr>
<td>2</td>
<td>250:300</td>
<td>25.6</td>
<td>60.6</td>
</tr>
<tr>
<td></td>
<td>(PH 5.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>500:100</td>
<td>10.4</td>
<td>44.8</td>
</tr>
<tr>
<td>4</td>
<td>250:100</td>
<td>68.3</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>(PH 5.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>250:300</td>
<td>25.6</td>
<td>60.6</td>
</tr>
<tr>
<td>6</td>
<td>250:100</td>
<td>68.3</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>(PH 5.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>250:100</td>
<td>15.2</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td>(PH 4.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concerning the brown phenotype parents. Table (IV) shows that the black individuals increase with the increase of carbohydrate and protein (diet 1&2). The dark brown individuals increase with the increase of carbohydrate, when little amount of protein was used (diet 3&4). On the other hand the increase of protein from 100 gm to 300 gm increase the dark brown (diet 5&6). With PH 4.5 the black individuals increase. On the whole, one can conclude that the increase of carbohydrate and protein increase the black pupae and the increase of carbohydrate or protein increases the dark brown ones.

Diet components have little effect on the pupal colour with the black phenotype parents. This is clear from X² values (see table III, IV, V).

2- Temperature:

2-1 Pupal colour:

Low temperature does not affect pupal colour of progeny from dark yellow parents, when used high amount of protein, and has slight effect in case of increasing or decreasing carbohydrate and protein contents. It has great effect when high amount of carbohydrate with little amount of protein was used. The decrease of temperature and PH increase the yellow individuals (diet 5) (Table VI).
Table IV : Effect of carbohydrate, protein contents and PH of the diet on pupal colour of the mediterranean fruit-fly (brown parents)

<table>
<thead>
<tr>
<th>No.</th>
<th>C&amp;P per 1000 w.b. gm</th>
<th>Progeny %</th>
<th>x²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Y</td>
<td>DY</td>
</tr>
<tr>
<td>1</td>
<td>500:300</td>
<td>0.0</td>
<td>5.1</td>
</tr>
<tr>
<td>2</td>
<td>250:300</td>
<td>0.0</td>
<td>12.6</td>
</tr>
<tr>
<td>3</td>
<td>(PH 5.5)</td>
<td>0.0</td>
<td>15.8</td>
</tr>
<tr>
<td>4</td>
<td>250:100</td>
<td>4.6</td>
<td>17.7</td>
</tr>
<tr>
<td>5</td>
<td>(PH 5.5)</td>
<td>0.0</td>
<td>12.6</td>
</tr>
<tr>
<td>6</td>
<td>250:100</td>
<td>4.6</td>
<td>17.7</td>
</tr>
<tr>
<td>7</td>
<td>(PH 5.5)</td>
<td>0.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Table V : Effect of carbohydrate, protein contents and PH of the diet on pupal colour of the mediterranean fruit-fly (black parents)

<table>
<thead>
<tr>
<th>No.</th>
<th>C&amp;P Per 1000 w.b.gm</th>
<th>Progeny %</th>
<th>x²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Y</td>
<td>DY</td>
</tr>
<tr>
<td>1</td>
<td>500:300</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>250:300</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>(PH 5.5)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>250:100</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>(PH 5.5)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>250:100</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>(PH 5.5)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table VI : Effect of temperature during larval development on pupal colour of the mediterranean fruit-fly (dark yellow parents)

<table>
<thead>
<tr>
<th>No.</th>
<th>Temp. at °C</th>
<th>Diet components C&amp;P gm.</th>
<th>Progeny %</th>
<th>x²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>DY</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>500:300</td>
<td>3.3</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>18.8</td>
<td>75.2</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>250:300</td>
<td>25.6</td>
<td>60.6</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>25.0</td>
<td>66.2</td>
<td>8.8</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>250:100</td>
<td>68.3</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>84.5</td>
<td>9.5</td>
<td>6.0</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>500:100</td>
<td>10.4</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>29.1</td>
<td>55.4</td>
<td>9.1</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>250:100</td>
<td>15.2</td>
<td>77.0</td>
</tr>
<tr>
<td></td>
<td>18 (PH 4.5)</td>
<td>93.5</td>
<td>3.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

131
With brown parents, temperature has some effect (Table VII). The increase of temperature with the increase of protein and carbohydrate increases the black individuals from 5.4% at 18°C to 26.1% at 25°C (diet no 1). Also at pH 4.5 temperature at 18°C decreases the black individuals from 43.9% to 0.0% (diet no 5).

Table VII: Effect of temperature during larval development on pupal colour of the mediterranean fruit-fly (brown parents)

<table>
<thead>
<tr>
<th>No.</th>
<th>Temp</th>
<th>Diet Components</th>
<th>Y</th>
<th>DY</th>
<th>Br.</th>
<th>DBr.</th>
<th>Bl</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25°C</td>
<td>500:300</td>
<td>0.0</td>
<td>5.1</td>
<td>38.3</td>
<td>30.5</td>
<td>26.1</td>
<td>132.6</td>
</tr>
<tr>
<td></td>
<td>18°C</td>
<td></td>
<td>1.9</td>
<td>6.9</td>
<td>74.6</td>
<td>11.2</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25°C</td>
<td>250:300</td>
<td>0.0</td>
<td>12.6</td>
<td>12.6</td>
<td>63.7</td>
<td>11.1</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>18°C</td>
<td></td>
<td>3.4</td>
<td>6.2</td>
<td>15.5</td>
<td>65.0</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25°C</td>
<td>250:100</td>
<td>4.6</td>
<td>17.7</td>
<td>66.3</td>
<td>11.4</td>
<td>0.0</td>
<td>4.77</td>
</tr>
<tr>
<td></td>
<td>18°C</td>
<td></td>
<td>2.1</td>
<td>16.2</td>
<td>72.5</td>
<td>9.2</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>25°C</td>
<td>500:100</td>
<td>0.0</td>
<td>15.8</td>
<td>40.2</td>
<td>44.0</td>
<td>0.0</td>
<td>103.2</td>
</tr>
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<td>18°C</td>
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<td>2.4</td>
<td>9.5</td>
<td>11.0</td>
<td>64.6</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25°C</td>
<td>250:100</td>
<td>0.0</td>
<td>4.9</td>
<td>9.8</td>
<td>41.4</td>
<td>43.9</td>
<td>566.5</td>
</tr>
<tr>
<td></td>
<td>18°C</td>
<td>(PH 4.5)</td>
<td>0.0</td>
<td>0.0</td>
<td>72.7</td>
<td>27.3</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

X² values clearly indicate that with black parents the temperature has great effect in all the diets used. With high amounts of protein and carbohydrate contents, raising the temperature from 18°C to 25°C increases the black individuals from 3.5% to 86.3%. It is concluded that the high temperature, alone with the black parents affects pupal colour (Table VIII).

Table VIII: Effect of temperature during larval development on pupal colour of the mediterranean fruit-fly (black parents)

<table>
<thead>
<tr>
<th>No.</th>
<th>Temp</th>
<th>Diet Components</th>
<th>Y</th>
<th>YD</th>
<th>Br.</th>
<th>DBr.</th>
<th>Bl</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25°C</td>
<td>500:300</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>13.7</td>
<td>86.3</td>
<td>2053.6</td>
</tr>
<tr>
<td></td>
<td>18°C</td>
<td></td>
<td>0.0</td>
<td>1.0</td>
<td>88.2</td>
<td>7.3</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25°C</td>
<td>250:300</td>
<td>0.0</td>
<td>0.0</td>
<td>7.6</td>
<td>28.6</td>
<td>63.8</td>
<td>204.8</td>
</tr>
<tr>
<td></td>
<td>18°C</td>
<td></td>
<td>0.0</td>
<td>14.3</td>
<td>57.1</td>
<td>7.2</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25°C</td>
<td>250:100</td>
<td>0.0</td>
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132
2-2 Percentage emergence:
Emergence of different phenotypes of pupae dark yellow (wild type), brown and black are normal at 25 °C, (95.0% - 96.1% & 93.8% respectively), but when held at 18 °C percentage emergence of the black ones decreased to 16.7 the brown to 42.4 and the dark yellow to 78.9. Black pupae show high sensitivity to low temperature.

It is thus concluded that pupal colour of the Mediterranean fruit-fly is inherited through multiple genes in conjunction with such factors as nutrition and temperature.

References


Y: Yellow DY: Dark yellow Br: Brown DBr: Dark brown BL: Black
C: Carbohydrate (molasses)
P: Protein (brewer's yeast)
W.b: Wheat bran
GENETICS
AN ANALYSIS OF YOLK PROTEINS FROM THE CARIBBEAN FRUIT FLY, ANASTREPHA SUSPENSA

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Abstract

An analysis of the regulation of yolk protein (YP) synthesis in the Caribbean fruit fly, Anastrepha suspensa (Loew), has been initiated. The major polypeptide constituent of oviposited eggs and vitellogenic ovaries, having a molecular mass of approximately 48 kDa, was identified and isolated by gel-filtration and ion-exchange column chromatography, and SDS-polyacrylamide gel electrophoresis. A second less abundant polypeptide having a molecular mass between 47 and 48 kDa was detected also. The major site of synthesis of these polypeptides was the ovary with the first appreciable increase in synthesis occurring at 4 to 5 days after adult eclosion. The polypeptides also were produced by adult fat body and found in the hemolymph, but at markedly lower levels compared to ovaries. Minor denatured hemolymph polypeptides in males co-migrated with the YPs, but their identity awaits immunological analysis. Female and male abdomens isolated from 3- to 5-day-old adults were tested for YP synthesis in response to 20-hydroxyecdysone and a juvenile hormone analog. The synthetic rate of fat body and ovarian YP did not differ significantly between untreated and hormone treated abdomens.

Introduction

The yolk proteins (YP) of a variety of oviparous insects have been subject to considerable analysis with regard to their temporal, spatial, hormonal, and sex-specific regulation. These studies not only elucidate an important developmental process, but also have the potential to reveal mechanisms that may be used to manipulate the reproductive capacity of economically important insects.

In particular, isolation and identification of YP gene coding and regulatory regions may allow manipulation of the YP gene product or allow placement of a selectable gene product under YP promoter regulation. Such a YP promoter gene-chimera that offers conditional expression for selection could have potential use in a genetic sexing program. Identification, isolation, and analysis of the YP genes for molecular biological manipulation depends upon first identifying and isolating the YP gene product. Potential uses for the YP regulatory promoter region depend upon defining the physiology of YP synthesis in terms of time and sites of synthesis, sex-specificity, and hormonal control.

For most insects, the adult fat body is a major site of YP synthesis, releasing vitellogenin into the hemolymph for transport to the maturing oocytes where it is taken up and sequestered. Indeed, vitellogenin has been defined as those proteins found in the hemolymph
of reproductively active females that are precursors to the vitellin found in the egg (Telfer, 1954). Within the ovaries, the follicular epithelium also can be a site of YP production in some insects, either sharing the duty of YP synthesis with the fat body, as occurs in several Drosophila species (Brennan et al., 1982; Srdic et al., 1979) or producing unique proteins as occurs in the lepidopterans (Ono et al., 1975; Bast and Telfer, 1976; Shirk et al., 1984). In a variety of insects, 20-hydroxyecdysone (20-HE), juvenile hormone (JH), and neurohormonal factors are required to initiate and maintain the vitellogenic process, although the timing, function, and site of action of these hormones varies considerably, or is not understood clearly (Engelmann, 1983; Girardie, 1983; Hagedorn, 1985).

This report discusses our initial efforts to define the vitellogenic process in the Caribbean fruit fly, Anastrepha suspensa, with the ultimate aim of isolating and characterizing the YP genes. Thus, we placed a primary emphasis on characterizing the major yolk proteins. In contrast to other insects, the major 48 kDa yolk polypeptide found in oviposited eggs is synthesized predominantly by the ovary and not the fat body, and its synthesis is not promoted significantly by either 20-HE or JH, although females do contain high ecdysteroid titers after vitellogenesis has begun.

Methods

YP identification and isolation

One-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to O'Farrell (1975) with modifications and sample preparation described in Belote et al. (1985). Gels were either straight 10% acrylamide or 9-12% gradients as noted in the text. YP purification was achieved as described by Shirk et al. (1984). Crude egg extract proteins were precipitated in 75% ammonium sulfate and resolved by S-300 Sephacryl gel permeation chromatography. YP containing fractions were detected by UV absorbence (280 nm) and verified by SDS-PAGE. S-300 peak fractions were pooled, concentrated by dialysis, and applied to a DEAE Sepharose C1-6B ion-exchange column and eluted with increasing concentrations of KCl (80 ml steps of 50, 100, 180, and 500 mM KCl). YP containing fractions were detected by UV absorbence and SDS-PAGE. Immunoblots were performed according to Towbin et al. (1979) using antisera raised against Drosophila melanogaster egg extract (Dm anti-yolk; J. H. Postlethwait, unpublished). Relative measures of protein synthesis were determined by densitometry of autoradiograms.

Sample preparation

Samples were prepared from the hemolymph or tissues diluted or dissected, respectively, in insect Ringer's solution. Isolated abdomens were prepared by tying nylon monofilament between the abdomen and thorax, cutting away the thorax, and incubated as described by Handler and Postlethwait (1977, 1978). Animals or abdomens were injected with 0.5 to 1.0 µl of 20-HE or 35S-methionine dissolved in Ringer's. JH analog methoprene; (ZR515) was dissolved in acetone and topically applied.
Radioimmunoassay for ecdysteroids

The radioimmunoassay for ecdysteroids was performed according to Borst and O'Connor (1974) as described by Handler (1982), except that samples of approximately 100 mg/ml homogenized tissue in 70% methanol were prepared, with 0.15 ml duplicate aliquots assayed. DHS I-15 antiserum was utilized (Soumoff et al., 1981).

Results and Discussion

Yolk polypeptide identification and isolation

The major yolk polypeptide constituents of A. suspensa ovaries and oviposited eggs were identified by SDS-PAGE. Figures 1 and 3 show that the predominant polypeptide in both oviposited eggs and vitellogenic ovaries had an electrophoretic mobility corresponding to a molecular mass of approximately 48,000 Daltons. Several other polypeptides found in eggs, ovaries, female hemolymph and male hemolymph, also co-migrated on SDS-PAGE. While some of these may be minor yolk constituents, our present analysis focused on the major 48 kDa YP. Identification of vitellogenin in the Caribfly was confused by the finding that a

![Figure 1. Identification of YP on 10% SDS-PAGE stained for protein. Molecular weight standards (lane a), water soluble proteins from homogenized oviposited eggs (lane b), ammonium sulfate precipitated egg proteins (lane c), UV absorbance peak fraction (fraction 24) of precipitated egg proteins run on an S-300 gel filtration column (lane d), UV absorbance peak fraction of S-300 fraction 24 run on a DEAE ion-exchange column (lane e), hemolymph proteins from 3-4d males (lane f), hemolymph proteins from 3-4d females (lane g).](image-url)
female-specific 48 kDa polypeptide was not apparent in the hemolymph. A 48 kDa hemolymph polypeptide was observed in females and males (Fig. 2), but only as a minor constituent and there was no apparent immunocross-reactivity with the Dm YP antiserum (Fig. 3, see below). If the female hemolymph polypeptide is indeed vitellogenin, then either it is a minor contribution to the yolk, or it fails to accumulate due to rapid uptake.

Initial efforts to purify the YP are represented as SDS-PAGE samples from crude soluble protein egg extract, ammonium sulfate precipitation, and fractions from S-300 Sephacryl and DEAE ion-exchange column chromatography (Figs. 1 and 3). A single band was resolved in DEAE fractions 51 (Fig. 3f) and 52 (not shown) although when combined and concentrated, a doublet was observed (Fig. 3e). These fractions eluted between 180 and 500 mM KCl on a step gradient. Resolution should be increased by repeating the purification with a continuous linear KCl gradient.

Figure 2. Hemolymph polypeptides identified on 9-12% gradient SDS-PAGE stained for protein. Hemolymph from 5-6d females (lanes a-b), hemolymph from 4-5d adult males (lane c-d), soluble proteins from oviposited eggs (lane e).

In an effort to further define the YPs and determine if they share antigenic homology with D. melanogaster YPs, which have similar molecular weights (Warren et al., 1979), immunoblots were performed using Dm anti-yolk antisera against A. suspensa YP from SDS-PAGE. Figure 3B is an immunoblot of identical samples stained for protein in
Figure 3A. The *Drosophila* antibody bound with an array of polypeptides extracted from *Drosophila* ovaries, with greatest cross-reaction with the three YPs. Cross-reactivity was also significant with the *A. suspensa* YPs, including those from ovaries, egg extracts and purified protein fractions. Both members of the polypeptide doublet from the combined DEAE fractions cross-reacted. Interestingly, the minor 48 kDa polypeptide from female hemolymph did not show cross-reactivity, nor did any polypeptides of the same molecular mass from male hemolymph. We observed significant binding of the Dm anti-yolk antiserum to Caribfly YP, yet the Dm anti-yolk antiserum had higher binding affinity for the *Drosophila* YPs. This suggests some, though not total, homology between the YPs of the two species. We are encouraged to attempt the use of cloned genomic YP DNA from *Drosophila* (Barnett et al. 1980) to isolate the YP gene(s) from *A. suspensa* since the antigenic homology may indicate sufficient structural homology to allow for specific hybridization between the heterologous genes.

Figure 3. Identification of YP on 9-12% gradient SDS-PAGE stained for protein (A), or electroblotted onto nitrocellulose and cross-reacted with Dm anti-yolk antisera (B). *D. melanogaster* ovarian proteins (lane a), 4 d adult male hemolymph (lane b), 4 d adult female hemolymph (lane c), *A. suspensa* ovarian proteins (lane d), DEAE fractions 51 and 52 (lane e), DEAE fraction 51 (lane f), S-300 fraction 24 (lane g), ammonium sulfate precipitated egg proteins (lane h), soluble egg proteins (lane i), molecular weight standards (lane j).
Site of synthesis

The majority of insects studied thus far contain a hemolymph-borne YP or vitellogenin in adult females, that presumably is synthesized by the fat body (c.f. Kunkel and Nordin, 1985). For some Drosophila species, the ovaries also contribute YP, as was most conclusively demonstrated by in situ hybridization of cloned YP gene DNA to follicular epithelium RNA in D. melanogaster (Brennan et al., 1982).

Our SDS-PAGE analysis of female hemolymph proteins did not reveal a major YP constituent. To determine the site(s) of YP synthesis, this analysis was continued by measuring protein synthesis in vivo and in vitro tissue culture by incorporation of radiolabeled methionine. Figure 4 shows an autoradiogram of radiolabeled polypeptides resolved by SDS-PAGE. Lanes a-c shows in vivo radiolabeled proteins contained in pre-vitellogenic, early, and late vitellogenic ovaries taken from females that were injected with $^{35}$S-methionine. YP synthesis was first detected in early vitellogenic ovaries where the 48 kDa doublet was resolved (7% of total synthesis); extremely high levels of synthesis were observed 2 days later in late-vitellogenic ovaries where the doublet was obscured due to the massive amounts of YPs produced (24% of total synthesis). Hemolymph taken from the same animals showed synthesis of the 48 kDa polypeptide only in late-vitellogenic females (Fig. 4d-f), although at a rate considerably less than a higher molecular weight hemolymph protein (7% vs. 18% of total synthesis).

![Figure 4](image)

Figure 4. YP synthesis in vivo and in vitro indicated on a 9-12% gradient SDS-PAGE autoradiogram. PV, pre-vitellogenic 3 d adult females; EV, early vitellogenic 4-5 d adult females; V, vitellogenic 6 d adult females; M, culture media; Ov, cultured ovaries; FB, cultured abdominal walls containing adult fat body. Adult females were injected with 3 $\mu$Ci $^{35}$S-methionine, incubated for 3 hr and dissected. In vivo cultured ovaries (lanes a-c) and hemolymph (lanes d-f). In vitro culture of ovaries (lanes g-l) and abdominal walls (lanes m-r) with 15 $\mu$Ci $^{35}$S-methionine.
Ovaries dissected from the same age females and radiolabeled in vitro exhibited high overall total protein synthesis with significantly high YP synthesis levels at all three stages (Fig. 4h,j,l). Interestingly, the culture media from all three ages contained almost exclusively the 48 kDa YP doublet (Fig. 4g,i,k). Abdominal walls, which had large amounts of fat body attached in addition to muscle and cuticle, were also incubated in vitro. Total protein synthesis from these tissues was extensive, although only a single polypeptide (co-migrating with the lower ovarian polypeptide) was observed, whose synthesis was moderate and equivalent at all three ages (approx. 2-3% of total synthesis; Fig. 4m-r). The cultured fat body secreted an array of proteins, of which the 48 kDa polypeptide was a minor contribution (3-5% of total synthesis).

These experiments strongly suggest that the ovary is the major, if not the exclusive site of YP synthesis in *A. suspensa*. The 48 kDa polypeptide found in the hemolymph, presumably secreted by the fat body, may indeed be a YP constituent, but its contribution appears to be minor. Vitellogenic and pre-vitellogenic ovaries cultured in vitro are competent to support a high level of YP synthesis which can be secreted. While leakage cannot be excluded, the pre- and early-vitellogenic ovaries are not disrupted easily, and certainly other polypeptides are conserved within the ovary. The selective secretion of ovarian YP in vitro suggests that either this is an artifact of in vitro conditions (e.g. a lack of feedback inhibition) or re-uptake of YP in vivo occurs too rapidly for appreciable accumulation in the hemolymph.

Hormonal control of YP synthesis

In a wide variety of insect species, as well as oviparous animals in general, hormones have been found to have a regulatory influence on YP synthesis, as well as other aspects of the vitellogenic process. In *D. melanogaster* both 20-HE and JH apparently have equivalent regulatory influences on YP synthesis, although 20-HE only affects fat body synthesis (Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980). On the other hand JH affects both fat body and ovarian YP synthesis, as well as promoting YP uptake by the oocytes (Postlethwait and Handler, 1978; 1979). In another dipteran, *Aedes aegypti* (L.), both 20-HE and JH influence YP synthesis but in this species JH does not stimulate synthesis directly, rather it is a prerequisite for the more direct action of 20-HE (Hagedorn, 1983). In contrast to the positive hormonal influence in these species, in the lepidopteran *Plodia interpunctella* JH has no effect on YP synthesis, while 20-HE inhibits YP synthesis at concentrations above $10^{-8}$ M (Shirk and Brookes, 1986).

Analysis of hormonal regulation of YP synthesis in *A. suspensa* generally followed the protocol we established for a similar analysis in *D. melanogaster*. Abdomens were isolated from females previous to or at the initial appearance of vitellogenic oocytes (approximately 3, 4, and 5 days after eclosion), incubated for 24 hr, and then treated with either $10^{-4}$ M 20-HE or $10^{-3}$ M juvenile hormone analog (methoprene). At 6 to 8 hr after hormone treatment, the ovaries and body walls were dissected and incubated separately in vitro with 15 μCi $^{35}$S-met for 3 hr. Autoradiography of the proteins resolved by SDS-PAGE indicate relatively low levels of 48 kDa polypeptide synthesis in both untreated and hormone treated abdominal tissues, yet total protein synthesis in these samples was extensive (Fig. 5). This test fails to demonstrate substantial stimulation of YP synthesis by either 20-HE or JH.
Figure 5. Influence of 20-HE (E) and JH on ovarian or fat body (FB) synthesis of YP. Abdomens isolated from 3, 4, and 5 d adult females were treated with hormone for 6-8 hr, tissues were dissected and incubated for 3 hr in Ringer's solution with 15 μCi $^{35}$S-methionine. Tissues and media were dissolved in sample buffer, and run on 9-12% gradient SDS-PAGE which was autoradiographed. Tissues from abdomens untreated with hormone (lanes a,d,g,j,m,p); tissues from abdomens treated with $10^{-4}$ M 20-HE (lanes b,e,h,k,n,q); tissues from abdomens treated with $10^{-3}$ M JH analog methoprene (lanes c,f,i,l,o,r).

Specificity in terms of hormone concentration or times of hormone sensitivity were not tested; thus this negative result does not rule out hormonal control of YP synthesis in *Anastrepha*. However, it is apparent that the endocrine physiology of vitellogenesis in this species differs from that in *D. melanogaster*.

Ecdysteroid titers in adult animals

Ecdysteroids have been found to promote YP synthesis experimentally in various insects, and upon RIA analysis physiologically significant amounts of hormone have been found in adults systemically or in homogenates (Hagedorn, 1983; 1985). In some species females have higher ecdysteroid titers compared to males, which might be related to vitellogenesis. While ovarian secretion of hormone has been detected in some instances (Hagedorn et al., 1975), for other species ovaries accumulate ecdysteroids for use by the zygote during embryogenesis (Lagueux et al., 1981). In *Drosophila*, ecdysteroids have been found in ovaries, though their ability to secrete hormone is subject of debate (Handler, 1982; Bownes et al., 1984; Schwartz et al., 1985). The ovaries are clearly not necessary for YP synthesis by the fat body.