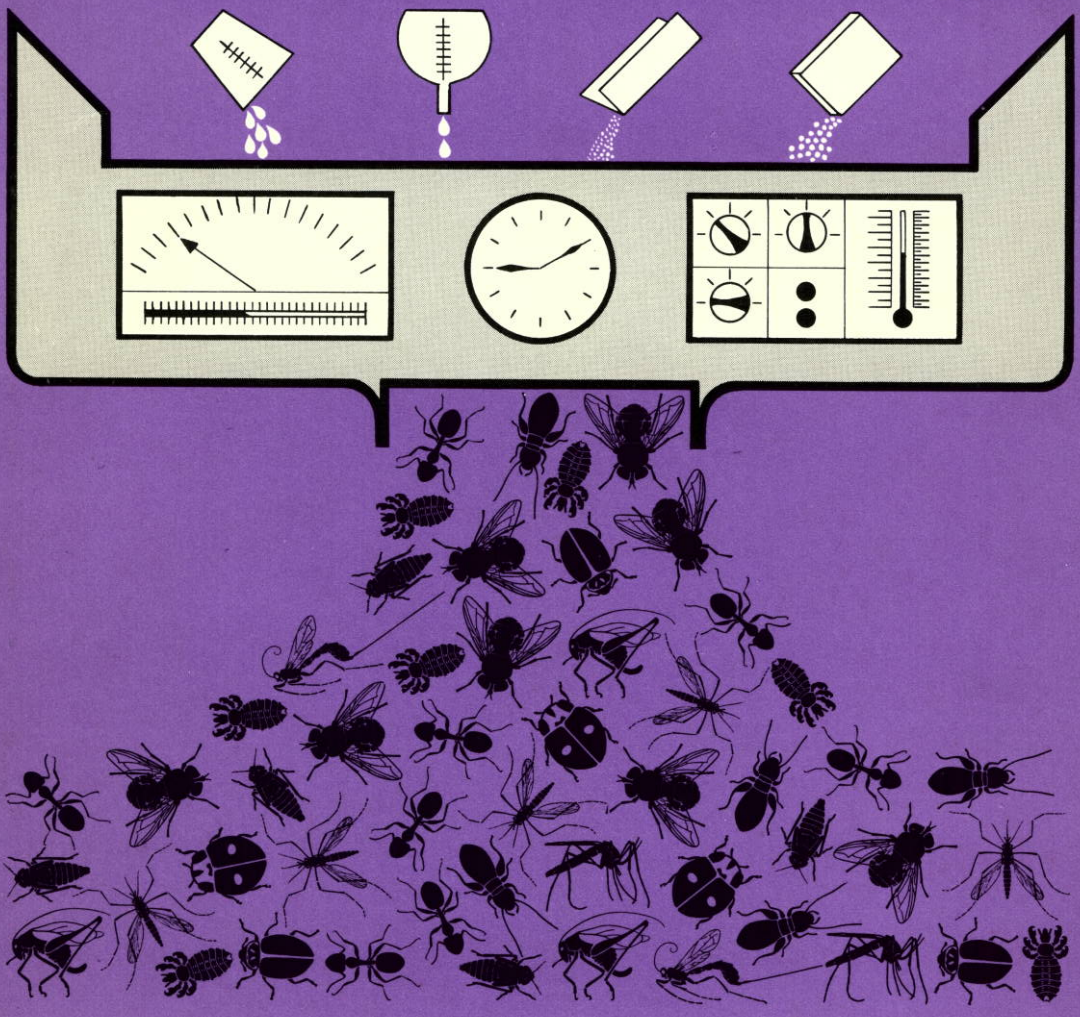
RADIATION, RADIOISOTOPES AND REARING METHODS IN THE CONTROL OF INSECT PESTS

PROCEEDINGS OF A PANEL, TEL AVIV ORGANIZED BY THE JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY IN FOOD AND AGRICULTURE



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1968

RADIATION, RADIOISOTOPES AND REARING METHODS IN THE CONTROL OF INSECT PESTS

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PANEL PROCEEDINGS SERIES

RADIATION, RADIOISOTOPES AND REARING METHODS IN THE CONTROL OF INSECT PESTS

PROCEEDINGS OF A PANEL
ON RADIATION, RADIOISOTOPES AND REARING METHODS
IN THE CONTROL OF INSECT PESTS
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY
IN FOOD AND AGRICULTURE
AND HELD IN TEL AVIV

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1968

RADIATION, RADIOISOTOPES AND REARING METHODS IN THE CONTROL OF INSECT PESTS
(Panel Proceedings Series)

ABSTRACT. Proceedings of a panel organized by the IAEA and held in Tel Aviv, 17-21 October 1966. Fifteen scientists from ten countries attended the meeting. This book presents a wide range of information on the subject, extending from data on fundamental nutrition to practical aspects of rearing millons of insects in a day or a week; the insects discussed included flies, moths, weevils and ticks.

The contents include, amont others, papers on rearing and irradiation of the tick, the nutritional requirements of diptera, mass-rearing techniques for the Mediterranean fruit-fly in Israel, progress report on rearing of the codling moth, and artificial rearing of the olive fly.

All the papers and the recommendations of the panel are in English.

(148 pp., 16 x 24 cm, paper-bound, 23 figures; 1968) Price: US $4.00; £1.13.4.

RADIATION, RADIOISOTOPES AND REARING METHODS
IN THE CONTROL OF INSECT PESTS, IAEA, VIENNA, 1968
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FOREWORD

Information on the sterile-male technique was given in "Advances in Insect Population Control by the Sterile-male Technique", published in 1965 by the International Atomic Energy Agency as Technical Reports Series No. 44. This report was the outcome of an IAEA panel held in Vienna in 1964.

It soon became apparent that there was a great need for the most complete and recent information on rearing the pest insects, and the IAEA convened a panel of experts on 17 - 21 October 1966 in Tel Aviv. The arrangement of the panel enabled a wide range of information to be presented, extending from data on fundamental nutrition to practical aspects of rearing millions of insects in a day or a week; the insects discussed included flies, moths, weevils and ticks.

The present book contains the papers and the recommendations of the panel. Another book in the IAEA Panel Proceedings Series, entitled "Control of Livestock Insect Pests by the Sterile-male Technique", has been published recently.

CONTENTS

REPORTS

Rearing and irradiation of the tick, Ornithodoros tholozani 3
A. Avivi, R. Galun and M. Warburg

Progress report on the rearing of torsalo larvae Dermatobia hominis (Diptera: Cuterebridae) 7
A.D. Banegas

Environmental and behavioural aspects of the mass rearing of plant-feeding lepidopterans 19
S.D. Beck and G.M. Chippendale

Biochemical requirements for mass rearing of plant-feeding lepidopterans 31
G.M. Chippendale and S.D. Beck

The nutritional requirements of diptera 41
W.G. Friend

Mass rearing of insects: its concept, methods and problems 59
R.T. Gast

Preliminary results in rearing the cherry fruit-fly (Rhagoletis cerasi L.) on a semi-synthetic medium 69
A. Haisch

Exploratory studies on the eradication of the Korean pine moth, Dendrolimus spectabilis Butler, by using gamma radiation 79
K.B. Lee, U.K. Hwang and K.S. Lee

Mass-rearing techniques for the Mediterranean fruit-fly in Israel 87
D.J. Nadel and B.A. Peleg

Improvements in mass rearing the Mediterranean fruit-fly, Ceratitis capitata Wied. 91
J. Monro

Progress report on rearing of the codling moth 105
A. Navon

Mass rearing of the Mediterranean fruit-fly in Costa Rica 107
B.A. Peleg, R.H. Rhode and W. Calderon

Application of the sterile-male technique on the onion-fly, Hylemya antiqua (Meig.) in the Netherlands. Progress Report..... 111
J. Ticheler and J. Ph.W. Noordink

Recent radiation work with the olive fly at the "Democritus" Nuclear Research Center, Greece 117
J.A. Tsitsipis and M.E. Tzanakakis

Artificial rearing of the olive fly. Progress Report 123
M.E. Tzanakakis, A.P. Economopoulos and J.A. Tsitsipis

Potential for control of the sugar-cane borer through radio-induced sterility 131
D.W. Walker

Recommendations 143

List of Participants 147

REPORTS

REARING AND IRRADIATION OF THE TICK, Ornithodoros tholozani*

A. AVIVI, R. GALUN AND M. WARBURG
ISRAEL INSTITUTE FOR BIOLOGICAL RESEARCH,
NESS-ZIONA, ISRAEL

Abstract

REARING AND IRRADIATION OF THE TICK, Ornithodoros tholozani. Data are presented on the natural and artificial rearing and irradiation of a tick, which is a vector for human relapsing fever. This tick is a candidate for control by the sterile-male release technique. Adult females were sterilized with 2 kR; males with 16 kR. These males were competitive with control males for at least six months during which time their fertility did not return.

INTRODUCTION

The tick Ornithodoros tholozani (Laboulbene and Megnin) which is a vector of human relapsing fever (Spirochaeta persica) occurs in isolated populations, mostly in caves, and is widely distributed in the Near East. These ticks usually stay in the upper layers of the soil, but in the prolonged absence of hosts, they burrow deep into the ground and may survive there many years of starvation. Thus they are inaccessible to the action of acaricides and insecticides.

The tick completes feeding within one hour, and drops off the host immediately after feeding. If the host moves, it drops before completing its meal. This behaviour precludes the transfer of the ticks by the host from one cave to another.

The biology of this tick makes it suitable for control by the radiation-induced sterility method.

MATERIALS AND METHODS

Culture on live rats

Ticks were reared in tin containers measuring 35 × 25 × 10 cm. These were filled with dustless dune sand to a depth of 5 cm, and about 500 adult ticks were placed inside to start a colony. The edges of the container were smeared with a sticky mixture of Tanglefoot (manufactured by the Tanglefoot Co., Grand Rapids, Michigan) and dimethylphthalate, to prevent the escape of the ticks. The repellent is used to prevent the ticks from touching the Tanglefoot. Additional precautions were taken by keeping all the tick colonies on racks placed on an iron net. The entire floor under the net was flooded with machine oil (Fig. 1). These measures were undertaken because ticks transmit spirochaetes transovarially; consequently, they are considered to be potentially infected.

* This study was supported by a grant from the Ford Foundation

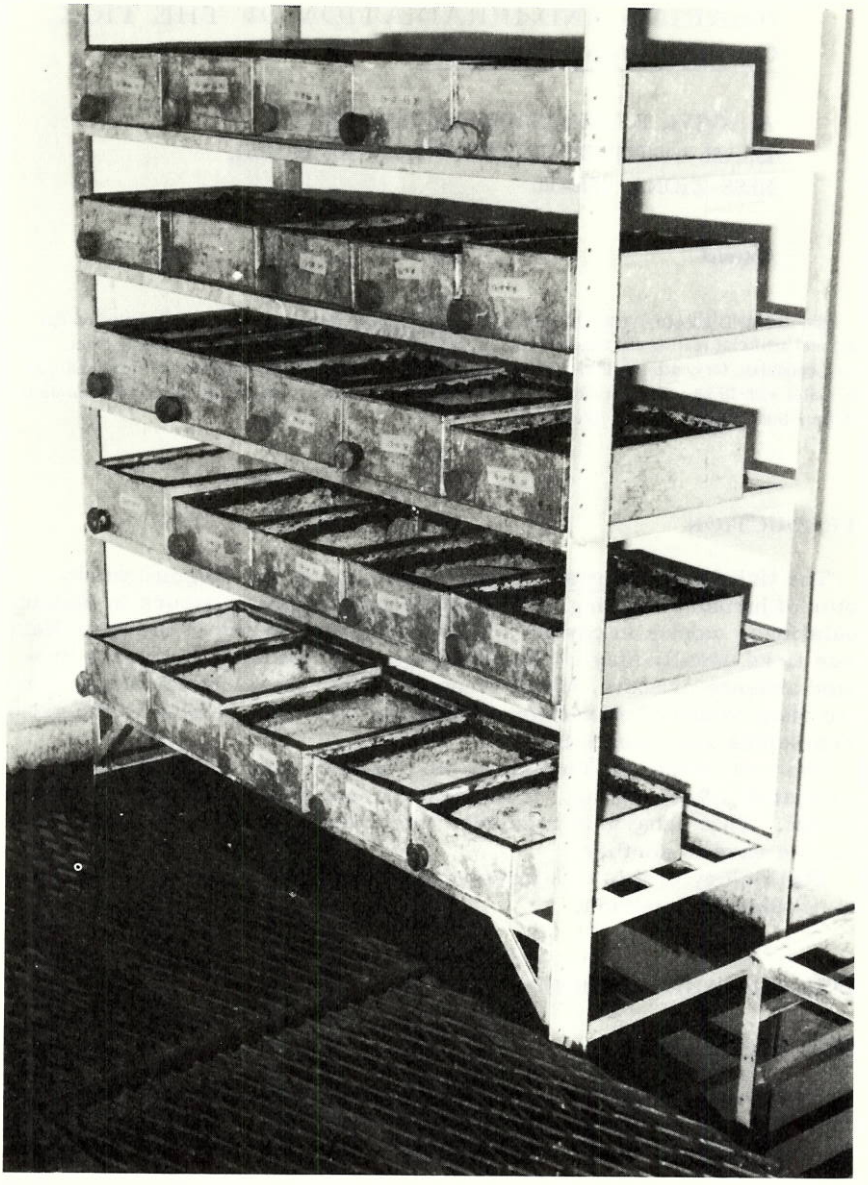

FIG. 1. Containers of tick colonies placed over oil.

The rearing room was kept at 25°C, 70 - 80% R H and in continuous darkness. The ticks were fed on rats every six weeks. The rats were confined to wire cones, specially constructed for this purpose (Fig. 2). After putting the rat into the cone it was plugged by a rubber plug which could be squeezed forward so as to keep the rat from moving around. The plug was held in place by a U-shaped iron wire, inserted through the cone.

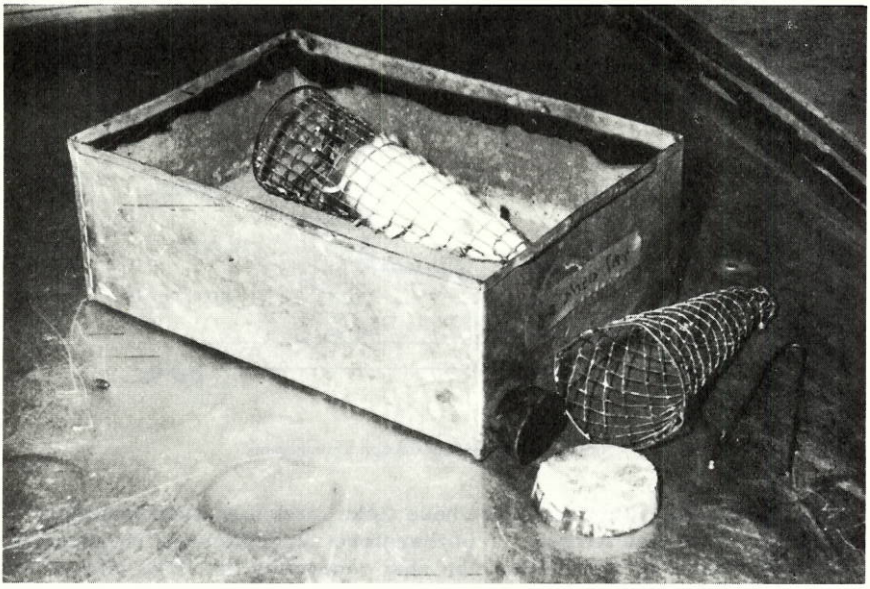

FIG. 2. Rearing container smeared with Tanglefoot and wire cones for confining the rats.

The rats were placed in the tick container on the sand for a few days. When the rats died, all the ticks dropped from it. The rats were removed and incinerated. When the number of ticks in a colony increased, up to four rats were used for one feeding.

The sand was emptied through a plugged hole (Fig. 2) and sieved through different wire meshes at regular intervals, in order to separate the young stages from the adults and to keep homogenous colonies.

A single colony yielded about 10 000 - 15 000 adult ticks a year. This method may be expensive for rearing the large numbers necessary for release in caves.

Membrane feeding culture

Attempts were made to economize on rats by feeding the ticks on beef blood through artificial membranes. This type of feeding was tried successfully on several species of soft ticks [1, 2], but no attempts to rear them were made.

The membrane was stretched on a glass ring, which was then partially submerged in blood kept at 38°C. The ticks were put inside the ring and allowed to probe through the membrane and imbibe (Fig. 3).

Successful feeding was obtained through parafilm membrane (Marathon, Menasha, Wisconsin) but, in general, the literature indicates that animal-membranes were superior to synthetic ones. Good results were obtained by using Baudruche membranes which are made of ox intenstine (Lang and Lang Co., Roosevelt Avenue, Belleville 9, New Jersey). Both these membranes are thin and tear easily. Therefore, for mass feeding, sturdier membranes were sought.

Rat or rabbit skin served well and could be used repeatedly. Skin membranes were prepared according to Tarshis [2]. Defibrinated beef blood was used. Citrated blood, though readily engorged, kills the tick within two weeks after the meal.

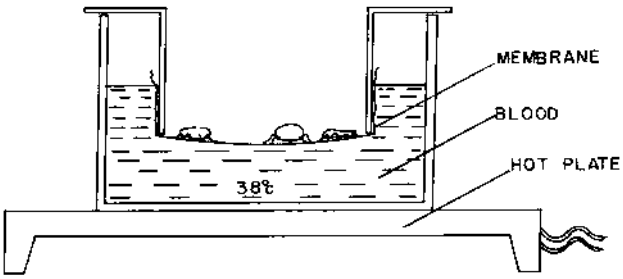

FIG. 3. Ticks feeding through a membrane.

Owing to strong thigmotaxis, these ticks feed only when touching the edges. For mass feeding, it is therefore suggested that the feeding area be increased by subdividing the ring containing the ticks into several smaller compartments. Ticks fed in this way moulted and laid eggs normally.

It is planned to use membrane feeding mainly for the adults and large nymphs, which gave about 100 mg of blood per meal. The younger stages will be fed on rats, as described at the beginning of this paper.

RADIATION STUDIES

The ticks were sterilized by irradiation from a ^{60}Co source emitting 7.7 kR/min. Nymphs irradiated with 2 kR and above did not moult if irradiated before feeding; emerging adults irradiated two weeks after feeding were sterile. The males obtained from the treated nymphs had no sperm.

Adult females irradiated with 2 kR became sterile. Adult males required 16 kR in order to induce 99% dominant lethality in the sperm. These males were competitive with normal males, for at least six months and there is no recovery of fertility. Blood feeding immediately after irradiation was not affected, but hardly any of the treated males took a second blood meal. A more detailed paper, concerning the irradiation effects, will appear elsewhere.

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PROGRESS REPORT ON THE REARING OF TORSALO LARVAE Dermatobia hominis (DIPTERA: CUTEREBRIDAE)

A.D. BANEGAS
ORGANISMO INTERNACIONAL REGIONAL
DE SANIDAD AGROPECUARIA
HONDURAS

Abstract

PROGRESS REPORT ON THE REARING OF TORSALO LARVAE Dermatobia hominis (DIPTERA: CUTEREBRIDAE). Heavy damage to the cattle industry in tropical Latin America by Dermatobia hominis (L. Jr.) furnished economic justification for research on the sterile-male approach to eradication of this species. The sterile-male method requires a mass-production of the flies; therefore, studies were initiated on rearing larvae in artificial media. A series of media based on biochemical and biological ingredients were tested and the preliminary results are presented in this report. Larvae were kept on artificial medium up to 40 d reaching the third of four instars.

INTRODUCTION

Dermatobia hominis (L. Jr.) commonly known as torsalo in Central America south of Guatemala (in Guatemala called Colmoyote), is an important parasite of livestock. Annual losses in the livestock industry caused by D. hominis were estimated at approximately 1.8 million US dollars for Honduras alone. The recapitulation of this figure is as follows:

Death loss of animals, mostly calves	US $1 200 000
Reduced value of beef animals going to market plus increased production cost	260 000
Reduced value of green hides	250 000
Losses in milk	100 000
	US $ 1 810 000

During the past eight years the systemic insecticide trichlorfon (2, 3 dimethyl (2, 2, 2 - trichloro - 1 hydroxyethyl) phosphonate) and more recently, Ruelene® (0-4-tert-butyl-2-Chlorophenyl O-methyl methyl-phosphoramidate) were commonly used. Good control of torsalo was obtained by applying these systemic insecticides, but their frequent use is costly and the terrain and the practices observed in producing live-stock in many localities are not favourable or economical to their regular application. Owing to this and to the alternative hosts of the ectoparasite, eradication based on insecticide application seems unlikely.

The success of the sterile-male technique in controlling the screw-worm created interest for similar techniques to control the torsalo. The usefulness of a research project comprising investigations in the biology

of the torsalo, to establish the feasibility of such an organized control measure was recognized by the Ministers of Agriculture of the Central American countries, Panama and Mexico, and at the XI OIRSA Meeting held in 1963. OIRSA (Organismo Internacional Regional de Sanidad Agropecuaria) was authorized to organize and initiate such a research programme in co-operation with the Ministerio de Recursos Naturales of Honduras.

The initial studies for the establishment of the research project were supported by the authorities of the United States Department of Agriculture (USDA) Livestock Insect Investigations Laboratory, Kerrville, Texas, and by the Food and Agriculture Organization (FAO) through their regional veterinarian for Latin America.

The torsalo laboratory was established in the San Jose Research Veterinary Laboratory owned by the Ministerio de Recursos Naturales of Honduras and located in Tegucigalpa.

In the sterile-male technique, one of the major features is a method for mass rearing the insect. Studies were undertaken to establish the feasibility of rearing D. hominis larvae (newly-hatched and older larvae which were obtained from bovines) on an artificial medium. This report concerns the preliminary results on the artificial rearing of torsalo larvae.

BIOLOGY OF Dermatobia hominis

D. hominis depends on other day-flying adult Diptera to carry their eggs. A mass of eggs is glued to the abdomen of the carrier insect, and the number of eggs laid on a vector vary from a few to more than a hundred.

When the eggs are ready to hatch, the larvae leave the eggs in response to the warmth from a potential host when this host is visited by the carrier. The larvae penetrate the skin and lodge in the subcutaneous tissue. The torsalo larvae do not migrate within the host, but encyst at the point of entrance. A breathing hole is maintained by the larvae throughout their development. The long period of development of D. hominis larvae (36 - 45 d) hinders rapid progress in the rearing programme. The full-grown larvae leave the host by crawling through the enlarged breathing hole, fall to the ground, burrow into the soil and pupate.

With optimum conditions the life cycle, egg to egg, is completed in about 80 d. Owing to climatic conditions, generations probably overlap, and perhaps four or five generations occur per year. Female adult flies were frequently observed on bovines, whereas, no male flies were captured. Matching habits in nature are poorly understood.

MATERIALS

Mature larvae that voluntarily left the host (bovines) were collected and placed in cardboard boxes containing moist soil. The larvae burrowed into the soil covering themselves in approximately 20 min. After 2 - 3 d, the puparium hardened. At 24 - 25°C and 70% RH, the pupal stage averaged about 34 d. Single pairs were established within 24 h after emergence. Each cage lighted with a 100-W light bulb was incubated at 28°C and 80% RH.

Flies were permitted to copulate during a period of 6 h. Females were separated and returned to the 24 - 25°C incubator. After 24 h the female flies were returned to the warm (28°C) and lighted incubator in cages with a number of house flies (Musca domestica L.) on which oviposition took place. After approximately 24 h, eggs were collected and placed in petri dishes at 28°C and approximately 100% RH. Eggs hatched after the fifth day. Infestation of the hosts was accomplished by placing the newly hatched larvae on their skin. Cattle appeared to be the best host, but goats and rabbits were used successfully.

Secondly, third and fourth instar larvae were obtained from bovines artificially infested at the San Jose laboratory, from fresh hides brought into a local tannery and from bovines obtained at a slaughter-house in Tegucigalpa.

METHODS AND RESULTS

Egg separation

Eggs adhere to vector insects by a glue-like substance. Because eggs could be better disinfected if they were separated, attempts were made to dissolve this substance. One to two per cent NaOH (sodium hydroxide) for 30 min, 1/10-N HCl (hydrochloric acid) for up to 30 min, and 0.25% Sure-Klean bleach (sodium hydrochlorite) for 20 min were unsuccessful for separation of eggs.

Eggs disinfection

Egg masses were removed from vectors collected in the field, placed in sterile tubes with 4-5 ml of formalin (1%) or Sure-Klean (0.25%) and agitated frequently during the 10-min test-period. Egg masses were removed with flamed forceps and washed in sterile water for 5 min. They were transferred to a shell vial containing cotton impregnated with a casein-yeast medium containing 100 units of penicillin and 0.4 mg of streptomycin per millilitre of diet. Diets were kept at room temperature for 24 h before egg distribution to ensure that the nutrient was sterile. Eggs treated with formalin appeared blackened, did not hatch and had heavy bacterial growth; consequently, no further tests were run. Eggs treated with 0.25% Sure-Klean survived, but the food was contaminated. Similar treatment for 20 min decontaminated the eggs and the larvae developed and hatched normally.

Larval disinfection

Larvae removed from their host could be more beneficially utilized for rearing if they could be disinfected. Dermatobia larvae placed in distilled water survived several days. If they survived for the same period or longer in a buffered saline solution combined with antibiotics, it might be possible to disinfect them including contaminants within their intestine.

The buffered saline solution contained: 90 ml 0.15 M KH_2PO_4, 286 ml 0.15M Na_2PO_4 and 376 ml 0.15M NaCl. This solution was autoclaved and the disinfectants were added in the desired amounts prior to use.

Larvae were pre-cleaned of excessive filth after extraction from the host and treated for 30 min (10 min in Sure-Klean). Larvae survived except in 0.2% Sure-Klean. (Table 1 part A).

TABLE I. DISINFECTION PROCEDURES APPLIED TO Dermatobia hominis LARVAE

A. Test 1

1.	Buffered saline			
2.	"	"	+	0.05% Sure-Klean
3.	"	"	+	0.20% Sure-Klean
4.	"	"	+	0.01% Formalin
5.	"	"	+	0.5 mg streptomycin/ml
6.	"	"	+	200 units penicillin/ml
7.	"	"	+	200 units penicillin/ml + 0.5 mg streptomycin/ml
				1000 units penicillin/ml + 1 mg streptomycin/ml

B. Test 2

1.	Buffered saline		+	200 units penicillin/ml
2.	"	"	+	0.5 mg dihydrostreptomycin/ml
3.	"	"	+	100 units mycostatin/ml
4.	"	"	+	100 units penicillin/ml + 0.4 mg streptomycin/ml
5.	"	"	+	200 penicillin/ml + 0.5 mg streptomycin/ml
6.	"	"	+	1000 penicillin/ml + 1 streptomycin/ml
7.	"	"	+	2000 penicillin + 2 streptomycin/ml (no pre-treatment)
8.	"	"	+	4000 penicillin + 4 streptomycin/ml

After subjection to test 1A, the larvae were placed in cotton-stoppered tubes with small amounts of buffered saline plus antibiotics and a fungistat (Table I part B), which covered the larvae but allowed them to protrude

their spiracles above the surface. After 24 h, the larvae were removed and the solutions cultured. Larvae were washed with sterile water and replaced in fresh solutions. The tubes were kept at 30°C.

Mature larvae (fourth instar) survived at least 7 d in solutions 1 through 6; other solutions were not tolerated. (Table I part B). These treatments did not disinfect the gut contents of the larvae.

Temperature

No specific tests were designed to analyse the effect of temperature; however, observations at various temperature are given for first instar larvae (Table II).

Dietary studies

Attempts were made to find a suitable diet on which first instar larvae would live and develop. Chemically defined media (Gingrich 1963) of screw-worm larvae served as a guide to develop a media for Dermatobia larvae.

1. Serum

Disinfected eggs with larvae beginning to hatch were put inside a vial about 1-2 mm above cotton saturated with bovine serum containing 1000 units of penicillin and 1 mg of streptomicyn/ml and kept in an incubator at 34°C. This test was repeated twice. Not all larvae moved to the food. Some larvae survived 4 d; however, 15 d later all larvae were dead and the medium was not contaminated.

2. Formalin - gelatin - albumin - gastric mucin

To the following diet either serum or distilled water was added in a 1:4 ratio.

Ingredients	(%)
Gastric mucin	0.2
Gelatin	2.0
Blood albumin	5.0
Yeast extract	0.5
Salts MO-185	0.125
Cholesterol	0.1
10% Formalin (bacteriostat)	0.1
Phosphate buffered saline	92.0
pH adjusted to neutral with NaOH	

Ingredients were dissolved at 70°C to avoid coagulation of the proteins.

Food in shell vials with cotton plugs was seeded with disinfected egg masses and newly-hatched larvae. Incubation was 34°C. After 24 h the larvae made mouth movements as if feeding.

After 4 d larvae on the diet containing serum were active; whereas, those on the diet containing distilled water were slightly flaccid. After the fifth day no larvae survived and after 9 d the media remained uncontaminated, odourless and moist.

TABLE II. TEMPERATURE EFFECTS ON FIRST INSTAR LARVAE OF Dermatobia hominis.

Temperature (°C)	Activity	Development	Survival (d)
28 - 30	sluggish	stopped	3-4
31 - 33	active	some	
34 - 36	active	some	up to 40
37 - 40			lethal

Formalin (0.1%) was a satisfactory bacteriostat but cotton as a supporting medium was too firm and therefore this test method was considered inadequate.

3. Peptone – egg - serum

Ingredients	(%)
Neopeptone	2
Dried whole egg (see text)	4-10
Yeast extract	0.5
Inorganic salts MO-185	0.125
Buffered saline (to complete volume)	
Serum was added in a 1:4 ratio	

Peptone, yeast extract and salts were dissolved in heated buffered saline. After cooling, egg was added as 4, 6, 8 and 10% of the medium. Six millilitres of the food were placed in shell vials, stoppered with cotton and autoclaved for 15 min at 121°C. When cool, serum was added.

The diets were seeded with disinfected egg masses and kept in an incubator at 34°C.

The larvae survived a few days but failed to grow because they could not penetrate the hardened denatured egg protein mass because of autoclaving but underneath, the medium was soft, easily penetrable and apparently an ideal consistency.

4. Formalin - serum - RNA - glycogen

Blood albumin, yeast extract, glycogen, inorganic salts, RNA and the cholesterol suspension were added to 44 ml of phosphate buffer saline solution and stirred thoroughly; serum was added:

Ingregients	(%)	(%)
Blood albumin	10.0	
Yeast extract	0.5	
Glycogen	1.0	
Inorganic salts MO-185	0.125	80

RNA (Ribose Nucleic Acid)	0.1	
Cholesterol	0.1	
Buffered saline	8.0	
Serum (1000 U. penicillin 1 mg streptomycin/ml)		20
Formalin (see below)		

Five shell vials containing 12 ml of the medium were given 0.05, 0.06, 0.12, 0.18, and 0.24 ml of formalin making 0.0025, 0.05, 0.01, 0.15 and 0.2%, respectively.

Each diet was divided into two shell vials containing cotton to take up the fluids and an untreated egg mass. In addition, 5-ml portions of diet without formalin were added to cotton in each of three shell vials. The vials were autoclaved and, after cooling, the serum and disinfected egg masses were added.

Hatching began after 24 h and 48 h later, larvae were observed in all vials. Some larvae tunnelled in the cotton and their spiracles were observed at the surface; others, submerged in the fluid, apparently had no access to atmospheric oxygen and were inactive. The protein coagulated in the diet vials that were autoclaved and the cotton was not penetrated by the larvae.

Formalin at 0.1% and lower did not prevent contamination. Larvae survived at least 8 d in 0.15 and 0.2% formalin; however, no growth was noted.

5. Formalin – gelatin - albumin - gastric mucin

This diet was prepared like diet No. 2, but contained semi-solid pieces of gelatin. Larvae survived 24 h after hatch and moved their mouth parts as if feeding. Therefore: (i) Agar; (ii) Wheat flour; (iii) Kaolin (hydrated aluminium silicate powder); or (iv) Regaliz (a dark flour from legumes) were added to determine which result was the preferred semi-solid medium.

Ingredients, except for gastric mucin and albumin, were dissolved by heat in a phosphate-buffered saline solution and after cooling, the albumin and gastric mucin were added. The 0.8% agar made the final medium semi-firm.

Three millilitres of this medium without agar were added to 2 g of wheat flour, kaolin, or regalize. Eggs hatched in all media. In the flour medium, one larva made a hole in the surface of the medium for breathing. Larvae were alive 3 d later in the agar media, but no survivors were found on flour, kaolin and regaliz.

Media of this type (except agar) are unfit for rearing unless water loss can be controlled. Agar appeared to give satisfactory moisture but all larvae were dead after 5 h.

6. Meat serum (Medium A)

A diet (Table III, column 1.) similar to the one used for rearing screw-worm larvae was used in duplicate tests for rearing newly hatched torsalo larvae.

After five days, eggs hatched in all diets. The greatest hatch and most active larvae were found in tests C and D. Some larvae were resting

TABLE III. MEAT-SERUM MEDIA FOR REARING Dermatobia hominis LARVAE

Ingredients		1. (%)	2. (%)
Ground beef		40	40
Bovine serum (1000 units penicillin 1 mg streptomycin/ml)		20	30
Distilled water		40	30
Formalin	Test: A	0.15	0.2
	B	0.20	
	C	0.25	
	D	0.30	

with their posterior spiracles at the medium surface, apparently taking in air, others were submerged deep in the medium. Most of the larvae appeared abnormally turgid; perhaps the high water content rendered the medium hypotonic.

After 8 d, test A had heavy fungus growth; the other tests had no apparent contamination. After 19 d, eight live larvae approximately 4 mm long were found in tests B and C. (Newly hatched larvae are 0.75 - 1.00 mm long). Survivors were replaced in test C and approximately 0.25 ml of serum was added to the meat. The next day all the larvae were dead, perhaps as a result of handling.

Meat serum (Medium B)

In this medium serum was increased to 30% and distilled water decreased to 30%. (Table III, column 2.)

Out of 20 larvae three were alive after 7 d and grew to a length of 4.5 mm. The medium was renewed. One larvae survived for 15 d and grew to a length of 12 mm.

Meat-serum medium for large larvae

Larvae were obtained from local and laboratory cattle and from fresh hides. The larvae were decontaminated (see Larval Disinfection) and placed in a nutrient medium to complete larval development. Larvae collected from the slaughter house were placed in a medium containing two parts lean ground beef, two parts distilled water, one part serum and 0.3% formalin, but these larvae died within 24 h. Larvae which had not completed their final instar were collected from hides and placed on m media (Table IV). Replicates were incubated at 28 and 34°C.

Apparently 0.1% formalin is toxic to these third instar larvae (Table IV); whereas larvae on media containing 0.15% formalin from the time of hatch developed into third instar larvae which survived at least 8 d. Perhaps older larvae extracted from their host and put on medium do not readily

TABLE IV. DIETS FOR LARGE LARVAE OF Dermatobia hominis AND THEIR SURVIVAL
All diets contained 50 units of mycostatin per gram of food

Diet	Ratio Meat:serum:water			Penicillin streptomycin[a]	Formalin 0.1%	Larvae/ maximum survival in days
A	2	1	2	no	yes	all/1
B	2	1	2	yes	no	all/1
C	3	1	1	no	yes	1/4
D	3	1	1	yes	no	1/7
E	4	1	0	no	yes	1/2
F	4	1	0	yes	no	3/3

[a] 500 units penicillin, 0.5 streptomycin/g

adapt to the change. Media with these quantities of formalin and antibiotics for rearing mature larvae were contaminated within 24 h.

Tests are being developed with second, third and fourth instar larvae to investigate the value of citrated bovine whole blood, serum and plasma, for rearing larvae extracted from bovines artificially infested at the laboratory.

7. Meat - serum - plasma - citrated whole blood

The encouraging results obtained with the meat-serum media for rearing newly hatched Dermatobia larvae suggested new areas for investigation in the larval rearing work.

Blood, serum and plasma were tested individually. Formalin (0.2%), and mycostatin (50 U/ml) kept the diets free from contamination at least 7 d. Each vial was seeded with 20 newly hatched larvae and observed daily.

Tests were designed to study various ratios of ground lean beef to citrated whole blood, serum, and plasma (Table V). Survival and growth data for these tests are also given in Table V. The larvae cultured on the blood media appeared healthier than those on the serum media. When third instar larvae develop, growth was abnormal. To test further the meat-serum media, we are investigating medium consistency, supplements to improve dietary nutritional requirements, handling methods, etc.

8. Meat - citrated whole blood

A medium similar to the one used by Melvin and Bushland for rearing screw-worm, Cochliomyia hominivorax, was prepared in the following proportions:

Ingredients	(%)
Ground lean beef	60
Distilled water	15
Whole citrated bovine blood	25
Formalin	0.2
Mycostatin 100 U/g	

Ten grams of the diet were placed in each of nine shell vials seeded with 15 newly hatched larvae. Incubation was 34-35°C. Within 24 h the larvae penetrated the media with their posterior spiracles at the surface. After 7 d 3 out of 135 larvae survived. They were 5 - 6 mm long and were transferred to fresh media. One larvae which survived for 25 d measured 13 mm and was considered a third instar larvae as compared with larvae of the same age reared on bovines.

TABLE V. CULTURE MEDIA AND OBSERVATIONS ON SURVIVAL AND GROWTH OF Dermatobia hominis LARVAE

Ratios			Observation on days:		
Blood Meat:serum:water plasma			7	12	17
2	1	2	up to 30%[a]	4-4.5 mm[b]	0%
2	1	1	up to 30%		
3	1	2	0%		
4	1	0	0%		
1	2	1	0%		

[a] % Refers to survival

[b] Length of larvae

DIETARY CONSISTENCY STUDIES

The consistency of the artificial medium seems an important factor for the penetration, growth and survival of newly hatched torsalo larvae.

Natural food

A disk of lean beef 8 cm in diam. and 0.5 cm thick was placed in a petri dish and incubated at 35°C for one hour, then disinfected egg masses ready to hatch were added. After 30 min incubation, larvae hatched and approximately 80% penetrated the meat, especially the crevices, and had

their posterior spiracles at the surface. Serum and distilled water (1:1) containing 0.25% formalin and fungizone (4 μg/g) were added so as not to overflow the posterior spiracles.

Three days later a few larvae survived and some growth occurred, but the medium was severely contaminated. No migration was observed once the larvae adapted their position.

Artificial food and use of agar

Triplicate tests of 20 newly hatched larvae per vial were placed on a meat-serum-distilled water medium (2:1:1 ratio) containing 0.2% formalin, mycostatin 100 U/g and various amounts of agar to test penetration, growth and survival. After 24 h dead larvae were found on the media, on the walls of the vials and in the cotton stoppers.

TABLE VI. FOOD CONSISTENCY TESTS FOR *Dermatobia hominis* LARVAE AND THEIR SURVIVAL AND GROWTH

Agar (%)	Dead larvae after 1 day (%)	Survival (%)	After 7 d growth (mm long)	Consistency of medium
10	80	0	---	Compact and dry
8	38	0	---	Compact and dry
6	25	3.32	1.5	Semi-compact still moist
4	50	1.66	3	Semi-compact still moist
2	50	0	---	Granulated- had good moisture
0	60	1.66	5.5	Had free liquid

Greatest growth was observed in the medium that had no agar (Table VI), although survival was low, indicating newly hatched larvae consume liquid nutrients more readily. There were no survivors in the medium that had 2% agar; 6% had the greatest percentage of survival but the larvae were much smaller than those at 0%; 8 and 10% had no survivors.

SUMMARY

Dermatobia hominis (L. Jr.), commonly known as torsalo in Central America south of Guatemala, is a very important parasite of livestock. The life cycle is discussed but natural habits are poorly understood. A laboratory colony was established on natural hosts in

order to obtain samples for experiments, such as, separation and disinfection of eggs, larval disinfection and artificial rearing techniques. Egg separation with sodium hydroxide, hydrochloric acid and sodium hypochlorite were unsuccessful.

Eggs were decontaminated with Sure-Klean (sodium hypochlorite) when treated with a 0.25% solution for 20 min and viability was normal. Apparently the optimal temperature for artificial rearing of torsalo larvae is 34-36°C. Several artificial media are presented and the survival and growth of torsalo larvae are discussed. Some larvae grew in a diet containing ground lean beef, serum, and water and one grew to a length of 12 mm in 15 d. Larvae survived 25, 40 and 12 d, respectively, on a meat and water medium supplemented with citrated whole blood, serum and plasma. Larvae cultured on blood and serum developed into their third instar. Further dietary studies with mature larvae are being developed to test the value of whole citrated blood and plasma for rearing Dermatobia.

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ENVIRONMENTAL AND BEHAVIOURAL ASPECTS OF THE MASS REARING OF PLANT-FEEDING LEPIDOPTERANS*

S.D. BECK AND G.M. CHIPPENDALE
UNIVERSITY OF WISCONSIN,
MADISON, WIS., UNITED STATES OF AMERICA

Abstract

ENVIRONMENTAL AND BEHAVIOURAL ASPECTS OF THE MASS REARING OF PLANT-FEEDING LEPIDOPTERANS. Effective mass rearing of plant-feeding lepidopterans requires the development of nutritionally optimum dietary media and general culturing conditions that: (1) Permit the maintenance of an indefinitely self-perpetuating population without attenuation of either viability or reproductive capacity; and (2) produce individual insects that are fully capable of competing with members of wild populations in respect to growth rate, vigour, and behaviour characteristics.

A high degree of asepsis is important to mass rearing in order to minimize the risk of decimating epizootics, and to maintain stable dietary media and standardized conditions for larval growth. Some of the problems associated with the maintenance of aseptic culturing conditions are discussed.

Mass-rearing methods must meet the insect's behavioural requirements as well as its purely nutritional needs. Larval feeding behaviour is promoted by chemicals acting as attractants, incitants, and stimulants via the larval chemoreceptor system. Larval host-plant specificity is determined primarily by the presence or absence of feeding incitants and suppressants. Feeding stimulants and deterrents play an important role in larval feeding behaviour, with many dietary substances acting as feeding stimulants as well as important nutrients.

Efficient mass rearing requires that the incidence of diapause be controlled. In most mass-rearing situations, the elimination of diapause will be desirable. The concept of "obligatory diapause" is discussed, and its validity is considered to be open to question. Photoperiod and environmental temperature are considered to be the major environmental factors governing the manifestation of genetically controlled diapause phenomena. Some general approaches to the problem of diapause elimination are discussed.

Modern knowledge of insect nutrition has stemmed from pioneer investigations carried out in the early 1940s. This investigative thrust was spear-headed by the brilliant work of G. Fraenkel and his co-workers, dealing with the specific nutritional requirements of a number of stored product insects. Biochemical isolation, characterization, and synthesis of individual vitamins, amino acids, and other nutritionally important substances gave increased impetus to the whole topic of animal nutrition, including the study of insect dietary requirements (for a review of early work, see Ref.[1]).

Plant-feeding insects have proved to be somewhat more difficult to study than the stored product infesting species. The development of the techniques for laboratory culturing and nutritional studies on plant-feeding insects has been relatively laborious. Indeed, the first successful rearing of a phytophagous hemipteran was not reported until 1957, with the work of Scheel et al. [2] on two plant-sucking forms. And prior to

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the success of Mittler and Dadd [3], aphids had been quite intractable to artificial culturing. The mandibulate larvae of lepidoptera proved to be easier to culture than did haustellate forms, and some rearing techniques were developed relatively early. An agar-cellulose substrate was formulated by Bottger [4] in an attempt to rear larvae of the European corn borer, Ostrinia nubilalis. Nutrients were added to the agar-cellulose carrier, and some larval growth was observed. Later investigation [5] showed that corn borer larvae would not grow on Bottger's diet in the absence of microbial contamination; the larvae apparently obtained required nutrients from bacteria and fungi thriving on the medium. The first successful meridic diet for a plant-feeding lepidopterous species was that of Beck et al. [5] for the European corn borer. The medium was based on the agar-cellulose carrier of Bottger, but with adequate nutrients added. From the dietary formulation developed, an aseptic mass-rearing method was reported [6].

The mass-rearing method of Beck and Stauffer was subsequently modified and applied to problems of culturing a number of other lepidopterous species. The medium and method was used by Matsumoto [7] with the oriental fruit moth Grapholitha molesta with some success. Ishii [8, 9] reported culturing the rice stem borer, Chilo suppressalis, and other species on the medium. In our laboratory, we found that the red-banded leaf roller, Argyrotaenia velutinana, could be reared quite readily on the borer diet [10]. A number of other species were also tried, with varying success.

The next important advance in techniques for rearing lepidopterous larvae was the development of the so-called "wheat-germ diet" by Adkisson et al. [11], in a study of the nutritional requirements of the pink bollworm, Pectinophora gossypiella. From a nutritional standpoint, the wheat-germ diet was not greatly different from the earlier corn-borer diet. In terms of form, consistency, and behavioural acceptability, the wheat-germ diet represented a most significant advance. This culturing medium has proved to have relatively wide applicability to rearing lepidopterans. As a result, slight modifications of the original dietary formulation have resulted in successful rearing of a good number of plant-feeding species.

GENERAL REQUIREMENTS FOR MASS REARING

In the present discussion, we are not concerned with the specific biochemistry of insect nutrition. Mass rearing involves a great deal more than providing a diet that meets the immediate nutritional requirements of the insect, and we wish to examine some of the problems associated with large-scale culturing.

A large-scale mass-rearing programme, in which thousands or hundreds of thousands of insects are to be produced according to a production schedule, requires the use of a nutritionally adequate dietary medium. The practical problems in respect to the formulation of the medium are quite different from those encountered in a small-scale purely nutritional project. Mass-rearing media should be relatively inexpensive and simple to prepare. Nutritional research, on the other hand, usually involves chemically defined culturing media that are very expensive and laborious to prepare. Mass-rearing programmes may require serious consideration of the cost per insect and the possible utilization of automation in the production of very large numbers of individual insect forms. We have

not been involved in problems of cheap diets or automated production methods in our research, and so tend to view such problems as being questions of economics and engineering that lie outside our areas of competence. But it is obvious, nevertheless, that mass rearing must necessarily be based on a sound understanding of the nutritional requirements of the species. The whole programme must also rest on a sound biological foundation, the biological characteristics of the insect species need to be understood and fully exploited if the mass-rearing programme is to operate with reasonable effectiveness. Evaluation of the effectiveness of a mass-rearing medium should include consideration of two very important biological criteria: (1) Indefinite perpetuation of the population; and (2) ability of individuals to compete with members of the natural population of the same species.

The requirement that the cultured population be capable of indefinite perpetuation by mass rearing is an extremely important factor. In some of our early work on the European corn borer, the dietary medium was quite satisfactory for larval growth, adult production, and reproduction. But the laboratory population became attenuated after four or five generations, and the culture died out. Such a loss of viability was interpreted as meaning that the medium was not optimal, and we continued to work on its improvement. Our present rearing medium has allowed continuous rearing through more than twenty generations without any apparent reduction in vigour, viability, or fecundity. In the absence of continuous culturing capability, it is necessary to renew the rearing population by periodic input of field-collected individuals. This is not only expensive and unreliable, but also increases the danger of disease and the difficulty of controlling the incidence of diapause. The use of rearing media that are suboptimal and that support growth for only a small number of generations may also lead to an undesired genetic selection. After a few generations, the surviving insects may show growth and behavioural characteristics that are quite different from those of a wild population. We have been concerned with this problem in our work with the European corn borer. With our present rearing methods, we have been unable to detect any adverse effects as the result of many generations under artificial conditions; growth, feeding behaviour, photoperiodic responses, and fecundity of the laboratory population have proved to be quite normal. In respect to body weight and egg deposition, the laboratory strain appears to be hardier and more vigorous than the field population.

The ability of mass-reared insects to compete with natural field populations in respect to flight, mating, and other behaviour patterns is of great importance where the objectives of the programme include field releases. This factor would be involved in field releases as part of sterile-male control efforts and in large-scale insect dispersion and migration studies. Attention also should be paid to this characteristic in rearing programmes that are part of insecticide and control studies, host plant specificity and resistance studies, and sex attractant or other behaviour investigations.

IMPORTANCE OF ASEPTIC CULTURING CONDITIONS

Truly axenic "germ-free" culturing conditions are probably unnecessary in most mass-rearing situations. It is, however, quite important that

microbial contamination be rigidly controlled. To accomplish this control, essentially aseptic culturing conditions can be attained without the necessity of instituting extreme precautions to ensure the absence of symbiotes and gut tract flora. The use of antimicrobial agents in the dietary media will have the effect of minimizing the influence of any accidental contamination by an occasional bacterial or fungal spore. The maintenance of essentially aseptic conditions has two major advantages. First, the absence of microbial growth in the dietary medium permits the maintenance of uniform dietary conditions, in which the diet is not subjected to degradative changes and need not be renewed or replaced during the larval feeding period. The second advantage of asepsis is that it prevents the build-up of pathogenic forms to outbreak levels. An outbreak of a disease in the laboratory population can be very quickly controlled and eliminated by virtue of the fact that the growing larvae have been isolated from the source of pathogenic contamination. To use non-aseptic methods in which reliance is placed solely on antimicrobial agents incorporated in the medium is to run a very high risk from the standpoint of decimating epizootics.

Essentially aseptic culturing conditions are not difficult to maintain. Glassware, cages, and other rearing equipment can be surface sterilized by the use of an autoclave or by chemical treatment. Sterilization of dietary media can usually be accomplished by autoclave, filtration, or a combination of the two. In our work with the corn borer and the cabbage looper, the use of the autoclave (15 min at 17 lb/in^2) has proved satisfactory. Some dietary constituents may be degraded by such harsh treatment. Sugars are partially converted into furfurans, and some vitamins may be destroyed. Ascorbic acid, for example, is notoriously unstable. Similarly, thiamine, riboflavin, and pantothenic acid may be partly destroyed by the high temperatures involved. These several substances may be added to the diet in excess to the insect's nutritional requirement; so that sufficient amounts remain after sterilization. The vitamins thiamine, riboflavin, pantothenic acid and ascorbic acid are partially protected under slightly acid conditions, and our diets are usually made up to have a pH of from 5.8 to 6.0. Cysteine is employed as a stabilizer to prevent excessive loss of ascorbic acid.

The incorporation of antimicrobials into the dietary medium serves to guard against the effect of an occasional contaminant. The use of such substances is particularly desirable where relatively large numbers of larvae are confined to each rearing container. A number of workers have used a combination of antibiotic and fungistat, such as Aureomycin plus a benzoate, sorbic acid, and/or formalin. We have found a low concentration (0.16 g/100 g diet) of sorbic acid to be adequate [12,13]. Sorbic acid has the advantage of good solubility and low toxicity. The most commonly used benzoates are methyl-p-hydroxybenzoate and n-butyl-p-hydroxybenzoate [14-18].

With surface sterilized rearing vials or other rearing containers, and with aseptic dietary media containing the added protection of a low concentration of antimicrobial compounds, we still have to deal with the most serious source of contamination – the insect itself. Because of the difficulty of freeing the insect from all surface contamination, internal flora and fauna, and possible symbiotes, the ideal of truly axenic culture is not attainable on a large, mass-rearing scale. Nevertheless, a great deal can be done to ensure reasonably aseptic rearing and to minimize the hazards of contamination. Of the several growth stages involved in

the lepidopteran life history, the egg is by far the easiest to deal with from the standpoint of disinfection. The chorion is a tough impervious system of membranes, making the egg capable of surviving surprisingly harsh treatment. In the later embryonic stages, the larval cuticle adds another protecting membrane within the egg, which may also render the insect resistant to harsh chemical treatment.

Disinfection of the external surfaces of the egg appears to be a relatively simple problem. Where eggs are laid in imbricated masses, such as is the case with the European corn borer, effective surface sterilization can be accomplished only after the egg mass has been dissociated. We have taken advantage of the fact that the cement substance between the eggs is proteinaceous, and have used a protease to effect separation. A 2% solution of partially purified trypsin (1 : 300) in 0.05 M phosphate buffer at pH 8 has proved to be quite effective [6]. The trypsin solution must be freshly prepared, because it becomes very toxic upon standing for more than a few hours. And we have found that the eggs must be rinsed very thoroughly to remove the last traces of the enzyme from the egg surfaces. Our laboratory routine calls for six water rinsings of the separated eggs. The corn-borer eggs are surface sterilized with mercuric chloride. For this purpose, the eggs are placed in a 0.1% aqueous solution of mercuric chloride for from 3 to 5 min, followed by a brief washing in 70% ethanol. We have found the hatchability of separated and disinfected eggs to be satisfactory when late stage eggs are used. Hatching is greatly reduced if the eggs are processed before the so-called "black head stage" at which time the dark larval head is visible through the chorion.

In contrast to the European corn borer, the cabbage looper deposits its eggs singly. Because of the danger of surface contamination by a polyhedrosis type pathogenic virus, the cabbage looper eggs are routinely disinfected with a solution that will destroy virus as well as bacteria and fungi. The eggs are held for about 30 min in a 0.04% aqueous solution of sodium hypochlorite to which a few drops of emulsifier has been added [13, 19]. Following this treatment, the eggs are rinsed with 70% ethanol. Looper eggs are routinely disinfected very early in the incubation period, usually within 24 h after deposition.

The principal pathogen to be dealt with is a virus in the cabbage looper, but in the case of the corn borer the principal pathogen is a microsporidian parasite that is transmitted in the embryo. This microsporidian is Perezia pyraustae, a protozoan that attacks the gut tract epithelial cells and the Malpighian tubules. Continuous laboratory culture of the borer requires elimination of this pathogen. We have accomplished this by the technique of Raun [19a]. This method calls for exposing the borer eggs to a rather high temperature (43°C) for 30 min. This rather drastic treatment is quite effective. We treated the first three generations of field population descendants, and have eliminated Perezia from our culture. Separation of the egg masses and surface sterilization of the eggs should follow rather than precede the heat treatment.

We have found that eggs treated by any of these several methods become quite susceptible to desiccation, and must be handled very carefully during subsequent incubation. Treated eggs of both our species are transferred aseptically from the last alcohol rinse on to sterile agar slants. These slants are prepared in very large test tubes (28 × 200 mm) and the substrate is a non-nutritive 4% agar. The drop of alcohol in which

the eggs are transferred quickly evaporates. The agar provides a moist substrate, but its surface should have no drops of standing water, as the newly hatched larvae may become trapped in such condensation. From the agar slants, the larvae are transferred to the culturing media. This is accomplished by means of sterile nichrome wire needles under the conditions of an aseptic transfer chamber. Experiments in which larvae have been allowed to walk across the surface of nutrient agar plants (potato-dextrose and peptone media) have shown that the larval feet were aseptic.

The maintenance of aseptic conditions in the larval-rearing containers is not difficult to maintain, if the cultures are left undisturbed during the entire growth period. Opening the cultures to replace dietary media or to introduce or remove larvae greatly increases the hazards of contamination. For these reasons, the best method is one in which the larvae are started on a dietary medium that is both quantitatively and qualitatively adequate for the larval growth period. With the European corn borer, we have found the most satisfactory rearing container to be a glass shell vial (23 × 85 mm), with a cylindrical block of diet, two borer larvae, and a cotton plug. Although borers may be reared in groups contained in cotton-plugged Erlenmeyer flasks, cannibalism is sometimes a problem, and the likelihood of microbial contamination seems to be greater. We have also found larval growth to be much more uniform with two larvae per vial than with twenty or more larvae in larger containers. The cabbage looper, on the other hand, is routinely reared in groups of about 10, using half-pint jars as rearing containers. In nutritional studies, the cabbage loopers have been reared in shell vials, with either one or two larvae per vial; under such conditions larval growth has been fully satisfactory.

IMPORTANCE OF PHYSICAL CHARACTERISTICS OF MASS-REARING MEDIA

The physical characteristics of a culturing medium is quite different from those of the insect's normal host plant. A question sometimes arises concerning whether or not a culturing medium should simulate the host plant in respect to form and texture. Of the species so far successfully reared on meridic and artificial media, none have been found to display rigid requirements in this regard. A possible exception is represented by species in which the caterpillars assume a very characteristic feeding stance in which the edge of the leaf of the host plant is straddled while the larva feeds on the edge of the leaf. Some of the saturniids have this feeding habit. Hyalophora cecropia and a few other Saturniidae species were reared on an agar-based artificial diet by Garrison et al. [20]. The dietary media were pressed into thin sheets, enabling the caterpillars to feed normally on the edges of the "artificial leaves".

Some larvae appear to be unable (or unstimulated) to begin feeding on a smooth agar surface. Feeding on an agar-based diet may thus be inhibited, especially if the dietary medium was poured into the rearing container so as to form a continuous layer. This difficulty can be overcome by scratching the surface of the medium with a sterile pointed instrument. The larvae will invade the medium at the furrows so produced.

Neither water content nor pH of the medium has proved to be highly critical to the acceptability of the diet. In general, any pH at which the

agar will gel normally will also allow normal larval feeding. As discussed earlier, pH influences the stability of certain dietary constituents and may require standardization. Water content also influences the consistency of the media, in that excessive water causes the agar gel to be soft and a low water content causes the medium to be relatively hard. Mass-rearing media with a water content between 65 and 85% are satisfactory, and we have found that if the water content of the media is outside that broad range, larval feeding and growth may be adversely affected. Some agar-based media may tend to dry somewhat during incubation, particularly if larval growth requires many weeks or months. Growth may be rather severely suppressed by such dietary desiccation.

IMPORTANCE OF FEEDING BEHAVIOUR TO MASS REARING

A satisfactory rearing medium must meet the insect's behavioural as well as its nutritional requirements. Quite obviously, the insect must be induced to ingest the diet, and to ingest amounts sufficient to meet its metabolic and developmental needs. And this consideration brings us to a discussion of the problem of host-plant specificity and the relationships between host-plant specificity and nutritional requirements. Since we are concerned only with larval specificity because we forcibly put the larvae on the diet, adult responses need not be taken into account. This simplifies our problem quite considerably, because much of host-plant specificity can be attributed to the specific behavioural patterns of the ovipositing female. The adult European corn borer, for example, has a somewhat restricted range of acceptable host species. The borer larva is polyphagous, however, and will feed readily on a very wide range of plant materials. Larvae of many species are more catholic in their feeding than is reflected by adult ovipositional behaviour. Another factor that influences natural host-plant specificity is that of the anatomy and growth pattern of the plant; this factor, too, is eliminated under mass-rearing conditions. Phenology and other ecological factors have also been eliminated under our artificial laboratory conditions. We have reduced the problem to its bare essentials – the relationship between the stereotyped behavioural patterns of the larva and the dietary medium which must provide the chemical and physical releasers required to elicit the insect's feeding. A wide range of feeding specificity is to be observed, ranging from virtually polyphagous forms such as the European corn borer to monophagous species such as the Catalpa sphinx, Ceratomia catalpae.

Larval feeding is a sequential pattern of separate behavioural responses, each component of which may display some degree of specificity in regard to releasing stimuli [21, 22]. The behavioural sequence may be described as: (1) Orientation to the possible food source; (2) an attempt to feed (biting response); (3) maintained feeding; and (4) cessation of feeding. Both the chemical and the physical characteristics of the rearing environment may influence these responses.

Orientation of the larvae to the medium is influenced by the insect's phototactic, geotactic, and thigmotactic responses as well as by chemotactic factors. The chemical factors involved in orientation can be classified as attractants and repellents. A satisfactory rearing medium should provide any attractants required for normal orientation, and obviously repellents should be absent. Attractants have not proved to be very important to the

species with which we have dealt. A rearing container such as a shell vial represents a rather confined situation in which the larvae do not have to search for the medium or to choose a favourable feeding site. Some of the dietary components may even have a slightly repellent effect, but the larvae become conditioned to the situation and will feed on the medium. Larvae with highly specific feeding habits may require the presence of specific attractants, however. The silk-worm, Bombyx mori, is a case in point, and according to the work of Hamamura et al. [23] citral and hexenol must be present in the medium as larval attractants.

Once oriented to the dietary medium and in contact with it, the larvae must be stimulated to initiate feeding. An incitant must be present in order to release the biting response. With the European corn borer, we have found that moisture and surface characteristics serve as biting incitants, and the insect requires no specific chemical stimulation. With less polyphagous species, the situation is not this simple, and rather specific incitants must be incorporated in the diet. The host-plant specificity of larval stages is probably determined primarily by the presence of incitants evoking a positive biting response and by feeding suppressants that inhibit the biting response. The incitants required for larval feeding behaviour have been identified in a number of cases. Larvae of the diamond-back moth, Plutella maculipennis, require the presence of sinigrin or some closely related substances for the initiation of feeding [24]. Beta-sitosterol and isoquercitrin were found to be required incitants for larvae of Bombyx mori [23]. Nayar and Fraenkel [25] reported that larvae of the Catalpa sphinx would not attempt to feed in the absence of catalpin, a glycosidic alkaloid found in foliage of the Catalpa tree. Mass rearing of many oliphagous and monophagous species will require inclusion of specific incitants in the rearing medium.

The biting response is followed by active feeding and ingestion. Feeding may be of very short duration or it may be maintained for relatively long periods. Caterpillars have a reputation as voracious feeders, and feeding may be almost continuous from one moult to the next. Chemical factors control the duration of these feeding periods, however. Feeding stimulants tend to prolong the duration of feeding and feeding deterrents tend to shorten or curtail active feeding. A satisfactory mass-rearing medium must provide the feeding stimulants needed to maintain a normal high rate of ingestion. In contrast to incitants, feeding stimulants are mainly nutrients. Sugars, amino acids, proteins, sterols, phospholipids, and fatty acids have all been found to act as feeding stimulants in one or many species [10, 26, 27]. The identity of many feeding stimulants as nutrients tends to simplify the problem of mass rearing, because a nutritionally well-balanced medium is also likely to satisfy the larval need for feeding stimulants. We have found that larval feeding is influenced by nutrient ratios and combinations, and some of these considerations may be significant in the development of mass-rearing diets. Several amino acids were shown to be feeding stimulants for larvae of the corn borer, but their stimulating effects could be detected only if the dietary medium contained a stimulating sugar such as sucrose or dextrose [26].

In some recent, still unpublished work on feeding behaviour of cabbage looper larvae, Gothilf and Beck have observed some interactions between nutrients and feeding stimulants that are of interest. The cabbage looper requires a dietary fatty acid for normal growth, but does not require a phospholipid [28]. The addition of wheat-germ oil to the rearing medium

satisfies this nutritional requirement. Wheat-germ oil also acts as a feeding stimulant, and experimentation with chemical fractions of the wheat-germ oil showed that the phospholipid fraction contained the feeding stimulant. But further study led to the finding that dietary inorganic ions exerted an effect on feeding stimulation by triglycerides and fatty acids. In the presence of potassium chloride in the medium, the neutral lipid fraction of wheat-germ oil exerted a feeding stimulation. In the absence of potassium chloride, no stimulation was observed. Cellulose, on the other hand, has been shown to be a feeding deterrent; its presence in the dietary medium greatly reduced the effectiveness of sugars and proteins as feeding stimulants.

DIAPAUSE AND MASS REARING

Diapause may pose a serious problem in attempts to carry out a mass-rearing programme. If the occurrence of diapause cannot be prevented or controlled, the rearing programme is limited in respect to the number of generations produced per year. The intervention of diapause at a particular growth stage in every generation will cause added time and financial expenditures associated with the storage and treatment of the diapausing forms. The synchrony between mass production and seasonal phenology required in field release programmes may be seriously disrupted by diapause. A further disadvantage imposed by the lack of diapause control lies in the resulting difficulty in the maintenance of a self-perpetuating laboratory population, necessitating the regular introduction of field-collected forms into the mass-rearing programme.

There is no question as to the desirability of controlling the incidence of diapause in mass-reared insect populations. The crucial question is whether or not such control is possible in a given mass-rearing programme. The concept of "univoltine" and "multivoltine" species or races within a species is now known to have little meaning in the absence of information on the environmental conditions involved. The temperatures and day-lengths under which the insects are grown are of particular importance in the determination of the number of generations produced per year. Similarly, the general concept that diapause may be "obligatory" in one species but "facultative" in another has been greatly weakened by research reported during the past decade. Numerous examples of so-called obligatory diapause – i.e., diapause that occurs in every generation regardless of rearing conditions – have been demonstrated to be fundamentally facultative. If the manifestation of diapause is influenced by environmental factors or is susceptible to experimental manipulation, the diapause in question must be considered to be facultative in nature.

One of the earliest demonstrations of the facultative character of a supposedly obligatory diapause was that of Harvey [29], who was able to establish and perpetuate a non-diapause strain of the spruce budworm, Choristoneura fumiferana. As long as this strain was cultured under long-day photoperiods, the larvae did not go into diapause. If exposed to short-day conditions, the newly hatched larvae diapaused. Similar demonstrations of the facultative nature of diapause have been reported for a large number of species. The most recent addition to the list is probably Hyalophora cecropia [30]. As better understanding of the in-

trinsic and extrinsic factors controlling diapause is acquired, the concept of obligatory diapause will probably become progressively less tenable.

Diapause is genetically determined, but its expression is a developmental response of the genetic system to environmental factors. Very marked geographical population differences in diapause induction have been described [31-34]. Continuous mass rearing without the intervention of diapause might be attainable by either maintaining the population under conditions that are conducive to non-diapause growth and development, or by the selection of genetic strains that are capable of continuous propagation under the standardized rearing conditions employed.

Within the mass-rearing situation, whether on a small laboratory scale or a large mass-production scale, a number of controllable factors contribute to the determination of diapause. Photoperiod is now recognized as the most important extrinsic factor in the determination of diapause. In most species, diapause is induced by a short-day type photoperiod (about 12 h of light per day), and is averted by long-day photoperiods (16 h or more of light per day). In some species, such as the commercial silkworm, the converse photoperiodic effects prevail. Similarly, species that display a summer aestival diapause may be committed to diapause by the influence of long day-lengths and high temperatures. In most cases, however, laboratory rearings in which diapause is undesirable should be carried out under conditions of controlled photoperiod and temperature. A very long day-length (about 18 h) in which the light intensity is relatively low (1-5 lux) and the temperature is towards the high end of the optimum range (25-30°C) will generally result in a relatively low incidence of diapause. Temperature tends to modify the photoperiodic effects, in that low temperatures tend to promote the short-day response and high temperatures augment the long-day effect. Rearing under continuous darkness prevents diapause in some, but by no means all species. Continuous illumination may also reduce the incidence of diapause, but continuous illumination sometimes has adverse effects on larval growth.

The environmental conditions experienced by the parents sometimes determine the incidence of diapause among the progeny. For this reason, it is important that the temperature and photoperiods to which the insects are exposed should be controlled at all stages of development, and such control must be maintained in every generation. If the photoperiod-sensitive growth stage of the species can be identified, the problem of diapause control may be simplified.

There is some evidence that the diet may influence the determination of diapause. Dietary water and fat concentrations have been implicated in the determination of diapause, but these factors appear to play no more than a minor modifying role. The results of Bull and Adkisson [35] with the pink bollworm, for example, showed that the incidence of diapause tended to be directly proportional to the fat content of the diet. To demonstrate this effect, however, the photoperiod employed had to be very close to the critical day-length for diapause determination. It may well be that, under conditions that are borderline between diapause and nondiapause, dietary factors may determine the direction of development. Compared with the influence of temperature and photoperiod, dietary effects have so far proved to be of relatively minor importance.

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BIOCHEMICAL REQUIREMENTS FOR MASS REARING OF PLANT-FEEDING LEPIDOPTERANS*

G.M. CHIPPENDALE** AND S.D. BECK
DEPARTMENT OF ENTOMOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WIS., UNITED STATES OF AMERICA

Abstract

BIOCHEMICAL REQUIREMENTS FOR MASS REARING OF PLANT-FEEDING LEPIDOPTERANS. Nutritional requirements of the European corn borer and cabbage looper illustrate general aspects of the dietary requirements of plant-feeding lepidoptera. This study concerns nutritional effects of ascorbic acid, fatty acids and minerals.

The mass rearing of plant-feeding lepidopterans on artificial diets poses special problems associated with behavioural response since many have developed intimate relationships with their host plants. Consequently, in many cases an artificial diet, besides being nutritionally adequate, must also provide suitable tactile and chemical stimuli for the insect [1]. However, the detailed nutritional requirements of the insect need not be known, if it will grow well on some readily available natural product. A few plant-feeding lepidopterans can be reared in such a way. For instance, Shorey and Hale [2] have developed a cheap, easily-prepared medium with lima beans as a major component for rearing nine species of Noctuidae. In many cases, however, it is neither convenient nor practical to rear plant-feeding insects on their natural food in the laboratory. For these, acceptable artificial diets, based on a knowledge of the nutritional requirements of the species, have to be developed. To date, satisfactory artificial media have been devised for several plant-feeding lepidopterans.

Beck et al. [3] reported the first successful aseptic rearing of a phytophagous lepidopteran, the European corn borer, Ostrinia nubilalis, on a meridic[1] diet which was unsatisfactory without the addition of plant material containing the so-called "corn leaf factor" (CLF). Also, Ishii and Urushibara [4] were able to rear the rice stem borer, Chilo suppressalis, on a modification of this diet which contained a rice stalk supplement. This stalk tissue supplied a necessary dietary component for normal development of the female moths.

Several attempts have been made to rear the monophagous silkworm, Bombyx mori, on an artificial diet [5, 6]. Some success was achieved

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** Present address: Department of Biochemistry, University of Leeds, England.

[1] The terms holidic and meridic were coined by Dougherty [8]. A holidic diet is one in which the constituents, other than purified inert materials, have a known chemical structure before compounding, whereas a meridic diet has a holidic base to which at least one substance of unknown structure is added.

when the diet was supplemented with mulberry-leaf extracts, which apparently contained both feeding stimulants and nutrients. Recently, Kato and Yamada [7], reported that a component of these leaves, chlorogenic acid, promoted larval growth when incorporated in the dry diet at a level of 1%.

For mass-rearing purposes, modifications of the diet originally developed for the pink bollworm, Pectinophora gossypiella, have proved especially useful. Vanderzant and Reiser [9] first formulated a meridic diet for the pink bollworm without a plant adjuvant. Further investigations, aimed at simplifying the rearing procedure, showed that the pink bollworm would grow on a medium supplemented with whole wheat germ [10]. This wheat-germ medium, with modifications, has been used to rear several other plant-feeding lepidopterans, including the bollworm, Heliothis zea [11]; the cabbage looper, Trichoplusia ni [12-14]; O. nubilalis [15]; the spruce budworm, Choristineura fumiferana [16]; the large white butterfly, Pieris brassicae [17]; the Douglas-fir tussock moth, Hemerocampa pseudotsugata [18]; the gypsy moth, Porthetria dispar [19]; and the codling moth, Carpocapsa pomonella [20].

TABLE I. COMPOSITION OF REARING MEDIA FOR THE EUROPEAN CORN BORER AND CABBAGE LOOPER

Component	European corn borer[a]	Cabbage looper[b]
Whole-wheat germ	20.0 g	30.0 g
Casein, vitamin-free	25.0 g	21.0 g
Dextrose	25.0 g	21.0 g
Powdered cellulose	8.0 g	18.0 g
Fibrous cellulose	10.0 g	--
Agar (Bacto)	18.0 g	15.0 g
Wesson salts	2.5 g	6.0 g
Sodium alginate	3.0 g	3.0 g
L-Ascorbic acid	3.0 g	3.0 g
Cysteine	0.6 g	--
Cholesterol	1.2 g	1.8 g
Wheat-germ oil	--	1.6 g
Corn oil and 1% alpha-tocopherol	1.6 g	--
Sorbic acid	0.72 g	0.8 g
Choline chloride	0.56 g	0.55 g
Vitamin solution	50.0 ml[c]	60.0 ml
Distilled water	610.0 ml	450.0 ml

[a] Modified from medium of Chippendale and Beck [15]

[b] Chippendale and Beck [14]

[c] An aqueous solution containing (mg/litre) myo-Inositol 4600, calcium pantothenate 860, niacin 330, para-aminobenzoic acid 155, riboflavin 48, pyridoxine HCl 37, thiamine HCl 28, folic acid 11, biotin 8, and B_{12} 2.

The nutritional research reported in this paper grew from the readiness of the European corn borer and cabbage looper to develop on wheat-germ supplemented diets (Table I). The nutritional requirements of these members of the Pyraustidae (corn borer) and Noctuidae (cabbage looper), will be used to illustrate general aspects of the dietary requirements of plant-feeding lepidopterans, and the practical application of the results to problems of mass rearing will be considered. The main areas of research to be discussed include the nature of the CLF for the borer and the attempts which have been made to characterize growth-promoting factors in wheat germ for both species. This study, therefore, concentrates especially on an investigation of the nutritional effects of ascorbic acid, fatty acids and minerals.

L-ASCORBIC ACID

Though all plant-feeding insects do not require ascorbic acid [9, 20a], Dadd's discovery [21] of the dietary indispensablilty of the vitamin for the desert locust, Schistocerca gregaria, prompted several investigations on ascorbic acid as a feeding stimulant and nutritional requirement for phytophagous insects. Ito [22] suggested that ascorbic acid was both a feeding stimulant and essential nutrient for B. mori. Vanderzant et al. [23] have also shown that the boll weevil, Anthonomus grandis, H. zea, and the salt-marsh caterpillar, Estigmene acrea, all fail to develop on meridic diets lacking ascorbic acid.

Research with the cabbage looper and the corn borer further demonstrated the dietary necessity of ascorbic acid for some lepidopterans, at the same time revealing different deficiency symptoms for the two species. A complete absence of ascorbic acid in the cabbage looper's diet resulted in total larval mortality at an immature stage [24], whereas in a corn borer's diet a similar deficiency resulted in a prolonged larval growth rate and the emergence of non-fecund adults [15].

A further analysis of the effect of this vitamin on the corn borer led to the conclusion that ascorbic acid was in fact the CLF. This interpretation was derived from nutritional results reinforced by a comparison of the wheat-germ supplemented diet and the alfalfa supplemented diet previously used for borer rearing [25], and an analysis of some of the chemical properties of the CLF [26]. As much as 500 mg % of ascorbic acid was required in the diet to allow normal development of the borer larvae. This amount was necessary to supply the larval needs and allow for some loss of activity through oxidation since ascorbic acid is very unstable in solution, especially under conditions which permit gaseous exchange with the atmosphere [27]. Some loss of ascorbic acid can be prevented by using freshly prepared media with a slightly acidic pH to which ascorbic acid is added after autoclaving. The addition of reducing agents such as cysteine, or reduced glutathione, and decreasing the level of ferric and cupric ions in the medium also assist in preventing the breakdown of the vitamin.

Since ascorbic acid is so unstable in solution, an excess should be added to diets for mass rearing insects which require the vitamin. For many plant-feeding insects, a level of 500 mg % of the diet seems satisfactory. In the past, failure to rear many phytophagous insects on diets

without plant materials was at least in part due to the absence of dietary ascorbic acid.

FATTY ACIDS

The observation that a dietary supplement of wheat germ was necessary for good development of both the looper and borer led to a series of experiments in which the growth stimulatory effects of various fractions of wheat germ were tested [24]. Whole-wheat germ is such a rich nutritional source that it is difficult to determine the specific nutrients it contains. The material is likely to be rich in both feeding stimulants and nutrients and therefore fractionation can create difficulty in an accurate interpretation of nutritional results. For example, wheat-germ oil has been shown to contain feeding stimulants for two species of grasshoppers [28]. They showed that lecithins and phosphatidyl inositol isolated from wheat-germ oil evoked feeding activity from old nymphs and adults. Thus, the fractionation of wheat germ may result in the omission of feeding stimulants from the diet and either delay or reduce larval feeding, even in cases where the diet is nutritionally adequate.

The fractionation results [24] showed that the looper required at least two nutritional factors contained in wheat germ. The first, a factor in wheat-germ residue, after chloroform:methanol (2:1 v:v) extraction, was required for normal larval growth. The second, a factor in wheat-germ oil, was required for both larval growth and normal wing development.

This wing deformity symptom of the looper was studied in some detail. Lack of the wing development factor resulted either in various gradations of wing deformity or even in total failure of moths to emerge from the pupal exuviae. The necessary factor was found to be contained in the saponifiable portion of wheat-germ oil. As a result, several fatty-acid methyl esters were tested. It was found that a dietary supplement of methyl linoleate promoted larval growth and normal pupation, but did not produce adults with normal wings. Of several others assayed (oleate, linolenate, arachidonate, eicosapentaenoate, and docosahexaenoate), only methyl linolenate (500 mg % of the diet) allowed both normal larval growth and wing development [29].

Somewhat different results were found for the borer. Although a series of experiments with this insect showed that a wheat-germ supplement was necessary for good larval growth, methyl linoleate or linolenate were unnecessary for adult emergence [30]. With either of these fatty acids in the diet, however, the larval growth-rate was slightly improved.

A dietary requirement for polyunsaturated fatty acids has been demonstrated for several other lepidopterous and orthopterous species. With some exceptions, linoleic or linolenic acid can fulfil this requirement. In the genus Ephestia, the flour moths, linoleic or linolenic acids promoted larval growth and normal wing development [31]. Arachidonic acid improved the growth-rate of Ephestia, but had no positive effects on wing development. Linoleic acid allowed normal wing development of P. gossypiella but because lower levels were active, linolenic acid was considered more effective [32]. Normal wing development of the greater wax moth, Galleria mellonella, and the red-banded leaf roller, Argyrotaenia velutinana, were promoted with linoleic or linolenic acids, but not with arachidonic acid [33, 34]. For the smaller tea tortrix,

Adoxophyes orana, however, linolenic acid was necessary for adult emergence, giving results essentially similar to those obtained for the looper [35].

Yet, despite all this nutritional research, investigations of insect fatty-acid metabolism are still in their infancy [36]. For example, the biochemical effect of unsaturated fatty acids on wing development has not been determined. Fraenkel and Blewett [31], speculating on this effect, suggested that the unsaturated fatty acids may be associated with the production or action of the moulting fluid, which in this case acts as a lubricant in the separation of the adult wing from the pupal cuticle.

Most meridic diets for plant-feeding lepidopterans contain corn oil or wheat-germ oil as relatively cheap sources of fatty acids. Since linolenic acid satisfies the requirements for the species investigated, linseed oil (containing about 50% linolenic acid, [37]) would appear to be a more suitable source.

MINERALS

Very little research has been conducted on the mineral nutrition of plant-feeding lepidopterans [38]. This paucity of information results mainly from mineral contamination of too many of the meridic dietary components, making detailed investigations of mineral requirements impractical. Even at present, many meridic diets for phytophagous lepidopterans use minerals supplied by Wesson's salt mixture, which was originally developed for vertebrate nutritional research [39]. It is therefore not necessarily optimal for use as a dietary supplement for plant-feeding insects. The use of a mineral mixture resembling more closely the mineral composition of their host plants would seem more appropriate.

Some attempts have been made to study the mineral requirements of the corn borer. It was found that a dietary supplement of the ash of the non-lipid portion of wheat germ (wheat-germ residue) stimulated larval growth. Though the exact nature of the minerals in the ash responsible for this effect are not yet fully known, the observation led to the development of a more satisfactory salt mixture than Wesson's, and to the formulation of a diet without plant adjuvants, which permitted excellent larval growth.

The composition of the new salt mixture for the corn borer is shown in Table II. It was slightly modified from a mineral mixture developed for the confused flour beetle, Tribolium confusum [40]. The composition was based on elemental analyses of various diets for the beetle, including those containing whole-wheat ash, and on the known mineral requirements of other insects. Our changes in the mixture were the use of mono and dibasic potassium phosphate to control the pH at about 6.7, and the replacement of zinc chloride by the less deliquescent zinc acetate.

The composition of the diets used to test the effect of this new salt mixture on the larval growth-rate of the borer are shown in Table III. In diets 2 and 3, additional supplements of vitamin-free casein and cellulose powder were used to replace the bulk of the wheat-germ residue. The results (Table IV) show clearly that the larval growth-rate on the diet supplemented with the new salt mixture was superior to that which

TABLE II. COMPOSITION OF NEW SALT MIXTURE FOR THE EUROPEAN CORN BORER
(modified from Medici and Taylor [40])

Constituent[a]	% in mixture	% in diet
K_2HPO_4	49.75	0.172
KH_2PO_4	18.00	0.062
$MgSO_4$	16.00	0.055
$Ca(H_2PO_4)_2 \cdot 1H_2O$	8.00	0.028
NaCl	5.00	0.017
$Fe_2(SO_4)_3 \cdot 6H_2O$	2.00	0.007
$MnSO_4 \cdot 3H_2O$	0.50	0.0017
$Zn(OOCH_3)_2 \cdot 2H_2O$	0.50	0.0017
$CuSO_4 \cdot 5H_2O$	0.25	0.0009
	100.00	0.3453

[a] Reagent or analytical reagent grade

TABLE III. COMPOSITION OF DIETS TO TEST THE EFFECT OF NEW MINERAL MIXTURE ON GROWTH-RATE OF THE EUROPEAN CORN BORER[a]

Component	Diet 1	Diet 2	Diet 3
Wheat-germ residue	4.0 g	--	--
Casein, vitamin-free	5.0 g	6.4 g	6.4 g
Dextrose	5.0 g	5.0 g	5.0 g
Agar	3.6 g	3.6 g	3.6 g
Fibrous cellulose	2.0 g	2.0 g	2.0 g
Powered cellulose	1.6 g	3.0 g	3.0 g
Sodium alginate	0.6 g	0.6 g	0.6 g
L-Ascorbic acid	0.6 g	0.6 g	0.6 g
Wesson salts	0.5 g	0.5 g	--
New salts	--	--	0.5 g
Corn oil and 1% alpha-tocopherol	0.32 g	0.32 g	0.32 g
Cholesterol	0.24 g	0.24 g	0.24 g
Sorbic acid	0.14 g	0.14 g	0.14 g
Cysteine	0.12 g	0.12 g	0.12 g
Choline chloride	0.11 g	0.11 g	0.11 g
Vitamin solution[b]	10.0 ml	10.0 ml	10.0 ml
Distilled water	122.0 ml	122.0 ml	122.0 ml

[a] For details of preparation, see Chippendale and Beck [15]
[b] See Table I, footnote

TABLE IV. EFFECT OF NEW SALT MIXTURE ON LARVAL GROWTH-RATE OF EUROPEAN CORN BORER[a]

Performance on 17th day at 30°C	Diet 1 Wheat-germ residue, Wesson's salts	Diet 2 Casein, cellulose, Wesson's salts	Diet 3 Casein, cellulose, New salts
Moribund larvae (No.)	3	11	0
Larvae (No.)	5	30	4
Pupae (No.)	48	8	37
Pupation (%)	91	21	90

[a] Larvae reared aseptically in pairs in cotton-plugged shell vials [15]

resulted on the diet supplemented with Wesson's salts. This improved salt mixture for the European corn borer should be useful in mass-rearing studies of other plant-feeding lepidopterans, as it contains relatively few components and is easy to prepare.

SUMMARY

The results of nutritional experiments on the European corn borer, Ostrinia nubilalis, and the cabbage looper, Trichoplusia ni, were used to illustrate several important points about mass rearing plant-feeding lepidopterans. Although the chemical and physical nature of the diet for each plant-feeding insect has to be worked out individually, some general conclusions about likely dietary requirements can be drawn.

Several plant-feeding lepidopterans, including the borer and looper, require dietary ascorbic acid for normal development. Where such is the case, sufficient ascorbic acid must be added to the diet to allow for some loss of activity through oxidation. In the past, failure to rear many plant-feeding insects satisfactorily on meridic diets without plant-adjuvants can in part be attributed to a lack of dietary ascorbic acid.

Several workers have shown that many plant-feeding lepidopterans require a dietary unsaturated fatty acid for good development. In our study, the cabbage looper required dietary methyl linolenate for larval growth and wing development, whereas for the borer dietary supplements of methyl linoleate or linolenate only had slight stimulatory effects on larval growth-rate. Since linolenic acid will satisfy the requirement for the species studied to date, a dietary supplement of linseed oil is suggested as a relatively cheap source of this chemical.

In a study of mineral requirements, it was found that the larval growth of the borer on a diet supplemented with a new salt mixture (based on one developed for the confused flour beetle) was significantly better than on a diet supplemented with Wesson's salts. The latter salt mixture, commonly used in meridic diets for plant-feeding lepidopterans, is not necessarily optimal since it was developed for vertebrate nutritional research. The new salt mixture described for the borer, on the other hand, would appear to be more suitable for plant-feeding insects because it more closely approximates the mineral composition of their host plants.

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THE NUTRITIONAL REQUIREMENTS OF DIPTERA

W.G. FRIEND
DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF TORONTO,
TORONTO, ONTARIO, CANADA

Abstract

THE NUTRITIONAL REQUIREMENTS OF DIPTERA. Qualitative and quantitative nutritional requirements for 19 dipterous larvae are reviewed.

This review is restricted to a critical appraisal of the nutritional requirements of the larvae of Diptera. It includes only work done under axenic (sterile) conditions on holidic (chemically-defined) diets. Reviews of insect nutrition giving much broader coverage are available [1-10]. Special attention is given to publications that appeared after 1962. The literature up to 1962 has been recently reviewed by House [8].

Dipterous larvae are well adapted for feeding on agar-based diets. Such diets are easy to sterilize and handle. The larvae tunnel in the diets, and consequently they cannot be easily weighed or measured. Normal nutritional-balance studies are also almost impossible to conduct because of the difficulties of collecting excretory products. Most workers have determined the effectiveness of various diets by measuring the time taken to reach various stages of development.

Qualitative nutritional requirements have been determined by omitting one or several nutrients from the diet and comparing development rates, numbers of insects reaching a certain stage, and/or reproductive efficiency. Single-deletion tests may yield misleading results because of the interconvertibility of certain nutrients. In Drosophila melanogaster for instance, folic acid can be partly replaced by thymidine, by purine, by serine or any combination of these [11]; biotin can be partially replaced by citrulline [12], glutamic acid, or any amino acids that degrade to glutamic acid [11]. Aspartic acid and glutamic acids play an important role in transamination reactions of insects in general [10, 13] and D. melanogaster in particular [14]. These amino acids, like methionine and cystine, are interconvertible in dipterous larvae [15], and consequently single-deletion techniques will fail to demonstrate the alternate requirements.

Quantitative requirements have usually been determined by varying the relative concentrations of nutrients in the diet, supplying the diet ad libitum, and measuring development rates, or the resulting weights of pupae or adults. Sang [16] has noted that such determinations give no direct measure of the quantities of each nutrient required; they define only the relationships between particular requirements. The animal under test may compensate for a lower concentration of a given nutrient per unit volume of diet by eating more. Under these conditions, the development rate may be slowed because the test organism has to metabolize other nutrients which it is getting in excessive amounts. To minimize these

effects, Gordon [17] has suggested the elimination from the diet of all non-essential nutrients in order to attain a minimal diet. House (personal communication) points out that data concerning quantitative requirements would be much more useful if these were expressed as the amount of nutrient eaten per day, or per unit of body weight, or per calorie of intake. These measurements would be extremely difficult to make with dipterous larvae that live and develop in the experimental diets.

One outstanding feature of the physiology of dipterous larvae that affects their nutritional requirements is the extraordinary speed at which they grow. D. melanogaster larvae show nearly a thousandfold increase in size in about 4 d. This growth is supported by metabolic reactions that have extremely high rates. Although purine biosynthesis is very fast in D. melanogaster when compared with other animals, it appears to be the metabolic reaction that limits growth, and dietary supplies of this nutrient must be supplied if maximum growth rates are to be attained [17]. Other dipterous larvae also require RNA or purines in their diet for maximum growth rates [6]. Apart from dipterous larvae, the only other insect that has been shown to require RNA is the beetle Sciobius granosus, [18].

REQUIREMENTS FOR PROTEIN OR AMINO ACIDS

No one has yet shown that any dipterous larva requires specific proteins or large polypeptides in their diet. The tripeptide glutathione was found to be necessary for larvae of Aedes aegypti larvae grown on a holidic diet containing amino acids, and greatly improved larval development on a similar diet containing casein [19]. Larval development was abnormally slow on both diets, however, and more recently Akov [20] has found that glutathione was not necessary when A. aegypti were fed another holidic diet with casein included as a source of amino acids.

When casein is replaced by a mixture of L-amino acids in the diet of D. melanogaster [21, 22], larval development is slowed. Erk and Sang [23] speculate that "casein provides the essential protein precursors, and perhaps includes as well some components, such as polypeptides, that are used to bring the larval development times close to those on live yeast cultures. The optimum amino acid balance has still to be determined for any Drosophila species."

Most of the amino acid requirements have been determined by omitting single amino acids from a mixture. This technique can give results which are misleading. It was originally reported [24] that larvae of Phormia regina required all the 10 essential amino acids except methionine, and that proline was essential. Deletion of two or more amino acids at a time showed that this insect required either methionine or cystine, and either glutamic acid or aspartic acid [15]. Later work by Kasting and McGinnis [25] showed that proline, isolated from larvae of P. regina injected with glutamic acid -U-C^{14}, had the highest specific activity of the amino acids studied; this indicated a high rate of proline synthesis. Re-investigation of the proline requirement, using the single-deletion method and a local strain of P. regina, showed that these insects did not require proline in the diet. Henry and Block [26] demonstrated that P. regina larvae could not synthesize methionine from cystine or sulphate, which indicates a dietary requirement for either methionine or a suitable thiomethyl precursor.

Dipterous larvae, when studied critically, have all been shown to require the same 10 amino acids that are essential for the rat, namely; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. There are conflicting reports concerning the glycine requirement of larvae of A. aegypti. Golberg and De Meillon [27] claim it is essential, and Singh and Brown [19] claim that it is not. When high concentrations of glycine (4.8 mg/g of diet) were incorporated into the diet of Calliphora vicina (= C. erythrocephala), the toxic effect of certain D-amino acids was reduced [28]. Hinton et al.[21] found a similar effect in D. melanogaster. Glycine, serine or formate are converted to ethanolamine by larvae of P. regina [29].

Non-essential amino acids are usually included in holidic diets because they stimulate the rate of larval development, growth, or egg production. The addition of alanine, glycine, serine and tyrosine improved the diet for Agria (= Pseudosarcophaga) affinis [30], as did the addition of hydroxyproline, proline and serine in the diet of A. aegypti [19].

Protein quality is determined by its digestibility, and the relative concentration of amino acids that are its constituents. Casein is the protein most often used in insect diets and its optimum concentration varies as to species: 5% of the diet for D. melanogaster [16], 1% for A. aegypti [20, 27]. Gordon [17] postulated that egg albumen would be the best protein source for rapidly growing insects, but most amino acid mixtures tested on dipterous larvae so far have relative concentrations of amino acids that resemble casein. When amino acids are substituted for protein, the "protein quality" can be varied. House [31] has demonstrated that larvae of Musca domestica are sensitive to changes in the relative concentration of amino acids in their diet. He tested four amino mixtures; the first resembled a casein hydrolysate, the second was the most successful amino acid mixture developed for Hylemya antiqua, the third approximated the amino acid composition of a liver hydrolysate, and the fourth resembled the hydrolysate of a yeast protein. Each amino acid mixture contained 19 amino acids. None of these diets showed normal development, but the third mixture was superior to any of the other three.

Replacing proteins in the diet with amino acids may affect larval development adversely, because osmotic effects may make it impossible to establish high enough amino nitrogen levels [19, 24, 32]. However, House reports that amino acid mixtures as high as 20% could be fed to larvae of A. affinis, and the larvae grew well. House [33] states: "This work also shows that A. affinis can be reared on diets of high nutrient concentrations regardless of whether the osmotic pressure of its food medium is greater than that of its blood. Apparently the limitations that seem to be imposed by osmotic pressure relationships, and that may occur if only the level of amino acids increased [32], are overcome even at high nutrient levels provided that some degree of satisfactory nutrient balance is maintained."

Free amino acids have various distinctive tastes and some may make the diet unappetizing [17].

THE LIPOGENIC NUTRIENTS

Sterols

Only one insect has been shown to be able to synthesize cholesterol, Ctenolepisma sp. [34]. All other insects studied critically require and cannot synthesize sterols [10]. The utilization of sterols by insects has recently been reviewed [35], and Levinson [36] has described the function of dietary sterols in phytophagous insects. With the notable exception of Drosophila pachea, cholesterol has been shown to be nutritionally superior or equal to other sterol analogues for all dipterous larvae that have been tested critically. Minor changes in the structure of the cholesterol molecule can greatly affect the nutritional activity. This indicates that the specificity of these substances is very high, a supposition supported by Robbins [37], who found that sparing sterols such as cholestanol could not entirely replace the requirement of M. domestica for cholesterol. The nutritional effects of variation in the cholesterol molecule varies from species to species; the recent work of Levinson and Bergmann [38, 39, 40], Robbins [37] and Kaplanis et al. [41] describe these effects. The hydroxyl group at C-3 is very important. Changing it from the β to the α position destroys its activity for Musca vicina. 3-β-cholestanol also allows some larval growth for this species, but 3-α-cholestanol is completely inactive [40]. The hydroxyl group can be esterified and still retain some nutritional activity. Acetate, laurate, and oleate esters of cholesterol were nutritionally active for M. vicina. Cholesteryl acetate is also well utilized by A. affinis [8]. Dipterous larvae probably have esterases that can hydrolyse these esters, releasing free cholesterol [40]. Support for this view comes from two sources. Robbins [37] found that M. domestica larvae esterified some of their dietary cholesterol, and most of these reactions are reversible. Cholesteryl fluoroacetate is non-toxic when injected into tissues lacking lipases, but causes rapid mortality when fed to M. vicina [40]. The absence of the hydroxyl groups at C-3 renders the molecule inactive, and its substitution by chlorine causes it to inhibit the growth of M. vicina larvae, even when adequate quantities of cholesterol are present in the diet [38]. The importance of the hydroxyl group at C-3 is further emphasized by the finding that alkyl ethers of cholesterol are inactive, and thiocholesterol is toxic for M. vicina [40].

The addition of a double bond between C-7 and C-8 does not remove its nutritional activity, although it reduces its effectiveness by about half for M. vicina [38]. This double bond is essential for D. pachea, which cannot use cholesterol [42]. Δ^7-dehydrocholesterol is well utilized by larvae of A. affinis [8].

Ergosterol, which differs from Δ^7-dehydrocholesterol by having a double bond in its side chain, is well utilized by D. melanogaster [43], A. aegypti [44], A. affinis [8], M. vicina [44] and P. regina [45]. D. pachea, however, cannot use either ergosterol or Δ^7-ergosten-3β-ol, [42].

The presence of a branched side chain such as is found in β and γ sterol, does not inactivate side chain containing a double bond as in stigmasterol, does not inactivate the molecule for M. vicina [38], or for D. melanogaster [43]. β-stigmasterol and sitosterol are also well utilized by A. affinis [8], A. aegypti [44], and P. regina [45]. M. domestica also can utilize β-sitosterol, and its pattern of utilization and metabolism has

been found to be almost identical with that of cholesterol. There was, however, no detectable conversion of β-sitosterol to cholesterol in this species [37], whereas the two are interconverted by M. vicina [36]. β-sitosterol is only about one fourth as efficient as either campesterol or cholesterol for supporting larval growth in M. domestica, and allowed less than 2% adult emergence [41]. Both β-sitosterol and stigmasterol were inactive for D. pachea, but larval growth and maturation was produced by Δ^7-stigmasten-3β-ol and $\Delta^{5,7}$-stigmastadiene-3-β-ol, again showing that the double bond at C-7 is essential. This latter compound, $\Delta^{5,7}$-stigmastadiene-3-β-ol, did not, however, give fertile females [42].

Bergmann and Levinson [38, 40] have reported on an extensive series of modifications to the cholesterol molecule, which either eliminates or severely affects the growth-promoting activity of this molecule for larvae of M. vicina. Saturation of the double bond between C-5 and C-6, as found in 3β-cholestanol allows some larval growth, but no pupation. 3α-cholestanol had no activity. Introduction of a hydroxyl or a keto group at C-7, or removal of the side-chain inactivates the molecule.

General statements based on the few species of Diptera that have been studied thus far would be of little value. M. domestica and M. vicina differ in the way they metabolize β-sisterol [40, 41]. D. pachea lacks enzyme systems found in other Diptera in that it cannot convert Δ^5-3β-sterols or C-24 methyl-substituted sterols to metabolically active compounds. It can only utilize sterols with a double bond at C-7 and requires either a hydrogen atom or an ethyl group at C-24. An additional double bond at C-5 can be tolerated in the cholestane series, but it causes non-fertile females in the stigmastane series.

Fatty acids

Most insects can synthesize fatty acids from the carbon chains of amino acids or carbohydrates. Certain Lepidoptera require linoleic acid for normal growth, wing development or adult emergence [8]. Linoleic and linolenic acids had no nutritional value for A. aegypti [44]. Only two species of Diptera have been shown to benefit from the incorporation of fatty acids into holidic diets. The growth rate of C. vicina larvae was significantly improved when a mixture of sesame and sunflower oil or hydrogenated coco-nut oil was added to the diet [28]. House and Barlow [46] have shown that the growth rate of A. affinis grown aseptically on a holidic diet is accelerated by the addition of certain fatty acids. Oleic acid was the most effective single fatty acid, but a mixture of palmitic, stearic, oleic, linoleic and linolenic acids gave the maximum growth rates. At the levels tested, 0 to 0.5% of the diet, the need for oleic acid was shown to be independent of any interaction with biotin or cholesterol. Previous work [47] demonstrated that the requirement for fat was independent of a need for carbohydrate. Barlow [48] surveyed the fatty acids in 30 species of insects including five Diptera: A. affinis, M. domestica, D. melanogaster, A. aegypti and Hylemya brassicae. He found a high concentration of palmitoleic acid in all these fly larvae. Barlow [49] states "there may be some relationship between the accumulation of C 16:1 in the fats of A. affinis and their apparent lack of requirement for the polyunsaturated fatty acids". In a later study, Barlow [50] determined that the characteristically high palmitoleic acid content of A. affinis, when fed on diets lacking fatty acids, was reduced when oleic acid was

fed. The body fats still contained unusually high proportions of palmitic, palmitoleic, and oleic acids even when a mixture of fatty acids was fed. Development of M. domestica larva was slightly accelerated by fatty acids in their diets [51], and the concentration of lipids in their body was directly related to the amount of fatty acid in their diet. In this, M. domestica differs from A. affinis, which maintained the same lipid content even when fatty acids were omitted from the diet. Lack of oleic acid in the diet of M. domestica gave the same results as omitting all fatty acids. Oleic acid in the diet causes a reduction in the concentration of palmitoleic and oleic acids in M. domestica fats. The effect is similar to that found in A. affinis, but more pronounced. Linoleic acid could not be detected in the body fats unless it was fed [51]. When C. vicina is fed acetate-1-C^{14} in a holidic diet under axenic conditions the larvae synthesized higher fatty acids, chiefly palmitic, on a large scale [52]. Later work by Miura et al. [53] showed that when this species was reared on a semi-synthetic diet containing acetate-1-C^{14}, the saturated fatty acids were twice as radioactive as the unsaturated fatty acids. The authors suggest that unsaturated fatty acids cannot be formed by dehydrogenation of saturated fatty acids in this species.

Analysis of 50 inbred lines of D. melanogaster showed that diet greatly influenced the total lipid content, the relative proportion of the classes of lipid, and the fatty acid distribution within a class [54].

The thermal resistance of A. affinis can be raised if larvae are fed on a diet that contained a high proportion of saturated fatty acids. The degree of saturation of body lipids was directly influenced by the degree of saturation of the dietary lipids [55].

Fat soluble vitamins

The only dipterous larva that has been shown to require any of the fat soluble vitamins is A. affinis. Recently, House [56] has demonstrated that vitamin E, alpha-tocopherol, is an essential nutrient for reproduction, and that vitamin A accelerates growth and development. Previous to this, Cohen and Barker [57] showed that M. domestica contained vitamin A when fed β-carotene, but on a vitamin-A-free diet, none of vitamin-A aldehyde, alcohol, or retinene could be detected in the insects.

Inositol

No dipteran has yet been shown to require inositol, a compound that acts as a water-soluble lipotropic agent in mammals.

Lecithin

Lecithin has been reported to have a growth stimulating effect on A. aegypti [44], but is inactive in M. domestica [58]. Sang [16] reports that lecithin is not an essential nutrient for D. melanogaster, but does increase the rate of larval development. Lecithin stimulated development even when an optimal supply of choline was present in the diet, and therefore must supplement some synthetic process other than those involving choline. Choline can entirely replace lecithin in Drosophila subobscura, Drosophila ambigua and Drosophila obscura. It is also not an essential

nutrient for Drosophila funebris and Drosophila immigrans [59]. Lecithin has not been found to be an essential nutrient for any dipterous larvae that has been studied thus far.

Choline

Gordon [17] classified choline as a lipotropic agent instead of a water-soluble vitamin because it is required at much higher dietary levels than are the vitamins, and it does not act as a coenzyme, but as a structural part of complex lipids, along with ethanolamine, serine, glycerol and fatty acids. Choline is an essential nutrient for A. affinis [30], C. vicina [28], D. melanogaster [16], D. subobscura, D. ambigua, D. obscura, D. funebris, D. immigrans [59], Drosophila simulans [23], H. antiqua [60], M. vicina [61], P. regina [24], A. aegypti [19], and M. domestica [55].

In 1964, Hodgson and Dauterman [62] reported on the effects of substituting 15 analogues of choline in diets fed to P. regina larvae. The criterion was weight gain after 7 d growth at 27°C. Lengthening the carbon chain of choline rendered the molecule incapable of supporting adequate growth. Substituting one of the N-alkyl groups with other chain lengths up to n-butyl gave compounds that were excellent substitutes for choline. When two of the N-alkyl groups are other than methyl, only the diethyl methyl analogue was effective. When all the N-alkyl groups differ from methyl, only the triethyl analogue had any effect, and it gave less than half the weight gain shown by choline. Previous work had shown that the following compounds are effective choline substitutes for P. regina larvae: 2, 2-dimethylaminoethanol; carnitine; γ-butyrobetaine [63]; methyl choline; 2, 2-dimethylaminoisopropanol [64]. On the basis of these findings, Hodgson and Dauterman conclude that, in order to be an effective substitute for choline in P. regina, the compound in question must have :"... at least two N-methyl groups (the only exception to this is the diethyl methyl analogue); the third alkyl group can vary, at least up to n-butyl, or it can be missing completely..... the hydroxyl group must be on the second carbon from the nitrogen or, alternatively stated, if a terminal hydroxyl group is present the carbon chain must be two carbon atoms long."

Geer and Vovis [65] have tested 23 structurally related compounds as substitutes for choline in the diet of D. melanogaster. Their criteria for effectiveness were the development times from hatching till pupation, percentage of larvae to pupate and to emerge as adults. They found that none of the substitutes, which included carnitine, could completely replace choline in the diet. The intact choline molecule is essential for maximal growth action. As found with P. regina, there was a progressive loss in choline activity as the N-methyl groups were replaced with ethyl groups; monoethyl choline was almost as effective as choline; diethylcholine gave reasonably good larval growth and about 90% pupation, but only a few adults emerged; triethylcholine gave almost no choline effect. Removal of the methyl groups, without the substitution of ethyl groups, showed that the ability to support growth to pupation was proportional to the number of methyl groups present; 2-dimethylaminoethanol was two-thirds as effective as choline, 2-methyl-aminoethanol, one-third, and 2-aminoethanol was almost inactive. Feeding betaine, a known methyl group donor, with these compounds did not improve larval growth, and from this the authors conclude that the methyl groups of choline are not used for transmethylation. Both sets of workers found that the spatial relationships between the methyl groups and the nitrogen

atom, and the maintenance of a protonated nitrogen atom, were important. Replacement of the nitrogen atom with sulphur, and reducing the number of methyl groups to two, as a sulphocholine, reduces the growth activity. Geer and Vovis agree with Bieber and Newburgh concerning the importance of the hydroxyl group on the second carbon from the nitrogen. Homocholine, in which the hydroxyl group is three carbons away from the nitrogen, only allowed between 66 and 86% emergence in D. melanogaster, the adults lived only a few days, and half of them had abnormally developed wings. The importance of the hydroxyl group indicates that the action of choline probably depends on its esterification. D. melanogaster larvae can utilize esters of choline for growth very effectively. D. melanogaster can use carnitine, deoxycarnitine and β-methylcholine in place of choline, apparently by converting deoxycarnitine to carnitine, and then to β-methylcholine. D. melanogaster can also convert betaine aldehyde to choline [65].

These two extensive studies show that there are more similarities than differences in the way larvae of P. regina and D. melanogaster can utilize analogues of choline.

Choline has been shown to be an important structural element of the characteristic phospholipids found n Diptera; for example, the fat body of Sarcophaga bullata was shown to contain the following proportions of phospholipids: 51% phosphatidylethanolamine, 21% phosphatidylcholine, 14% lysophosphatidylethanolamine and 13% lysophosphatidylcholine. No sphingosine - containing lipids were detected [66]. This ratio of phosphatidylethanolamine to phosphatidylcholine is characteristic of Diptera [67].

Bridges et al[68] have shown that the choline in the phospholipids of the housefly M. domestica can be replaced by other bases. Larvae reared axenically on holidic diets incorporate β-methylcholine into phospholipids in place of choline, when either carnitine or acetyl-β-methylcholine are substituted for choline in the diet. When monomethylaminoethanol or dimethylaminoethanol were added to the larval diets, these bases replaced choline, and partially replaced ethanolamine, in the phospholipids. Replacement of choline by carnitine or β-methylcholine enabled the larvae to develop normally, pupate, and give adult flies which still retained the abnormal phospholipid pattern.

REQUIREMENTS FOR WATER-SOLUBLE VITAMINS

Nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine are probably essential nutrients for all immature insects [8]. Biotin and folic acid may also fall into this category, although there are some conflicting reports concerning these two vitamins. Determination of vitamin requirements usually has been done by the single omission technique. Since vitamins, particularly biotin, are required in such minute quantities, contamination of the diet with vitamins, or materials with the ability to replace or spare vitamins, seriously complicates the situation. For example, House [30] found that larvae of A. affinis would grow normally under axenic conditions when any one of the following nutrients was omitted from the holidic experimental diet: pyridoxine, folic acid, para-aminobenzoic acid, inositol or vitamin B_{12}. Later, however, Barlow found that these larvae had significantly lower transaminase activity than normal when grown on the "pyridoxine-free" diet, and that some of the components

of the diet contained enough pyridoxine, or chemicals that could act in its place, to sustain development [69]. He concludes that A. affinis requires pyridoxine. M. domestica larvae, grown axenically on the same "pyridoxine-free" diet, failed to develop unless additional pyridoxine was added, indicating that this latter species has a higher pyridoxine requirement. House [8] notes that his failure to demonstrate the essentiality of folic acid for A. affinis may be due to folic acid contaminants in the diet, folic acid reserves in the embryo, or to the sparing action of thymidine, purine, or serine, all of which were present in the diet. Sang [11] has shown that D. melanogaster can synthesize some folic acid, and that it can be partially replaced by thymidine, purine and serine, singly or in combination. M. domestica larvae require folic acid in their diet [70]. House and Barlow [71] were unable to demonstrate this need for M. domestica on a holidic diet containing RNA and serine.

One of the most noteworthy findings concerning the vitamin requirements of Diptera is that D. simulans is apparently capable of normal development on diets lacking biotin, or on diets containing from 0.005 to 0.04 μg of desthiobiotin per 5 ml of diet; this substance acts as an anti-metabolite for biotin in mammals [23]. Sang [72] had previously shown that some D. melanogaster larvae could survive on a diet containing 4% casein and no biotin. When the protein content of the diet was raised to 7% casein, 0.1 μg of biotin per 5 ml of diet was required for normal growth, 0.01 μg would not support growth. Sang claims that biotin is not required for the metabolism of amino acids, but that it functions in fat synthesis, since Tween 80, which contains oleic acid, could partially substitute for biotin. Royes and Robertson [59] report survival of less than 5% of controls on biotin levels of 0.001 mg/l of diet in D. melanogaster, 0.01 mg/l in D. funebris, and 0.02 mg/l in D. immigrans. Erk and Sang [23] cautiously conclude, " In view of its need by all other species of Drosophila studied thus far, it seems unlikely both that an extraneous source of biotin is not required by D. simulans and that biotin mediation of biochemical functions has been dispensed within this species. The significance of the observation that desthiobiotin has no effect on the development of D. simulans is not clear and it may be that this anti-metabolite is not effective in Drosophila. In any case, the biotin requirement for D. simulans remains to be demonstrated".

Antivitamins have been used in conjunction with aseptic rearing techniques and holidic diets to determine the vitamin requirements of dipterous larvae. The first studies on which these were used on Diptera was the testing of folic acid analogues. Aminopterin ([N-p-(2, 4-diamino-6-pteridinyl) methylaminobenzoyl glutamic acid]) and its methyl analogue, amethopterin, both inhibited larval development in D. melanogaster [73, 74], and the housefly M. domestica [75]. Since then intensive studies have been made by Levinson and Bergmann [61] on vitamin deficiencies in M. vicina produced by antivitamins; Perry and Miller [70] on the effect of antimetabolites of folic acid on the growth and metamorphosis of M. domestica; and by Akov and Guggenheim [76, 77] on the effects of analogues of pyridoxine and nicotinic acid on larvae of A. aegypti.

Larval growth of M. vicina was severely hampered by the addition of neopyrithiamine, but this effect could be completely reversed by additions of thiamine to the diet. Similarly, addition of pyridoxine reversed the inhibitory effects of desoxypyridoxine [61]. The development of larvae of

A. aegypti was inhibited by three analogues of pyridoxine. As in M. vicina, the antagonism of 4-deoxypyridoxine was reversible by pyridoxine, but not competitively. ω-methylpyridoxine could replace pyridoxine partially at low concentrations, probably because of its conversion to ω-methyl-pyridoxal phosphate. At higher concentrations (more than 2.0 μg/ml), this analogue became inhibitory. Isoniazid had the lowest toxicity of the three tested; it could not spare pyridoxine, and its toxicity was not affected by the pyridoxine level of the diet [76].

The following analogues of niacin inhibited the development of larvae of M. vicina: 3-acetylpyridine, α-picolinic acid and pyridine-3-sulphonic acid. Addition of nicotinic acid only partly relieved the inhibitory effect [61]. In A. aegypti, small doses of 3-acetylpyridine could replace nicotinamide, but it was only one-tenth as effective, and it became toxic when it was added at the level of 200 μg/ml of diet. α-picolinic acid, isonicotinic acid hydrazide, pyridine-3-sulphonic acid, and 6-aminonicotinamide all inhibited development [77].

Pantothenic acid would only partially correct the inhibitory effects of pantothenol or pantoyltaurine in the diet of M. vicina. The growth-inhibiting effect of avidin could be corrected by heating, or by additions of biotin [61].

Folic acid analogues, 5-fluorouracil, azaserine, 3-aminotriazole and fluoroacetamide, strongly inhibit larval development in M. domestica. Aminopterin and pyrimethamine reversibly inhibit the folic acid reductase reaction in which folic acid is reduced to tetrahydrofolic acid, and this in turn impairs larval growth and metamorphosis [70]. Aminopterin also inhibits the development of larvae of M. vicina [61].

In general, insects respond to antivitamins more like other metazoans than like micro-organisms.

REQUIREMENTS FOR CARBOHYDRATES

No dipterous larva has been shown to have a specific requirement for carbohydrate. As might be expected, utilization of polysaccharides varies with the insects ability to digest it, and on the degree to which the products of digestion can be absorbed. Maltose, sucrose, fructose and glucose are well utilized by most insects [8]. Some Diptera have been shown to require no carbohydrate in holidic diets: A. aegypti [20], P. regina [45], M. domestica [58], C. vicina [28], and A. affinis [47].

REQUIREMENTS FOR MINERALS

Contamination of diet components by trace amounts of minerals greatly complicates determinations of the mineral requirements of insects [78]. House [8] states, "Investigation of mineral requirements in insect nutrition is probably the most neglected area of research as well as a difficult one in which to work". D. melanogaster larvae have been shown to require potassium, phosphorus, magnesium and sodium; no requirement for calcium could be demonstrated [16]. Calcium is required by larvae of A. aegypti [1]. House and Barlow [79] found that changing the salt mixture in their holidic diet for A. affinis from U.S.P. XII No. 2 to a mixture resembling the elements found in the ash of pork liver greatly

accelerated the larval growth rate. The body weight and the protein content of the larvae reared on the diet with the new salt mixture equalled that of larvae reared on pork liver. One gram of the new salt mixture contained the following salts in grams: $FeCl_3.6H_2O$, 0.0287; K_2HPO_4, 0.6000; $Na_2HPO_4.12H_2O$, 0.0838; $MgSO_4.7H_2O$, 0.2100; $MnSO_4.H_2O$, 0.0006; $CoCl_2.6H_2O$, 0.0077; $CaCl_2$, 0.0488; $CuSO_4.5H_2O$, 0.0090 and $ZnCl_2$, 0.0114. House and Barlow [79] agree with Brooks [80], who stated, "For insects the use of mineral mixtures designed for feeding vertebrates is illogical".

REQUIREMENTS FOR NUCLEIC ACID, PURINES AND PYRIMIDINES

Although nucleic acids or their component purines or pyrimidines have been shown to be essential nutrients only for certain mutant strains of D. melanogaster, which have lost their ability to synthesize purines or pyrimidines, particularly adenine [12, 81], the addition of RNA to the diets of dipterous larvae usually accelerates the growth rate. Gordon [17] states that purine synthesis is the limiting reaction for dipterous larvae developing on holidic diets lacking RNA or its components. DNA has been found to inhibit larval growth in D. melanogaster [82]. Inclusion of RNA or DNA in the diet of P. regina promoted pupation, but had no significant effect on larval growth [45]. DNA could not substitute for RNA in a diet for A. aegypti [20] or for D. melanogaster [83], whereas either DNA or RNA will meet the dietary requirement of A. affinis for growth [84].

Derivatives of nucleic acids, when included in holidic diets and fed to various species of dipterous larvae, differ in their effects. D. melanogaster utilized the nucleotides adenylic acid and guanylic acid better than the nucleosides adenosine and guanosine [85]. A. affinis could utilize the nucleotides adenylic acid, guanylic acid, cytidylic acid or uridylic acid when fed singly, or in any combination of different pairs, although the development was not quite as good as when RNA was included in the diet.

The corresponding nucleosides did not stimulate larval development at all [84]. D. melanogaster did not utilize pyrimidine nucleotides or nucleosides when fed alone, and only combinations of nucleotides that included adenylic acid increased the growth rate [85]. Sang concluded that D. melanogaster primarily requires adenylic acid, and especially adenine. House [84] concludes that A. affinis requires nucleotides with any base, but cannot phosphorylate nucleosides to form nucleotides. Royes and Robertson [59] compared the requirements of D. melanogaster, D. funebris and D. immigrans for RNA. On diets lacking RNA or any of its components, the larval period for D. funebris was extended 20%, the larval size was reduced 25%, and there was no reduction in survival. In D. melanogaster, the larval period was extended 35%, the larval size was reduced 35%, and survival was only 50%. D. immigrans was the most seriously affected: the larval period was doubled, larval size reduced by 70%, and survival reduced about 75%.

The reactions of D. funebris and D. immigrans to various combinations of nucleic acid components were compared [59]. Purine bases were fed as the nucleosides adenosine and guanosine in equimolar amounts. Pyrimidine bases were supplied as the nucleoside of uracil, and the nucleotide cytidylic acid. To facilitate comparison with RNA figures, all concentrations were reported as milligrams of nucleotides per litre of diet. On

diets lacking RNA, the larval period of D. funebris was shortened as purine-based nucleosides were added to the diet, up to a concentration of 0.35 mg/l. This effect was shown whether pyrimidine bases were present or not. If pyrimidine-based compounds were present in the diet, there was a concomitant increase in larval body size as the purine concentration was increased, but a decrease in larval body size if pyrimidines were absent. If no purine-based chemicals were present, increases in the concentration of pyrimidine-based chemicals had no effect on body size, but development times were increased.

In D. immigrans, increased concentrations of purine-based chemicals caused a reduction in larval development time up to 75% at the 1.0 mg/l of diet level, when pyrimidine-based chemicals were present. Only a 25% reduction in development times could be obtained if pyrimidine-based chemicals were not present. If pyrimidine-based chemicals were present, increases in the concentration of purine-based chemicals caused an increase in larval size. In the absence of pyrimidine-based chemicals, larval size diminished as the concentration of purine-based compounds was increased. In the absence of purine compounds, increases in pyrimidine compounds prolonged the larval development period, and became lethal at concentrations of 0.25 mg/l of diet. Royes and Robertson [59] conclude that, either processes determining the duration of the larval period may have a lower pyrimidine requirement and a higher purine requirement than those which determine body size, or, increasing purine in the absence of pyrimidine may cause larvae to pupate at a relatively smaller body size.

Geer [86] has compared the utilization of RNA and its components in five wild-type strains of D. melanogaster; Canton-S, Oregon-R, Riverside, Bikini and Oakland. When RNA or its components was omitted from the holidic diet, the insects showed striking inter-strain differences in survival and development times. Only 3.5% of the Canton-S larvae pupated under these conditions. Pupation of Oakland, Bikini and Oregon-R was better than 60%, and Riverside 35%. Canton-S and Riverside strains were compared in their reaction with the nucleotides that comprise RNA. Addition of pyrimidine-based uridylic or cytidylic acids singly, or in combination, increased the percentage survival by more than 50% in Canton-S, and 30% in Riverside, but had little effect on development times. Addition of guanylic acid, a purine-based nucleotide, gave no measurable effect in Canton-S, but caused reduced survival and increased development time in Riverside. Addition of guanylic acid to diets containing uridylic and cytidylic acids also caused reduced survival and increased development times. Combinations of uridylic acid, guanylic acid, cytidylic and adenylic acids, in the proper proportions, could replace RNA. Geer [86] concludes that RNA synthesis in Drosophila is marginal, and speculates that this condition would not seriously affect these insects because of the abundant supplies of RNA found in the normal diet. Dissimilar responses to diet by particular strains are actually responses to different environments.

NUTRIENT BALANCE IN LARVAL DIETS

The adequacy of a diet depends on the proportions of the nutrients in the diet. Each nutrient must be present at a level that will satisfy the

direct needs of the test organism for structural materials, and that will allow the efficient metabolism of other nutrients in the diet. Gordon [17] states "A deficiency of any one essential nutrient lowers the rate of utilization of many other nutrients (and so, in effect, lowers the nutritional requirement for them). The balance of essential nutrients is the dominant quantitative factor in any diet; it is likely that an organism must destroy surplus essential nutrients until they are restored to optimal balance with the most deficient essential nutrient ". Sang [11] has shown that there can be many more than one optimum diet for D. melanogaster, if the balance of protein and sugar is altered, and the vitamin content of the diet is changed to permit optimum metabolism. Because of this interdependence of nutrients, determinations of the optimal or minimal requirements for any one nutrient must be interpreted in the light of the levels of the remaining nutrients in the diet. This makes comparisons of the minimal or optimal levels of nutrients required by different species almost meaningless. The problem is further complicated by the fact that the larvae which are feeding ad libitum may adjust their total food intake [72]. House [56] has shown that larvae of Celerio euphorbiae, a lepidopteran, will increase its food intake on sub-optimal diets to obtain the amounts of nutrients required for satisfactory nutrition.

The most penetrating studies of the effects of nutrient balance on insects have been conducted on D. melanogaster by James Sang [11, 16, 72]. In 1956 he developed a holidic diet and aseptic rearing technique that allowed larvae of D. melanogaster to complete larval development in 4.4 d, as compared with 4.1 d when the same strain is reared on an optimal supply of killed yeast. He then determined the dose-response for all the main dietary constituents under conditions in which interactions between them are minimized. The following optimum amounts were determined: protein, as casein, 5%, carbohydrate, as fructose 0.75 g%, cholesterol between 0.01 and 0.05%, nucleic acid, as RNA, between 0.3 and 0.6%, vitamins, as μg/5 ml of diet, thiamine 1 μg, riboflavin 4 μg, nicotinic acid 6 μg, pantothenic acid 10 μg, biotin 0.32 μg, pyridoxine between 1.2 and 3.0 μg; folic acid is synthesized by the larvae but if RNA is not included in the diet 4 μg/5 ml of diet is required, choline 200 μg. Greater amounts of the vitamins could be used without detrimental effect. Later [11] it was determined that larval growth was maximal at 5.5% casein, at 4.0% growth was retarded but if 0.035% L-serine was added, growth became near optimal. This amount of serine is only one-third the amount that would be present in the additional 1.5% casein. The addition of 0.025% glycine had the same effect if the folic acid level was raised 400%. Casein is therefore deficient in serine and glycine for D. melanogaster and at the 5.5% level many other amino acids must be present in excess. Their destruction to establish acceptable amino acid balance places the larvae under metabolic stress. In 1962 Sang [72] tested the relationship between protein supply and the requirement for the water soluble vitamins in larvae of D. melanogaster. The protein, casein, was fixed at 4% and 7% levels. The requirement for thiamine or riboflavin did not increase as the protein level was raised from 4 to 7%, the requirement for pyridoxine increased fourfold. The requirement for nicotinic acid increased very little as the protein level was raised from 4 to 5%, but increased by a factor of 3 at the 7% protein level; this increase was involved in the metabolism of extra tryptophan and glutamic acid, since supplementing the low-protein diet with either of these increased the requirement for

nicotinic acid. At the 7% protein level, twice as much pantothenic acid was required, possibly due to increased fat synthesis from ketogenic amino acids. Fructose increased, and oleic acid spared, the requirements for this vitamin. Biotin requirements increased with increased protein, but oleic acid had a strong sparing action. Folic acid requirements went up about five times, most of this being involved in the conversion of glycine to serine, and the further metabolism of serine. Addition of the extra amount of glycine present in the 7% protein diet (0.025% glycine), greatly increased the folic acid requirement, and 0.2% glycine carried the requirement beyond the test range of 12.8 μg of folic acid per 5 ml of medium. Sang [72] concludes that the importance of taking into account the total pattern of metabolism resulting from dietary changes, particularly with respect to determinations of minimal supplies of vitamins, is often overlooked.

Erk and Sang [23] have compared the quantitative nutritional requirements for larval growth, metamorphosis and eclosion for D. melanogaster and D. simulans. This work is important, because it illustrates the differences that can occur between two closely related species. The concentrations of nutrients that permitted optimal larval development times were similar for protein, fed as casein, carbohydrates, fed as sucrose, cholesterol, nucleic acid, and the vitamins pantothenic acid, folic acid and choline. Differences in the requirements for nicotinic acid, thiamine, pyridoxine, riboflavin and biotin were found. D. simulans required more nicotinic acid (20 vs. 12 μg/5 ml of diet), less thiamine (2.0 vs. 3.0 μg), pyridoxine (1.8 vs. 2.5 μg) and riboflavin (3.5 vs. 6.0 μg). No biotin requirement for D. simulans could be shown, even in the presence of 0.04 μg of desthiobiotin per 5 ml of diet. This study shows that quantitative, and perhaps qualitative differences in nutritional requirements can exist between very closely related species.

This type of study has been extended by Royes and Robertson [59], who determined the nutritional requirements and growth relations of five species of Drosophila using variations of Sang's medium C [16]. The following species were compared: D. subobscura, D. ambigua and D. obscura, all the obscura group; D. funebris and D. immigrans. The members of the obscura group are adapted to a lower temperature range than are D. funebris or D. immigrans. These latter two species grow to about two-and-a-half times the body weight of D. melanogaster. All the obscura group were similar in their requirements for RNA, casein and choline, but all required much less casein and choline than D. melanogaster; all required about as much RNA as D. melanogaster. D. funebris, D. immigrans and D. melanogaster show similar requirements for casein. D. funebris requires only one third of the RNA needed by D. melanogaster or D. immigrans. D. funebris and D. immigrans require less cholesterol, lecithin and/or choline in their diet than does D. melanogaster. D. immigrans requires more riboflavin, pantothenic acid, thiamine, nicotinic acid and pyridoxine than does D. funebris, whose requirements in turn are much higher than those of D. melanogaster.

Geer [86] has studied the ribonucleic acid-protein relationship in five wild-type strains of D. melanogaster: Canton-S, Oregon-R, Riverside, Bikini and Oakland. The results of the nucleic acid studies are reported elsewhere in this paper. Geer also found that the amino acid balance was extremely important, and that there was an interaction between

dietary supplies of RNA and proteins. Even when strains within a species are studied, dietary balance is extremely important. Geer [86] concludes, "These experiments illustrate two points which should be considered in all nutritional studies. Variability in a dietary requirement may be quite prevalent within a species. What may seem to be an absolute requirement may only be a characteristic of a particular individual or strain. Also, as in the present experimental work the composition or balance of the diet may have a marked effect upon a requirement. Changing the balance of the diet may result in a corresponding change in the requirement".

Nutritional studies, such as those reported in this review, are progressively defining the metabolic activities of larvae of the Diptera. The plasticity of these activities is great, for example Sang [11] states that he could probably select a strain of P. regina that would have the same vitamin requirements as D. melanogaster although these animals differ markedly in the food that they normally eat. Many workers who have attempted to rear dipterous larvae on artificial diets have found that, through selection, a strain could be developed which was well adapted to the rearing conditions. These animals may be very different from the wild population and in many cases data obtained with such animals may not be applicable to the normal condition.

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MASS REARING OF INSECTS: ITS CONCEPT, METHODS, AND PROBLEMS

R. T. GAST
ENTOMOLOGY RESEARCH DIVISION,
AGRICULTURAL RESEARCH SERVICE,
UNITED STATES DEPARTMENT OF AGRICULTURE,
STATE COLLEGE, MISS., UNITED STATES OF AMERICA

Abstract

MASS REARING OF INSECTS: ITS CONCEPT, METHODS, AND PROBLEMS. Mass rearing has the single purpose of producing acceptable insects at the lowest possible cost. Criteria of acceptability will depend on the use made of the insects and may be very different for insects used in a sterile-male release programme as compared with insects used for pathogen or parasite production. Once criteria have been established, it is necessary to develop strains of insects to meet these standards, and maintain them under conditions that will ensure no changes in the population during succeeding generations.

After an acceptable insect strain has been colonized, the most important phase of the programme is producing the insects at the lowest possible cost per insect. Mechanization of techniques, less costly diets, and making the insects perform their own labour are the chief methods of reducing labour costs. Ideas can be borrowed from other industries, principally food-processing plants, that will greatly improve the efficiency of mass rearing. Methods used in mass rearing depend on the individual species of insect, but in most instances, the basic design of rearing factories are similar. Examples of equipment and their adaptation for rearing are numerous and some methods are applicable to widely dissimilar species. Concern over percentage yield of egg to adult is most often a dominant influence in development of a rearing method. Percentage yields, however, are unimportant except where they affect the final cost of production. It is better to accept a drastic reduction in percentage yield if, in doing so, the cost of production is reduced by even a small amount.

The problems encountered in mass rearing are generally similar to all species. Three of the most common are genetic changes in populations after many generations, contamination of the diets by microorganisms, and pathogenic diseases of the insect colony. Methods for combatting these problems vary with each particular species. Unique problems in sterile-male release programmes are lack of sterility, loss of aggressiveness or mortality due to the sterilizing agent. Problems are not insurmountable if sufficient effort and financial support are available.

The mass rearing of insects has become a major weapon against insect pests. Millions of mass-reared insects have been used to combat pest species as parasites, predators, host material for pathogen production, and as a source of sex attractants used to lure the pest species into traps; however, the most effective use of insects has been in the sterile-male release programmes. This idea was based on work by Knipling [1] for the use of sexually sterilized male insects to control the screw worm Cochliomyia hominivorax (Coquerel). This pest has been eradicated in the United States of America by overwhelming the normal population with millions of sexually sterilized male flies. Because of the success of this programme, numerous other insect pests are now being considered for this type of control. The first requirement for such a programme is the ability to mass-produce millions of insects at an economically practical cost.

CONCEPT OF THE MASS REARING OF INSECTS

Mass rearing of insects has a simple objective — to produce an acceptable insect at the lowest possible cost. The major differences between laboratory rearing of insects and mass rearing is one of economics. In laboratory rearing, maximum yields of insects are of major importance for studies on genetics, nutrition, adaptation, and methods for sexual sterilization; and costs of 15 to 20 ¢ per insect may not be excessive. For sterile-male releases, such costs would be prohibitive. The cost per insect is the most important factor in mass rearing provided the insect produced is acceptable for the project.

The term "acceptable insect" must not be confused with "normal insect". The moment an insect is colonized for mass production, it is different from its predecessor in a field population. Standards for acceptability must be established, based upon the ultimate use made of the insects. To obtain sex attractants or to produce insect pathogens or parasites, a large sluggish insect may be acceptable; for dispersal studies or for a sterile-male release programme, the insect must be active and sexually aggressive. Insects may also need genetic markers to facilitate evaluation of the programmes. Such standards of acceptability must be developed in direct co-operation with ecologists, geneticists and other specialists in biology. It is the responsibility of the rearing personnel to incorporate such characteristics into the mass culture.

Once the mass-reared insect is considered acceptable for a programme, the total effort must be directed towards reducing the cost per insect. Far too frequently, personnel involved in mass rearing are obsessed by percentage yields of eggs to adult insects. Percentage yields are frequently used as a basis for evaluating the efficiency of mass-rearing programmes when actually they are meaningless. For example, under the present system of mass rearing the boll weevil Anthonomus grandis Boheman [2], it is more efficient to obtain 20% yields of adults from eggs than 40% (Table I). The reason is that the egg cost is relatively low (US $0.10/1000 eggs) while the cost of media and its preparation is high. Greater utilization of the diet by over explanting eggs results in a lower cost per adult reared (Fig. 1). By careful hand planting of eggs, yields of 70 to 80% are possible, but the cost per insect increases to nearly $100 per 1000 insects. If an insect can be produced at a cost of 1/10 ¢ each with a yield of only 10%, such rearing is far more efficient than yields of 80% with the insects costing 1/5 ¢ each. For one million insects, those yielding 10% would cost $1000 while those yielding 80% would cost $2000. Such a saving makes any reduction in yield justifiable — providing the insects are acceptable for projects involved.

METHODS OF MASS REARING AND COST REDUCTION

The insect species and its requirements will determine the best method of reducing production costs. In each case, the total production costs should be analysed and divided into categories such as labour, equipment, and supplies. If labour costs are high, then the greatest cost reduction can be realized by mechanization. However, if labour costs

TABLE I. COST OF PRODUCING BOLL WEEVILS AT 20% AND 40% YIELDS

	40% yield (US $)	20% yield (US $)
Material	0.09	0.09
Labour	0.04	0.04
Eggs	0.01 (100 eggs)	0.03 (300 eggs)
Total cost	0.14	0.16
Weevils/plate	40	60
Cost/1000 weevils	3.50	2.70

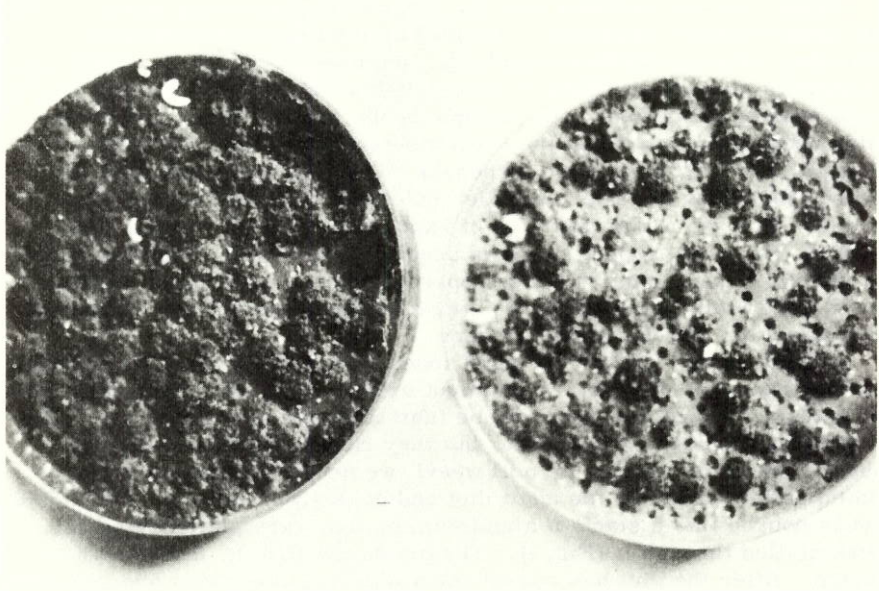

FIG. 1. Petri dishes containing larval cells in diet. Dish (left) with 60 cells represents 20% yield and dish (right) with 40 cells represents 40% yield.

are only a small part of the total, a major saving cannot be achieved by mechanization.

Cheaper diets are often one of the easiest ways to reduce production costs in mass rearing.

Keller, formerly of the IAEA, reduced the cost of rearing Mediterranean fruit flies from $80 to $4/million by substituting cottonseed meal and sugar-cane bagasse for dehydrated carrots and yeast. The cost of rearing the oriental fruit fly in Hawaii was reduced from $30/million to $16.50/million by substituting grey wheat shorts and middlings for

dehydrated carrots. Production costs of rearing the screw worm were reduced from $1345 to $800/million flies by substituting a hydroponic mixture of blood, fish meal and milk solids for horse meat. The facility in Mission, Texas produces over 150 million screw worms per week; thus, there is a saving of 1/3 million dollars per month by this diet substitution.

The best diet for mass rearing may not always produce the highest yields. Boll weevil adults fed on fresh cotton bolls will produce nearly twice the number of eggs per female as weevils fed on artificial diets [3]; however, the cost of producing eggs on bolls is $0.40/1000 compared with $0.10/1000 on artificial diet.

Regardless of the insect species, some mechanization of the rearing procedures is necessary to mass-produce the insect efficiently. Even with Diptera, where labour costs are relatively low, great savings can be accomplished by mechanical devices. The greatest example of mass rearing of an insect is the screw worm. The economical production of this insect could not have been done without the high degree of mechanization used at the Mission, Texas, plant.

Mass-rearing factories are like large food-processing plants, and many valuable ideas can be gleaned by visiting candy factories, bakeries, canning and freezing plants, and meat processors. Often a machine is already available to accomplish a time-consuming and difficult rearing procedure. An example is the small jelly or cream cup filler used by Burton [4] of the Corn Insects Laboratory in Tifton, Georgia. By placing a worm dispenser on this standard machine, he can fill 3000 cups per hour with diet and infest them with first instar larvae. This job would take 12 h of hand labour. With a slight modification, the worm hopper could be used for bollworms, coddling moths, or any other insect that requires separate rearing containers. Other readily available items are large steam kettles, hoppers for dispensing dry materials, and large food mixers.

Unfortunately, mass rearing of insects is a rather specialized field and no catalogues of special equipment exist. Much equipment falls into the category of gadgets that must be fabricated for specific needs. The gadgets are usually quite simple, but they result in great savings in labour. In mass rearing the boll weevil we need thousands of wax-coated pellets daily for the adult diet and oviposition site [5]. Hot liquid diet is poured into a stack of aluminium plates, each plate having 225 holes drilled through it (Fig. 2). The plates are 0.5 in. thick and 8 in. square. After the diet has cooled and solidified, the plates are separated, thus shearing the diet into 0.5-in. lengths. A plate with retractable needles is used to pick the pellets from the plates and dip them in hot wax. The needles are then retracted and the waxed pellets released.

While this operation is suitable for production of half a million weevils per week, it is not suitable for larger numbers. A machine used by candy manufacturers to produce centres for chocolate-covered candies is now being modified for making diet pellets. The machine drops uniform-sized pieces of diet on to a conveyor belt where they are rolled into spherical shapes. A second conveyor belt is used to coat the pellets with wax. The pellets will thus be produced in a continuous stream rather than by the batch method.

The diet pellets are placed in $\frac{1}{4}$-in. mesh trays in the weevil cages. The pellets are changed twice a day by shaking the weevil from the pellets,

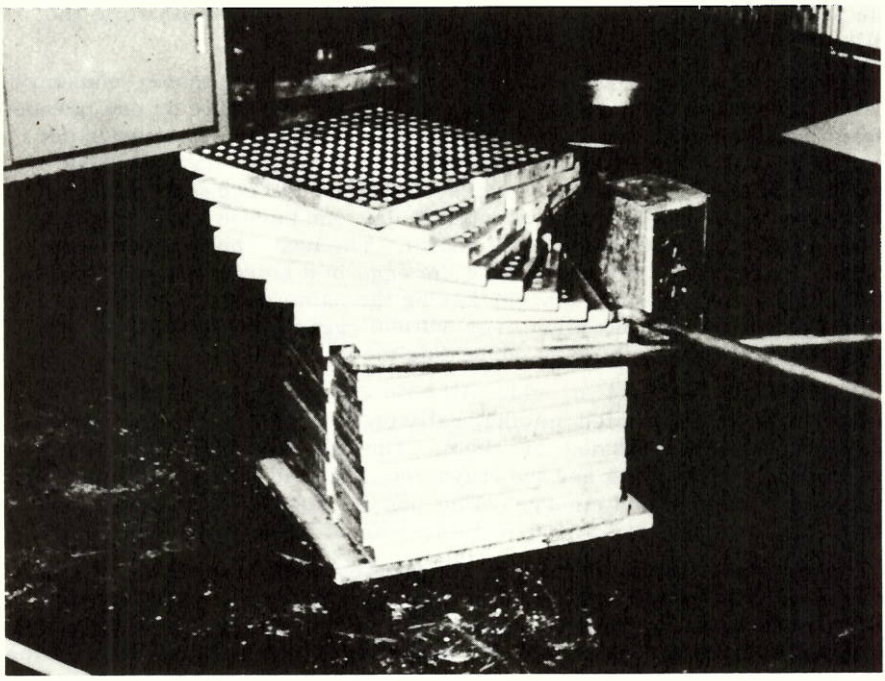

FIG. 2. Stack of aluminium mould plates for making adult boll-weevil diet pellets.

through the tray, and replacing the pellets with fresh ones. The cages have screen bottoms and solid tops and sides. Since the weevils crawl towards light, they remain on the bottom of the cage where the food is. In cages with screen tops, the weevils stay near the top of the cage until they almost starve. The use of cages with screen bottoms has increased egg production by 25% compared with conventional cages [3].

During egg extraction the wax covering is removed from the pellets with another simple machine [2]. A blender was tried, but caused considerable damage to the eggs and since many pellets were not broken, repeated grindings were necessary. A set of concave revolving discs is now used. The diet pellets and water are fed into a cavity in the centre. As the discs revolve, the pellets are rolled to the periphery, removing the wax from each pellet.

Another useful item is a screen cylinder for removing water from the crushed pellets. A tall 45-mesh screen cylinder allowed the diet particles and eggs to settle and the water to drain off. This piece of equipment reduced labour time from 4 hours to half an hour.

The adult oviposition diet contains germinated cottonseed. The original method was to squeeze the pulp from each germinated seed by hand; one person could process only 0.5 - 1.0 lb seed per hour. A set of 6-in. rollers was made to squeeze the seeds [6]. The flake-like pulp was washed through screens that retained the hulls, and one person could then process 20 to 30 lb/h. The addition of a motor and automatic feed hopper, and autoclaving the germinated seeds for 5 min at 20 lb pressure, now enables one person to extract over 150 lb of seed per hour.

The paste-like pulp adheres to the rollers and is scraped off while the hulls are discharged out the bottom.

Implanting eggs in larval media can be a time- and money-consuming task. By hand methods, only 700 eggs/h can be implanted by one person. These eggs generally yield from 50% to 70% adults, or 490 weevils per hour of labour. The use of an implanting machine [6] containing 100 hypodermic needles enabled one person to place 100 eggs per dish simultaneously. Yields were about 40%, but one person could implant 60 000 eggs/h for a yield of 24 000 adults. The next improvement was a spray method [7]. By suspending the eggs in a sterile viscous liquid (18% sucrose and 2% corn starch) having the same specific gravity of the eggs, one person can plant over 300 000 eggs/h in petri dishes and over 3 000 000 eggs/h in trays containing media.

Trays 3 ft long by 4 in. wide will hold the equivalent of 18 petri dishes. The trays are fed automatically on to a conveyor belt where a ribbon of media is extruded into them. The eggs are sprayed on to the surface of the media and the trays are placed in large cabinets where the weevils develop and emerge. The use of trays has reduced the cost of rearing weevils from $2500/million to about $1000/million.

Insects are themselves one of the greatest labour savers. When possible, let the insects do their own work. The screw-worm factory uses insect labour in two operations. First, the mature larvae separate themselves from the spent media by crawling off the edges of the rearing trays and dropping into a flume of water where they are conveyed to a collecting station. Second, after the majority of the larvae have pupated, the pupae and larvae are placed on a lighted conveyor belt. The larvae crawl to the edge of the belt and fall into boxes of sawdust to pupate; the pupae are carried to the irradiation section.

Adult boll weevils were formerly collected from petri dishes one by one with forceps. Only 1000 insects per hour could be collected in this manner. Now the open dishes are placed in emergence boxes where the insects collect themselves in screen traps [2]. One person can collect 40 000 to 50 000 insects in 15 - 20 min. This method is successful because a strain of weevils was developed that emerge more uniformly in time. Originally, for eggs laid the same day, it required 8 to 10 d from the time the first adult emerged until 90% emergence occurred. During this 10-d period the dishes were open to microbial contamination, and by the 5th or 6th day moulds and bacterial growth generally grew over the plate and killed the weevils. By using as oviposition stock (weevils that emerged the 1st day), the time for 90% emergence to occur was reduced to 3 d. This resulted in considerable saving in equipment and space since 70% fewer emergence boxes are needed to produce the same number of weevils.

An added beneficial effect of selecting the breeding stock from the adults that emerge earlier is that the larval developmental period can also be reduced. With the boll weevil, the development time has been reduced from 16 to 13 d. This shorter life-cycle has an obvious advantage in mass rearing.

PROBLEMS IN MASS REARING

Problems encountered in mass rearing are continuous. The insects may stop laying eggs, the eggs will not hatch, the larvae will not develop, or the adults die suddenly or are deformed. The most probable causes are contaminated supplies or malfunction of the environmental control of the rearing facility. Problems of greater concern are: (1) Genetic changes in the population; (2) pathogenic diseases of the insect colony; and (3) contamination of the diets by microorganisms.

Insect populations maintained in a laboratory for many generations undergo changes due to selective pressures. The strain of boll weevils currently being maintained at the Boll Weevil Research Laboratory has lost the natural ability to diapause. This change makes the insect more suitable for a sterile-male release programme because weevils unable to diapause cannot survive the winter.

The male boll weevils produce a sex pheromone that attracts the female, and there is some indication that male weevils from the laboratory culture are not as attractive to the females as wild males. This may be caused by the diet of the laboratory strain, but it is also possible that selective genetic changes have taken place that make the males less attractive. In nature, weevils are generally widely separated, and thus the most attractive males would be the most likely to father future generations. In the laboratory where thousands of insects are confined in small cages, the attractiveness of the male is meaningless. The present culture has been maintained for 9 yr in the laboratory without introduction of new germ plasma. When a sterile-male release eradication programme is attempted, it may be necessary to introduce new insects into the culture to increase the attractiveness of the males. Laboratory-reared males are equal to wild males in sexual aggressiveness; however, their effectiveness in a release programme would be lessened if they did not attract females in the same ratio as wild fertile males.

In another example, there is concern that maintaining the screwworm adults in nearly complete darkness for the past few years has produced a strain of flies that is partially blind and that does not react in nature in the same way that wild flies do. The flies are reared in darkness to prevent excessive flight in the oviposition cages, which causes the flies to injure themselves. Periodically, new wild strains are introduced into the population. The wild strains are allowed to increase on living animals by artificially infesting wounds with eggs. Five or six goats used on a three-month rotation will produce sufficient insects to influence the genetic make-up of the mass colony when they are introduced into it.

Mosquitoes, which normally have a wide flight area in nature, have been successfully colonized in the laboratory by gradually reducing the size of the oviposition cages during many generations. A strain of these insects was finally selected that could be maintained in small cages. However, when these insects were used in a field sterile-male release study, they would not fly far enough from the release point to be effective.

Introduction of wild strains into a laboratory culture, while a means of eliminating one problem, may cause an equally serious problem of disease. Although some pathogens are introduced on contaminated food or equipment, these can usually be eliminated by treatment of all items

brought into the rearing facility. Diptera, in general, do not have a severe disease problem, but Lepidoptera and Coleoptera are subject to devastating epidemics. Introduction of wild strains into a laboratory culture is one of the easiest ways to introduce pathogens.

There was no known disease of the boll weevil until this insect was mass reared. To date, four diseases have been found [8], three of which caused the complete destruction of the culture. Of the four, three were traced to the introduction of new insects. The fourth disease was caused by bacteria, and the method of entrance into the culture is still undetermined. The quarantine and isolation of a new strain of insects is effective only in interception of known pathogens. In the boll weevil, the four pathogens were unknown and only discovered after they had infected the culture. For the insect pathologist, this is an excellent method of obtaining new disease organisms; for mass rearing, it means disaster.

Once a pathogen becomes established in a culture, the most effective control is to destroy the entire culture and sterilize the rearing facilities. Unless the pathogen can be completely eliminated, it is not practical to try to live with the disease. Diseased insects do not act the same as healthy ones and would probably be ineffective in a sterile release programme.

The third problem is contamination of the rearing media with microorganisms. With some insects, as most Diptera, which can live in media with a low pH, the addition of an acid usually eliminates the contamination. Dipterous larvae may also utilize the microorganisms as food. However, with Coleoptera, Lepidoptera, and most other insects, the media must be near a pH of 7, and the choice of microbial inhibitors becomes very important. Usually the inhibitor is toxic to the insects as well as to the microorganisms, and a strain of insects must be selected that will tolerate the inhibitor. The microorganisms also build up tolerance to the inhibitor, and after several years it is frequently necessary to switch to a different inhibitor.

The best control for all microorganisms is to exclude them from the media. This requires a rigid programme of sanitation and, quite often, filters that can remove bacteria and spores from the air. Boll weevils are reared on media in covered dishes, and the only time the dishes are opened is in the hood with filtered sterile air. Although the materials placed in the hood may have bacteria and mould spores on them, these spores, if dislodged, are blown out of the hood and thus cannot fall on the media and contaminate it. If and when we obtain a special rearing facility, all the air will be filtered to remove microorganisms.

Problems are never eliminated completely but if sufficient effort is expended, they can be kept at a non-economic level.

SUMMARY

The main difference between mass rearing of insects and laboratory rearing is one of economics. In mass rearing the cost per insect is the most important consideration while in laboratory rearing cost factors are usually not important. Methods of reducing costs in a mass programme can be best achieved by mechanization and use of less expensive

diet ingredients. Problems encountered in mass rearing include genetic changes in the insect population, diseases of the insect and microbial contamination of the media. These problems are never completely solved and continuous effort is needed to prevent any of them from destroying the culture.

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PRELIMINARY RESULTS IN REARING THE CHERRY FRUIT-FLY (Rhagoletis cerasi L.) ON A SEMI-SYNTHETIC MEDIUM

A. HAISCH
BAYERISCHE LANDESANSTALT FÜR BODENKULTUR,
PFLANZENBAU UND PFLANZENSCHUTZ,
MUNICH, FEDERAL REPUBLIC OF GERMANY

Abstract

PRELIMINARY RESULTS IN REARING THE CHERRY FRUIT-FLY (Rhagoletis cerasi L.) ON A SEMI-SYNTHETIC MEDIUM. The economic importance of the cherry fruit-fly injuries to fruit, difficulties in combatting by insecticides and the danger of noxious residues of insecticides on treated cherries, were the reasons for attempting control or eradication by the sterilization technique. The following report deals with the difficulties of rearing the cherry fruit-fly.

1. INTRODUCTION

Many Trypetid species are insect pests of great economic importance. It is understandable that people try to control or eradicate them by all possible methods including the sterile-male release. This technique is very promising if specific conditions are fulfilled, especially if the species can be mass cultured.

It is difficult to eradicate the cherry fruit-fly by insecticides. In the Federal Republic of Germany are large areas planted with cherries in which a regular control has been carried out by aerial spraying since 1952. Despite this, a fruit infestation of 1-4% is not uncommon. Up to 10% of all fruits are infested if the insecticide application is not very carefully carried out [1]. Cherries with more than 4% infestation can no longer be exported and are therefore used only for distillation purposes. Thereby, profits are reduced by half. This economic factor and the danger of toxic residues from insecticides were the reason to try the sterilization technique against the cherry fruit-fly.

2. GENERAL BIOLOGICAL DATA

This insect pest is mainly indigenous to Europe as shown by Fig. 1 [7]. However, the distribution of the cherry fruit-fly is not as even as shown by the map. It occurs in numerous but relatively small areas, and not in all regions with cherry cultivation [5, 10]. Because of its low flight range the cherry fruit-fly does not extend beyond such small infested areas for many years. This behaviour is considered to be favourable to the application of control by the sterilization technique.

There is only one generation per year of the cherry fruit-fly. The adults emerge if the soil has reached a certain temperature [2, 10, 14].

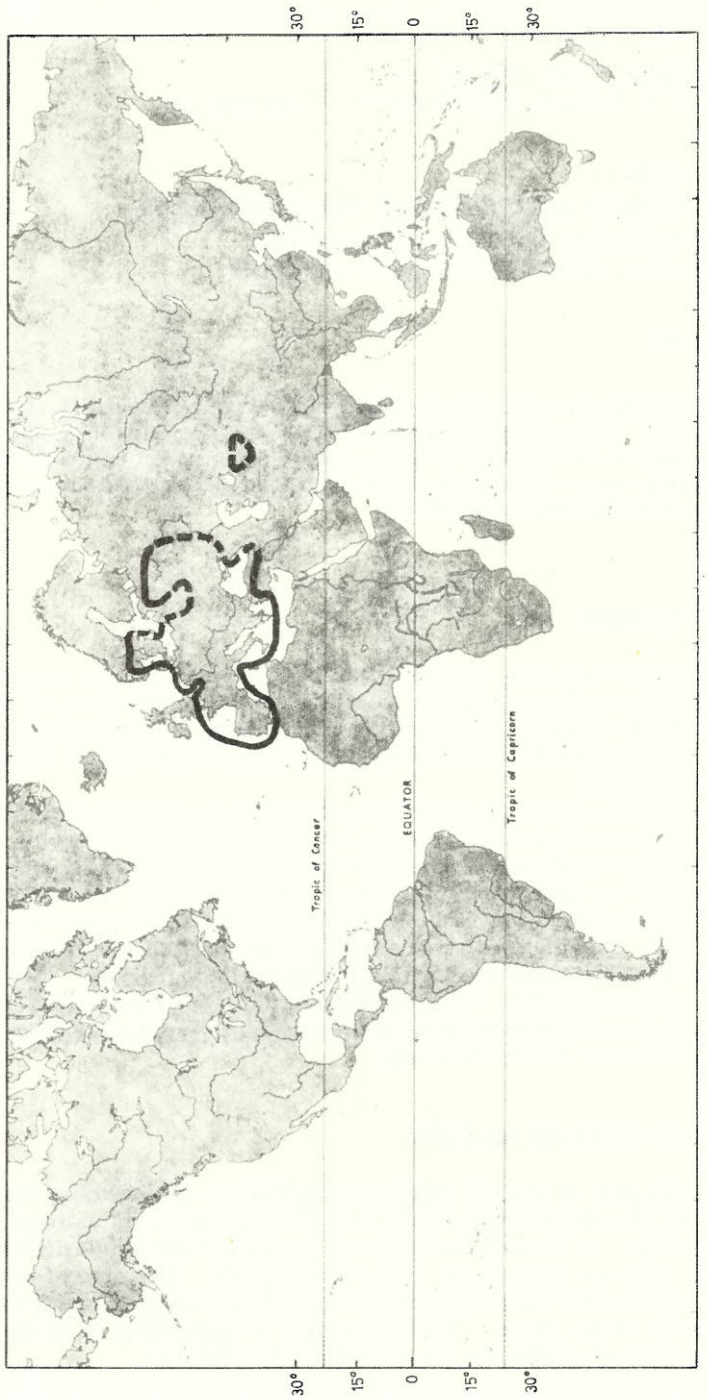

FIG. 1. Distribution map of insect pests — here showing the distribution in Europe of the Rhagoletis cerasi L. (cherry fruit-fly of Europe). Hosts: Cherry, Lonicera spp. (See Ref. [7] : Commonwealth Institute of Entomology, distribution maps of insect pests, Series A, Map 65 (1956)).

Emergence and oviposition are separated by 6-9 d in nature [4]. The eggs are deposited individually into the cherries. Before becoming mature to oviposit, the female stings the cherries with her ovipositor. In doing so a small drop of juice overflows which she consumes. It is still unknown whether this food is essential in adult nutrition. Besides cherries the cherry fruit-fly attacks some other fruits, for instance Symphoricarpus sp., but only to a very small degree. It can be characterized as a monophagous insect. One female is able to deposit more than 400 eggs, but on an average not more than 200 eggs [3]. The lifetime of flies may be approximately four to six weeks. The larval development lasts about three weeks in nature [12]. Pupation takes place in the soil. Subsequently, pupae remain in diapause for six months. If this diapause is to be broken, the temperatures must be kept between 0 and 7°C during this time. This long diapause greatly hinders rearing experiments, but later in mass-production it may facilitate the stocking of pupae. Problems of cherry fruit-fly rearing under laboratory conditions can be considered under the following three headings:

(1) Adult rearing
(2) Collection and handling of eggs
(3) Larval nutrition

3. ADULT REARING

To get material for the rearing experiments, heavily infested cherries were collected and kept until the larvae had left the fruits to pupate. The experiments were carried out in a glass-house compartment. The temperatures did not fall below 23°C. In the summer the maximum was 37°C with a mean of 25°C to 27°C. In winter, the natural short day was prolonged to 16 h by supplemental illumination with light within the short-wave range. The cages used consisted of plastic gauze covered by a plastic disk (Fig. 2). They were 28 cm high with a cross-section of 15 cm^2.

Tap water flowed into the cage through a hole in the cover, wetting a pad. Thereby the flies were able to drink. A glass tube drawn from a capillary controlled the water flow. Through a second hole, a sponge soaked with the liquid food was placed in the cage. The fly flood consisted of a mixture of water, sugar and brewer's yeast in proportions 10:8:4, which was renewed twice a week.

A single cage held 20 to 30 pairs of flies. Under those circumstances, the lifetime of the flies was eight weeks. After 30 to 36 d, 50% of all flies were still alive. In one instance of overcrowding – 98 flies within a single cage – only 39% of the flies were alive after 22 d. Overcrowding apparently reduces longevity. The life span of the other few flies was normal. Under the conditions described, three days lapse from emergence to mating. Of course it was not demonstrated that fecundation took place. Oviposition begins between the fifth and the tenth day. The average fecundity per female was 45.4 eggs in a population of 374 females.

4. EGG COLLECTION

Cherry fruit-fly females do not deposit their eggs in containers through drilled holes as do other Trypetids. For want of cherries, the female

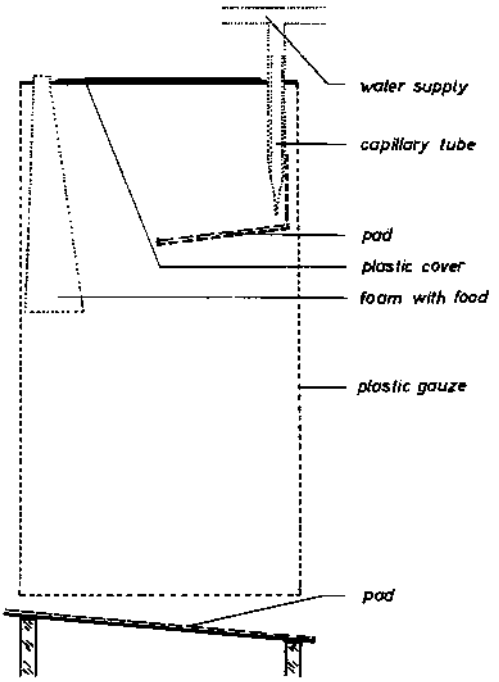

FIG. 2. Cage for keeping Rhagoletis cerasi L.

attaches her eggs to all smooth surfaces (for instance glass) or drops her eggs. Normally such eggs very quickly dry out. Therefore, little wax domes were tested, similar to those used by Hagen [8] for the olive fly. The amount of eggs deposited in such domes varies widely, depending on the thickness of the wax wall. The cherry fruit-fly is smaller and weaker than many other fruit flies. Therefore, the female has difficulties in boring with her ovipositor through a thick material. It is not easy to make suitable, i.e. very thin-walled, egg containers. Only one out of ten domes was usable. For this reason an artificial cherry was made consisting of a foam ball 2 cm in diam. covered by thinly stretched parafilm. By this method, three tests were conducted between November 1965 and March 1966 to evaluate the usefulness of this cherry dummy, using as a criterion the proportion between deposited and dropped eggs. Results are shown in Table I.

Eggs laid within the dummies made up only one-third of the total number of eggs and, moreover, this was not constant. In other tests during the subsequent season, it was no longer possible to collect eggs within the dummies; all the eggs were dropped. This observation may be explained by a slight change in temperature and light conditions.

To increase their attractiveness, the cherry dummies were coated with extracts of cherry fruits: ethanol, diethyl-ether, dioxan and dichlormethane. However, in no case could the attractiveness or the number of eggs laid be raised. Therefore, in subsequent experiments only dropped eggs were used. These fell through the gauze bottom of the cage on to a wet filter paper (Fig. 2), where they were collected once a day.

TABLE I. THE ATTRACTIVENESS OF OVIPOSITION DUMMIES

Mode of oviposition	Percentage of eggs laid		
	1st test	2nd test	3rd test
Within dummies	8.2	29.3	11.3
Dropped	91.8	70.7	80.7
Total number of eggs	294	1433	911

As already mentioned, on an average one female deposits 45 eggs in her whole life span. A dissection of the ovaries of all dead females revealed many developed and undeveloped eggs. This is probably the result of not having a suitable egg receptacle.

5. EGG DEVELOPMENT

Before using the above-mentioned cages, fertile eggs could not be obtained in the laboratory. Now 23.7% of 114 000 eggs hatched. It was believed that the cause of this high egg mortality might be a temperature decrease owing to evaporation on the egg pad. To clarify this question, an experiment with different egg incubation temperatures was carried out. Eggs were carefully rinsed from the filter paper, on which they had been dropped, and transferred to other clean filter papers in small petri dishes (3 cm diam.). The atmosphere in the dishes was nearly water saturated. One batch of 1443 eggs was incubated at 20°C, a second one of 1764 eggs at 25°C, and a third one of 936 eggs at 30°C. Figure 3 shows the results of this experiment expressed as the relative, daily hatching rate of the larvae at the different temperatures.

The total hatching percentages at 20, 25 and 30°C, respectively, were: 17.4%, 23.4% and 24.0%. An analysis of variance showed no significant difference between the two underlined values (0.01 level). The interaction

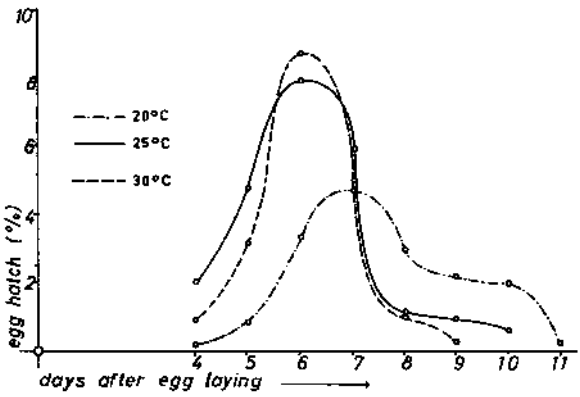

FIG. 3. Effect of temperature on the egg development of the cherry fruit-fly (rate of hatching).

between the causes of variances "time of development" and "temperature" was also not significant. Therefore, it can be said that under the given conditions, the egg development took up to 11 d, and that the bulk of the larvae hatched between the sixth and the seventh day. On the whole, the influence of temperature upon egg development could be demonstrated; however, it seems not to be responsible for such a high rate of egg mortality. In another trial, the hatching rates were compared between eggs dropped and eggs deposited within foam balls covered by parafilm. The incubation temperature was 25°C. Table II shows the results.

TABLE II. DEPENDENCE OF HATCHING PERCENTAGE ON MODE OF EGG COLLECTION

Mode of egg collection	No. of eggs	Hatching			
		After 6 days (Total No.)	(%)	After 11 days (Total No.)	(%)
Dropped	3210	341	10.6	715	22.3
Deposited within dummies	1241	268	22.0	361	29.1

The mode of egg collection has a significant influence upon the hatching rate: this is higher for the eggs deposited within the dummies than for those dropped. Besides, the former develop faster than the latter. It can be inferred from Table II that, after 6 d incubation, a maximum of 47.7% of the dropped eggs had hatched as opposed to 74.2% of those deposited within the dummies. Oviposition within dummies is apparently more favourable and more similar to natural conditions.

In a third trial (which was more of an observation than an exact experiment) the females were allowed to deposit their eggs into cherries. At 25°C incubation temperature 45 out of 50 counted eggs had already hatched after 4 d.

The first of the trials just mentioned shows that high egg mortality is not due to evaporation cooling. Likewise, deficient adult nutrition seems to have no bearing on the phenomenon, because eggs develop well in their natural environment. The results of trial 2 suggest that eggs, which have remained up to one day within the dummies, were less exposed to the detrimental influences of their unnatural environment, e.g. infection by pathogens. In fact, fungal infestations have been frequently observed. Eggs turning red could be a symptom of an infection by Serratis marcescens [6, 11].

6. LARVAL REARING

Preliminary experiments were based upon the idea that larval nutrition on something similar to their natural food might be the easiest way of rearing. Materials like powdered cherries, cherry jam, juice in various mixtures have been tested. Naturally this idea seems to be

wrong, because not all organic substances can be preserved. Moreover, in fruit flies, symbionts may play an important role and therefore, if possible, one should attempt to preserve them. The total number of media tested was approximately 150.

Yeast was the essential food component of all media tested. While no exact trials were made, it is possible to say that no significant difference between brewer's and torula yeast was recorded. Torula yeast, however, is much cheaper in Germany. In trials with different yeast concentrations (from 3 to 10%) the highest content was the best one. Other nitrogenous nutrients were also tested, eg. bacto peptone, bacto casein, proteose peptone, meat peptone and hydrolysates of cotton seeds, soya and brewer's yeast (2% and 3%). Fatty acids were supplied by peanut butter (2% and 3%). No significant development acceleration has been recorded with any of these additives. However, the addition of 5% Pseudomonas sp. had a definite beneficial effect. The exact classification of this microorganism has not yet been made. The bacteria were isolated from cherries infested by cherry fruit-fly larvae and cultured by the microbiological section of the Bayerischen Landesanstalt. Only killed bacteria were used. Table III shows the development of the larvae on various substrates all containing 10% yeast and 12 - 15% carrot powder (depending on the consistency of the medium).

TABLE III. DURATION OF DIFFERENT DEVELOPMENTAL STAGES OF CHERRY FRUIT-FLY LARVAE FED VARIOUS MEDIA

HCl	+	-	-	-	-
Organic acids	-	+	+	+	+
Pseudomonas sp.	-	-	+	+	+
Myo-inositol	-	-	-	+[a]	+[b]
	Duration of development (d)				
Instar I	5-6	6-7	6-7	3	3-5
Instar II	-	7	2-3	3-4	2-3
Instar III	-	7	-	6-9	6
Total	-	20-21	-	12-15	11-13
Percent pupae	-	3.8	-	13.0	1.5

[a] 3% myo-inositol
[b] 1% myo-inositol

All three substrates with Pseudomonas sp. allowed quick development during the second instar. Sucrose was used at the 2.5 and 5% levels; there was no difference in the effects of these two concentrations.

Linoleic acid and the sterols cholesterol, -sitosterol and stigmasterol were also tested without any effect (0.05%). However, the addition of 1 and 3% myo-inositol was found beneficial by shortening larval development to 12-15 d and 11-12 d, respectively (Table III). Myo-inositol is a sexvalent,

cyclic alcohol. Remarkable amounts of inositol phosphatides can be found in tissues, especially in microsome lipids [9]. Many microorganisms are not able to synthesize inositol and depend on supply. In view of the quantity of inositol which was effective, it becomes doubtful whether this was utilized as a micronutrient by the organism. It seems more likely that inositol served as a secondary source of carbohydrates.

In addition to food and carrier components of a larval medium, preservers are also important. In nearly all experiments these were 0.05% benzoic acid and 0.15% or 0.25% nipagin. To acidify the medium to pH 4.0 - 4.3, (this pH can be observed in ripening cherries) hydrochloric acid was used at first; the pH was tested with indicator paper. However, the substitution of HCl by organic acids, DL-malic acid and citric acid (Table IV) resulted in a higher rate of larval survival (Table III). This interesting phenomenon cannot yet be explained.

TABLE IV. COMPOSITION OF A MEDIUM FOR FEEDING LARVAE OF THE CHERRY FRUIT-FLY (% p.w.)

Substance	(%)
Water	61.10
Carrot powder	13.55
Benzoic acid	0.05
DL- malic acid	1.20
Citric acid	0.80
Nipagin	0.25
Sugar	5.00
Torula yeast	10.00
Pseudomonas sp.	5.00
Cholinchloride	0.05
Myo-inositol	3.00

The combination of the preservers used still needs to be improved because its efficacy is too short. If it could be shown that the medium afforded good and fast larval development, perhaps the present preserver combination might even be satisfactory. The composition of the best medium so far is shown in Table IV.

In preparing the medium, all solid ingredients were very well mixed and then put into solution. The mixture was stirred well until an even consistency had been obtained. The media were tested in little petri dishes 3 cm in diam. These small petri dishes were put in larger ones containing a wet filter paper so as to protect the medium from drying out.

Every petri dish was filled with 5.0 g medium. The number of larvae inoculated varied between 40 and 80 or so, depending upon availability. Only neonate larvae were used and each test was replicated twice. The quality of a medium was appraised according to the amount of feeding,

TABLE V. MEASUREMENTS OF CHERRY FRUIT-FLY PUPAE REARED IN DIFFERENT FOODS

Rearing	Length (mm)	Width (mm)
In cherries	3.47	1.70
In cherries and in a medium [a]	3.39	1.61
In a medium	2.92	1.53
Standard deviation ± s	0.032	0.028

[a] Transfer of larvae at the 3rd instar on a medium with 3% yeast and carrot powder

crawling and tunnelling of the larvae. Another, more precise standard of evaluation is the speed of development, i.e. the time lapse from one ecdysis to the next (Table III). Of course, in the last resort, larval mortality is decisive and this is still high (Table III). The pupal yield and the speed of development are not the sole criteria, since unsatisfactory nutrition may result in pupation under duress. The quality of the pupae is also important. It seems more reasonable to measure pupal length and width rather than their weight because young pupae especially lose water. Table V shows the size of pupae grown in cherries and in artificial media. The test also included the transfer of third instar larvae from cherries into a medium containing carrot powder and brewer's yeast in which these larvae pupated. The differences between the measurements of the pupae were statistically significant at the 5% level. The size of the pupae reared exclusively on an artificial medium were only a little below the natural values. Because of the long-lasting pupal diapause, nothing can be said concerning the viability of the pupae.

Over the entire rearing cycle, the highest mortality rate occurs during the first instar, immediately after the newly hatched larva has been transferred. While rearing the olive fly (Dacus oleae Gmel) Hagen [8] also observed the biggest loss of larvae at that time. Some of these young larvae are apparently not able to bore in, others make tunnels and die within the medium. If the larvae quickly develop during the first and second instar, the probability of growing to pupae is high. Only a few cases of diseases could be observed. The turning of larvae to a red colour suggests an infection by Serratia marcescens [6]. Every decomposition of the medium by microorganisms results in a slowing down of the development and often in the death of the larvae.

7. DISCUSSION

The method described above allows the cherry fruit-fly to be reared under laboratory conditions on a small scale. A mass cultivation is possible if difficulties in egg handling are overcome and the medium for feeding the larvae is improved. By working as much as possible under aseptic conditions, egg mortality can be reduced. The use of dummies made of wax for egg collecting corresponds more to natural conditions

than the dropping of eggs. To be economic, mass rearing of the larvae of the cherry fruit-fly requires low-cost food, carrier and preservers. In this connection a cheap substitute for Pseudomonas sp. is necessary, since culturing of this microorganism on a large scale is expensive and complicated. In addition an improvement of the larval medium is imperative. This appears to be possible since rearing has succeeded. Moreover, it can be expected that rearing will improve after some generations have been grown under laboratory conditions by an adaptation and selection process. An example of this is the melon fly (Dacus cucurbitae). This species could not be grown on the medium used for rearing Ceratitis capitata and Dacus orientalis. Suddenly, after five years of continuous rearing, all three species could be grown on the same "Ceratitis-medium" [12].

ACKNOWLEDGEMENTS

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EXPLORATORY STUDIES ON THE ERADICATION OF THE KOREAN PINE MOTH Dendrolimus spectabilis Butler BY USING GAMMA IRRADIATION

K.B. LEE, U.K. HWANG AND K.S. LEE
BIOLOGY DIVISION,
ATOMIC ENERGY RESEARCH INSTITUTE,
SEOUL, KOREA

Abstract

'EXPLORATORY STUDIES ON ERADICATION OF THE KOREAN PINE MOTH Dendrolimus spectabilis Butler, BY USING GAMMA RADIATION. This project had two objectives. First, to determine the radiation effect on the pine-moth, Dendrolismus spectabilis Butler, in connection with sterilization irradiation and, secondly, to make an exploratory study on rearing the insect by artificial diets.

Irradiation of the pupae of the pine-moth with 15000 R of gamma-rays induced a complete arrest of fertility without harm to the reproductive behaviour, adult emergence, and longevity.

The larvae of the earlier instar stages were much more sensitive to gamma radiation than those of the later instar stages. The mortality is dose-dependent.

Field-cage tests, using normal male and normal female at a 1:1 ratio, have shown that the egg hatchability of 54.9%, and at a ratio 2:1, was 47%. In the experimental group with the ratio of irradiated male to normal female at 1:1:1, the egg hatchability was 37.1%.

The sterile males do not seem to compete equally in mating with normal females. The nutritionally essential amino acids for pine-moth were determined by using the indirect method. The present experiment shows that it is feasible to rear the larvae of the pine-moth on artificial diets.

INTRODUCTION

Since the successful eradication of screw-worm flies in the south-eastern United States of America by the sterile-male technique, this method has been widely employed in the control of a number of harmful insects all over the world [1]. In Korea the pine moth, Dendrolimus spectabilis Butler, is a major forest pest causing serious damage to pine trees which are economically important. Great efforts have been made to control the insect but the results of conventional field control methods have not always been satisfactory.

After reviewing a number of characteristics, especially those of reproductive behaviour, we decided to examine the feasibility of applying the sterile-male technique for the eradication of the insect by gamma-irradiation. The present communication deals with the first experiments in which the radiosensitivity of the larvae and the fertility of the irradiated pupal male were studied.

MATERIAL AND METHODS

Life cycle of the pine-moth

Larvae were collected in the field from August through November and reared in laboratory cages at 25°C on fresh pine twigs. The duration of the life cycle was determined.

Radiosensitivity of the young larvae

The larvae of the 1st through 5th instars were collected in August and November from the branches and bark (for hibernating 5th instars) of pine trees. Between 30 and 50 larvae were placed in a cylindrical metal mesh cage (height 60 cm, diam. 20 cm). The cages were located in a large room (25°C, 60% relative humidity) and direct sun was avoided. The material in the cages simulated a natural environment, as fresh pine twigs were continuously supplied until irradiation several days later. Doses ranging from 0 (control) to 600 kR were given. The ^{60}Co irradiator (1000 Ci) delivered 2500 R/min at a target distance of 7 cm. The irradiated larvae were returned to the cages to continue development. Mortality was recorded each day until 10 d after the treatment.

Fertility of the adult male irradiated during the pupal stage

The larvae of 7th and 8th instars were collected during April and July and reared as described above. On pupation the males were separated from the females. At 10 to 13 d of pupal age, the males were divided into eleven experimental groups, each consisting of 40 to 80 pupae, and the irradiation dose ranged from 0 to 100 kR. The irradiated male pupae were returned to the cage and 7 to 10 d later emergence occurred. Upon emergence, occurring in late afternoons, each individual male adult moth was placed in a smaller cage containing a virgin female adult. Within a few days after mating was observed, eggs laid on pine twigs were collected daily into pyrex test tubes (15 × 2.5 cm) and counted. The tubes, covered with nylon net, were incubated to determine egg hatchability.

Sexual competitiveness of the irradiated male pine-moth

Irradiated (sterile) and normal males and the normal female with the ratio of 1:1:1, were placed in a metal mesh cage (5 × 5 × 5 m). Egg hatchability per female was recorded. Doses of 15 and 25 kR were used.

RESULTS AND DISCUSSION

Observation on the life cycle of the pine-moth

The duration of the developmental stages are illustrated in Fig.1. The findings on reproductive behaviour compared with studies of previous authors [2, 3]. The larval period is the longest part of the

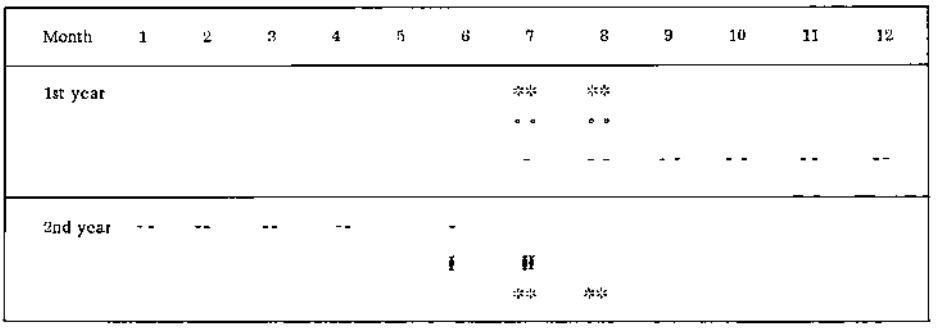

FIG. 1. Life cycle of the Korean pine-moth, Dendrolimus spectabilis Butler.

* Adult (life span average 7 d; copulation soon after emergence.
o Egg (hatching in 10 d)
--- Larval instars – 1st 10 d
2nd 18 d
3rd 15 d
4th 15 d
5th 25 d (hibernation)
6th 16 d
7th 23 d
8th 34 d
9th 15 d (End of larval period identified by change of body colour from blue-red to black-brown; body-length: male av. 7.8 cm, female av. 9.5 cm)
Pupa (20 d) - male is smaller than female.

cycle and consists of eight instars. About half the larval period, however, is spent in hibernation during winter months. The life span of adult moths of both sexes is approximately 6 d. Females are essentially monogamous.

Radiosensitivity of young larvae

The results on the radiosensitivity in terms of mortality at 5 and 10 d post-irradiation of larvae are shown in Figs. 2 and 3. In each of the radiated 1st through 4th instars, the mortality was dose-dependent and the radioresistance increased with larval age. At 5 d after irradiation (Fig. 2), the dose of 50 kR, which resulted in about 30% mortality in the 1st instar, produced no effect in the 4th instar. A mortality of 100% was observed at 175 kR in the 1st instar, and approximately 0% in the 4th instar. At 250 kR a slight mortality (4%) of the 4th instar occurred. An intermediate result was noted with both 2nd and 3rd instars, although the former had a higher mortality. Up to about 125 kR, these two irradiated instars showed slight differences in mortality, but for greater doses the difference became greater at 200 kR mortality, which was 90% in the 2nd instar and less than 40% in the 3rd instar. In the 1st instar, with 250 kR, 100% mortality occurred within 24 h post-irradiation treatment. Usually, a loss of vitality preceded larval deaths.

At 10 d post-irradiation, although the individual values of mortality were naturally higher than those at 5 d after irradiation treatment,

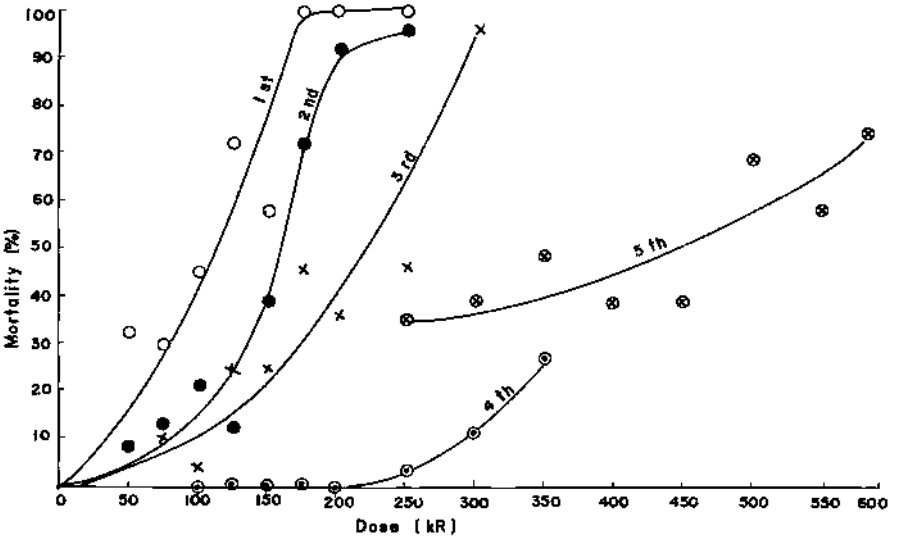

FIG. 2. The mortality of the irradiated larvae of the pine-moth at various instar stages at 5 d post-irradiation.

○ - 1st instar
● - 2nd "
× - 3rd "
⊙ - 4th "
⊗ - 5th "

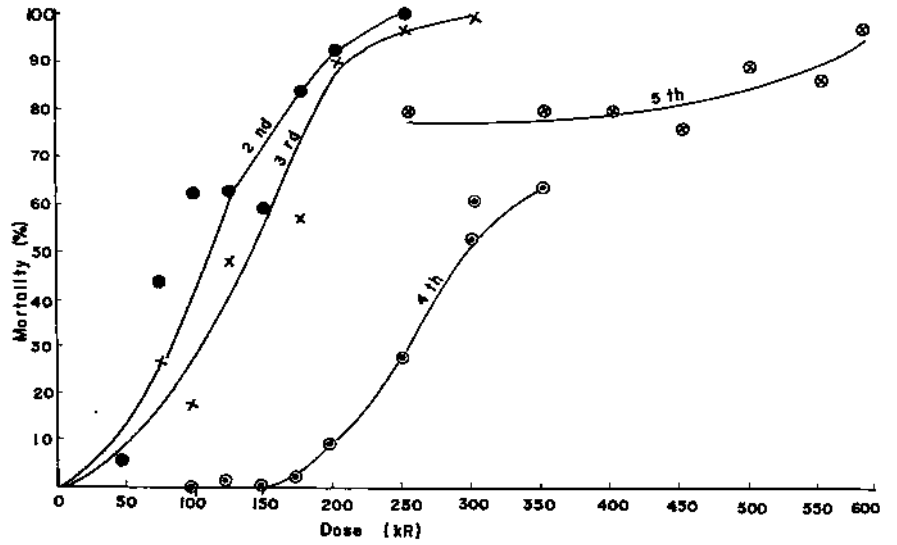

FIG. 3. The mortality of the irradiated larvae of the pine-moth at various instar stages at 10 d post-irradiation.

○ - 1st instar
● - 2nd "
× - 3rd "
⊙ - 4th "
⊗ - 5th "

essentially similar patterns of the mortality for the instars were observed (Fig. 3). No result was available for the 1st instar because of high mortality before 10 d post-irradiation. The mortality of the 2nd instar is greater than that of the 3rd instar at every dose, but the differences between them at higher doses were not great indicating a higher death rate of the 3rd instar between 5 and 10 d post-irradiation.

When a comparison was made between the data of 5 and 10 d after the irradiation, at doses less than 200 kR, the mortality of the former was approximately half that of the latter. The result with the hibernating larvae (5th instar) was completely different from those of younger larvae. Not only did the hibernating larvae appear more radiosensitive, but also the mortality remained within a very narrow range irrespective of the doses, even though extremely high doses of 250 to 600 kR were used. However, the mortality 10 d following irradiation was nearly twice that of 5 days following irradiation as observed in the younger larvae at low doses. Whether this peculiarity of nearly constant mortality with high doses is due to the lower rate of metabolism during the hibernating, has not yet been studied.

These results indicate that larvae up to the 5th instar showed radioresistance dependent on the stage of development. The irradiation of eggs and larvae in order to produce sexually sterile moths was attempted but undesirable side effects resulted [1, 4].

Fertility of the irradiated male pupae

The average number of eggs laid by a normal female mated with a normal male was 312, similar to that reported in earlier data [2]. In each group of males irradiated as pupae then mated with normal females, regardless of the doses between 1.5 and 100 kR, the average numbers of eggs were not markedly different from those of the control (Table I). No adverse effects were observed on pupal and adult life duration, on copulatory and ovipositional behaviours, or on vigour. Egg hatchability showed a clear-cut effect of the irradiation. Hatchability was normal up to 5 kR. At doses between 15 and 100 kR, complete male sterility was noted. Hatchability was reduced to about 20% by 10 kR. At 3 kR, the approximate 17% increase of hatchability was considered a stimulatory effect, similar to that found in the rate of emergence in the granary weevil [5].

Individuals in the second half of pupal development appeared suitable for sterilization by radiation because no adverse side effects were seen. Irradiation of 5-d-old pupae was not successful. The latest pupal stage as an effective irradiation time was previously recommended [1]. Comparable effects were observed in pink bollworm, codling moth, cut-worm moth and Colorado beetle with the doses of 55, 40, 9-11 and 8-10 kR, respectively [1, 6].

Sexual competitiveness of the irradiated males

When the male:female ratio was 1:1, hatchability of the eggs produced by control matings was 54.9% (Table II); however, a ratio of 2:1 gave a hatchability of 47.7%. When the males irradiated with 15 kR

TABLE I. THE FERTILITY OF THE ADULT MALE PINE-MOTH GAMMA-IRRADIATED DURING THE PUPAL STAGE

(Statistical analyses were omitted)

Dose (kR)	Number of pairs (RM × NF)[a]	Average number eggs per female	Hatchability (%)
0	80 (NM × NF)[a]	312	55.8
1.5	76	330	54.4
2.5	79	313	55.1
3	71	297	73.0
5	60	286	54.8
10	58	309	19.8
15	46	267	0
25	51	328	0
50	75	316	0
75	59	338	0
100	39	287	0

[a] NM: Normal male; RM: Irradiated male; NF: Normal female.

TABLE II. THE REPRODUCTIVE COMPETITIVENESS OF THE ADULT MALE PINE-MOTHS IRRADIATED DURING THE PUPAL STAGE

Dose (kR)	Ratio RM : NM : NF[a]	Number of irradiated adult males	Hatchability (%)
0 (control)	1 : 1	500[b]	54.90
	2 : 1	500[b]	47.70
15	1 : 1 : 1	500	37.51
25	1 : 1 : 1	500	31.99

[a] See footnote Table I

[b] Normal males

were placed with normal males and females with the ratio 1:1:1, the hatchability was 37.5%, which is less than the normal value of 47.7%. Similarly, males irradiated with 25 kR further reduced hatchability to 31.9%. Since theoretical hatchability for equal competitiveness of irradiated and unirradiated males was 23.8%, the degree of reduction in hatchability indicated that the sexual competitiveness of the irradiated males was about half that of those unirradiated. A similar response was shown for various flies [1] and pink bollworm [6].

SUMMARY AND CONCLUSION

The radiosensitivity of the 1st through 4th instar larval stages of Korean pine-moth (Dendrolimus spectabilis Butler), increased with the instar age. Doses of 100 kR or less did not produce mortality in the 4th instar.

The number of eggs laid by the normal female mated with the irradiated males treated with 1.5 to 100 kR during the pupal stage was unchanged. Hatchability, however, was reduced to zero by doses of 15 kR or more. With 5 kR or less hatchability was similar to that of control (55.8%); while 10 kR resulted in a partial reduction.

The sexual competitiveness of irradiated male with 15 and 25 kR was about half that of the control males.

The use of the sterile-male method in the eradication of the pine-moth might be feasible if mass rearing of the larvae could be accomplished. Studies on rearing are in progress.

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MASS-REARING TECHNIQUES FOR THE MEDITERRANEAN FRUIT-FLY IN ISRAEL

D.J. NADEL* AND B.A. PELEG**
BIOLOGICAL CONTROL INSTITUTE
CITRUS MARKETING BOARD,
REHOVOT, ISRAEL

Abstract

MASS-REARING TECHNIQUES FOR THE MEDITERRANEAN FRUIT-FLY IN ISRAEL. A rearing technique for the Mediterranean fruit-fly is described, which is convenient for productions of over one million pupae per day. The method was tested in 1965 when some 100 million pupae were reared with a maximum production reaching two millions per day. Usually only one man was involved in this production. A more efficient method of egg collection than that reported previously has been developed, which involves a minimum of maintenance of the adult flies and also prevents their escape from the cages. The larval-rearing medium is cheap and its cost per million pupae is between US $5 and US $10 depending mainly on the cost of the dry brewer's yeast, its most expensive constituent. It is envisaged that the method described could be easily adapted to provide material for a large-scale sterile-insect release programme.

INTRODUCTION

In 1964 we reported on a method of Mediterranean fruit-fly production [1] whereby one million pupae could be reared per week with the expenditure of eight man-hours and at a cost of about US $5 for the larval medium. An egging system was also described which was an improvement over previous methods in efficiency and reduced the danger of fly escape from the rearing room. However, in preparation for increasing the scope of field studies in the sterile-insect release method it was necessary to increase production. The modified system is described below.

MATERIALS AND METHODS

Oviposition cage

The cage was of wooden frame construction, the floor and sides being reinforced by plywood sheeting and the top and rear fitted with a standard mesh nylon fly screen. The front of the cage, which provides the oviposition site, was covered with white silk or nylon cloth having a loose weave and smooth finish [1]. The cage measured 210 cm long × 30 cm wide × 50 cm high, and was conveniently handled by one man. Adult fly food consisted of a mixture of one part Fleishman's Type-M yeast and

* Present address: Division of Research and Laboratories
International Atomic Energy Agency
Kärntner Ring 11, 1010 Vienna

** Present address: OIRSA, Apartado Postal 3628, San José,
Costa Rica

three parts sucrose, and was presented to the flies in a shallow plastic dish on the cage floor, or preferably from the outside by inverting on the screened top. Drinking water was supplied by placing cotton-wool on the screened top of the cage moistened as necessary and covered from above by a plastic cup to reduce evaporation. From 25 to 50 thousand pupae were loaded per cage[1] and the cages were stagger-stacked three high to provide one oviposition unit. The female flies oviposited through the cloth material and the eggs fell into shallow collecting trays set below the unit. Sufficient water was provided in the trays to prevent drying out of the eggs during the 24-h collection cycle. Over a 14- to 16-d egging period at 25°C and 60% RH the three-cage unit produced about 350 ml of eggs after which the remaining flies were destroyed and the cage cleaned and prepared for a new egging cycle. The cloth facing was also replaced. Continuous fluorescent lighting was supplied from the ceiling in front of the cage unit. Lighting was uniform to prevent attraction and crowding of the flies in the cage corners. With the exercise of some care this egging method did not permit fly escape and cages were checked for possible leaks before reloading with pupae. The eggs were collected once daily and their numbers estimated volumetrically. There are about 20 thousand eggs in a cubic centimetre.

Larval rearing

The larval medium based on wheat bran has been reported previously [1]. Shallow plastic trays measuring $25 \times 35 \times 1.5$ cm were flush loaded with 1.75 kg of medium. Each tray received about 1.5 ml of eggs evenly distributed over the medium by means of a table salt-shaker. The shaker was loaded with 7.5 ml of eggs, enough for five trays, and sufficient water was added as a carrier. After loading, the trays were stacked, six to a level, in a wooden-framed larval-rearing cabinet with a total capacity of 114 trays. At this time, the trays were sealed by the addition of a second inverted tray to maintain the high humidity necessary for larval hatching. Subsequently, usually after 4 d, the covers were slightly opened to permit aeration and heat dispersal. One day before initial emergence of mature larvae, the covers were removed. For pupation, the larvae made their way to the edge of the tray from which they jumped or fell to large collecting boxes set underneath the rearing unit. A thin layer of sawdust was added to the box to prevent clumping of the larvae and to encourage pupation. The pupae were usually collected at least once daily.

Each tray returned about 20 thousand pupae with an average weight of 10.68 mg at time of collection. Over a 3-d collecting period the larval-rearing unit returned about 2.2 million pupae. There were about 55 000 pupae to a litre. Two days after collection the pupae were separated from the sawdust by means of a sieve and measured. At this time pupae were set aside for mother stock replenishment. This did not amount to more than 10% of total production.

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[1] With a single loading of 25 thousand pupae a return of about 258 ml of eggs is reasonable per egging unit over a period of 14 egging days. However, by adding 25 thousand pupae from which flies are to emerge 7 d after emergence of the first lot, the egging days can be extended to 16 and a yield of about 366 ml is expected.

Space requirements

The oviposition room measured 6 × 6.5 m and had a low ceiling which limited the number of cages stacked to three per unit. We estimated that up to 10 million eggs could be produced daily in this space and to take care of eventualities we preferred to produce twice the number of eggs for the number of pupae finally required.

The larval-rearing rooms measured 4.5 × 5 m and could hold four rearing cabinets each permitting a continuous rearing of at least one million pupae per day per room.

TABLE I. TIME SCHEDULE FOR THE PRODUCTION OF THREE MILLION MEDFLIES PER WEEK

Task	Time (min)	Repetitions/week	Total
Egging			
Collection (six units)	15	7	105
Volumetric measuring	5	7	35
Cleaning collecting boxes	20	2	40
Larval production			
Medium preparation (40 units)	60	3[a]	180
Distribution of medium on trays, egg dispersal, sealing and stacking for 20 units	45	7	315
Slit opening of trays	10	7	70
Full opening of trays	10	7	70
Pupae handling and measuring	10	7	70
Cleaning plastic trays	10	6	60
Egging cages (three cages = one unit)			
Preparation of adult food	20	1	20
Loading one unit	30	1	30
Cleaning one unit	20	1	20
		Total	1025 min (17 h)

[a] The larval medium may be prepared once a week if cold storage (5°C) facilities are available.

Costs

Depending on the cost of the dry brewer's yeast, which is the most expensive constituent in the larval diet, one million pupae could be reared at a material cost of about US $5 to US $10. Table I indicates the work schedule we found necessary for a production of three million pupae per week.

The method has the advantage of not requiring a high capital outlay. Cages and rearing cabinets are cheap and easily constructed. Except where extensive airconditioning is required, housing requirements are minimal.

IMPROVEMENTS IN MASS REARING THE MEDITERRANEAN FRUIT-FLY, Ceratitis capitata Wied.

J. MONRO
DIVISION OF RESEARCH AND LABORATORIES,
INTERNATIONAL ATOMIC ENERGY AGENCY,
VIENNA, AUSTRIA

Abstract

IMPROVEMENTS IN MASS REARING THE MEDITERRANEAN FRUIT-FLY, Ceratitis capitata Wied. This paper summarizes the results obtained with systems of mass-rearing at the IAEA laboratory, Seibersdorf, Austria. The aim was to develop cheap and efficient methods of rearing Ceratitis which would be immediately applicable to large-scale factory production. In particular, attempts were made to cut handling costs and costs of larval media and to diversify these for use in different countries.

ADULT BEARING

This was developed from the method described by Nadel [1]. Pupae were sealed into the rearing cage and the adults were fed and watered through the roof of the cage. Females laid their eggs through a wall of fine terylene gauze. The cages measuring 100 cm × 50 cm × 30 cm were made of aluminium or PVC sheet, the roof of plastic fly-screen, and the long wall which faced the light source was of terylene gauze. Each cage was loaded with 30 000 pupae and kept in a room at 25°C, 70-75% RH and a day-length of 14-16 h. Food consisting of a paste of four parts sucrose and one part enzymatic hydrolysate of yeast was smeared on paper towels and placed on the roof. A strip of filter paper lying on the roof received water automatically (using a time-switch and solenoid-valve) for one minute in every thirty. Food and the watering pad were changed weekly. The eggs fell from the terylene gauze wall into a trough of water from which they were filtered at 4 p.m. each day.

An automatic egg-collector

To reduce labour the eggs dropping from the cages may be mechanically collected by means of a conveyor belt discharging into a hopper filled with water. A small-scale model was built and tested successfully. The conveyor belt was two-layered; an inner driving band of solid rubber and an absorbent outer layer of sponge-rubber, which took up water from the hopper and prevented the eggs from drying. The band ran automatically for one minute in each half-hour and carried the eggs into the water-filled hopper through which they dropped into a burette ready for "seeding" on the larval medium. A full-scale egg-conveyor would be useful in a large factory in countries where labour costs are high.

TABLE I. CHIEF COMPONENTS (in grams) OF MEDITERRANEAN FRUIT-FLY MEDIA DEVELOPED AT THE IAEA LABORATORY, SEIBERSDORF, AUSTRIA[a]

Medium	Wheat germ	Brewer's yeast	Peanut paste	Sucrose	Solvent extracted cotton seed	Sawdust	Dried cellulose pulp	Dried extracted sugar cane (Bagasse)	Dried maize stalks	Wheat bran	Dried extracted sugar beet
SYP	-	200	50	300	-	360[b]	-	-	-	-	-
CeYP	-	200	50	300	-	-	200[b]	-	-	-	-
BaYP	-	200	50	300	-	-	-	-	-	-	-
MYP	-	200	50	300	-	-	-	-	200[b]	-	-
BrYP[c]	-	200	50	300	-	-	-	-	-	600[b]	-
BaCoP	-	-	50	300	500	-	-	175[b]	-	-	-
MCoP	-	-	50	300	500	-	-	-	175[b]	-	-
BrGYP	150	200	50	300	-	-	-	-	-	500[b]	-
BeYP	-	200	50	300	-	-	-	-	-	-	350[b]

[a] These components are mixed with 1500 ml of water and 3 g each Nipasol and Nipagin as mould inhibitors. pH brought to 4.0 - 4.5 with HCl.
[b] These components were chiefly carriers. Therefore, the quantities shown were varied when necessary to achieve a firm consistency.
[c] This is a variant of Nadel's medium [1].

LARVAL CULTURE

Handling

The best methods of handling are the open-tray methods described by Nadel [1], and Peleg during this Panel.

Larval media

Our objective was to develop a wide range of inexpensive larval media. The constituents of these media (Table I) were chosen in such a way that at least one of the media can be readily made up with materials available within almost any country from the tropics to the temperate regions. All these media gave better than 50% yield of pupae, and in the medium most removed from earlier media, BaCo P, five generations of healthy flies were successfully reared. It should be remembered in starting a new medium that early difficulties tend to disappear because of selection after a few generations. The constituents of such media may be classified as follows:

(a) Carriers

These are materials rich in cellulose or other absorbent substances. We found that pine sawdust, paper-pulp, extracted sugar-cane, sugar-beet residues[1] and maize stalks, as well as the combined carrier-nutrients, bran [1] and dried carrot [2], were suitable for adjusting the consistency and free water content of larval media.

(b) Major nutrients

We continued to use commercial sucrose as a source of carbohydrate [1] and brewer's yeast as a source of protein.

(c) Minor nutrients

Peanut paste in small amounts (2-4%) accelerated growth, probably because it contributed fatty acids and fat soluble vitamins. Yeast is the other source of minor nutrients.

(d) Minerals

We did not add any minerals, but relied on the natural mineral content of the constituents.

(e) Mould inhibitors

Nipasol (propyl p-hydroxybenzoate) and nipagin (methyl p-hydroxybenzoate) with HCl to set the pH at 4-4.5, gave good control of moulds assisted by natural fungistatic properties of Ceratitis larvae at a density of 20 eggs/g of medium.

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[1] Also independently suggested by Drs A. Haisch and I. Moore

(f) Starters

We found that carotene accelerated the growth of very young larvae and therefore we sprinkled a thin layer of dried carrot over the surface of the medium before "seeding" it with larvae.

Forced pupation

To have uniform batches of pupae for irradiation we selected Medfly larvae which pupated after 7-8 d. Pupation was forced as soon as mature larvae began to leave the medium. After two to three generations this procedure resulted in a strain which pupated within 24 h. This procedure had the added advantage of breaking the life cycle of Drosophila, a serious contaminant of the Medfly larval medium.

The method of washing the larvae from the medium and spinning off excess water in a washing machine [3] did not work with coarsely divided carriers. Therefore, we used infra-red lamps on a small scale to drive the larvae through a screen into a pupation tray. Though effective, this method requires much labour and space. Larvae may also be forced from food by blowing heated air over the trays and allowing the larvae to fall past the source of heated air into cool trays of dry sawdust for pupation.

Pupal storage

After 24 h the larvae pupated and were placed at 25°C and 70-75% RH. The number of pupae was estimated volumetrically on the second or third day, after the sawdust had been sieved off. Pupae were stored in layers about 2 cm deep in shallow trays to await irradiation or entry to the adult cages.

TOWARDS A FLUID MEDIUM FOR MEDFLY

Existing methods of rearing the Medfly are adequate for countries where labour costs are low. However, mechanization of rearing would be easier with a fluid or thin slurry larval diet. The semi-solid or "mash" diets are not flexible enough for pouring. Moreover, the spent medium is difficult to discard. A medium in the form of a fluid or slurry could be pumped, piped, shaken and drained. Its level and composition could be easily controlled and varied. The spent medium could be discharged into a normal sewage system.

To provide an experimental basis for such media we investigated the uptake of major nutrients from liquid diets during larval development. A more extended account will be published elsewhere.

A standard fluid medium known as Diet A (Table II) was used initially. Larvae were reared in small petri dishes in a thin film of medium which was changed every 3 d. The optimal density for survival and growth was 600 larvae started in 5 ml of medium and changed to 25 larvae in 5 ml of medium from the sixth day onwards. Having established these methods of rearing, we used them to measure the apparent uptake of protein and carbohydrate and to study how various components of the diet influenced growth-rate (change in wet body weight) and survival.

TABLE II. BASIC EXPERIMENTAL LIQUID DIET FOR MEDITERRANEAN FLY

Cholesterol	- 0.3 g
Sucrose	- 100 g
Brewer's yeast	- 50 g
Nipagin	- 0.8 g
Nipasol	- 0.8 g
Water	- 500 ml
HCl - to pH	- 4.0
Vitamin pill [a]	- 1

[a] Contents of vitamin pill	
Vit. A	5000 i.u.
Vit. D_3	500 i.u.
Vit. E	0.5 mg
Vit. K	0.05 mg
Vit. B_1	5 mg
Vit. B_2	5 mg
Vit. B_4	5 mg
Vit. B_6	0.5 mg
Vit. B_{12}	0.5 µg
Folic acid	0.5 mg
Nicotinamide	50 mg
Vit. C	75 mg
Minerals (mg)	
Cobalt	0.5
Copper	1
Fluorine	0.2
Iron	20
Magnesium	25
Manganese	2
Potassium	0.2
Sodium	1
Zinc	1

pH

Survival and growth were maximal with a starting pH of 4.0. All larvae were killed if started in media with a pH less than 3.0 or greater than 5.0.

Comparison of "fluid" with "mash" diets

Survival rate was only 50% after 3 d in the fluid diet compared with 80-90% in the standard carrot-yeast medium [2] and medium BrGYP (Table I). Thereafter, survival rates were the same for all media. These early deaths were probably caused by drowning and were reduced if the young larvae were kept on thin sheets of paper dampened with medium rather than in a film of liquid.

Growth-rates on the carrot-yeast and BrGYP media were higher than on Diet A (Fig. 1). Apparently some nutrients in these media, particularly in carrot, gave a much faster rate of growth.

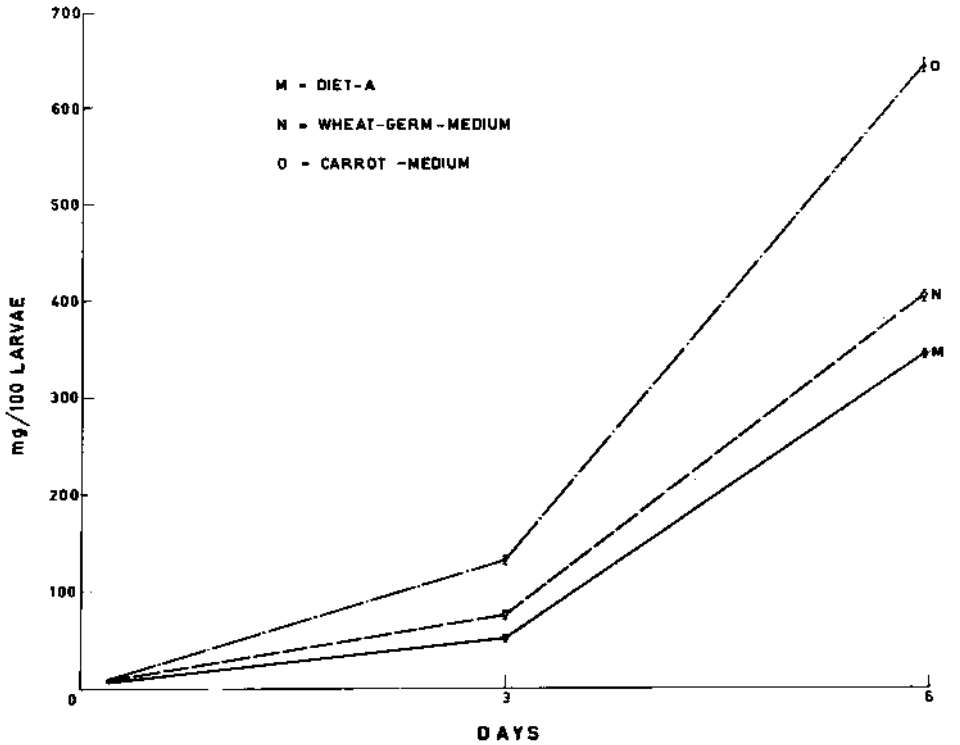

FIG. 1. Growth of Ceratitis larvae on a fluid diet compared with two "mash" diets.

Addition to Diet A

In Diet A either 5% wheat-germ or 5% dried carrot were incorporated. Survival was not significantly changed by these additions but growth-rate was considerably increased (Fig. 2).

Further experiments showed that growth and survival were better on Diet A (10% yeast) plus 5% dried carrot (Figs 3 and 4, respectively). Carotene seemed to be the growth accelerator in carrots. β-carotene at a concentration of 0.190 gave about the same acceleration as a 5% concentration of dried, water-insoluble residue of fresh carrots (Fig. 5).

Uptake of protein and carbohydrate

Relative percentages of total protein and sucrose taken up by larvae from Diet A became equal between 6 and 9 d (Fig. 6). This suggested that media, in which the ratio of sucrose:yeast decreased with larval age, might give better results than those of fixed composition. In Diet A conversion by microorganisms of sucrose to protein was minimized by acid pH, the fungistatic benzoates and frequent renewal of the medium.

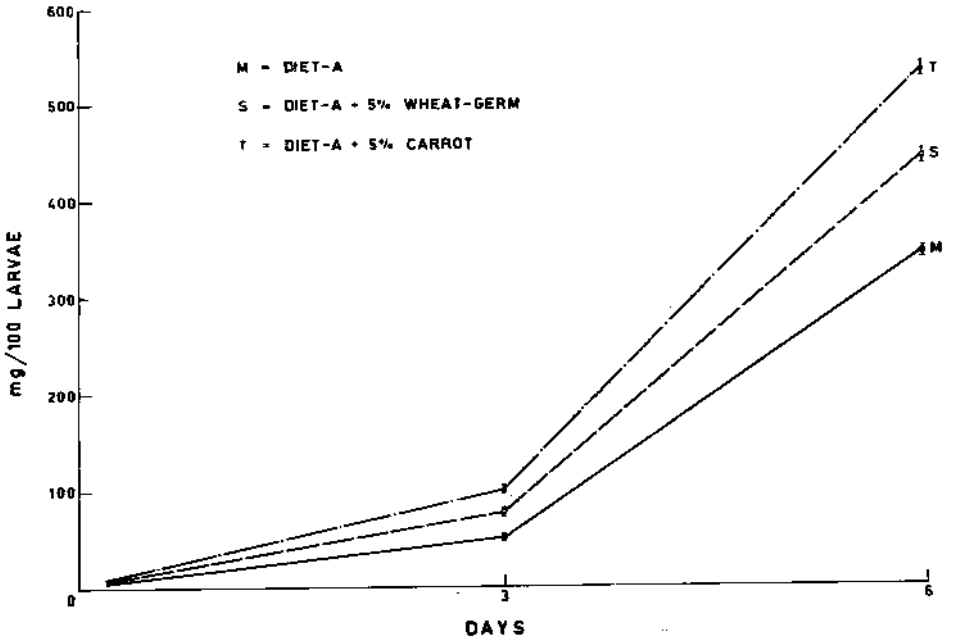

FIG. 2. Growth of Ceratitis larvae on enriched fluid diets.

In ripe fruits such as peaches the ratio of carbohydrate to protein is 14.8 : 1.0. This implied that in fruit carbohydrate must be converted to protein to supply the needs of the more mature larvae.

Growth and survival in sterile and contaminated peach-slice

Larval survival and growth at initial crowding of 200 larvae on 10-g peach-slices were compared (Figs 7 and 8 respectively) with survival and growth at optimal crowding on Diet A. In the sterile treatments peach slices were surface sterilized with u.v. and the Medfly eggs washed in 1% Zephirol (Zephiran). Sterile peaches were not adequate. Larval growth "took-off" faster on contaminated peaches than on Diet A (Fig. 8). This slow start on Diet A may be due to the high ratio of protein to carbohydrate, again suggesting the potential advantage of flexible fluid diets. Apparent uptake (based on loss from the medium) of sucrose (Fig. 9) and protein (Fig. 10) indicated that the older larvae failed to keep pace with microbial production of protein. Sterile peaches infested with larvae maintained a pH of 3.8 to 4.1 for 9 d. However, the pH of non-sterile peaches infested with Medfly larvae changed from 3.8 to 7.3 in the 9-d experimental period. The pH of non-sterile peaches without larvae changed from 3.8 to 7.1 during the same period. By contrast Diet A was acid throughout.

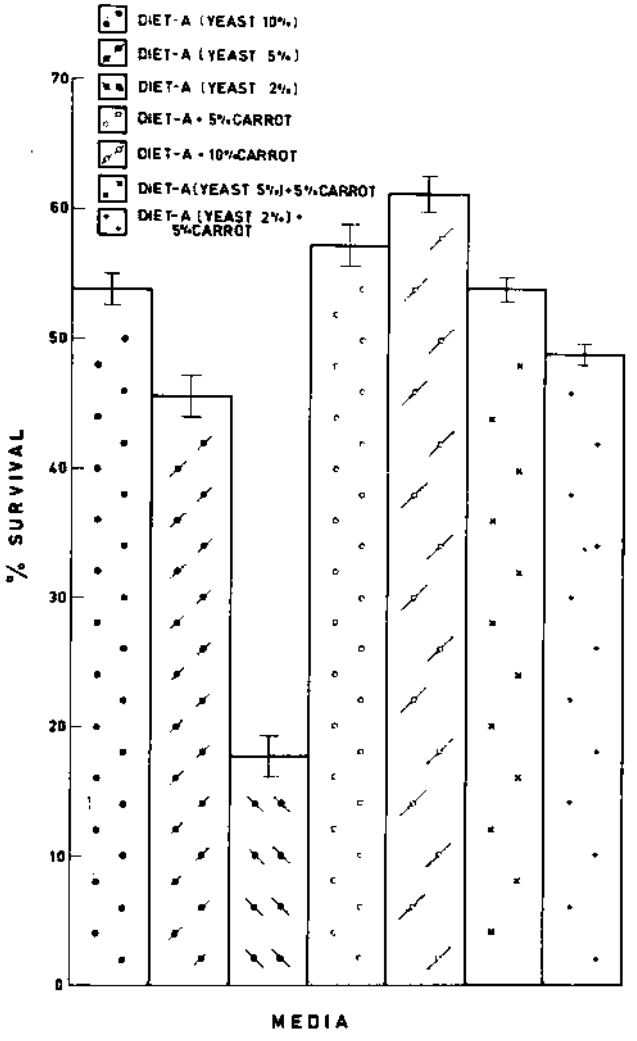

FIG. 3. Survival of Ceratitis larvae on fluid diets at different concentrations of yeast and dried carrot.

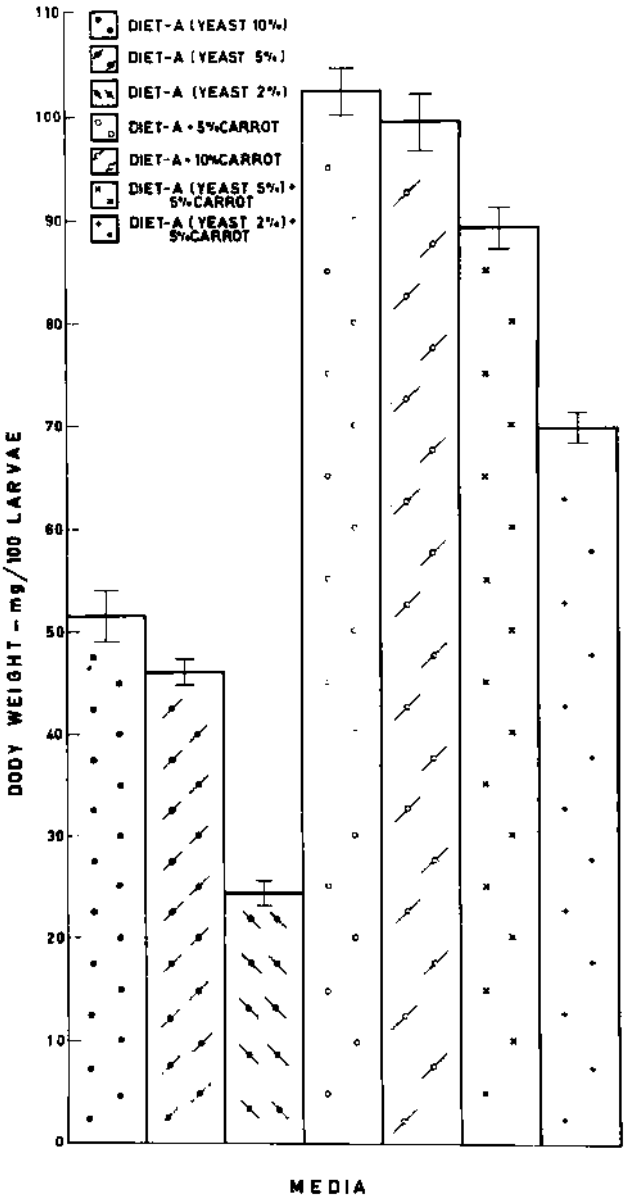

FIG. 4. Growth of Ceratitis larvae on fluid diets at different concentrations of yeast and carrot.

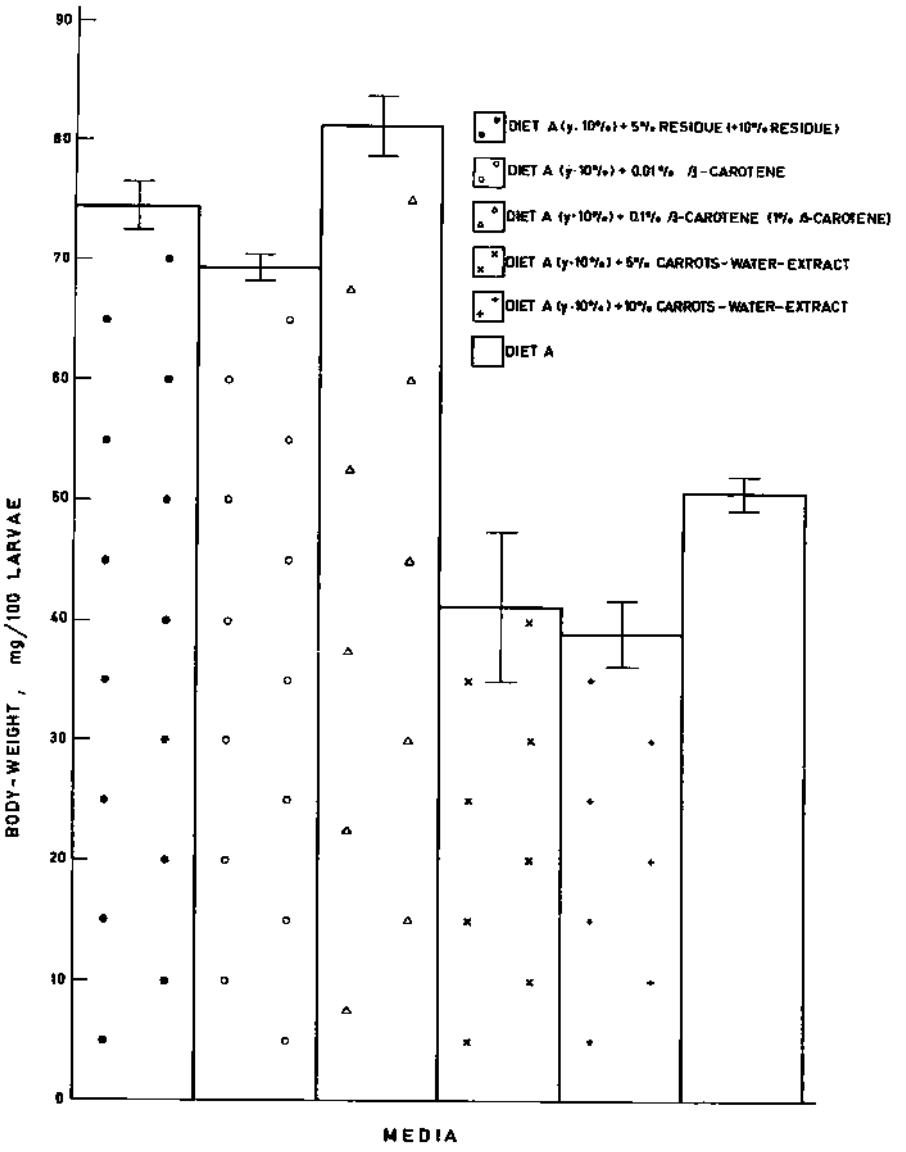

FIG. 5. Growth of Ceratitis larvae with addition of carrot extract and β-carotene to the fluid diet. (10% residue) means that 10% of dried water-insoluble extract of fresh carrots gave the same response as 5%. (1% β-carotene) means that 1% β-carotene gave the same response as 0.1%.

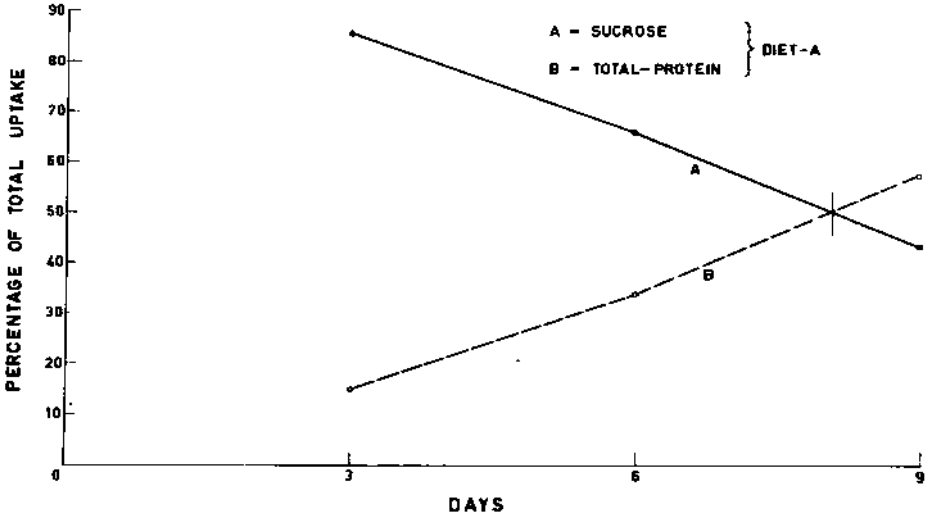

FIG. 6. Relative uptake of protein and sucrose from Diet A.

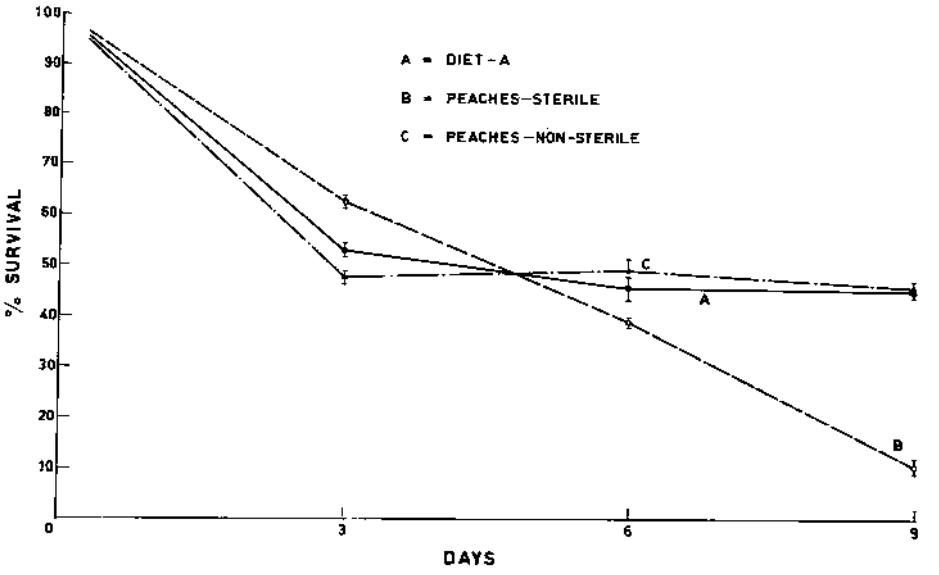

FIG. 7. Survival of Ceratitis larvae on sterile and contaminated peaches and on Diet A.

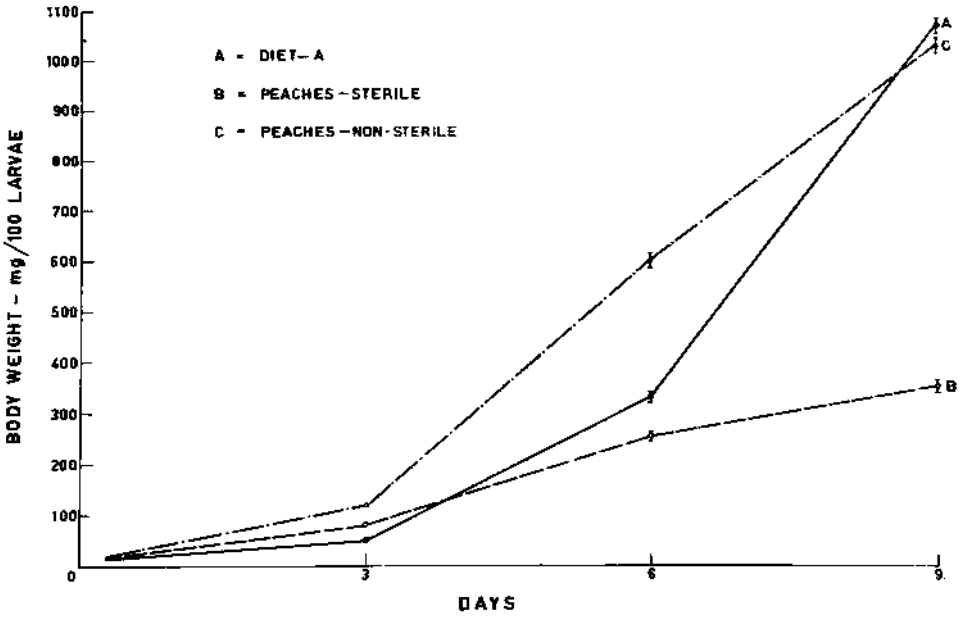

FIG. 8. Growth of Ceratitis larvae on sterile and contaminated peaches and on Diet A.

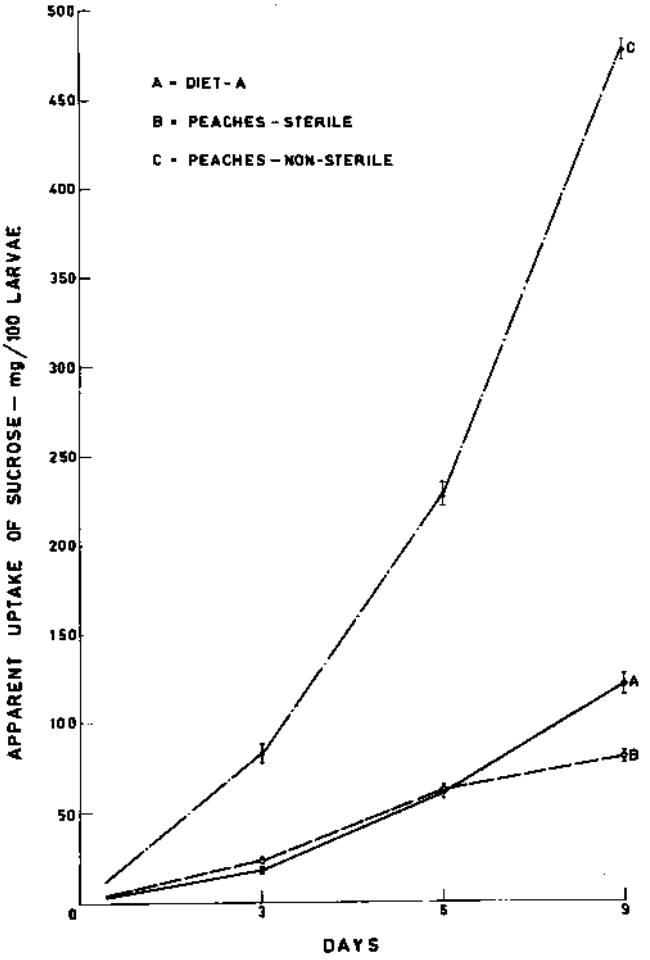

FIG. 9. Apparent uptake of sucrose from sterile and contaminated peaches and Diet A by Ceratitis larvae.

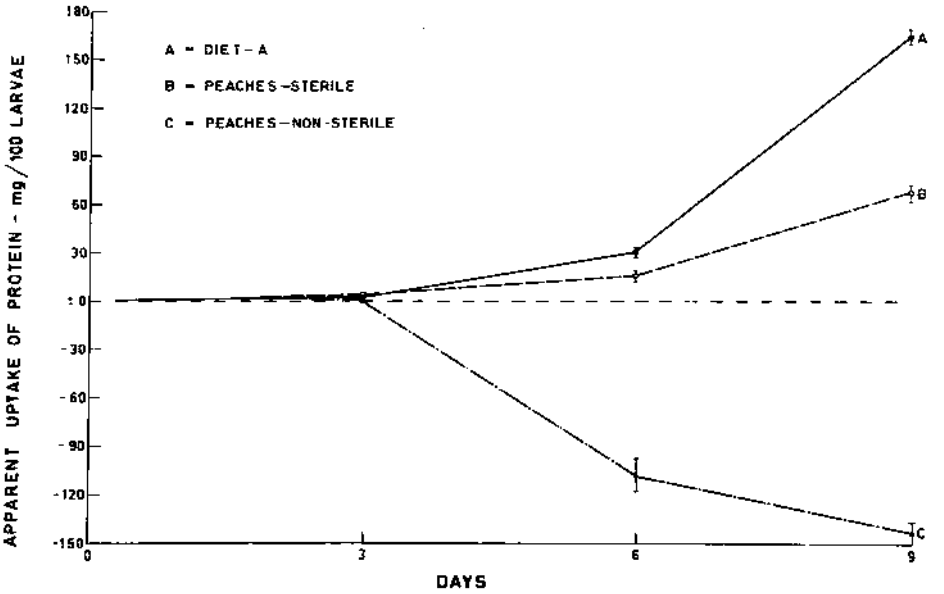

FIG. 10. Apparent uptake of protein by Ceratitis larvae on sterile and contaminated peaches and on Diet A.

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PROGRESS REPORT ON REARING OF THE CODLING MOTH

A. NAVON
THE VOLCANI INSTITUTE OF AGRICULTURAL RESEARCH
REHOVOT, ISRAEL

Abstract

PROGRESS REPORT ON REARING OF THE CODLING MOTH. Research on the artificial rearing of the codling moth (Carpocapsa pomonella L.) was initiated. The purpose of this work was to develop an economic medium for the mass rearing of the codling moth.

INTRODUCTION

Attention was paid to the following details:

(1) Individual rearing of larvae to avoid cannibalism
(2) Use of inert carriers to test nutritional requirements
(3) Control of bacterial and fungal contaminants.

MEDIUM COMPOSITION

Although the Codling moth attacks pears and quinces, it mainly damages the apple. It seemed, therefore, reasonable to use the chemical composition of that host as a guideline for making up an artificial medium. The ingredients and their quantities were as follows:

1.	Full fat soy meal (fine mesh)	24.0 g
2.	Sucrose	48.0 g
3.	Brewer's yeast	18.0 g
4.	Apple sarcocarp	100.0 g
5.	Ascorbic acid	0.3 g
6.	L-malic acid	4.0 g
7.	Methyl p-hydroxybenzoate	1.5 g
8.	Agar-agar (flakes)	24.0 g
9.	Cellulose powder (alphacel)	60.0 g
10.	Citrate buffer (pH 3.4)	120.0 ml
11.	Distilled water	480.0 ml

Full fat soy meal was included because it contains the following important nutrients: (1) High-quality proteins, (2) phytosterols, (3) saponifiable fats and phospholipids, (4) minerals, (5) carbohydrates, (6) part of the vitamin-B complex.

Preliminary tests showed that the addition of apple sarcocarp (seeds and pericarp excluded) improved the larval growth rate. Agar-agar and cellulose powder provided a texture similar to apple tissue; however, free moisture due to syneresis occasionally caused larval mortality by drowning.

Carriers as methyl cellulose and carboxy methyl cellulose were also tested, but their use was discontinued because they made the texture

viscous and this prevented the larvae from feeding. Methyl p-hydroxybenzoate at a concentration of 0.25% w/v gave some control of bacteria but mainly prevented fungus growth. Citrate buffer, which adjusted the pH of the medium to 3.4, prevented bacterial growth. No contamination was observed in any of the media used, even when no aseptic precautions were taken. The soy meal, nipagin, sucrose, and brewer's yeast were blended with a heavy-duty waring blender. This homogenate was autoclaved in order to destroy the antitrypsin factor of the soy, and to effect sterilization.

The agar-agar was melted separately in boiling water after which the cellulose powder was stirred into it. This fraction was then incorporated into the sterilized homogenate by shaking. Just before jelling (approx. 45°C), the apple scarcocarp, which was blended to a purée, and citrate buffer solution containing the malic and ascorbic acids, were added. After the medium had set, it was kept under refrigeration until used.

BREEDING TECHNIQUE

Neonate larvae were used in small-scale individual rearing in test tubes with cotton wads wrapped in tissue paper. The total amount of food available to a single larva was about 5 g. Food was offered every 10 d. The larvae tunnelled inside the medium and moved to new food by themselves. Pupation took place in the cotton plugs of the test tubes, whereas in collective rearing it occurred in corrugated paper.

Larval rearing was carried out at 27 ± 1°C and 70-75% R.H. The room was illuminated continuously with fluorescent light in order to inhibit diapause. Emerging adults were transferred to mating-oviposition cages. Each cage consisted of a cylindrical metal frame wrapped in wax paper. The bottom of the cage was covered with cheese cloth for ventilation; the top was closed by ordinary opaque paper. Both these covers were fixed with rubber bands. In the centre of the paper, on top, a hole was made over which a plastic box supplying water was put upside down. The open end of this box was covered with perlon cloth fixed by the original plastic lid into which a hole had been made. The adults could drink and there was no water spillage.

The cages were kept at 23 ± 1°C and 70% R.H. They were illuminated with a 2.5-V small red light-bulb placed about one metre to the side of them, in order to induce mating. Oviposition took place on the wax paper only. Adults were transferred to new cages every two days and wax papers with the egg batches attached to them were kept for larval hatching.

RESULTS

Two successive generations have been obtained so far, and the biggest yield in terms of adults per neonate larvae was 20-30%. However, the adults obtained were of normal size. These very preliminary results direct us to concentrate our future efforts primarily on the following steps:

(1) The gaining of more information on the essential nutritional ingredients and their relative concentrations in the medium; and
(2) Improving the physical nature of the medium.

MASS REARING OF THE MEDITERRANEAN FRUIT-FLY IN COSTA RICA

B. A. PELEG, R. H. RHODE AND W. CALDERON
ORGANISMO INTERNACIONAL REGIONAL DE SANIDAD AGROPECUARIA (OIRSA)
APARTADO POSTAL 3628, SAN JOSE,
COSTA RICA

Abstract

MASS REARING OF THE MEDITERRANEAN FRUIT-FLY IN COSTA RICA. Procedures developed in Israel and the IAEA laboratory (Seibersdorf) and modified to meet local conditions replaced those of the USDA (Hawaii) as regards Mediterranean fruit-fly egg collection, egg and larval handling, the larval medium and pupal recovery techniques. These changes have resulted in higher yields and lower production costs under our conditions.

INTRODUCTION

The International Atomic Energy Agency through the United Nations Development Programme Special Fund, is engaged with the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) in a joint effort to determine the feasibility of eradicating the Mediterranean fruit-fly Ceratitis capitata (Wied.) in Central America by the sterile-insect release method.

To meet the programme's production objective of releasing fifty million sterile flies per week the development of efficient and economical rearing methods became of immediate concern.

Until 1966 rearing procedures used by OIRSA followed closely those developed by the United States Department of Agriculture's fruit-fly laboratory in Hawaii. However, production costs were high and the yield unsatisfactory under local conditions.

The system of rearing Mediterranean fruit-fly developed in Israel by Nadel [1] and Nadel and Peleg [2] proved more satisfactory for our purposes and involved making basic changes in technique in regard to egg collection, egg and larval handling and pupal recovery. We have made further modifications to utilize local equipment and to adapt the method for local conditions.

MATERIALS AND METHODS

1. Egg collection

For anticipated production needs the egg collection method utilizing perforated plastic oviposition containers now in use in Hawaii involves excess manual labour. In addition, egg quality varies because of relative humidity fluctuations during presentation of the containers. Nadel's egg-collection system, where the cloth facing on the cage serves as the oviposition site, overcomes these disadvantages. We modified our Hawaiian

fly-stock cages, measuring 122 × 61 × 34.5 cm by covering one side with a suitable white cloth. Five cages forming an oviposition unit are stacked so that each cage is slightly inset from the one above. Thus the eggs deposited through the cloth fall directly into a water-filled receptacle placed on the floor at the base of each unit [1, 2].

The cloth-covered faces of the cages receive light from a fluorescent source on the ceiling. Each cage is set up with 450 cm^3 of pupae (approximately 25 000 flies) and supplemented with an additional 200 cm^3 of younger pupae timed to emerge about a week following the original emergence [2]. Each fly-stock population is held for a total of 18 d, 14 of which are effective egg-producing days.

Because the flies were conditioned to the plastic oviposition containers, initial egg deposition through the cloth was very poor, only 14 cm^3 from five cages. After four generations oviposition through the cloth reached a satisfactory level. From each five-cage unit about 300 - 350 cm^3 of eggs are obtained throughout the 14-d oviposition period.

The fly stock room is kept under continuous artificial illumination. Temperature and relative humidity range from 24 to 34°C, and 45% to 95% respectively. Food consists of a mixture of three parts sucrose, one part Fleichman's type-M yeast and a small amount of orange-juice crystals. A source of drinking water is also supplied.

2. Larval medium

Although a cheap larval rearing medium was first developed and used successfully in Israel [1] its main constituent, wheat bran, was not readily available in Costa Rica and a substitute diet based on bagasse and wheat germ was developed by Monro [3] for the project. This replaced the more costly Hawaiian diet which is based on dehydrated carrot powder. The relatively inexpensive dried, ground sugar-cane bagasse is obtained from haciendas where no insecticides are used in the growing of sugar cane. The addition of wheat germ was found to be essential for satisfactory larval development and for providing a suitable medium texture.

After testing various combinations of ingredients, we adopted the formula given in Table I for use in large-scale medfly production:

The mixing process follows the sequence shown in Table I. No heating of the water is necessary to dissolve the preservatives as when the Nipagin-Nipasol combination is used [1]. Because of slight variations in particle size and moisture content, the amounts of bagasse must be adjusted to obtain a very loose mixture. If the water content of the medium is too low, subsequent heating occurs when the larvae near maturity. The pH of the mixture ranges from 4.3 to 4.5.

3. Pupal recovery

Using the Hawaiian system the mature larvae had been recovered from the carrot medium by washing and were thus not conditioned to jump or fall into collecting boxes as practised in Israel [1, 2]. Although initially some larvae jumped from the new medium, the majority either pupated or died in the trays. It was found that this situation could be prevented if the mature larvae were stimulated to leave the medium by inverting the trays. Development of recovery methods based on this principle thus required

TABLE I. FORMULA FOR THE PREPARATION OF ONE TRAY OF ABOUT 7300-ml CAPACITY

Material	Amount in grams	Costs in US $
Water	5500.00	0.000
Nipagin	11.25	0.075
Sodium benzoate	11.25	0.017
HCl 1N	66.00 (56 ml)	0.024
Sucrose	1135.00	0.185
Torula yeast CF-2	680.00	0.302
Wheat germ	620.00	0.064
Bagasse	675.00	0.058
Total	8698.50	0.725

procedures and equipment different from the simpler system used in Israel.

A metal frame of slightly larger dimensions than the tray and covered with $\frac{1}{4}$-in. mesh hardware cloth is placed over the top tray. The tray and frame are inverted and placed in the recovery unit. Both tray and plastic liner are then removed leaving the medium resting on the hardware mesh screen. (Two doors are provided for convenience in cleaning.) The unit is fitted with 13 shelves each separated by 11.5 cm and has a capacity for 26 trays. Under each shelf except the bottom one are wooden guides upon which two plywood panels each measuring $89 \times 40 \times 0.3$ cm can be pushed into position. The plywood panels are inclined downwards from the centre of each shelf at an angle of about 7° towards both sides. A space of 2 cm remains between the outer edges of the panels and the sides of the cabinet. The ends of the panels are separated by about 3 cm from the cabinet doors. A triangular section of wood is attached to the ends of the panels facing the front. This fits against the edge of the shelf and prevents the larvae from jumping out when the cabinet door is opened.

The larvae leaving the medium drop upon the plywood panels and by jumping reach the outer edge and fall. Near the bottom, tin wings deflect the falling larvae into a vermiculite filled drawer on the cabinet floor.

4. Rearing procedures

Eggs are collected once a day and distributed directly over the medium. Because of some yet undetermined reason, better results are obtained with this method than when the eggs are incubated in petri dishes.

A 250-ml plastic bottle with a perforated cap is used to distribute the eggs [1]. The bottle is filled with 56 ml of eggs with water as a carrier

and the contents are sprinkled evenly among eight trays of medium. Thirty trays are placed in each screened rearing cabinet which measures 205 × 84 × 94 cm and held in the larval-rearing room at a temperature of 27 - 29°C and 70 to 90% relative humidity. When properly prepared the interior temperature of the media on the last day of larval development ranges between 28 - 33°C. On the ninth day (6 d as larvae) the trays are removed and placed in the recovery unit. After a holding period of four days, the media are discarded. Maximum larval recovery takes place on the tenth day. The pupating larvae are collected once or twice a day according to the numbers recovered. Larval yields range from 1 800 000 to 2 500 000 for each unit.

DISCUSSION

Under local conditions the present rearing methods have definite advantages over the former technique. A higher quality product is obtained with less labour by collecting the eggs through cloth than by using the plastic oviposition containers. The egg eclosion period is more uniform. Development under water is temporarily suspended resulting in eggs laid at the beginning of a 24-h period hatching at about the same time as the last egg laid. Also, increased viability results because the eggs do not dry out as with the plastic oviposition containers.

The bagasse-wheat-germ medium yields higher larval recoveries than the carrot powder. From bagasse individual tray recoveries range from 70 000 to 90 000 compared with about 20 000 to 40 000 for carrot. These increased recovery rates compare favourably with those obtained in Israel using wheat bran, and give an efficient use of equipment, space and manpower.

The inverted tray method eliminates the washing-out process which resulted in about a 35% loss of immature forms. Although a small number of larvae fall from the medium, especially within the first hour after overturning, the majority leave only after having completed their development. Pupal colour, size and weight are satisfactory and adult emergence rates average about 95%.

Depending on larval recovery, production costs per million pupae using carrot powder normally average about $65.00. With bagasse-wheat germ, the cost is about $8.05 to $10.40.

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APPLICATION OF THE STERILE-MALE TECHNIQUE ON THE ONION-FLY, Hylemya antiqua (Meig.), IN THE NETHERLANDS. PROGRESS REPORT

J. TICHELER AND J. Ph. W. NOORDINK
INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK,
WAGENINGEN, NETHERLANDS

Abstract

APPLICATION OF THE STERILE-MALE TECHNIQUE ON THE ONION-FLY, Hylemya antiqua (Meig.), IN THE NETHERLANDS. The onion-fly causes millions of dollars damage each year to onion crops. This fly was controlled by Dieldrin but, since 1962, it has developed resistance to this and related insecticides. This fly serves as a test case for the application of the sterile-male release technique.

The topics considered in this paper include, general biology, rearing on artificial diets, sterilization by radiation dispersal, and mating behaviour of the onion-fly.

INTRODUCTION

Annually in the Netherlands, approximately 6000 ha are planted with onions – mainly seeded onions – with a production of about 200 000 t valued at roughly US $13 million. The onion-growing is confined to limited areas, chiefly in the south-western part of the country. The most important pest is the onion-fly, Hylemya antiqua (Meig.). If no control measured were applied the fly would often cause a complete failure of the crop, particularly on the lighter soils. Until 1962, the insect was satisfactorily controlled by means of a seed-dressing with Dieldrin. But since then the fly has become resistant to Dieldrin and related compounds including DDT, which was formerly used. Thus one cannot rely solely on the organo-phosphorous insecticides, which could replace the chlorinated hydrocarbons. Of the recent approaches to insect control, the sterile-male technique seemed the most promising. The onion-fly serves as a test case for the method. The same arguments apply for the cabbage-fly, Hylemya brassicae (Bché), the seed corn maggot, H. cilicrura (Rond.), and the carrot rust fly, Psila rosae (F.). Onions are grown in most parts of the world and yield eight million tons a year. The onion-fly is known to occur in countries yielding 46% of the total production, and the fly is resistant to insecticides in countries producing 23% of the total. (Production figures from FAO, 1964, Ref. [1]).

BIOLOGY

The onion-fly emergence from the over-wintered pupae starts at the end of April. After a pre-oviposition period of a week, the adult female lays eggs close to the onion plants. The larva tunnels in the underground parts of the onion plant and kills it. As the seeded onion plants are still very small, the larva moves from one plant to the next. Maan [2] showed

that for every plant primarily attacked as many as seventeen may be killed later. The fly has three generations a year, of which the first is the most important economically. Pupae enter diapause as in the first generation but in proportions increasing with time. Quantitatively, this phenomenon must be checked. Mating of the fly has seldom been observed and persists for a few seconds (our laboratory data). Under laboratory conditions, flies may live three months and produce as many as 600 eggs.

REARING OF THE INSECT

For the maintenance of our stock, we used onions [3]. The adults fed on honey, water and soy hydrolysate. The latter proved as good as brewer's yeast for egg production and has the advantage of not drying rapidly and of keeping better. The food was changed once a week. Mixing the ingredients as indicated for the rearing of the olive-fly (10 parts soy hydrolysate : 40 parts honey : 50 parts water) reduced the egg production to a quarter.

To obtain quantitative data on egg production, the flies were offered a small slice of onion put on moist, loose silver sand in a petri dish as an oviposition site. The greater part of the eggs was laid in the sand. Eggs were picked from the slice and separated from the sand by stirring in water and filtering. We wanted to eliminate the onion slice and obtained partial success by using n-propyl disulphide as an oviposition stimulant [4]. Using 1.5 mm of this substance on 100 g sand, we obtained an egg production of 37% of the standard method. It is hoped that this can be improved.

In connection with the sterilization trials, we determined egg-hatchability. Satisfactory results were obtained from eggs put on moist filter paper in a petri dish covered with parafilm and put in a 25°C incubator for two days.

A start was made on a semi-artificial medium for the larvae. The mixture for fruit flies [5], modified by Christenson et al. [6], was used. Young (not neonate) larvae gave 40% pupation. As Feron et al. [7] claimed a better result, their medium was tried, but the yield of pupae dropped considerably. This led us to believe that the preparation method of the carrot powder was of importance because Feron worked with steamed carrot powder dried by atomization, whereas our carrot powder was dried in lumps and ground later. The preservatives, methyl p-hydroxybenzoate, potassium sorbate and HCl, of Finney were compared with the benzoic acid and HCl of Feron. The former gave the better results: 63 pupae against 33. Replacing a quarter of the carrot powder with wheat germ improved the medium as well: 63 pupae against 33. Brewer's yeast was as good as a mixture of this yeast and soy or yeast hydrolysate. These experiments were started with neonate larvae and a yield of 10% pupae was obtained. Pupae developed into normal flies. Experiments are under way to improve this medium which contains:

500 mg methyl- p-hydroxybenzoate	75 g carrot powder
500 mg potassium sorbate	25 g wheat germ
30 cm^3 $\underline{N}$ HCl	15 g brewer's yeast
500 cm^3 water	

STERILIZATION EXPERIMENTS WITH THE ONION-FLY

In mid-1965, preliminary experiments were carried out to establish the radiation dose required to induce dominant lethals in the male onion-fly.

Because the effect of radiation differs with age, individuals were collected daily during the 17-d-long pupal stage. The dosages were 10, 20 and 40 krad. Each dose was given to 1700 pupae divided in 17 groups of 100 pupae. The radiation was given by means of the electron generator of the Institute of the Application of Atomic Energy in Agriculture (I. T. A. L.) at Wageningen. Pupae failed to hatch after 20 and 40 krad. Most pupae irradiated in the last stage of their development with 10 krad hatched, but 65% of these were deformed.

Clearly, the required dose was less than 10 krad and should be applied during the last part of the pupal stage. In 1966 the experiment was repeated with dosages of 2 and 5 krad. The pupal stage of insects collected in the field lasted 17 d but the duration was shortened to 14 d if the larvae were raised under laboratory conditions with a temperature of 21°C ± 1°C and a relative humidity of 45% ± 5%. Thus, in our experiments, irradiation took place when the pupae were 11, 12 and 13 days old.

During a period of six weeks eggs were collected from cages containing five irradiated and five unirradiated flies. Each time the eggs were placed in an incubator at 25°C. After two days, 85-90% of the control eggs hatched. The experiment had four replicates. The results are presented in Fig. 1. The data given in the column on the right of the figure can be compared with the other columns for hatchability but not for oviposition.

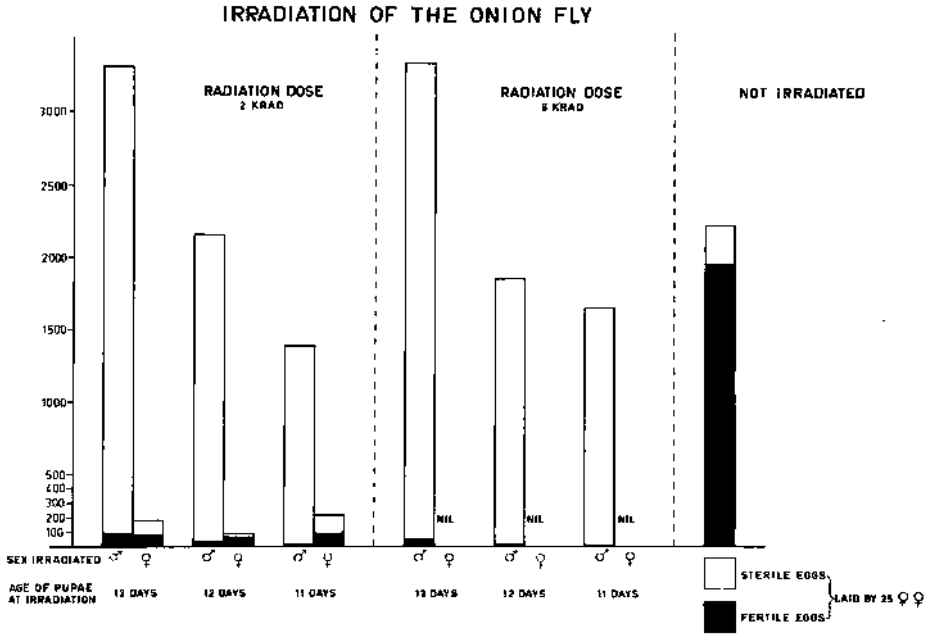

FIG. 1. Effects of γ-radiation of onion-fly pupae of different ages on the oviposition and egg-hatchability in reciprocal crosses between irradiated and non-irradiated adults.

Irradiation of the female pupae with 2 krad reduced the oviposition to 4.1% of the number of eggs laid by normal females mated to males irradiated as 13-d old pupae. Of these eggs 49.4% hatched. Treated with 5 krad the females laid no eggs.

Normal females mated to treated males laid a considerable number of eggs irrespective of the radiation dose received. The age at which the male pupae were treated, however, had a marked effect on the oviposition of the normal females mated to them. In both treatments the number of eggs deposited by the females decreased when the males had been treated earlier in the pupal stage. This is clear from the results of an analysis of variance of the data obtained. The calculated F-values are for: doses < 1; linear component of age 4.43 ($p =< 0.05$); quadratic component of age < 1; interaction of doses $\times$ age < 1.

The rate of fertility of these eggs ranges from 0.4 to 2.7%, compared with 88% in the control (see Table I).

TABLE I. PERCENTAGES OF HATCH OF THE EGGS FROM FEMALES MATED TO IRRADIATED MALES

Age of pupae at irradiation (d)	Dose applied:	
	2 krad	5 krad
11	0.9	0.4
12	1.6	1.2
13	2.7	1.6

Neither recovery of the fertility of the irradiated flies, nor effects on longevity were observed during the six weeks' post-irradiation.

To explain the different effects of irradiation on males and females, it was important to realize that at the time of treatment the reproductive organs of the male pupae were more developed than those of the female pupae. This was clear from the fact that, upon hatching from the pupa, the male had developed sperm and was ready to copulate; whereas the female had no developed ovaries and required a pre-oviposition period of 7-10 d before she oviposited. It was assumed that irradiation caused permanent damage to the spermatogonia and oogonia. Dominant lethals must have been induced in the gonial cells beyond a certain stage of development, in this case present in the males only and giving rise to sterile eggs after mating with normal females.

The relation between the number of eggs laid by females after mating with irradiated males and the age at which the male pupae were treated (see the decreasing number of eggs in Fig. 1) lead us to believe that the oviposition stimulus due to the male was affected more seriously if male pupae were irradiated at an earlier stage of development. Whether the explanation was due to the amount of sperm present at the time of irradiation, which is probably smaller in younger pupae, or to another factor, will be clarified in future research.

The same phenomenon was deduced from the data of Flint [8]. Normal females of the eye gnat (Hippelatus pusio), when mated to males

irradiated as adults of 24-36 h old, laid an average of 232 eggs; however, when mated to males irradiated as pupae two days before emergence they oviposited only 171 eggs. The difference between these two averages is significant ($p =< 0.01$) using the Wilcoxon test.

DISPERSAL STUDIES

Field experiments determined the flight range of the adults. Several attractants were used, of which beer and n-propyl disulphide proved the most effective. In future experiments, however, only n-propyl disulphide will be used since beer was not selective. The flies were labelled in the laboratory by mixing their food and water with ^{32}P in a concentration of 10 μCi/ml. After three days, the average radioactivity amounted to 4000 cpm counted with a thin-window G-M tube. Of the 11 000 flies released, only 24 specimens were captured because of unsatisfactory trapping methods. The maximum flight range observed so far was 160 m.

MATING BEHAVIOUR

In preliminary experiments, a virgin female fly was placed in a plastic cage with a young male. After 10 d, oviposition started. Over several days the eggs were collected and put into an incubator to hatch. Viability of the eggs was normal, indicating successful mating. The male was then replaced by a young male made radioactive by mixing its food and water with ^{32}P in a concentration of 2 μCi/ml. After one week of feeding, the radioactivity of 10 males ranged from 6000 to 32 000 cpm. Subsequently, eggs were collected and the radioactivity established by means of autoradiography. Polygamy of the female fly was proved by the fact that 74% of these eggs were radioactive. The females were anaesthetized, counted and found to be radioactive up to 2000 cpm.

Perhaps this method could be used to study the mating vigour between treated and normal males as well as the competition between sperm of sterilized and normal males.

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RECENT RADIATION WORK WITH THE OLIVE FLY AT THE "DEMOCRITUS" NUCLEAR RESEARCH CENTER, GREECE

J. A. TSITSIPIS AND M. E. TZANAKAKIS
"DEMOCRITUS" NUCLEAR RESEARCH CENTRE,
ATHENS, GREECE

Abstract

RECENT RADIATION WORK WITH THE OLIVE FLY AT THE "DEMOCRITUS" NUCLEAR RESEARCH CENTER, GREECE. A dose of 8000 rad of gamma radiation was given to olive-fly pupae, which developed into sterile adults. Sterile and control males showed similar mating competitiveness.

The purpose of the radiation work with the olive fly, Dacus oleae (Gmelin) described here was to establish gamma-ray doses which, when given to pupae, would permanently sterilize both sexes without affecting the mating ability and longevity of the males.

The proper radiation dose, reported elsewhere [1], was substantiated in earlier work by Italian colleagues [2, 3], according to whom 8000 to to 12 000 rad were satisfactory doses. In addition, 6000 rad were also shown to be adequate. During most of 1965 and 1966 only the 8000-rad dose was used. The insects were laboratory-reared on olives at 25± 1°C. They were irradiated as advanced pupae, i.e. upon the appearance of certain colour characteristics, which were found to be associated with a certain stage of pupal development [4].

QUESTION CONCERNING HOW AND TO WHAT EXTENT AFTER THE ADDITION OF STERILIZED MALES TO PREVIOUSLY INSEMINATED FEMALES THE NUMBER OF PROGENY ARE REDUCED

Thirty pairs of newly emerged normal flies reared as larvae on olives were divided equally between three cages and maintained together for 8 d. During this period most of the receptive females were assumed to have mated at least once. On the ninth day the normal males were removed from the cages and an equal number of radiation-sterilized males of the same age were added to each cage. In a similar set of three cages, the normal pairs were together for 15 d, after which time the normal males were replaced by sterile ones. The data (Fig. 1) show that the reduction of egg hatchability to low levels was slow and took place after the majority of the eggs had been laid. A 50% reduction in hatchability occurred after the fourth week in the early replacement group and after five weeks in the later one. Unfortunately, by mistake, no control data were recorded. We know, however, from many other control cages maintained during various periods that the hatchability of the eggs generally does not show a substantial drop from the beginning to the end of the oviposition period. Therefore, the reductions in egg hatch shown in Fig. 1.

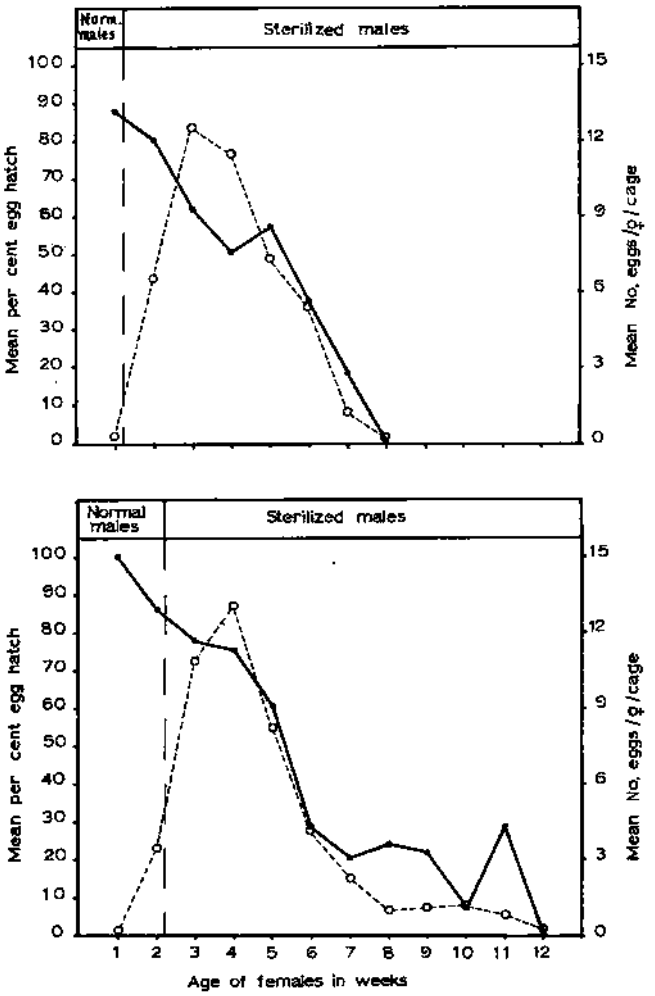

FIG. 1. Egg production (o- -o- -o) and hatchability (●——●——●) by 30 females of Dacus oleae, which were maintained at 25 ± 1° C and 59-62% RH, first with normal, then with radiation-sterilized males. (N = 3).

should be attributed to defective sperm from irradiated males. On the basis of this assumption it seems that after insemination by normal males, the replacement of such males by sterilized ones may not reduce the viability of the eggs to the desired degree, and that the reduction did not take place before some weeks had passed. Another experiment in which the females were first mated with irradiated males then with normal males gave similar results. Here the per cent of the egg hatch did not increase from practically zero to high levels until a few weeks after the replacement of irradiated males by normal ones.

The mating ability of radiation-sterilized males

This mating ability was tested in three limited experiments. In the first experiment, normal and radiation-sterilized males were maintained

TABLE I. MATING ABILITY OF RADIATION-STERILIZED VERSUS NORMAL MALES OF Dacus oleae, AS EXPRESSED BY THE PERCENTAGE OF INSEMINATED NORMAL FEMALES

(Sex ratio 1:1; 25± 1°C; 59-62% RH; November 1965)

	Number of ♀♀ held with:		Percent ♀♀ inseminated when held with:	
	Normal ♂♂	Irradiated ♂♂	Normal ♂♂	Irradiated ♂♂
5-d-old ♀♀ held for 3 d	30	30	83.3	100.0
with 3-d-old ♂♂	30	25	90.0	80.0
Newly emerged ♀♀ held for	30	30	86.7	83.3
8 d with ♂♂ of same age	30	29	56.7	86.2
Newly emerged ♀♀ held for	29	27	100.0	92.6
15 d with ♂♂ of same age	26	30	100.0	96.7

with an equal number of normal females, for the periods shown in Table I. There were two replicates for each combination. At specified dates, the females were dissected to determine insemination. In the two other experiments, which involved a small number of flies, the males were maintained for a given number of days with 2.5 to 5 times as many females (Table II).

Under the conditions of these experiments the results show that generally radiation-sterilized males are no less able than normal males to inseminate females.

Mating competitiveness of radiation-sterilized males

Radiation-sterilized males were mixed with normal males and females in the proportions shown in Table III and maintained together for the first 7 d of their life. On the eighth day the females were removed and placed in cages without males for the rest of their life. After the removal of the females, new virgin unirradiated females were added to the cages containing the males and were kept for 7 d. This was done to detect possible changes in mating competitiveness of the same males as they grew older. In interpreting the data of Table III, one should bear in mind that male mortality was not recorded and this may have changed the sterilized-to-normal male ratios. We concluded that irradiation of the male pupae with 8000 rad of gamma-rays generally did not make these males less competitive than normal males in the presence of normal females. Experiments along these lines are continuing.

TABLE II. MATING ABILITY OF RADIATION-STERILIZED (S) VERSUS NORMAL (N) MALES OF Dacus oleae, AS EXPRESSED BY THE INSEMINATION RATE

(Varying sex ratio and holding periods: 25 ± 1°C; 59-62% RH.)

Age of flies at start (d)		Number of flies per cage		Days together	Inseminated females per male
♂	♀	♂	♀		
4	7	S 4	N20	2	0.75
"	"	N 4	N20	"	0.25
"	"	S 3	N 9	"	1.00
"	"	N 3	N 9	"	0.67
4	4	S 4[a]	N16	7	2.33
"	"	N 2	N 9	"	2.00
"	"	S 4	S16	11	0.75
"	"	N 4	S16	"	1.50
"	"	N12	S30	"	1.08

[a] On the day both sexes could mate, only three sterilized males were alive in this cage.

ACKNOWLEDGEMENTS

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TABLE III. MATING COMPETITIVENESS OF RADIATION-STERILIZED AND NORMAL MALES, AS SHOWN BY THE PER CENT HATCH OF THE EGGS OF NORMAL FEMALES MAINTAINED FOR THE FIRST 7 d OF THEIR LIFE, WITH VARIOUS NUMBERS OF STERILIZED (S) AND NORMAL (N) MALES

(25 ± 1°C; April 1966)

No. adults per cage			Egg hatch during the first 8 weeks of the ♀ life (%)			Replications.	Total No. eggs laid
S ♂	N ♂	N ♀	Expected	Observed: Mean	Range		
			Males in the first week of their life				
5	5	5	50	33.0	16.2 - 52.9	3	1135
5	0	5	0	0	0 - 0.1	3	2426
0	5	5	100	90.5	80.9 - 100.0	3	758
8	2	5	20	72.8	71.3 - 74.3	2	615
2	8	5	80	77.9	54.3 - 94.0	3	1189
0	10	5	100	73.0	67.3 - 78.0	3	543
			Same males in the second week their life				
5	5	5	50	30.2	26.2 - 36.8	3	2365
5	0	5	0	0.3	0 - 1.3	3	928
0	5	5	100	77.6	-	1	407
8	2	5	20	1.7	-	1	467
2	8	5	80	83.6	75.0 - 90.4	3	1423
0	10	5	100	90.5	89.5 - 91.6	2	381

ARTIFICIAL REARING OF THE OLIVE FLY
Progress Report*

M. E. TZANAKAKIS, A. P. ECONOMOPOULOS AND J. A. TSITSIPIS
"DEMOCRITUS" NUCLEAR RESEARCH CENTER,
ATHENS, GREECE

Abstract

ARTIFICIAL REARING OF THE OLIVE FLY: PROGRESS REPORT. Larval and adult diets for mass rearing the olive fly are formulated and the costs are detailed.

INTRODUCTION

The authors initiated research on the rearing of the olive fruit-fly - Dacus oleae (Gmelin) at the Democritos Nuclear Research Center in early 1965. In the course of this work, efficient artificial larval diets were developed and limited research was carried out on aspects of ecology and behaviour relevant to the rearing of the olive fly.

The following describes briefly the most important results obtained as well as some recent work not yet published.

LARVAL DIETS

To our knowledge, 10 publications or reports have appeared on the rearing of larvae of the olive fruit-fly on artificial diets since the first paper on the subject by Moore [1]. Those prior to July 1965 have been touched upon in another paper [2].

The two oligidic diets (designated M and P in Table I), both proved satisfactory for the continuous rearing of Dacus oleae. As a result, for the past eight months our group has not relied on olives for the production of flies. To date, consecutive generations have been reared on diet M, and four on diet P. The latter medium contains no agar and, therefore, needs no boiling or heating during its preparation. It can also be diluted with water a fact which allows the collection of pupae by flotation.

The other diets described in Table I have not been tested for more than one generation but, because of their nutrient composition, and the quality and number of pupae produced on these media, we feel they may also prove satisfactory.

Steiner [3] stresses that larval diets intended for mass production should be tested and evaluated at levels near growing. He suggested densities of 10 or more eggs/ml food (private communication). To our knowledge such larval conditions have not been tried yet with D. oleae.

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TABLE I. COMPOSITION OF SOME LARVAL DIETS
(liquid components in ml, solid components in g).

	Proved satisfactory		Believed to be also satisfactory				
	M	P					
Tap water	66.0	55.0	55.0	55.0	55.0	55.0	47.0
Agar fine powder	2.0	-	0.5	2.5	-	-	-
Cellulose powder	-	26.0	20.0	-	25.0	25.0	24.0
Brewer's yeast	9.0	7.5	7.5	7.5	10.0	10.0	10.0
Soya hydrolysate	3.5	3.0	3.0	3.0	4.0	4.0	4.0
Roasted peanuts	6.0	6.0	5.0	5.0	6.0	8.0	8.0
Sucrose	2.4	2.0	2.0	2.0	3.0	3.0	3.0
Olive oil	2.4	2.0	2.0	2.0	2.0	3.0	3.0
Tween-80	0.8	0.75	0.75	0.75	1.0	1.0	1.0
Nipagin	0.2	0.2	0.15	0.2	0.2	0.2	0.2
Potassium sorbate	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Streptomycin sulphate	-	-	0.01	0.01	-	-	-
2N HCl	3.5	3.0	3.0	3.0	3.0	3.0	3.0
Per cent yield in pupae (1 egg/ml food)	41.0[a]	44.0[a]	64.4[b]	40.0[b]	56.0	38.0	47.0
Pupae/ml food	0.41	0.44	0.76	0.52	0.56	0.38	0.47
Mean pupal wt. in mg	5.24-6.42	5.33-5.46	5.5	6.0	5.89	5.68	5.23
Mean duration of larval stage (d)	9.6	9.9	11.8	11.1	11.1	11.5	11.1

[a] From Tzanakakis, M. E., and Economopoulos, A. P., unpublished rep., 18 August, 1966.
[b] Ref. [2].

The density we have been using as the standard was 1 egg/ml food. The change in the number of pupae obtained in diets M and P when the number of eggs per millilitre of food is more than 1. (See Table II).

Substitution of soy hydrolysate by non-hydrolysed casein

Hagen et al. [4], as well as all those who subsequently modified the Hagen larval diet, used enzymatically hydrolysed soya or casein as the main source of nitrogenous nutrients. Orphanidis et al. [5] also used a partially hydrolysed protein. Hagen et al. had poor results when they used unhydrolysed protein, and the flies did not reproduce. On the contrary Moore [1, 6] obtained adults which could reproduce when he used unhydrolysed casein, and Moore and Navon [7] completed up to three generations of D. oleae on a larval diet, which had peanut butter instead of casein.

Although no satisfactory continuous rearing of D. oleae has, to our knowledge, been yet achieved on diets containing only unhydrolysed pro-

TABLE II. PUPAL YIELD AT 25 ± 1°C, ON LARVAL DIETS M AND P AS A FUNCTION OF THE NUMBER OF EGGS PER ml FOOD [a]

Season	Larval diet	Eggs/ml of food	Mean yield in pupae		Container
			Per cent over eggs	Per ml of food	
Oct. 1965	M	1	42.0	0.42	50-ml cups
Oct. 1965	M	2	35.4	0.70	50-ml cups
Oct. 1965	M	4	27.5	1.10	50-ml cups
Oct. 1965	M	6	18.0	1.08	50-ml cups
June 1966	P	1	44.0	0.44	50-ml cups
June 1966	P	4	20.0	0.80	50-ml cups
June 1966	P	4.8	21.8	1.04	170-ml boxes

[a] From Tzanakakis, M. E., and Economopoulos, A. P., unpublished report 18. VIII. 1966

tein, we substituted the soy hydrolysate in larval diet P by the same quantity of casein. The mean yield in pupae, with 100 eggs/50 ml food, was 25.6% (range 21-30%) and the adult emergence 58.5%. The larval stage, at 25 ± 1°C, lasted 12-19 d i.e. longer than with soya hydrolysate, and the mean weight of the pupae was small (4.04 mg). Yet the adults started laying eggs on the 10th day and 4 d later were laying 0.9 eggs per living female per day. This egg production is roughly one fourth of that expected from females reared on one of the larval diets containing hydrolysed protein. However, these adults were produced under conditions which are considered to eliminate the symbiotic bacteria of the digestive tract which are present in flies developing in olives. Therefore, the direction Moore has followed, i.e. to use inexpensive non-hydrolysed proteins, may prove worthy of further investigation.

ADULT DIETS

A 1:4:5 mixture of yeast hydrolysate, sucrose and water, with or without choline [1, 4], is today used as the standard adult diet by most investigators.

It has been reported [4, 8] that the adult olive fly does not feed at all or very little on solid protein hydrolysates, so that such diets cannot be relied upon to achieve a substantial egg production. It was found in our laboratory that this is not always true. With olive flies from certain relatively dry areas of Greece, the total fecundity of the standard liquid diet group was 87% greater that that of the solid diet group. However, fecundity on solid food was by no means negligible, and some females oviposited more than the most fecund females of the liquid-diet group. A selection is currently being carried out in order to increase the percentage of the flies which respond to solid protein hydrolysates.

One of the main advantages of using a solid diet for adults is that it does not need frequent renewal [3]. For some other fruit-fly species

the solid diet does not need renewal during the 4-5 weeks that the flies are kept in the cages [9]. Moore [1] added new liquid food to D. oleae adults every second day and had a satisfactory fecundity. Hagen et al. [4] recommend the addition of fresh liquid food to the fly cages at least twice a day; this involves a lot of labour. To reduce adult feeding costs, Lopez [10] suggested a technique whereby liquid food would not solidify for 2-3 d. In the past we renewed liquid food daily and fecundity was satisfactory; however, we recently found that renewing this food twice a week was enough. Our data and Moore's suggest that further work is necessary to determine whether the standard liquid diet can be renewed even less frequently.

With flies reared as larvae in olives, modifying the standard diet by the addition of 2% egg yolk, 6% olive juice (ripe, cold stored) and the replacement of half the sucrose by honey, did not increase fecundity in two groups of 10 and 16 couples fed in this manner. However, flies of similar origin and fed the standard diet did not live as long, and laid their eggs within a shorter period than the enriched-diet flies. Thus, approximately twice as many eggs per day of the females' lives were laid by the standard-diet flies. In another case 15 females reared as larvae on diet P, and which as pupae weighed from 6.0 to 7.0 mg were given egg yolk or olive fruit juice in their diet. Preliminary results over the first six weeks at 25±1°C and 55-65% RH are given in Table III.

TABLE III. PRELIMINARY RESULTS WITH NO MODIFICATION OF STANDARD DIET

Diet	Mean No. eggs per initial female	Egg viability (%)
Standard diet	90.3	55.7
Standard diet + 7% egg yolk	303.5	72.6
Standard diet + 20% juice of green olives	116.0	71.3
Standard diet + 20% juice of ripe olives[a]	184.7	44.1

[a] Picked in February 1966 and cold-stored for three months before the juice was expressed.

Tentatively, the data in Table III suggest that egg yolk has a beneficial effect when added to the standard diet of flies reared as larvae on the artificial diet P and not in olives.

COST OF REARING

An estimate of the cost, in drachmae, of pupal production under our conditions with larval diet P is given below. The production depends on the upkeep of 200 adults in 50 cages, with an egg turnover of 2000/d of which 17% are used for the maintenance of the mother stock (see Table IV).

This cost is very high. It is approximately 280 times higher than the cost of mass-producing Dacus cucurbitae Coquillet in Hawaii [9].

TABLE IV. COST ESTIMATE OF PUPAL PRODUCTION WITH LARVAL DIET P

	Current cost in drachmae	
	At 1 egg per ml of food	At 4.8 eggs per ml of food
Eggs (Drach. 122.15/1000 eggs)	122.15	586.32
Larval food ingredients (1 kg)	40.00	40.00
Preparation of food (1 man-hour)	11.70	11.70
Placement of eggs on food	2.34	11.23
Collection and handling of pupae	5.85	5.85
TOTAL	182.04	655.10
Expected mean No. of pupae	440	1040
Cost per 1000 pupae in drachmae	414	630
Cost per 1000 pupae in US $	13.80	21.00

We believe that even at the present low rate of production and with the present facilities, the cost can be reduced by reducing the man-hours of preparing the food, and placing the eggs on it; however, a major reduction of the cost of rearing will result only when the method of obtaining eggs is simplified. Until the cost of obtaining eggs is greatly reduced, it will not pay to overload the larval diet with eggs.

POSSIBILITIES OF MASS REARING

Today we can rear the olive fruit-fly continuously on a number of larval diets at a rate of 10 000 - 15 000 flies per week. However, achieving an efficient mass production, i.e. of around half to a few million per week, is not just a matter of more space, facilities, staff, and funds. We need a fairly simple method whereby large numbers of flies will be produced in a medium-sized facility, and at a reasonable cost. To this end, some of the main points which, in our opinion, need investigation and improvement are as follows:

(1) Preparation and servicing of oviposition shells. (The present method is far too costly).

(2) Adult holding, servicing of cages, and number of adults per cage space.

(3) Increasing pupal yield per unit larval food. (e.g. by improving the larval diet so that it can nourish and maintain more larvae).

(4) Illumination for good egg production.

When these major points have been sufficiently improved, olive-fly mass-production at a reasonable cost will become possible.

Excessive crowding may result in smaller individuals, and we recently observed that light females reared as larvae on diet P had a lower egg production than heavier females of the same group. If smaller males are not substantially less efficient in mating than normal ones, one can envisage one larval density or diet for the production of mother flies, and

another more crowded or cheaper for the production of flies for sterilization and release.

ECOLOGICAL AND OTHER CONSIDERATIONS

Optimal temperatures for larval development

Constant temperatures of 20, 22.5, 25, 27.5, and 30°C under continuous darkness were recently tried from the second half of the embryonic stage to pupation (larval diet P). The results given in Table V indicate that while 30°C was unfavourable, the other temperatures all gave similar yields in pupae of acceptable weight. However, at 20°C it took almost twice as long as at 25°C for the larvae to reach pupation. This makes 20°C unsuitable for the efficient rearing of larvae.

TABLE V. EFFECT OF VARIOUS TEMPERATURES ON THE LARVAL AND PUPAL STAGES OF THE FLY (Medium P: 50 eggs/50 ml food: continuous darkness; 3 replications)

Temperatures (°C)	Mean pupal yield as a percent of: Eggs	Viable eggs	Mean pupal weight in mg	Duration of larval stage in days (Range)	Percent adult emergence
			29. VII. 66		
Constant					
20	40.0	58.3	5.57	18-19	-
22.5	45.0	57.4	5.66	11-13	-
25	28.8	38.4	5.81	9-12	-
27.5	30.7	40.4	5.31	8-13	-
30	5.3	9.1	3.62	11-13	-
			2. IX. 66		
Constant					
22.5	27.3	35.9	5.37	11-18	78.0
25	26.7	40.4	5.24	10-16	50.0
27.5	38.7	58.6	4.83	9-16	27.6
Alternating 18 hrs 20° / 6 hrs 25°	24.7	32.5	5.62	11-17	75.6
18 hrs 22.5 / 6 hrs 27.5	22.7	27.0	5.48	9-14	82.3
18 hrs 27.5 / 6 hrs 22.5	12.7	18.7	5.35	9-14	73.6

Alternating temperatures were also tried under continuous darkness (Table V). No data on adult fecundity, or on the pupal yield at a higher egg density are available for a final evaluation of the above temperatures. Furthermore, this performance of diet P in continuous darkness was below the average performance in other parts of our laboratory with 25+1°C and a daily photoperiod of 16 h. Therefore, further work is needed to show which temperature in the 21-27°C range is best for late embryonic and larval development on certain larval diets.

The necessity of a 1:1 sex ratio for good egg production

In two groups of 100 adults each, the one with a 1:5 ratio had a higher total egg production, and approximately the same egg viability as that with the 1:1 sex ratio.

The necessity for the continuous presence of males for good egg production

At a previous panel (1964) we reported that, at 25°C, females of D. oleae after separation from the males, laid fertile eggs for up to 15 weeks. Recent work in our laboratory showed that females which mated only once, or were maintained with males during only the first 10 d of their life, had as good an egg production as the females which were kept with males throughout their life. Egg viability was good in all cases.

A separation of the sexes of individuals reared on larval diet P, as well as on green olives was found possible. This was done in the advanced pupal stage by detecting the setal fringe on the sides of the male's abdomen, as described by Cunningham for Dacus dorsalis Hendel and D. cucurbitae [11]. Although this method does not seem practical for separating the sexes of D. oleae on a mass-production operation, it certainly can be useful for small-scale work.

Free amino acids in olive fruit before and after cold storage

Complete development, in cold-stored green olives, of the progeny (larvae) of adults which had received streptomycin in their diet [12, 13], indicated that the nutritive value or the assimilability of fruit for the larvae changed after cold storage and subsequent maintenance for 5 d at 25°C. Work by C. Savvas-Dimopoulou and E. Fytizas was recently carried out at our Center to detect rough differences in the free amino acids present in fruit picked in October 1965 and extracted before and after two months of storage in closed containers at 4-6°C.

Preliminary data with two-dimensional paper chromatography showed that: (1) At the concentrations tried, 12 or 14 free amino acids were found in non-stored fruit, and 17 or 18 in cold-stored fruit; (2) three amino acids were present in cold-stored fruit in quantities at least 18 times greater than in non-stored fruit. Rf values showed that two of these amino acids were leucine and isoleucine. Details of this work will soon be reported elsewhere.

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POTENTIAL FOR CONTROL OF THE SUGAR-CANE BORER THROUGH RADIO-INDUCED STERILITY

D.W. WALKER
PUERTO RICO NUCLEAR CENTER,
MAYAGUEZ, PUERTO RICO

Abstract

POTENTIAL FOR CONTROL OF THE SUGAR-CANE BORER THROUGH RADIO-INDUCED STERILITY. A project was undertaken to determine whether this species could be effectively sterilized by gamma irradiation and, if so, what stage in the life cycle is most suitable for producing the maximum sterility effect with minimum somatic damage and minimal behaviour change. In addition an attempt was made to develop a suitable method for culturing the insect in the laboratory under artificial conditions, and other biological studies were made that are relevant to a programme of eradication by population overflooding with sterile reproductives.

The sugar-cane borer, Diatraea saccharalis (Fab.) (Crambidae, Lepidoptera), is a serious pest of sugar cane in the new world, particularly between the latitudes of 35° N and 5° N. This includes the cane-producing areas of the southern United States of America, Mexico, Central America, the northern portion of South America, and the West Indies. This pest causes damage by tunnelling into the cane stem or terminal bud in the larval stage, thus causing weight loss as well as providing an entry point for plant diseases and secondary pests. In young cane plants larval feeding kills the terminal bud, called dead hearts. In addition, the larvae cause inversion of sucrose to glucose and fructose in mature cane stalks due to feeding and secondary bacterial growth.

The infestation rate in Puerto Rico was 4.4% in 1961 [1]. This equalled an annual loss of approximately three million dollars. Comparable figures for the continental United States of America were 13.0% joint infestation rate with an annual loss of six million dollars for 1958-63 [2].

Endrin emulsifiable was used for control in Puerto Rico when infestations exceeded 10%. In Puerto Rico the infestation was usually maintained below this level by parasites, predators and disease. The rate of parasitism of eggs by Trichogramma minutum Riley (Trichogrammidae, Hymenoptera) was generally high (12 to 40%), and the rate of larval parasitism by Lixophaga diatraea Townsend (Tachinidae, Diptera) was also high (10 to 30%). These are the two most important parasites of Puerto Rico. Parasitism rates vary considerably during the year being higher during the dry seasons. In addition, there was heavy predation on egg clusters by the ubiquitous ant solenopsis geminata (Fab.), as well as other ants. Little is known about the etiological agents of the naturally occurring disease of this insect in Puerto Rico. Van Dine [3] reported that infestations by the fungus Cordyceps barberi were common in Puerto Rico. We found that larval mortality in cane was approximately 10% due to disease during the wet season. The cumulative effect of these biological factors often accounts for more than 70% of a larval population reduction in the sugar-cane of Puerto Rico.

I. RADIATION-INDUCED STERILITY

The 2000-Ci ^{60}Co source in a pool was described by Linden and Teas [4] The dose-rate was 2000 rad/min.

A. Pupal Stage: The pupal stage of most insects is relatively immobile; thus, it can be easily handled. In many lepidoptera the larval stage leaves the feeding site and tunnels into the ground before pupating. This species pupates in the plant stem.

Hensley [5] irradiated pupae at various dosages. He found that young pupae were very radiation sensitive and that resistance increased with age. He reported that mortality of pupae 3 to 6 d old was high at 10 to 30 krad. We found a similar mortality; however, the tolerable dosage range was 9 krad for the oldest pupae (4-5 d), and younger pupae were killed at dosages above 9 krad (2-3 d). Pupal mortality was 90% at the 9-krad exposure for pupae less than 5 d old. The few pupae that emerged from these exposures had crumpled wings and were incapable of mating. Adults were slow to emerge after irradiation when given doses as low as 3 krad as pupae.

B. Larval Stage: Lavae were more sensitive to gamma radiation than pupae. First, second and third instars were killed by 3 krad. Fourth instar larvae given 6 krad developed into adults which failed to mate.

C. Adult Stage: The adult stage was the only stage that could be sterilized by gamma irradiation without producing high mortality.

Males were sterilized at a higher exposure than females, and females were less subject to somatic damage when exposed to doses higher than the sterilizing dose.

The reduction in egg hatchability was not apparent when the male parent received less than 12 krad. From 12 to 35 krad the relation between dose and egg hatch was exponential for radiated males; 30 krad produced 99.99% sterility in males. Adult males irradiated at 50 krad were capable of mating, but had a reduced lifespan.

Females were sterilized at 30 krad. Reduction in egg hatchability was not apparent when females received less than 8 krad. From 8 to 25 krad hatchability was exponential. Females given 120 krad mated; other females, given 240 krad did not mate and died within four hours after irradiation.

Preliminary studies showed that males and females sterilized by exposure to 35 krad were normal in their courtship behaviour, and that they competed with unirradiated adults.

D. Cytogenetic aspects: Virkki [6] found that the chromosome number was smaller in this species than in other lepidoptera: n equals 17. Comparison of n numbers of other lepidoptera listed by Spector [7]:

Bombyx mori	28
Lymantria dispar	31
Pieris brassicae	15
Telea polyphemus	30

Virkki found that the meiotic division in male pupae is asynaptic, which, although reported for other insects including lepidoptera and Coccidae, is not common. He discussed the possible types of radiation effects on developing sex cells of Diatraea in detail. Since the centromeres are diffuse, he speculated that the chromosome fragmentation by itself should not cause difficulties, providing that after gene duplication there is equal segregation of chromosomes, and chromosome fragments go to

each of the daughter cells. The combined effect of chromosome breaks with unequal segregation may provide the explanation of the actual damage observed as egg hatchability of the F_1 larvae. Whatever the effect is, the radiation resistance in these dividing mitotic or meiotic cells is very low; whereas, that of non-dividing somatic cells, such as those found in the adult stage, is very high.

A study of the cytogenetic changes in sex cells caused by radiation presents an interesting challenge in this species because of the unique features of diffuse centromeres and asynaptic meiosis.

II. BIONOMICS

A. Lifespan (Table I)

The female begins depositing eggs on the first evening after emergence. At first she only lays a few eggs, with few eggs per cluster, but with many clusters. After mating, she lays larger numbers of eggs with a greater number of eggs in each cluster. Some clusters have up to 250 eggs. The eggs hatch six days later. The average egg production was 350 per female [8] and extremes were 0 and 750 eggs per female.

The larvae emerging from the eggs were 2 to 3 mm long. In nature, these larvae feed on the blade of the leaf. The first larval instar is usually 3 d long. During the early part of the first instar the larva spins a thread and lowers itself to a new leaf where it feeds on the inside surface of the leaf axil where it usually passes the second instar which lasts about 5 d. The third instar, usually 5 to 7 d long, feeds on the stem and towards the end of this stage the larva begins to tunnel into the stem. The fourth instar spent in the tunnel formed in the stem is often the last larval instar. Some of the longer tunnels are 20 cm long. Before pupating the larva forms an exit hole covered by silk by eating through the stem to the outer epidermal layer. The pupal stage lasts 6 to 8 d. Adults emerge in the late afternoon.

B. Mating

Perez-Perez [9] in Louisiana found that mating takes place between 1.0 and 4.0 a.m., and that virgin females attract males by a pheromone. Traps baited with caged virgin females, or with a filter paper treated with an extract from the excised abdomens of virgin females were more attractive to males than u.v. light. He dissected 137 females captured by u.v. light trap from a Louisiana cane field: 68% mated once, 15% twice, 1% three times, and 16% had not mated.

Walker [10] described the courtship and reported that mating took place from 8.0 p.m. to 3.30 a.m. in Puerto Rico. In this laboratory colony there were individual females that mated four times. Some females in this colony normally mate once. About half the females mated when the sex ratio of males to females was one to one, with one or two pairs per 4-oz. plastic cup. Some males mated four times. In the laboratory males never mated more than once each 24 h, even when kept in constant dark.

It is not known how many times males mate in nature. Judging from the male lifespan, the limited period of darkness during the diurnal cycle,

TABLE I. LIFESPAN OF D. saccharalis IN THE LABORATORY

Stage	Lifespan of stadium (d)	Length (mm)	Activity at this stage	Main cause of death
Egg	4 to 9[a] (5)	0.6 to 0.8	Embryonic development	Mould
First larval	4 to 18 (5)	2 to 4	Surface feeding	Desiccation and starvation
Second larval	3 to 18 (6)	3 to 7	Tunnel forming	Bacteria
Third larval	3 to 18 (7)	6 to 18	Tunnel forming	Mould and bacteria
Fourth larval	5 to 21 (11)	15 to 29	Tunnel forming, stops feeding before pupating	Mould and bacteria starvation
Pupal	5 to 10 (6)	18 to 27	Quiescent in tunnel	Accidental cannibalism by tunnelling larval, desiccation, bacteria
Adult				
male	1 to 11 (3.5)	15 to 25	Rests during day, nuptial flight and mating at night	Old age, desiccation mould, starvation
female	1 to 13 (5.2)	18 to 29	Same as males, oviposits during day and night.	Old age, desiccation, mould, starvation
Total	25 to 107	2 to 29	----	----

[a] Range and mode in parentheses

and the female frequency of mating, it can be assumed that the average male mated once or twice and rarely more than four times.

C. Ecology

Holloway et al. [11] stated that the mature larva wintered in Louisiana. Some larvae diapaused in our laboratory culture in Puerto Rico. We collected fourth instar larvae from the field in an arrested state of development. Usually the sclerites of these larvae were lighter coloured than normal. In one case a larva remained in a dormant state for over five months, but we did not observe its entire lifespan.

Mating frequency and female fertility appeared related to the lunar cycle. There was considerable variation in the length of time required to complete larval development, which might be related to day-length, temperature, and/or rainfall. Western Puerto Rico has a wet season from July through September, a dry period from October to February, and a second wet season from March through June. During the remaining months the rains are not as frequent or as heavy as those during the wet season.

In nature the abundance of larvae and adults is extremely variable throughout the year but peak populations usually occur during July and August. In the United States of America there are three to four generations per year during May to October. In Puerto Rico the length of adult lifespan and time required for eggs to mature before hatching appeared to change slightly throughout the year.

III. ARTIFICIAL REARING METHODS

Four-ounce polyethylene sputum cups are used for adults [12]. Although convenient, they have the disadvantage of having an adverse effect on mating frequency. Because of the convenience of cleaning and handling, the cups are preferred to inflated polyethylene bags. Larvae were removed easily from cups but not from bags. High humidity was maintained in cups by moist cotton.

Larvae were transferred by a moistened artist's brush to the food immediately after hatching. Five to ten larvae were placed in each 32-ml glass vial. If the weather was humid, or if the food moulded, each larva was washed in a dilute solution of sodium hypochlorite (0.3%). Young larvae withstood exposure of three minutes to this treatment.

Often the food became mouldy before the larvae reached the pupal stage. When this happened the larvae were removed and were then transferred to fresh food. Pupae were removed from the food with a small stainless-steel spatula. We experimented with a vacuum aspirator for transferring larvae and for removing pupae from the vials, and hope to improve this method for transferring larvae since considerable labour was involved in this operation.

Laboratory environment

After adults were transferred to the cups, they were kept in the dark at 85° F and 70% relative humidity for at least 24 h. After the 24 h the

TABLE II. COMPARISON OF DEVELOPMENT ON DIFFERENT DIETS

	Diet number				
	1	2	3	4[a]	5[b]
Survival from egg to adult (%)	95	90	90	<30	<40
Average length of larval life span, (d)	25	22	23	>90	50
Range, larval life span	18-32	18-35	18-33	90->210	40-65
Average length of adult life span: (d) ♂	8	9	8	> 4	3.5
(d) ♀	10	10	10	> 5	5.2
Average number of eggs produced per female	600	500	550	360	550
Range, egg production	300-800	300-850	300-850		
Number of moults	5	5	5	7	5 or 6
(time between moults (d))	3-5	3-5	3-5	>20	5-6
Tunnelling	excellent	excellent	excellent	poor	good
Feeding	excellent	excellent	excellent	fair	excellent
Texture	good	good	good	liquifies	poor
Mould and bacterial inhibition	good	good	good	fair	very poor
Colour changes in medium during larval growth	slight	slight	slight	fair	---
pH stability	good	good	good	poor	poor
Odour changes	none	none	putrifies	slight	putrifies

[a] Diet by Miskimen [12]
[b] Rearing on cut sections of corn stalk.

cups were examined, and if a female started to lay large clusters of eggs it was assumed that she had mated. If the females did not mate, the cups were left in the dark for an additional 24 h. This environment was superior to the normal diurnal cycle for promoting mating.

After the females began to lay fertile eggs, the cups were kept in partial shade at outside temperature. Larvae were kept at 85° F and 70% humidity with 14 h light (florescent day-light type) and 10 h dark. After removal from food, the pupae were kept in plastic cups containing moistened cotton at room temperature (approximately 75° F and 30 to 40% relative humidity).

Diet

One hundred diets were tested by Walker et al. [13]. These diets were artificial, but not synthetic. The ingredients used in the diets were essentially preparations of the normally preferred host-plant material with the addition of supplementary nutrient material. Comparisons of development on five different diets are presented in Table II. Table III presents the three most promising diet formulae developed in our laboratory. Even on diet 1, there was considerable variation in larval lifespan: 19-84 d, mode 34 d.

It was possible to rear up to 10 larvae per vial: whereas, diets previously used supported one larva per vial. This provided considerable economy in time, yield, and convenience. A high degree of efficiency was possible with the improved diets, and it is believed that they will provide a suitable method for rearing sufficient quantities of adults for a small field test at this laboratory. Adequate space, personnel, and equipment to rear sufficient quantities for population overflooding of a large land mass are not available.

Handling methods

Larvae were transferred to 8-dram vials (25-mm diam. and 95 mm long) after hatching. These larvae readily entered the food, and pupated before the diet became mouldy. Pupae were recovered from the vials, after which the sex of each individual pupa was determined. Males were isolated from the females. Data were kept of the genetic lines with regrowth speed of development. This ensured the prevention of brother-sister matings. Upon ecdysis, the virgin males were irradiated and transferred to a small 6-oz. sputum cup. A virgin female was placed in the same cup. Each mating pair was given a separate code number and a record was kept of egg hatchability and longevity of each adult. After the female died her abdoment was dissected to determine the number of spermatophores in her bursa copulatrix.

The cycle was completed when the new eggs hatched and the F_1 larvae were placed on new food.

Containers for mass-rearing

Thirty different types of containers were tested with the object of developing a manageable method of mass rearing and the most suitable was the cotton-stoppered glass vial. This container was not satisfactory

TABLE III. THREE DIETS FOR LABORATORY REARING OF THE SUGAR-CANE BORER, Diatraea saccharalis (Fab.)

Ingredients	Diets 1	2	3
Corn fibre (blended) (g)	20.00	20.00	20.00
Carrot powder (g)	10.00	10.00	10.00
Agar (g)	2.00	2.00	2.00
Brewer's yeast (g)	15.00	15.00	10.00
Casein (enzymatically hydrolised) (g)	1.00	1.00	2.00
Sodium benzoate (g)	0.30	0.30	0.30
Methyl-p-hydroxybenzoate (g)	.05	.05	.05
Ascorbic acid (g)	.32	.32	.50
Distilled water (ml)	------	125.00	125.00
Corn filtrate (ml)	125.00	-----	-----
Hydrochloric acid (1.0 N) (ml)	----	10.00	10.00
pH	5.80	5.80	5.80

because a large amount of manual labour was required to transfer larvae, remove pupae and fill vials. Additional work is needed to improved handling and management procedures before a satisfactory mass-rearing method is realized.

Field test

Competition between sterile males and normal males, sterile females and normal females, and the effects of releasing combinations of both sexes in field cages are being studied. Corn was planted in the cages and when the plants were sufficiently large the adults were released. Populations were measured by harvesting the corn and counting the number of

larvae. This gave a relative index of the number of individuals in the F_1 generation. Similar tests were run in the laboratory to measure competition.

Field cages, 40 × 40 × 10 ft, were made of metal tubing and covered by 40-mesh saran screens. Eight cages were set in an isolated field at the University Farm in Mayaguez.

IV. ADDITIONAL FACTORS TO BE CONSIDERED

1. The long larval lifespan required that the food retain its nutritional value over a long period. The rate of food consumption by larvae increased with the growth of the larva, but the nutritional value and palatability decreased. Thus, we have the dilemma of needing the best from the food at the very time when the food is in its poorest condition. A solution to this problem would greatly improve our possibilities for mass rearing. Potential solutions to this problem are:

(a) The incorporation of chemicals in the food for inhibiting growth of bacteria and moulds. However, effective chemical preservatives are usually at least mildly toxic to larvae, and often the toxic effect is not easily recognized because the effect is chronic rather than lethal. Deliterious effects include: shorter adult lifespan, slower larval development, reduced vigour of larvae and pupae, high larval mortality, reduced pupal weight, reduced fertility, and reduction in mating activity.

(b) The frequent transferring of larvae to fresh food to avoid bacterial and mould growth, but the new food might develop these contaminants in transfer.

(c) The provision of a larval environment that can be frequently cleaned, and into which fresh food can be added. This method is being used in the culture of bollweevils by providing food in small pellets enclosed in wax.

2. The possiblity for the eradication of species by using female sex pheromone to attract males appears very good. Hensley et al. in Louisiana are attempting chemical analysis of this substance. If the chemical compound could be identified, it would be possible that the artificial sex pheromone could be synthesized.

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RECOMMENDATIONS

RECOMMENDATIONS

1. Since the mass production of the Mediterranean fruit-fly is feasible, a specific project could be most profitably investigated. Such a project should involve the use of the sterile-male technique for autocidal insect control so that island eradication and a quality control programme would be initiated. Such a programme is most strongly recommended, but support and encouragement of similar projects with other insects is also required.
2. The Joint FAO/IAEA Division should arrange for a formal mechanism for the annual collection and dissemination of information on the current state of mass-rearing projects throughout the world.
3. Investigations into the sterilization and release of blood-feeding insects and ticks should be intensified. This is needed to ensure that sterilized and arthropod insects do not produce harmful effects in the environment.
4. A Data-Book should be prepared to provide background information for future programmes. The following information should be given:
   (a) A Nutrition List that includes types of processed and non-processed food that have been used for rearing insects artificially.
   (b) Recommended factory design for insect mass production including quarantine and sanitation. Types of mechanical equipment and food-processing equipment as well as a list of inhibitors of undesirable microorganisms.
   (c) Important nutrients as well as symptoms of nutrient deficiencies: an annotated bibliography.
   (d) Methods of population reduction prior to a mass-release programme.
   (e) Quarantine principles in mass-release programmes.
   (f) Tagging methods and material.
   (g) Feeding behaviour, adult maintenance, mating conditions, diapause control and effects of non-rhythmic conditions.
   (h) Quality control criteria for best insect performance in the sterile-male technique.
   (i) A sequence of research for pest control by the sterile-male technique.

SEQUENCE OF RESEARCH IN APPLYING STERILE-MALE ERADICATION OR CONTROL

Experience with a number of species suggests that, in applying the sterile-male method against an insect pest, progress will be most rapid and economical if the work is divided into a sequence of three phases (see diagram in Fig. 1), each providing a basis for the next. The limiting factors most likely to prevent success should receive the most attention in early phases of the programme. Stated in simpler terms, the reasons for failure should be explored first, if these problems are solved, then the simpler problems should be attacked. This is particularly desirable as costs jump by a factor of about four or five times between Phases I and II, and by an amount decided by the size of the pest population, perhaps ten- to a hundredfold, between Phases II and III. The scale of the work also changes with each phase and corresponding changes in financing and organization will probably be necessary.

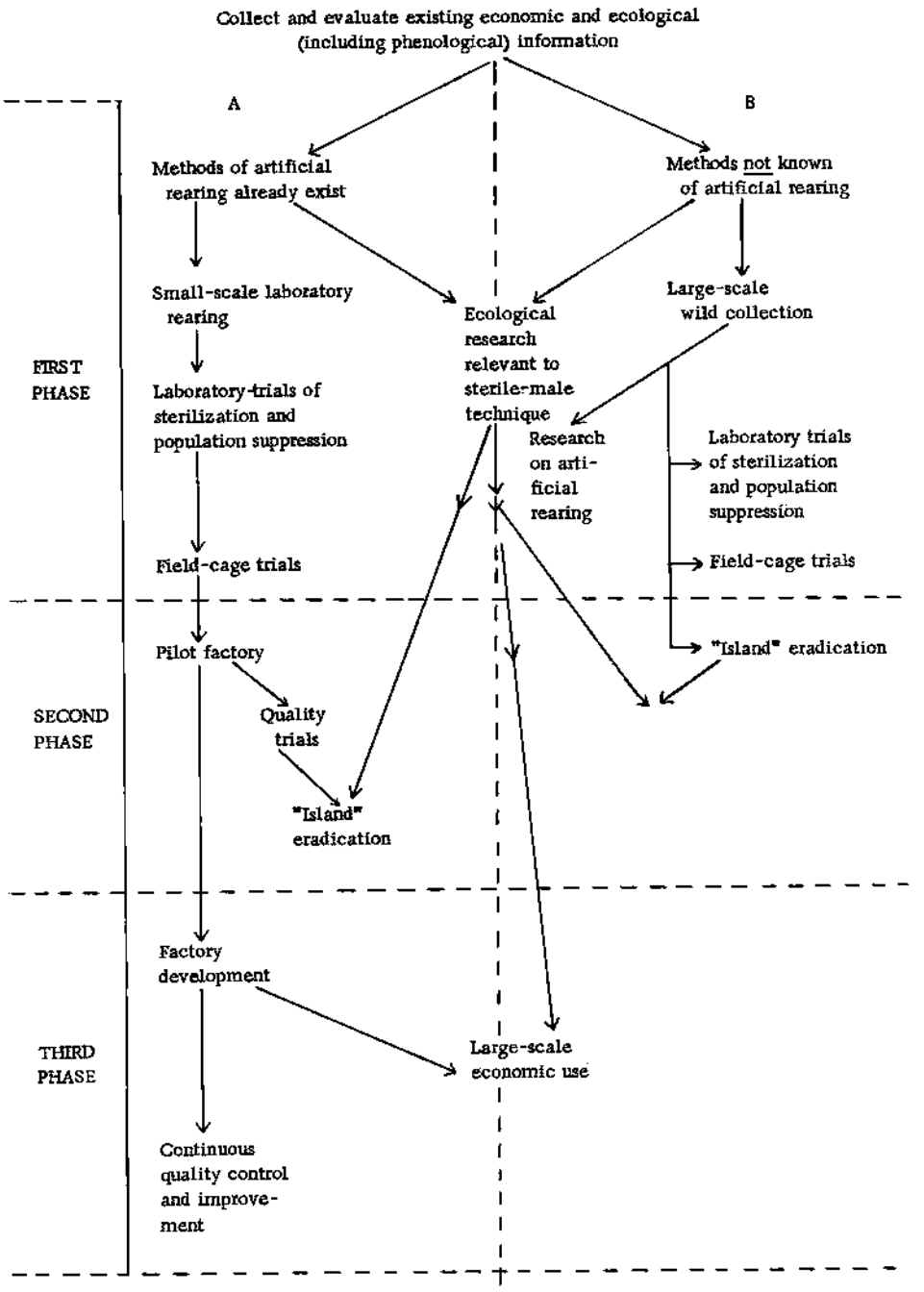

FIG. 1. Sequence of research (pest-control with sterile males)

FIRST PHASE

The sterile-male method is neither inexpensive nor simple to apply. Therefore, the pests against which it is applied should be carefully chosen, taking into account existing information on their ecology and the economic damage that they cause. Pests which are most suitable for control by sterile males have some or all of the following characteristics:

(a) Naturally occurring or artificially attainable small populations in some seasons or years
(b) Populations separated by natural (e.g. seas, mountains, deserts) or artificial (e.g. quarantine, sprayed areas) barriers from other populations of the same species
(c) A high rate of economic damage per adult individual of the pest-species
(d) The pest can be economically reared and sterilized without greatly reducing its life, rate of movement or mating competitiveness.

During the first phase the following ecological questions should be answered:

(a) What are the areas and patterns of distribution of the pest through the annual seasons over several years?
(b) How do the absolute sizes of pest populations fluctuate through the seasons and between years? This should give an estimate of the minimum sizes of populations and the rates at which such populations increase during the favourable part of the year.
(c) When and where do the adults mate?
(d) Do isolated populations exist or can populations be isolated artificially?

Because ecological research cannot be hurried, it should be carried out concurrently with the laboratory research during Phase I and not left until the field trials of Phase II are about to begin. If the pest has already been reared artificially, work can proceed as under A in Fig. 1. At this stage one should strive for reliable production and not to develop a mass-rearing method.

The insects produced by such a method should be enough for estimating the doses of radiation or chemosterilant, which confer sterility with the least reduction in sexual activity and length of life. The optimal treatments will be those that give the greatest reduction in birth-rate for a given sterile:fertile male ratio in laboratory and field-cages.

If there is no known method of artificial rearing, the first phase and part of the second may be carried out under B in Fig. 1. Here, as many questions as possible are answered using large numbers of the pest collected in the field. These may even be sufficient for the small-scale eradication experiment in Phase II. At the same time this material can be used for research on artificial rearing. In this way all stumbling-blocks are explored before entering the later and more expensive parts of the programme.

The development of a suitable larval diet may be a formidable obstacle, particularly if the pest is monophagous. No universal procedure can be laid down but successful diets often contain "carriers", which imitate the texture and water-content of the natural diet together with sources of macro- and micro-nutrients and substances that inhibit the growth of micro-organisms. One approach is to try to imitate the dietary composition of the natural food, another is to achieve satisfactory growth on minimal

chemically defined diets and to imitate this diet with less expensive materials. These approaches will be illustrated by individual contributions to this book.

SECOND PHASE

At this point the project usually grows in cost, complexity and staff. Suitably isolated island populations are chosen for treatment, the ecological research usually narrows to deal with these populations, but expands into developmental work on dispersing sterile males and evaluating their success.

At the same time laboratory rearing gives place to small-scale factory rearing. Economical diets and methods of handling that are appropriate to large numbers are developed and a regular production-schedule is established. Particularly in this second phase the quality of the product is evaluated in terms of its ultimate use by means of field-cage trials and trial-releases. Although economy should be sought by methods such as crowding and selecting for high productivity in the laboratory, this must form a compromise with the requirements of mating competitiveness, activity and longevity in the field.

To complete this phase one or more attempted field-eradications are carried out observing the rate of suppression of the treated populations and the ratio of sterile:fertile males in order to provide a guide to the requirements of the third stage. After eradication, methods of quarantine, warning-systems of traps and methods of suppressing incipient re-infestations should be tested.

THIRD PHASE

At this point the quality of the project changes. The biological research is over and the decision to use the method rests on economic arguments. Special organizations will probably need to be set up to build and operate the factory, to release the product, to maintain quarantine and to evaluate the results.

The total cost per effective sterile male released in the field is now the limiting factor. A balance must be struck between the costs of buildings, mechanical plant, operating costs, raw materials, labour and distribution. Biological efficiency may become less relevant in the factory; as Gast points out[1], if adults are cheaply maintained their offspring may be wastefully collected or reared provided this reduces the overall cost of production. The cost of distribution should also be taken into account, though it is often forgotten in discussions of the sterile-male method. There is little point in economizing in production costs if distribution-costs/sterile male are two to three times as high. Similarly there may be a point in, eg. doubling the effective life of sterile males at some slight increase in production-costs, if this means releasing only half as often and thereby cutting distribution costs.

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LIST OF PARTICIPANTS

BALDWIN, W. F.	Nuclear Research Center "Democritos" Aghia Paraskevi Attikis Athens Greece
BANEGAS, A. D.	Organismo Internacional Regional de Sanidad Agropecuaria Laboratorio San José Apartoado Postal 789, Tegucigalpa Honduras
BECK, S. D.	College of Agriculture University of Wisconsin Madison 6, Wisconsin United States of America
CHIPPENDALE, G. M.	Dept. of Biochemistry University of Leeds Yorks, United Kingdom
FRIEND, W. G.	Department of Zoology University of Toronto Toronto 5, Ontario Canada
GALUN, R.	Institute of Biological Research Ness-Ziona Israel
GAST, R. T.	Entomology Research Division USDA/ARS P. O. Box 5367 State College, Mississippi 39762 United States of America
HAISCH, A.	Bayer. Landesanstalt für Bodenkultur Pflanzenbau und Pflanzenschutz 8 Munich 38 Federal Republic of Germany
LEE, K. B.	Atomic Energy Research Institute Seoul Republic of Korea
MONRO, J.	Department of Research and Isotopes IAEA, Vienna Austria

NADEL, D.
Biological Control Institute
Citrus Marketing Board of Israel
27 Keren Kayemet Street
Rehovot
Israel

NAVON, A.
The Volcani Institute of Agricultural Research
P.O.B. 15, Rehovot
Israel

NOORDINK, J. Ph. W.
Instituut voor Plantenziektenkundig Onderzoek
Binnenhaven 12
Wageningen
Netherlands

TZANAKAKIS, M.E.
Nuclear Research Center "Democritos"
Aghia Paraskevi Attikis
Athens
Greece

WALKER, D.W.
Puerto Rico Nuclear Center
College Station
Mayaguez
Puerto Rico

SECRETARIAT

Scientific Secretaries:

CUTCOMP, L.K. — Joint IAEA/FAO Division of Atomic Energy in Food and Agriculture

MOORE, I. — Joint IAEA/FAO Division of Atomic Energy in Food and Agriculture

ERDMAN, H. — Joint IAEA/FAO Division of Atomic Energy in Food and Agriculture

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