



JOINT FAO/IAEA DIVISION OF ISOTOPE AND RADIATION
APPLICATIONS OF ATOMIC ENERGY
FOR FOOD AND AGRICULTURAL DEVELOPMENT



INTERNATIONAL ATOMIC ENERGY AGENCY –
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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INFORMATION CIRCULAR
ON
RADIATION TECHNIQUES AND THEIR
APPLICATION TO INSECT PESTS

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ANNOUNCEMENTS

1. A limited number of copies of the Book of Extended Synopses of the Symposium on the Sterile Insect Technique and the Use of Radiation in Genetic Insect Control are still available on a first-come, first-served basis. Please use the reply form attached to the outside of this Informaion Circular to request a copy.

2. We have received the notification that the International Fruit-Fly Symposium, organized jointly by the General Directorate for Agriculture of the Commission of the European Communities (CEC) and by the West Palaearctic Regional Section of the International Organization for Biological Control of noxious animals and plants (IOBC), will be held in Athens, Greece, on November 16-19, 1982. It is planned to publish the proceedings of the meeting. The Symposium will be organized in sections as follows:

- Biological and ecological aspects of fruit-fly populations.
- Relations fruit-flies and host plants.
- Biotechnical aspects of fruit-flies management.
- Genetic aspects.
- Integrated control programmes.
- Theoretical considerations in the evaluation and management of fruit-fly populations.

For further information contact the Chairman of the Symposium, Prof. R. Cavalloro, CEC Joint Research Centre, I-21020 Ispra (Varese), Italy, Tel: 0332-789736, Telex: 380042 EUR 1 or the Chairman of the Organizing Committee, Dr. A.P. Economopoulos, Demokritos Nuclear Research Centre, GR-Aghia Paraskevi (Attiki), Greece, Tel: 01-6511767, Telex: 21-6199.

3. We are happy to announce that a Regional Training Course and Seminar on the Use of the Sterile Insect Technique for the Control of Fruit Flies will be held at the Laboratory of the "Programa Moscamed", Metapa near Tapachula, Chiapas, Mexico, from 6 September to 8 October 1982. The training course is being organized under the joint auspices of the IAEA and the FAO in co-operation with the Government of the United Mexican States. All applications must be returned through official channels.

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PLEASE NOTE

The summaries of unpublished work often represent preliminary reports of investigations in progress and, therefore, such findings are subject to possible revision at a later date. The contents of this Information Circular should not be published or referred to in articles for publication without obtaining permission from the authors first.

1. The purpose of this study is to determine the effect of the new curriculum on the learning of the subject of Mathematics in the primary school.

2. The study was conducted in the primary school of the district of ...

3. The sample of the study consisted of ...

CHAPTER 2

2.1. Theoretical Framework

2.1.1. The concept of learning

2.1.2. The concept of curriculum

2.1.3. The concept of evaluation

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CHAPTER 3

3.1. The results of the study

3.2. The conclusions of the study

3.3. The recommendations of the study

A. Publication Policy of Information Circular

The policy of the Joint FAO/IAEA Division in publishing this Information Circular is to emphasize the results of recent research on the use of radiation and radioisotopes in entomology. Therefore, emphasis is placed on unpublished data. Please bear in mind that we cannot edit your contributions and that these are reproduced by a photographic process. Therefore, their appearance and content in the Circular will faithfully reflect your own care. Some of you have sent in reprints of published papers or long articles as contributions to the Information Circular. We include summaries of recently published data only. The length should be no more than one typewritten page when double-spaced (a form for this purpose is included in this copy of the Information Circular).

B. Professional Staff

Headquarters

G.C. LaBrecque	Head, Insect and Pest Control Section
E.D. Offori	Insect and Pest Control Section

BICOT (Biological Control of Tsetse by the SIT) - PO Box 76, Vom, Plateau State, Nigeria

R. Sarmiento	Project Director
M. Oladunmade	Project Co-Director
J. Greiling	Supervisor of Laboratory and Rearing Operations
T. Tenabe	Co-Supervisor of Laboratory and Rearing Operations
D. Bourn	Supervisor of Field Operations
(not filled)	Co-Supervisor of Field Operations

Seibersdorf Laboratory

R.E. Gingrich	Head, Entomology Laboratory
D.J. Nadel	Mediterranean Fruit Fly Investigations (retired 1 March 1982)
U. Feldmann	Tsetse Fly Investigations (FAO Associate Expert)
H-J. Hamann	Tsetse Fly Investigations
A. Van der Vloedt	Tsetse Fly Investigations
L. Gringorten	Isotope Investigations
M.E. Ruhm	Isotope Investigations
G. Kapatsa	Tsetse Fly Investigations (reported 18 December 1981)
P. Kaiser	Genetic Sexing Mechanisms

C. Entomology Laboratory

The IAEA has an international laboratory located at Seibersdorf, Austria, about 18 miles from Vienna. A part of this laboratory is devoted to the use of atomic energy in entomology.

The primary research objective of the entomological programme at the Agency's Seibersdorf Laboratory is to support and service the Joint FAO/IAEA Division's programme on insect control. This involves primarily the development of the Sterile Insect Technique (SIT) as a means of insect control or eradication. Because of the dependence of this technique on efficient, economical mass rearing of insects, much of the research at the laboratory involves rearing. Other major research areas include (1) methods of radiation sterilization for producing the best possible sterile insect (in terms of sexual competitiveness, longevity and quality), (2) handling techniques for large numbers of insects, and (3) field programme direction and/or supplying insects for field programmes. The laboratory's programmes are associated with existing field programmes and much of the research is concerned with the field problems that arise.

The general areas of research presently being pursued are:

- (1) Develop and improve mass rearing;
- (2) Improve radiation techniques;
- (3) Develop methodology for "fail-safe" radiation sterilization;
- (4) Develop laboratory methods for estimating "fitness" and sexual competitiveness of laboratory-reared, sterilized insects; study possible genetic changes taking place during colonization and mass rearing;
- (5) Develop methods of shipping insects as pupae, either before or after sterilization;
- (6) Develop release methods for large numbers of insects, both aerial and ground.

At the present time, the following species of insects are being reared at our laboratory:

- (1) Mediterranean fruit fly, Ceratitidis capitata (Wiedemann);
- (2) Tsetse fly, Glossina palpalis palpalis (Robineau-Desvoidy);
- (3) Brown planthopper, Nilaparvata lugens (Stal).

1. Medfly programme

- (a) Develop less expensive larval and adult diets with particular emphasis on locally available ingredients (non-imported) from various parts of the world.
- (b) Improve systems of rearing.
- (c) Develop laboratory and field quality control techniques.
- (d) Improve handling techniques for large numbers (100s of millions) of flies.
- (e) Improve methods of releasing sterile flies in the field from aircraft.
- (f) Provide emergency supplies of sterile medflies for field programmes.
- (g) Develop genetic and mechanical sexing mechanisms.

2. Tsetse fly programme

- (a) Improve rearing technology with reduced handling of flies.
- (b) Develop in vitro and in vivo feeding technology for mass rearing methods.
- (c) Develop methods for preserving blood (freeze-drying).
- (d) Use of blood additives for improving tsetse fly performance and offspring quality.
- (e) Develop synthetic diet for tsetse fly rearing.
- (f) Improve radiation sterilization techniques.
- (g) Develop methods of estimating fitness of laboratory-reared, sterilized flies; study possible genetic and/or behavioural changes taking place during colonization and mass rearing.
- (h) Conduct cross-breeding experiments with morphological mutants.
- (i) Develop laboratory and field quality control techniques.

3. Isotopes and Radiation in Integrated Pest Management programme

- (a) Predator-prey and host-parasite relationships.
- (b) Mass rearing.
- (c) Selectivity of insecticides.
- (d) Alternate hosts.
- (e) Adult population estimates for forecasting.
- (f) Pest dispersal.
- (g) Training.

D. Fellowships Awarded in Radiation Entomology (1979-1981)

Subject to quarantine regulations, availability of funds, etc., the laboratory can serve entomologists in developing countries planning or carrying out sterile insect projects. The laboratory also serves as a training institution for entomologists from developing countries. These trainees are handled under the Agency's fellowship programme and usually spend from one to six months at Seibersdorf.

Fellowships may be awarded for a period of several months to a maximum of twelve months. In certain exceptional cases, extensions of up to twelve additional months may be granted. Fellowships can be awarded as part of a comprehensive project or on an individual basis as a direct contribution to projects in the country's atomic energy programme, and provide opportunities for training there.

Applications for fellowships must be made to the Agency exclusively through official channels, and priority is given to requests associated with projects of direct benefit to individual Member States.

<u>Country and Name of Fellow</u>	<u>Host Country</u>	
BANGLADESH		
Muhammed Husain	USA	
Manjur A. Chowdhury	USA, Austria	
A.K.M. Qudrat-e-Khuda	USA, Austria	
EGYPT		
Istander M. Costandi	USA	
Soher M. Riad Souka	UK	
L.M. El-Gazzar	USA	
GHANA		
Delphina A. Adabie	USA	
Abdullah Essaka	Netherlands	
Rajainder Kumar	USA	
Henry Meier	USA	
Kojo Gyapia Montford	USA	
Jackson K. Akuamoah	Austria, UK, France	
GUATEMALA		
Zacarias Saenez Calderon	Spain, Israel, Austria	Scientific Visit
Jorge F. Benitez Coronada	Spain	Scientific Visit
Carlos A. Molina Urizar	Spain	Scientific Visit
Alvaro R. Klee Garcia	Spain	Scientific Visit
Salvador Sanchez Loarca	Spain	Scientific Visit
Sergio F. Morales Suarez	Spain, Israel, Austria	Scientific Visit
INDIA		
Amara Sambasiva Rao	USA	
Ohondiba H. Ranavavare	USA	
Gargadhar W. Rahalkar	Spain, Israel, Austria	Scientific Visit
Shankar Amonkar	USA	Scientific Visit

IRAQ		
Z.S.J. Al-Hakkak	USA	Scientific Visit
KENYA		
Philip A. Onyango	Austria	
MEXICO		
José Cisneros Luna	Spain, Austria	Scientific Visit
Raúl Arjona Granados	Spain, Austria	Scientific Visit
Cecilia Garcia Viesca	Spain	Scientific Visit
Servarda Lopez Benitez	Spain, Israel, Austria	Scientific Visit
J.L. Zavala Lopez	Austria	
NIGERIA		
Godis U. Okengwu	USA	
Emmanuel Ofodile	Austria	
Timothy Tanko	Austria	
Stephen O. Tenabe	Austria	
Moses Oladunmade	Austria	
PAKISTAN		
Sana Ullah Khan Khattak	USA	
SPAIN		
M.E. Riva Francos	Netherlands	
SRI LANKA		
D.M. Jayakody	India	
B.H. Rohitha	India	
SUDAN		
Said Mohamed Hussein	USA	
O.A.S. Mohammed	USA	
N. Sharaf El Din	USA	
THAILAND		
Manon Sutantawong	USA	
Ratana Poramarcom	USA	
Pravait Kaochoung	USA, Austria	
ZAMBIA		
Blackwell Kafwimbi	Austria, Nigeria	
Geoffrey G. Haangwanji	Austria	
Geoffrey M. Kapatsa	Austria	
S.M. Moobola	Austria	
K.H. Chisanga	Austria	

E. Technical Assistance Assignments, January - June 1982

<u>Name</u>	<u>Nationality</u>	<u>Location of Assignment</u>	<u>Major Responsibility</u>
G.C. LaBrecque	USA	Sri Lanka	To review the project on the control of the Red Palm Weevil (<u>Rhynchophorus ferrugineus</u>) using the SIT.
G.C. LaBrecque	USA	Egypt	To review status of Egyptian Medfly Project and to initiate administration and infrastructure

F. Recent Publications by the Joint FAO/IAEA Division

Symposia

STI/PUB/525 Isotope and Radiation Research on Animal Diseases and their Vectors. (1980) FAO/IAEA Symposium, May 1979, Vienna, Austria.

STI/PUB/595 Sterile Insect Technique and Radiation in Insect Control. Proceedings of a symposium, Neuherberg, 29 June - 3 July, 1981. Jointly Organized by IAEA and FAO.

Technical Reports Series

STI/DOC/10/61/2 Laboratory Training Manual on the Use of Isotopes and Radiation in Entomology - Second Edition. (1977).

J. Piedade-Guerreiro
J.I.C.U./Zoological Center
Integrated Pest Control Division
Lisbon, Portugal

J. Gomes da Silva
I.P.O.F.G. Radiotherapy (Dept. of Physics)
and Faculty of Medical Sciences (Dept. of
Biophysics)

X Ray High Energy (25 MeV) Radiation used
in order to Sterilize the Fruit Fly,
Ceratitidis capitata (Wied.)

1

ABSTRACT

The A.A. carried out a study about the utilization of X ray high energy (25 MeV) in order to sterilize the fruit fly Ceratitidis capitata Wied.

With this purpose they submitted several lots of 1, 3 and 7 days old pupa at different doses of this radiation between 10 and 110 Grays, with 20 Grays of interval.

The aim of this work is to establish the parameters of irradiation optimum to sterilize the males maintaining the behaviour pattern similar to that of untreated wild insects.

This is one approach to perfect and autocidal method in order to sustain this pest at a lower level of economic risk.

2

PRAVAIT KAOCHAUNG
CHETTACHAI BANDITSING
MANON SUTANTAWONG

Mating competitiveness of sterilized
and normal male Culex mosquitoes [Culex
pipiens quinquefasciatus (Say)]

OFFICE OF ATOMIC ENERGY FOR PEACE
BANGKOK 10900, THAILAND

Newly emerged male Culex mosquitoes, reared at $27 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ R.H., were irradiated with gamma radiation from Co^{60} source at 12 Kr. They were, then, put into $30 \times 30 \times 30$ cm. cages containing non-irradiated newly emerged male and female adults at ratio of 5:1:1 and 10:1:1 (irradiated ♂ : non-irradiated ♂ : non-irradiated ♀). Determination of hatchability was done for 16 continuous days.

The results of the experiment are that the hatchability in the 1:1 (non-irradiated ♂ : non-irradiated ♀), 5:1:1, 10:1:1 group were 80.67, 33.24 and 19.19 % respectively. That is by using sterilized male at the ratio of 10:1:1, decreased 58.39 % of number of mosquito eggs hatch.

MANON SUTANTAWONG
CHETTACHAI BANDITSING
PRAVAIT KAOCHAUNG

American Bollworm [Helicoverpa armigera
(Hubner)] Control by Gamma Irradiation

OFFICE OF ATOMIC ENERGY FOR PEACE
BANGKOK 10900, THAILAND

The experiment on Helicoverpa armigera control by gamma irradiation was conducted by irradiating 2-day-old egg, 13-day-old larva, 8-10-day-old pupa, and 1-day-old adult, reared at $26 \pm 1^\circ\text{C}$, 70-75 % R.H., 8-hour light duration, with 0-4, 0-12.5, 0-25, and 0-30 Krad respectively. The results are as follows : (1) in the males the sterility dose for egg, larval, pupal, and adult stage were 4(95 %), 5(100 %), 25(98.11 %), 30(99.52 %) Krad respectively while the females were 4(73.02 %), 5(76.45 %), 25(99.69 %) and 30(99.98 %) Krad respectively. (2) The mating frequency ratio between irradiated male : non-irradiated male from egg, larval, pupal and in adult stage were 0.39 : 1.38, 0.85 : 1.4, 0.4 : 1.45 and 0.85 : 1.1 respectively. The artificial media used in this experiment consisted of mungbeans, brewer's yeast, vitamin B-complex, and preservative.

LAWAN ANANTIKO
CHETTACHAI BANDITSING
CHITAPA KETAVAN*

Studies on the Life Cycle and Effects
of Gamma Radiation on the House Fly
(Musca domestica L.)

OFFICE OF ATOMIC ENERGY FOR PEACE
BANGKOK 10900, THAILAND

*DEPARTMENT OF ENTOMOLOGY
KASETSART UNIVERSITY, THAILAND

Studies on life cycle and the effects of gamma radiation on house fly (Musca domestica L.) fed with the mixtures of rice bran, fish mill, and water (1:1:1.5 by volume) and sugar solution soaked on cotton wick administered for adults only. They were reared at $30 \pm 3^{\circ}\text{C}$ and $75 \pm 6\%$ R.H. The results of this investigation were as follows:

- (1) The average female house fly oviposition was 122.68 eggs. The egg, larval (3 stages), pupal and male and female adult stages were 20.8 hours, 1.4, 2.3, 5.2, 5.3, 11.93 and 12.07 days respectively.
- (2) In general, there were reductions in number of eggs laid, egg weight and percentage hatchability from normal female mated with irradiated male house flies with gamma radiation obtained from Cs^{137} source at 500, 1,000, 2,000, 3,000, 4,000 and 5,000 rad. These reductions increased sequentially as the dosage increased. The number of eggs laid were no statistical difference but the egg weight obtained from irradiated flies were significantly difference from those non-irradiated ones. The percentage of hatchability was also found significantly difference between the irradiated and non-irradiated flies except in only 500 rad group. Percentage of the adult emergence in the third generation, descendants of the first 500 rad male generation, was 54.
- (3) The sterility dose for male house fly was at 3,000 rad.

The effects of gamma radiation on the sperm productions was found significantly difference between non-irradiated and irradiated flies except in the 500 rad males. However, irradiation did not alter the morphology of the spermatheca and the release of the sperms.

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Division of Entomology
CSIRO Screwworm Fly Unit
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Sterilization of the screwworm fly,
Chrysomya bezziana (Diptera: Calliphoridae),
by gamma radiation

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The effect of gamma radiation on the screwworm fly, Chrysomya bezziana Villeneuve, was studied to determine the optimum dose and stage for sterilization. Laboratory cultured flies were exposed in a gamma irradiator with a cesium-137 source. Treated flies were compared with untreated controls for adult emergence, survival, insemination and fertility. Puparia treated with 5 krad at 1-2 days before emergence were rendered infertile but not otherwise adversely affected. When flies were irradiated earlier in pupal development, adverse effects were apparent. When late puparia were irradiated with 1-8 krad, adult emergence, survival and rate of insemination were similar to controls. Complete sterility of males and females was achieved with 4 krad, although as low as 1.5 krad caused 90% sterility of both sexes. There was no larval survival from eggs when both parents were treated with 1 or more krad but larval survival was recorded from eggs laid by untreated females mated with 1-2.5 krad treated males and from eggs laid by 1-1.5 krad treated females mated with untreated males. The minimum safe dose for sterilizing C. bezziana was 3.0-4.0 krad, depending on the eradication strategy employed.

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RATANA PORAMARCOM
CHETTACHAI BANDITSING
NATHAPOL WANLEELAG*
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BANGKOK 10900, THAILAND

Studies on Gamma Irradiation in the
Control of Patanga succincta (L.)
and Some Effects on Biology and
Reproduction

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Patanga succincta (L.) nymphs started to feed after hatching 3-5 hours. Each molting period took about 25-35 minutes except for the last molt which took 30-45 minutes. Cannibalism was found in dense population of both nymphs and adults, in the condition of their food was not sufficient or they were reared with only rice bran.

Patanga succincta (L.) reared with fresh plants (corn seedling and banana leaves) and rice bran reached the adult stage in 38.74 ± 2.24 days and the average body weights of males and females were 1.58 and 2.26 gm but was 39.68 ± 1.74 days and 1.41 and 1.94 gm when they were reared with only fresh plants. Furthermore, there was no significant difference in body weights among these insects when they were reared with fresh plants and artificial media consisting of powder milk, Brewer's yeast and vitamin B complex.

The LD_{50} for 28 day-old eggs, 9 day-old-nymphs at 16 and 18 days after irradiation were 1,950 rad, and 3,100 and 2,200 rad respectively. In addition, the LD_{50} for 75-day-old and 12-day-old adults at 16 days after irradiation were 3,300 and 6,600 rad, but 1,600 and 5,100 rad at 18 days after irradiation.

Furthermore, the LD_{50} at 18 days after irradiation for 41-day-old male and female nymphs were 1,100 and 1,700 rad but in 12-day-old male and female adults were 2,950 and 6,150 rad.

The sterility dose for the male and female adult was 2,000 rad. The sterility at 700, 500 and 300 rad were 95, 92 and 36 % respectively.

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Sodium fluoride effects on fecundity
and longevity of Tyrophagus putrescentiae (Schränk) (Acarida: Acaridae)

Pol. Pismo Entomol., in press

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The research reported here was to determine (a) whether sodium fluoride can produce partial or complete sterility in Tyrophagus putrescentiae (Schränk) adults, and (b) if low dosages of the compound can stimulate increased egg production.

Sodium fluoride, NaF, is toxic to T. putrescentiae at doses higher than 0.5%. At these concentration it caused a high mortality of mites even during the first week of exposure. The males looked to be slightly more resistant than females. Higher concentrations of the salt added to wheat germ the lower the longevity and fecundity of the mites. At a 2% concentration of NaF in the diet, about 58% of the females failed to produce eggs. However, only 4-8% females ceased laying of eggs at the lower doses. Thus, it appears that an intoxication with sodium fluoride results in a suppression of fecundity rather than acting as a chemosterilant. The eggs produced by treated females were of normal viability.

In order to determine if NaF is a chemosterilant for T. putrescentiae, the females and males were fed diets with the salt for the first 3, 7, and 14 days after eclosion and then returned to an untreated diet. After an NaF-diet females feeding on untreated food produced fewer eggs than controls, i. e. mites never fed NaF. Viability of these eggs was not affected. The greatest reduction in fecundity was found in tests with 2.0% NaF. The few females which survived 7-14 day treatments at that level had greatly reduced life span and produced just a few eggs. The longer the exposure time to sodium fluoride and/or the higher concentration of the salt in diets the lower the level of recovery of reproductive abilities and the higher the mortality of mites. Thus, the doses of NaF which are required to effect complete sterility in T. putrescentiae and concentrations which cause a high mortality are very close, i. e. NaF is not good chemosterilant for T. putrescentiae adults.

Adults of T. putrescentiae were fed wheat germ for 3-14 days, and then they were exposed to a diet with 0.25%, 0.5%, 1.0%, or 2.0% NaF. In all cases, after the diets were changed the females laid fewer eggs than the controls. The higher the concentration of the salt in diet the lower the production of eggs. The period of feeding on an untreated food, however, did not affect the susceptibility of mites to NaF-diets. Mites of all combinations produced just a few eggs and soon died when they were transferred from wheat germ to a diet with 2.0% NaF.

Sodium fluoride is regarded as a strong poison for a number of insect pests. It causes a high level of mortality in cockroaches and the confused flour beetle, Tribolium confusum Duv., when they ingest or contact the compound. NaF is also toxic for T. putrescentiae. However, it exhibits rather mild acaricidal effects, although its toxicity is somewhat higher than the toxicity of boric acid and sodium borate. The lethal concentrations of NaF for T. putrescentiae are higher than 0.5%.

Suppression of egg-laying caused in T. putrescentiae females by sodium fluoride is not permanent, and it is recovered to some extent after a return to an untreated diet. Therefore, NaF seems to be a weak chemosterilant for T. putrescentiae like boric acid or sodium borate.

After the change of diets, females of T. putrescentiae given wheat germ produced fewer eggs than controls in all combinations. Thus, there was no increased fecundity in T. putrescentiae following low dosages of sodium fluoride. Johansson and Johansson (1972, J. Econ. Entomol., 65:356-357), however, reported stimulatory effects in some cases following exposure of the confused flour beetle to sublethal doses varying from 0.001% to 1.0% of sodium fluoride.

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Boric acid and sodium borate effects
on fecundity and longevity of Tyrophagus putrescentiae (Schränk)
(Acarida: Acaridae)

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Roczn. Nauk Roln., in press

The longevity of female and male mites was significantly affected by boric acid added to food at concentrations higher than 0.5%. These levels were lethal for Tyrophagus putrescentiae (Schränk). The higher concentration of the compound in diet the shorter the longevity and the lower the fecundity of the mites. The 0.5% level of boric acid in a diet had no significant effects on male or female longevity, but it lowered the production of eggs by 59.1%. However, females which were fed a diet with 2.0% boric acid produced no eggs and died during the first 1-2 weeks. It indicates that the concentrations of H_3BO_3 which are required to effect complete infecundity and concentrations which result in death are rather close.

Boric acid at a 0.5% concentration had a slight effect on egg mortality. In the other tests, however, it was impossible to obtain a proper number of eggs for accurate study on their viability.

Mites were given diets with 0.0-2.0% boric acid for 3, 7, and 14 days, then they were returned to an untreated diet. These tests were set up in order to determine if boric acid could induce permanent or temporary sterility in T. putrescentiae. It was found that a partial infecundity was recovered to some extent by mites exposed for 3 or 7 days to diets with 0.5-1.0% boric acid. Longer exposure, however, suppressed significantly egg-laying in T. putrescentiae. Females fed diets with 0.5% and 1.0% boric acid for 2 weeks laid 22.2% and 75.4% fewer eggs, respectively, when they returned to an untreated food.

Because of a high mortality, females produced no eggs in tests with 2.0% boric acid. Also, those mites that survived the treatment did not recover their reproductive abilities on an untreated food. In the other tests, longevity of both males and females did not differ significantly from the control. These results show that sterility induced in T. putrescentiae by boric acid was incomplete and only temporary. Moreover, they agree with the previous conclusion that doses of boric acid which are required to effect complete sterility in T. putrescentiae and concentration which cause a high mortality are close.

To determine if boric acid can suppress the egg-laying in females which already are producing the eggs, the mite pairs were fed wheat germ for 3-14 days, and then they were given diets with 0.0-2.0% boric acid. The results show that after the diets were changed the females laid fewer eggs than the controls. The higher the concentration of boric acid added to food the lower the number of eggs produced. However, the period of feeding on untreated food had no effect on susceptibility of mites to boric acid diets. Fecundity of females fed on an untreated food for 3 and 14 days, and then given a diet with 0.5% H_3BO_3 was lowered by 54.5% and 40.9%, respectively.

When an untreated food was replaced by a diet containing 2.0% boric acid, the mites produced a few eggs and soon died. In the other tests, the longevity of adults was not affected.

Tests with sodium borate, $Na_2B_4O_7 \cdot 10H_2O$, showed that 0.5-2.0% levels of the compound in the diet lowered the fecundity of mites by 65-99%. The higher the concentration of the salt in the medium the lower the fecundity of mites. The longevity of both males and females was not affected by concentration up to 1.0%. The adults fed a diet containing 2.0% sodium borate lived much shorter than the controls. The viability of eggs was not affected by the compound. In general, sodium borate was less toxic to the adults of T. putrescentiae than boric acid.

A suppression of fecundity caused by sodium borate was partially annihilated after the mites returned to an untreated medium. However, at the highest doses of the salt the fecundity was still lowered by ca. 70%. After the diets were changed the viability of eggs laid by T. putrescentiae females was similar to the control as it varied from 94 to 96%. In these tests, the longevity of adults was similar to the control, even in a case when the males and females were fed for 1 week on a diet containing 2.0% sodium borate and then given an untreated food. Therefore, sodium borate is less toxic to T. putrescentiae than boric acid.

The effects of electromagnetic field on living organisms have been studied for many decades. Lately the interest of research workers has extended also to nonionizing radiation - on RF and microwaves. In connection with increasingly broader use of electricity and electromagnetic field there is a danger that the electromagnetic field will become one of the factors which deteriorate the life environment.

In the experiments during which insects were exposed to microwaves ($f = 2375$ MHz) we have found out the following:

1) The electromagnetic field of 2375 MHz and power density ranging from 0,1 to 40 W/cm² induces irritation in insects. In species falling into cataleptic state when disturbed, the time of akinesis is prolonged if the field's power density is low. A low power density impaired the motility of all species tested. High power densities, different for individual species, enhanced the motility of the exposed insects which tried to escape from the field. A prolonged stay of the insects in the field led at first to the loss of coordination of movements and then to death.

2) The insects leaving the field move in most cases along the gradient of the field's power density. This ability is impaired after antennectomy, and is entirely lacking in some larvae.

3) Autotomy of the hind and middle pairs of legs was observed in adult stick insect, *Carausius morosus* at a low power density of the field.

4) The mechanism of orientated escape of insects from the maximum power density of the field may be explained by induction of electric current in antennae and its subsequent irritating effects on non-specific receptors or narrowed places. Insects can be affected by the electromagnetic field in the same way through their legs.

5) The following thermal effects of microwaves become apparent at higher power densities of the field:

a) Morphological changes in the next developmental stages in all cases of irradiation of larvae, praepupae and pupae. Morphological abnormalities probably arise due to interference with the histolysis of larval or pupal structures and destructions of imaginal discs and primordial tissues of new structures. In some cases, developmental changes are brought about by selective damage to or destruction of already determined tissues or layers of the organism. Damage to the endocrine system cannot be excluded.

b) Damage to reproductive organs, apparent in reduced fertility of irradiated individuals. The changes observed were caused by damaging of external genitalia and by disturbing of the water economy of the organism. We suppose, however, that damaging of sexual cells is possible even though we have not observed it yet.

c) Death by overheating at higher power densities or prolonged exposure. The lethal effect of the field depends, in order of importance on:

- mass, or "effective size" of the experimental organism; the dependence can be mathematically expressed;
- temperature, humidity and thermal conductivity of the environment in which irradiation occurs;
- orientation of the longitudinal axis of the experimental animal regarding the vectors of the electromagnetic field;
- physiological state of organism depending on the developmental stage and its age;
- on other properties of organism, determining its resistance to unfavourable life conditions.

d) All factors given under c) should be considered equal in evaluating the effects of microwaves on insects.

e) Extermination of pest insects in stores with microwaves is possible, but the cost - benefit ratio poses a problem.

6) Mutagenetic effects of electromagnetic field were not found, they are supposed to occur with chronic exposure and higher frequencies.

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MED-FLY CELL LINE: RESPONSE TO IRRADIATIONS WITH FAST NEUTRONS AND GAMMA-RAYS

in Revue Canadienne de Biologie "in the press"

10

Abstract

It has been observed that biological systems have different sensitivities to various radiation qualities. Moreover, the relative response depends on the dose level of the applied radiation.

In this context the radiosensitivity of Med-fly, Ceratitis capitata Wied., cells, to gamma-rays and neutrons, has been tested by observing cell population growth and mortality.

Fast neutron doses ranged between 1.5 and 25 rad, a region of particular interest in radiation protection studies; the ⁶⁰Co gamma-ray dose values were between 300 and 5000 rad.

The experimental results show a much higher radiosensitivity of these cells to fast neutrons than to gamma-rays, and indicate a dependence of the RBE values on the neutron-dose levels. Furthermore, there was a certain dependence on radiation quality for the morphological damage.

Dose-effect relationships are analyzed for both neutrons and gamma-rays and the results are discussed.

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Study of DNA polymerases α , β and γ
in Ceratitis capitata Wied. cells: Effect
of ionizing radiation on enzyme activity

11

ABSTRACT

Three different DNA polymerases have been described in eukaryotic cells: α , β and γ . They have distinctive properties and functions: α and β -polymerases are localized in the nucleus and are involved in the replication and repair of DNA, respectively; γ -polymerase is present both in nuclei and in mitochondria and is responsible for mitochondrial DNA replication.

A phylogenetic study has shown that α and γ -polymerases are present in all phyla so far examined (from animals to protozoa), whereas β -polymerases have not yet been found in insects and in unicellular organisms.

We have analyzed the three DNA polymerase activities in the cells of the Mediterranean fruit fly, Ceratitis capitata Wied., cultured "in vitro" and we have found, to our surprise, high levels of β -polymerase.

We have also followed the levels of these enzymes at different times during embryogenesis: α -polymerase increases of an early stage of egg development while β and γ tend to increase at late times.

The β -polymerase activity is resistant to N-Ethyl Maleimide and is much evident in crude extract, but is easily lost upon fractionation of sedimentation gradients. The activity is restored after addition of extract, thus suggesting the necessity of an activating factor.

DNA polymerases α and γ present sedimentation values close to those of the vertebrates (8 and 9 S, respectively); on the contrary the value for β -polymerase is much higher (about 7 S).

We have studied the effect of different doses of ionizing radiations on the three DNA polymerases, and we have observed that the levels of α and β -polymerases do not show significant variations, while the level of γ -polymerase increases of several folds with irradiation dose.

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RADIOSTERILIZATION OF MALE AND FEMALE
Sitophilus oryzae (L.)

12

SUMMARY

The sterilization curve for Sitophilus oryzae has been determined exactly by using 2-day old adult virgins exposed to gamma radiation. Following radiation treatments, appropriate crosses involving irradiated and untreated insects were made.

Females show a greater radiosensitivity than the males: doses of 95% and 100% sterility have been found to be 4 and 8 krad respectively for females, and 7 and more than 11 krad for males.

Moreover, starting from 6 krad there is a reduction in the lifespan of the irradiated adults.

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WHOLESOMENESS STUDIES WITH A FULL
DIET OF IRRADIATED DATES USING THE
INSECT Ephestia cautella (Walker) :
II. EFFECT OF HIGH DOSES OF GAMMA
RADIATION .

13

It has been previously reported that the dibis (date syrup) yield from fully ripen date fruits was highly increased when irradiated with high doses (275 - 2000 krad) of gamma radiation. Therefore, the present study was conducted to investigate the effect of dates treated with different high doses of gamma radiation and fed as a whole diet on the biology of the fig moth Ephestia cautella to provide more information on the safety of irradiated dates with low doses (up to 100 krad) for the purpose of insect disinfection as well as for dibis production in the future by using high doses of gamma radiation .

The following results could be concluded when the data obtained were statistically analysed :-

- 1- When incubating 200 E. cautella eggs for 30 days on date fruits (both Zahdi and Sayer varieties, separately) irradiated with either 625, 1250, 2500, or 5000 krad of gamma radiation the average numbers of larvae and pupae developed were not different from the control .
- 2- No significant effect could be detected on the percentages of emerged adults or their sex-ratio when developed during 60 days of incubating the eggs on irradiated dates of both varieties .
- 3- Although the average percentages of malformed moths showed somewhat a consistent increase as the radiation dose increased, the differences were not statistically significant .
- 4- The average number of eggs per female and the average percentage of egg hatch were similar in different crosses of either adults developed on irradiated or unirradiated dates, or between them and adults of the control .
- 5- Rearing insects on irradiated date fruits with such doses of gamma radiation has no significant effect on the fecundity and fertility of their F_1 progeny .
- 6- These extremely high doses of gamma radiation caused significant increase in the softness of date fruits of both varieties .
- 7- Significant delay of development of pupae caused by the highest doses of 2500 and 5000 krad. This could be attributed to the increased stickiness of these fruits as previously predicted and not for toxicological events in irradiated dates .

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WHOLESOMENESS STUDIES WITH A FULL
DIET OF IRRADIATED DATES USING THE
INSECT Ephestia cautella (Walker) :

III. EFFECT OF LONG TERM FEEDING .

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Our previous studies have shown that low doses of gamma radiation (Cobalt-60 source) are enough to kill all developmental stages of insects species that are economically important to date industry in Iraq . However, wholesomeness studies are needed in order to evaluate the safety of irradiated dates for consumption purposes . Therefore, in the present study the fig moth, Ephestia cautella , used as a test organism, were reared for five generations on a 100% diet of date fruits treated with 100 or 200 krad of gamma radiation . Each generation was checked for development from egg to adult stage . Furthermore, the developed adults at each dose were paired (20 pairs from each treatment), and female fecundity and mating frequency were compared . The average percentage of egg hatchability were also investigated for all the five generations as indicator for genetical effects .

The following results could be drawn when data at each generation were statistically analysed :-

- 1- No significant changes were observed in the average percentages of last instar larvae, pupae and adults produced from 400 eggs reared on irradiated dates , as compared with the unirradiated .
- 2- Mating frequency, as measured by the average number of spermatophore per female , and fecundity, as measured by the average number of egg per female were unaffected as a consequence of rearing on irradiated dates .
- 3- Rearing E. cautella from egg to adult on irradiated date fruits for 5 generations did not induce genetical effects (dominant lethal mutation) as estimated by the average percentages of egg hatch at each dose in each generation .

Effect of gamma radiation on various important amino acids was studied by taking whole body tissue extracts of different developmental stages of D. dorsalis and analysing them chromatographically for their amino acid contents. Tissue extracts of different stages of untreated insects showed the presence of almost all the essential amino acids. Ten ninhydrin positive spots identified were, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, methionine, phenylalanine, proline and valine throughout the post-embryonic development. Radiation damage was reflected by qualitative changes in amino acids pool. The concentration of free amino acids changed at specific exposure threshold and the intensity of ninhydrin positive spots was dose dependent. At 15 and 20 Krad the intensity of amino acids in larva, pupa and adult extracts was reduced. Phenylalanine and proline were not at all detected in the developmental stages under the influence of 15 and 20 Krad doses. Furthermore, treatment with 20 Krad reduced the amino acid content and also resulted in the loss of aspartic acid and valine in all the post-embryonic stages and alanine as well as cystine in adult stage, 10 days after irradiation.

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FOOD IRRADIATION AS A QUARANTINE
TREATMENT OF FRUITS

16

Quarantine treatments are needed to disinfect fruits, vegetables and other commodities in international trade. The presence of fruit flies and other pests may restrict export or prevent movement of host commodities to or from countries unless the commodities have been subjected to quarantine treatment. Procedures for such treatments have been approved for use, and their limitations have been established. Research has been conducted during the past 25 years on the effects of ionizing radiation on immature fruit flies and other pests of fruit in Hawaii and elsewhere. As a result of these studies the potential for use of irradiation as a quarantine treatment for commodities subject to infestation with fruit fly larvae or other pests has been recognized. Adverse effects or the lack of such effects on treated commodities have also been observed. The potential availability of irradiators for treatment of large quantities of fruit and engineering constraints on such equipment has been considered. Research has demonstrated that the use of gamma radiation is feasible as a quarantine treatment for commodities infested with fruit fly eggs or larvae. Further research may be needed to determine the feasibility of using irradiation treatment for other pests. The tolerance of commodities to the treatments and the methodology for their application also may require additional research.

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DISINFESTATION OF COMMERCIALY
PACKED DATES, ZAHDI VARIETY, BY
IONIZING RADIATION*.

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Disinfestation of packed dry dates, Zahdi variety, in standard carton boxes was accomplished by using gamma radiation or methyl bromide fumigation. The dose distribution of radiation from Gammabeam - 650 facility with a ^{60}Co source was calculated and the best feasible uniformity ratio (= 1.4) was followed where the average absorbed dose of 15 points was 75.65 ± 8.03 krad.

The results of examination indicated that a complete disinfestation was achieved in both methyl bromide - or radiation - treated boxes when stored for 25 d. The live insects found in the irradiated dates were genetically sterile and developmentally inactive. While on longer periods of storage (55 or 80 d), live, active and fertile insects have been found in the treated as well as untreated (control) boxes indicating reinfestation cases.

On the basis of the present results, the parameters of measuring the induction of full sterility and incapability of immature stages to develop, could easily be utilized as identification methods for scientifically sound quarantine measures as far as radiation disinfestation of foodstuffs is concerned. Also, insect - proof packages, which are possibly impermeable to chemical fumigation, should be tried in future disinfestation of dates by using gamma radiation.

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RADIODISINFESTATION OF CARNATION
TORTRICIDAE PESTS: II effects of
ionizing radiations on the eggs of
Epichoristodes acerbella Walk.
(Lepidoptera Tortricidae)

Boll.Zool.& Bachic., 14:123-132, 1978

Summary. The radiosensitivity of the eggs of Epichoristodes acerbella has been investigated in order to use ionizing radiations for treating infested cut carnation flowers.

Doses from 5 to 70 krad have been applied to the eggs at 7, 4 and 1 day before hatching.

The main results achieved have shown:

- the doses of 7, 20 and 70 krad respectively applied on eggs of 2, 5 and 8 days of age caused a mortality of 100%;
- a dose of 13 krad on the oldest eggs is sufficient to kill all insect in the cysalid stage;
- a dose of 10 krad induced a complete sterility of the adults obtained from treated eggs.

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Biological halflife of radioactive
phosphorus and sulphur in Oriental
fruit fly, Dacus dorsalis Hendel.

J.Nuclear Agric.Biol., 10(1981):96-97.

Laboratory experiments were conducted to label Oriental fruit fly, Dacus dorsalis Hendel by feeding third instar maggots on mango and guava fruits treated with radioactive phosphorus and sulphur, respectively @ 1 m Ci activity/fruit. At the time of emergence, radioactivity (counts/100 sec) in adults ranged from 1822 to 57980 (male) and 7364 to 19782 (female) in case of ^{32}P and from 2320 to 4760 (male) and 986 to 2920 (female) in the case of ^{35}S treatments. The method was thus found to be quite suitable for labelling the adults of D. dorsalis for ecological studies. Further studies revealed that the biological halflife of the isotopes in male and female flies was 66.07 and 44.77 days for ^{32}P and 14.12 and 10.87 days in case of ^{35}S . The isotopes were retained for longer period in the male flies. Data thus indicate that ^{32}P , in spite of its shorter physical halflife (14.3 days), was retained for longer duration in adult flies than ^{35}S (halflife: 87.2 days) and hence, is better suited for labelling the adults of D. dorsalis.

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Y-translocations of insecticide
Resistance in Anopheline
Mosquitoes

20

Strains of mosquito in which a gene for insecticide resistance is linked by a translocation to the Y-chromosome have been used as sexing-systems for the mass-production of males for release. Such translocations were sought in Anopheles stephensi with dieldrin resistance and in An. arabiensis with malathion resistance.

Homozygous resistant males were irradiated with 4 krad of γ -rays from a ^{60}Co source, and mated with homozygous susceptible females. The male progeny were backcrossed en masse to susceptible females, which were isolated for egg-laying. Egg-batches showing less than 70% hatch were reared, and the resulting adults were exposed to a dose of insecticide which killed susceptible homozygotes but not heterozygotes. Male linkage of insecticide resistance in this and subsequent generations indicates a Y-translocation of the resistance gene.

The table below shows that such translocations were obtained in An. arabiensis but none were found in a much larger sample of An. stephensi. The contrast is significant by Fisher's exact test, but its causes are not known.

Species	:Insecticide	No. egg-batches counted	No. with <70% hatch	No. Y-resistance translocations
<u>An.stephensi</u>	: Dieldrin	712	167	0
<u>An.arabiensis</u>	: Malathion	124	41	4

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Makisterone A identified as the molting
hormone in nymphal Oncopeltus fasciatus

21

The major ecdysteroids of last-stage Oncopeltus fasciatus nymphs were determined by radioimmunoassay (RIA) combined with high-pressure liquid chromatography. Contrary to the notion that 20-hydroxyecdysone is the universal molting hormone, it occurs in this species only at low or undetectable titres and its function is taken over by another ecdysteroid, makisterone A. The possible physiological role of makisterone A in other species, in which it can be detected by RIA, is now being investigated.

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THE ATTRACTION OF MALES AND FEMALES
OF THE CODLING MOTH TO MIXED TRAP.

22

The influence of feeding aggregate on the pheromonal traps on the attraction of the codling moth has been tested in the comparative field test. Are compared the "Ferocon" traps with pheromone alone, with the mixed traps with pheromone and the aggregate liquid yeast hydrolysate and too yeast hydrolysis alone. Female and males of the Laspeyresia pomonella are attracted preferentially to mixed traps with pheromone and yeast hydrolysate (in bottle). The second place occupied the pheromone alone traps and latest the yeast hydrolysis alone traps. The percentage relation between three type of traps are: 79 : 18 : 4 . The mixed traps are attracted the 60% codling moth and too some females of Grapholita molesta. This preference of the codling moth to feeding element may be useful in the monitoring service.

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The maintenance of Glossina palpalis
gambiensis Vanderplank 1949 (Diptera,
Muscidae) on different kinds of freeze-dried
blood and on defibrinated irradiated blood -
a comparison

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Freeze-dried blood for these experiments was obtained from the IAEA, Vienna, Austria (porcine blood), and from the EGIC Laboratory, Lisbourne, France (cattle blood). The defibrinated blood was collected in a local abattoir from different cattle breeds. Each kind of blood was irradiated with a dose of 50 krad in order to avoid bacterial contamination and thus a negative influence on the experiment. The gamma-irradiation took place in a ^{137}Cs source. 160 teneral females each were obtained from a colony with a mixed feeding regimen, i.e. 5 days on membranes, 2 days on rabbits. The experimental flies were maintained likewise using freeze-dried cattle or porcine blood and defibrinated cattle blood respectively. The material (silicone membranes, supports and fly cages) was the same as for the colonies. The performance of the flies was compared in terms of productivity, longevity, mean pupal weights, and eclosion rates. The experiment had a duration of 115 days, thus comprising as well the performance of the filial generations over a period of 70 days. Neither the freeze-dried porcine blood nor the freeze-dried cattle blood gave satisfactory results when compared with their control. Both experimental groups had a higher mortality and a lower reproductive rate which was characteristic for the filial generations as well. Due to the poor results, the female number was constantly decreasing in clear contrast to the control, which showed a regular growth. It was found that freeze-dried cattle blood was inferior to freeze-dried porcine blood. However, neither kind of freeze-dried blood offered a potential alternative for the replacement of freshly collected defibrinated cattle blood. Further, the expenses of producing 1 liter of freeze-dried blood place a severe limitation on its large-scale use in Africa. On the other side, the data obtained from a colony of G. palpalis gambiensis allow to recommend the large-scale use of defibrinated and irradiated cattle blood for the mass rearing of tsetse flies in Africa.

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Semisynthetic Culture Media for Raising
the Codling Moth (Laspeyresia pomonella
L.) (Lepidoptera, Olethreutidae) under
Laboratory Conditions

In: Ecology 8 (77-86) September 1981
Bulgarian Academy of Sciences

24

SUMMARY

The results of the tests of semisynthetic culture media based on wheat embryos and agar designed for laboratory raising of the codling moth are reported. Diets 2, 3 and 4 involve modifications of the Sender (1969) recipe which was used for comparisons. Caterpillars were raised in artificial apples 2.5 - 3 cm in diameter prepared with the corresponding media. Unripe apples were used as controls. The composition of the diets and the raising technique are described in Methods. Diapause, vitality of caterpillars and pupae, weight of pupae, duration of the caterpillar - adult period, sex ratio, duration of the adult stage, fecundity and hatchability were studied. Some factors influencing the reproduction coefficient (Kr) and the related reproducibility of the populations under conditions of mass raising are discussed. The data on intraspecific competition between two caterpillars in one artificial apple are results of preliminary experiments. The best results were obtained from diets No. 1 (standard) and 2, followed by diets 3 and 4. The actual reproducibility in all culture media was sufficient for practical implementation. Diets No. 3 and 4 are relatively inexpensive and the raw materials can be obtained easily.

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Semisynthetic Culture Media for Laboratory
Rearing of the Tortricid Moth Grapholitha
molesta Busck. (Lepidoptera, Tortricidae)
in: Ecology 9 (68-76) Sept. 1981

25

SUMMARY

Three semisynthetic diets for laboratory rearing of the moth were tested. These contained agar, wheat embryos, corn flour, apple flour and mashed peaches as the main components. Caterpillars were fed on spheres measuring 2.5 - 3.0 cm in diameter. Nonripe natural apples of approximately the same dimensions were used as controls. Spheres of diet No. 3 were tested for feeding 2, 4, 6 and 8 caterpillars in one sphere. The diapause, viability of larvae and pupae, intraspecific competition among larvae, pupae weight, duration of the larva-adult period, sex ratio, life expectancy of adults, fecundity and hatching were studied. The expected rate of reproduction as a function of the reproduction coefficient (Table 3) and the effectiveness of the diets tested is discussed. The diets are relatively inexpensive and involve easily available materials.

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The effect of a sulfonamide administered to
rabbits on the performance of Glossina
palpalis gambiensis

26

Rabbits served as hosts for two colonies of G. palpalis gambiensis. The rabbits were routinely treated with a sodium salt from Suldimeoxine, registered trade mark "Sunix", against coccidiosis. The accidental use of treated rabbits led to an increased mortality and a distinct reduction in the reproductive rate of the two colonies. In order to confirm the results obtained from the colonies, an experiment with Sunix was conducted. One rabbit was treated as usual on 3 consecutive days, the drug being applied to the drinking water. After a time lag of 2 days, three groups of flies were fed on this rabbit the following 3 days. The groups consisted of:

- 80 teneral females (A)
- 100 females with an age of 40 - 50 days (B)
- 100 females aged between 70 and 90 days.

Two untreated batches of flies, their age corresponding to the first two experimental groups, served as a control.

The longevity of all experimental flies was adversely affected during the period of observation of 50 days, the percentage of surviving flies ranging between 7 and 17.5%. This contrasted clearly to the control where the survival rates were 70 and 60%, respectively.

With the exception of A, productivity of the experimental females remained unchanged during the two subsequent ovulations after treatment. Thereafter, a sharp decline of the reproductive rate was recorded when compared with their control. For A, a 90% reduction of the reproductive rate was observed which did not correspond to the results of its control. The performance of the filial generations was likewise negatively affected. The puparia produced by A did not hatch. The F_1 generation of B had a poor survival rate and a low productivity. The filial generation of C did not produce at all, their survival rate resembling the results of the F_1 of B. The results of the experiment indicate that this sulfonamide could be a potential means of control in an integrated control campaign against tsetse flies.

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Comparative results of Glossina palpalis
gambiensis Vanderplank 1949 (Diptera,
Muscidae) maintained on defibrinated
and on heparinized cattle blood

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For all experiments, the flies derived from a colony maintained on a 5 days membrane/ 2 days rabbit feeding regimen. Two experiments were conducted. In the first, two groups of 360 teneral flies each were fed exclusively on defibrinated and on heparinized cattle blood being collected in a local abattoir. 400 I.U. of sodium heparin were added per 100 ml of blood. For all colonies, the blood was irradiated with a dose of 50 krad prior to its use to avoid contamination with bacteria and trypanosomes. The second experiment comprised 3 groups of 180 teneral females each. Two fly groups were offered a chance to feed on rabbits the first 4 consecutive days after eclosion, which was in contrast to the first test. Thereafter, they fed exclusively on defibrinated and on heparinized cattle blood respectively. The third group ran as a control and was maintained on a 5/2 feeding regimen. The results of the experiments were compared on the base of longevity, productivity, offspring size and eclosion rate.

The flies of the first experiment showed up with a poor performance, which was characterized by a high percentage of daily mortality (1.9 - 2.0%), low reproductive rate (2.4 - 3.1 puparia/initial female/90 days) and a low mean pupal weight (24.0 - 24.2 mg). The filial generations performed similarly. All the groups of the second test produced better results. The percentage of daily mortality was lower (0.7 - 0.8%), simultaneously their reproductive rate was higher (4.2 - 4.6 puparia/initial female/90 days) as well as the mean pupal weight (26.4 - 26.6 mg). The substitution of heparin did not improve the results nor was it found to be harmful to the flies. The experiments demonstrated the need of a rabbit supplement in the diet of G. palpalis gambiensis. A continuing fly adaptation and technical improvements of the membrane system were regarded as a pre-requisite to a future reduction of the rabbit supplement.

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Some mathematical methods in the control of a population of Ceratitis capitata Wied.

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SUMMARY

Mathematical models are proposed for the study of the control of a population of Ceratitis capitata Wied. in a citrus fruit agro-ecosystem, through the use of chemical products and biological agents.

The population dynamics, referred to two of the fundamental stages of development - that preimaginal (eggs, larvae, pupae) and the adult stage -, is described by ordinary differential equations with delay, that keep in mind the most relevant biological processes of the population and of the environmental characteristics.

Assigned the characteristic parameters of the described processes (fertility, mortality, time of development, receptivity and fruit production), referred to a particular agro-system, mathematical methods are proposed to determine:

- a) the efficiency of the phytosanitary intervention, resolving numerically the system of differential equations which describe the biological processes, from previously fixed economical thresholds;
- b) an optimal strategy of interventions by means of the combined action of larvicides and adulticides, resolving a problem of control optimum from a fixed economical function that represents the cost of the damage and that due to the intervention action.

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A Combinatorial Model for Evaluating the
Changes in Population Number Produced by
Radiation Sterilization Applied in the Control
of Harmful Insects
In: Horticultural and Viticultural Science
18(3): 24-32 (1981)

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SUMMARY

A combinatorial method was elaborated for use in evaluating the quantitative changes occurring in insect population after introducing into them individuals sterilized by radiation. On the basis of a complex of initial assumptions, the interactions between sterile and fertile insects are described in relation with their proportion in the population. Statistical expressions are deduced, which make it possible to foresee the probable development of the F_1 and F_2 generations and their respective variances. The gametogenetic or parthenogenetic types of propagation are examined in cases of inducing dominant lethal mutations sperm inactivation or aspermia. The effects of applying a sterile population or sterile male individuals only are compared. Results obtained are summed up also for the special case of polygamy.

The analysis proving the applicability of the model indicates that the complex of conditions introduced in it does not limit its use to laboratory experiments only. Certain deviations are possible, however, in case of natural conditions, due to various factors.

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A Model of Evaluating Imago Population of
the European Cherry Fruit Fly Rhagoletis
cerasi L. (Diptera: Tephritidae)

In: Horticultural and Viticultural Science
18 (2):18-27 (1981)

30

SUMMARY

The article presents results obtained in an investigation on the main factors determining imago numbers of R. cerasi in the cherry orchards. This information is intended mainly for the purpose of the sterile insect technique for control of the insect. The values of the following factors were determined: diapause I and II year, natural mortality of the pupae and parasitizing. No diapause was established in the III year.

A statistical method for determining the expected imago population numbers in the orchard is proposed, which includes the parameters: density of the pupae, area of projection of the tree crowns, numbers of trees, total number of pupae in the orchard, correction for no pupae imagination, correction for parasitizing and expected numbers of adult flies. The model was evaluated in respect to its generality, realism and accuracy. The experimental check-up by the aid of ³²P marked flies showed that its structure actually imitates the nature of the studied process. The application of the model for the prognosis of measures used in the control of the insect is discussed also.

Joint FAO/IAEA Division of Isotope and Radiation Applications
of Atomic Energy for Food and Agricultural Development

Coordinated Research Programme on
using radiation and isotopes to develop diets for mass rearing
Haemotophagous insects for sterile insect releases and to study
disease transmission by these vectors

Vienna, Austria

10-14 May 1982

AUTHOR(S): S. M. MOOBOLA

ORGANIZATION: NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

TITLE of Working Paper: Development of in vitro and in vivo feeding
method in mass-rearing of Glossina morsitans centralis for
use in SIT and ecological studies.

SHORT SUMMARY OF PAPER

This project is the first of a three-phased long term study that will eventually lead to the use of the sterile insect technique (SIT) to control tsetse flies in Zambia. It is being planned within a governmental land use programme aimed at reclaiming some of the tsetse fly infested pastoral and arable land. Because of the diffuse and extensive distribution of savanna tsetse fly species, control measures are being targeted at isolated tsetse foci and those tsetse flies which are re-invading areas from which they had previously been cleared. Thus, as a prelude to the use of SIT, mass-rearing of G. morsitans centralis, using in vitro and in vivo techniques, has been initiated.

Since the project has just been financed by an IAEA research contract, no immediate results are yet available. However, this working paper reports ^{on} future plans of action with a view to attracting input from the group in the form of recommendations that will serve as guidelines for the continuation of the project.

As per proposed work plan of the research contract for the first year of the project, the following aspects will be covered:-

- I (i) collection of female flies and pupae from the field to initiate a new colony of G. m. centralis.
- (ii) determination of the reproductive rhythm and the number of generations required to condition field collected material to laboratory conditions.
- (iii) investigations on mass-rearing of this species using guinea pigs and artificial membrane feeding techniques.

II Optimization of the general handling and holding procedures of laboratory reared flies.

Prior to the above plan of work, tsetse fly rearing facilities are being improved. So far, renovations and structural alterations to the insectary have been completed. Insectary climatic conditions will also improve after the arrival of equipment being awaited from IAEA.

After mass-rearing has been achieved, other two phases of this long term project will follow. These include induction of sterility in males by irradiation, field releases of sterile males, evaluation of the results of releasing sterile males and assessment of the long term effects of the use of SIT.

RESEARCH COORDINATION MEETING

Joint FAO/IAEA Division of Isotope and Radiation Applications
of Atomic Energy for Food and Agricultural Development

Coordinated Research Programme on
Tsetse Fly Control or Eradication by the Sterile Insect Technique
Vienna, Austria
10-14 May 1982

AUTHOR(S): Bauer, B. & Politzar, H.

ORGANIZATION: C.R.T.A., B.P. 454, Bobo-Dioulasso, Upper Volta

TITLE of Working Paper: The large-scale rearing of *Glossina palpalis gambiensis* in West-Africa

SHORT SUMMARY OF PAPER

Two self-supporting colonies of *G. palpalis gambiensis* were started in October 1980 and in March 1981. The feeding regimen was 5 days cattle blood/2 days rabbit and 6 days cattle blood/1 day rabbit respectively. The defibrinated cattle blood was collected twice per week in a local abattoir. The blood was routinely irradiated with 50 krad in a ^{137}Cs -source. The irradiation prevented an outbreak of bacterial diseases in the colonies. Over a period of 17 months no bacterial infection occurred. The irradiation interfered as well with the development of trypanosomes in the tsetse flies. The performance of the colony on a 5/2 feeding regimen was comparable to the performance of a colony maintained on rabbits only. Starting with 1069 individuals the number of females exceeded 20.000 within one year. The performance of the flies maintained on a 6/1 feeding regimen approached the results of the other colony. The number of rabbits required for the colonies with a mixed feeding regimen was at least 3 times lower than for a colony exclusively reared on rabbits. The results demonstrated that a large-scale rearing of this species is feasible under the conditions described.

RESEARCH COORDINATION MEETING

Joint FAO/IAEA Division of Isotope and Radiation Applications
of Atomic Energy for Food and Agricultural Development

Coordinated Research Programme on
Tsetse Fly Control or Eradication by the Sterile Insect Technique
Vienna, Austria
10-14 May 1982

AUTHOR(S): DISTELMANS, W. and D'HAESELEER, F.

ORGANIZATION: Rijksuniversitair centrum Antwerpen

TITLE of Working Paper: The susceptibility of untreated and gamma-irradiated
Glossina palpalis palpalis to infection with Trypanosoma congolense...

SHORT SUMMARY OF PAPER

It was recently demonstrated that colonized G. palpalis offered an infected meal (T. congolense TORORO/69/EATRO/1157 inoculated in guinea pigs; level of parasitaemia 10^8 tryp/ml) within the first 32 h after emergence, became infected. Results of the dissections (day 30 following infected meal) revealed similar levels of infection : 17/146 or 11.6% of Nigerian strain males and 14/139 or 10.1% of Nigerian strain females were infected whereas in the case of Zaire strain material 42/285 or 14.7% males and 23/285 or 8.2% female flies developed infection respectively.

Of particular interest was that none of the flies which were given the infected meal beyond the 32 h period (on day 2, day 3, day 5, day 10, day 15 and day 25 respectively) develop the infection, whereas the younger ones would cyclically transmit T. congolense to guinea pigs (Distelmans et al., 1982). Studies were continued in an attempt to compare the susceptibility of untreated and gamma-irradiated G. p. palpalis (puparial stage) to the same trypanosome strain. It can be concluded that the susceptibility to infection with T. congolense is not influenced by irradiation. Results are discussed.

PRELIMINARY STUDIES ON THE VECTORIAL
CAPACITY OF IRRADIATED MALE GLOSSINA PALPALIS
REARED AT BICOT LABORATORY, VOM. EXPERIMENTAL
INVESTIGATIONS UNDER NEAR RELEASE CONDITIONS

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One hundred and thirty-five male Glossina palpalis, irradiated at the dose rate of 96.9 krad/hr for 7.5 minutes using a γ - cell Cesium source, and 135 non-irradiated male G. palpalis were used to investigate the feeding behaviour (in vivo on mice and guinea-pigs) their longevity and vectorial capacity for Trypanosoma vivax Y58 (a mouse infective stock).

The surrounding laboratory temperature and relative - humidity obtainable when PVC cages were wrapped with wet towels were used for the studies. The latter conditions may be comparable to the environmental conditions at the proposed release site. The following protocol was followed:

- (i) 45 non-irradiated G. palpalis were fed on clean white swiss mice once and later maintained on guinea pigs
- (ii) 45 non-irradiated G. palpalis were fed on Trypanosoma vivax, Y58 parasitaemic mice (parasitaemia ~~test~~ by wet blood film examination) once and later maintained on guinea pigs.
- (iii) 45 non-irradiated G. palpalis were fed and maintained on guinea pigs.
- (iv) 45 irradiated male G. palpalis were fed on clean mice once and maintained on guinea pigs.
- (v) 45 irradiated male G. palpalis were fed on T. vivax, Y58 parasitaemic mice (parasitaemia ~~test~~ by wet blood film examination) - once and maintained on guinea pigs.
- (vi) 45 irradiated male G. palpalis were fed and maintained on guinea pigs only.

On days 12 and 15 post fly infection, surviving irradiated flies were fed on clean, susceptible mice to detect transmissibility of fly-infection (if any) in addition to fly dissection carried out on day 15 after feeding on mice.

It was observed that -

- a) Fly mortality occurred over the 24 hours feeding intervals and at feeding-times. The cumulative mortality as at day 5 averaged 83% for both irradiated and non-irradiated flies largely due to environmental fluctuations.
- b) A positive feeding response of 42.6% for flies feeding on mice as opposed to 82.5% for flies feeding on guinea pigs were recorded for both irradiated and non-irradiated flies at first blood meals.
- c) All flies that received T. vivax infective blood meals that died during feeding were immediately dissected. In addition, the eight surviving irradiated flies that received infective blood meals were also sacrificed and dissected on day 15 after feeding on clean susceptible mice.
- d) No developmental stages of trypanosomes were detected in the labrums and hypopharynges of irradiated and non-irradiated flies that were dissected.
- e) Fly-transmissibility studies have yielded no positive result as all mice offered to irradiated flies which had fed on infective blood meals have remained negative for trypanosomes for 3 consecutive weeks.

The results so far obtained are reflections of the generally low trypanosome infection rates observed in the literature for G. palpalis; the few number of flies surviving under the present trial conditions and the low fly infectivity of the stock of T. vivax used. However, the experience gained in handling the flies and the studies undertaken may provide strategies for more detailed investigations to follow.

The Sterile Insect Technique against Glossina morsitans morsitans
Westw. in Tanzania

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ABSTRACT

Sterile males of laboratory reared Glossina morsitans morsitans Westw. were released with a 200 square kilometre test area after initial reduction of the natural population with two aerial applications of endosulphan.

Sterile males which were distributed twice weekly averaged about 135 males per square kilometre per month in the test area and reduced the natural population by 90%. Although eradication was prevented by immigration of fertile flies across an ineffective barrier, the 90% control achieved was maintained at that level for over 15 months.

The cost of production for the sterile males using goats and rabbits as host animals was US\$ 220 per 1,000. Most of the production cost was for host animals maintenance.

Embryonic defects in eggs of Glossina palpalis palpalis (Diptera, Glossinidae) fertilized by sperm of gamma-irradiated males

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ABSTRACT

In most eggs fertilized by sperm of irradiated males, embryogenesis is inhibited in early cleavage division, when cleavage is over, lytic processes take place and are followed by extrusion of eggs. The above changes, occurring in about 95% of eggs, have been described in detail by Matolin and Van der Vloedt (1981). Former cleavage nuclei can be identified by characteristic, more deeply staining halos.

The results of previous experiments have shown that sterile eggs of females fertilized by sperm of gamma-irradiated males are later expelled (Van der Vloedt & Taher, 1978; Matolin & Van der Vloedt, 1981). In less than 5% of expelled eggs development can be observed up to advanced stages of embryogenesis. The same results were obtained after exposure to fast neutrons (Van der Vloedt et al., 1976). Normally, the first larviposition takes place between day 18-21, but the first sterile egg is usually extruded between the 14th - 20th day, which means that the egg is retained in the uterus for some time after the cessation of development. The present study deals with eggs aborted during a longer space of time between 14th - 56th day after emergence, i.e. eggs from first and several consecutive reproductive cycles. Many malformed embryos described in this paper were found in eggs expelled towards the end of the period.

Application of The Sterile Insect Technique for Tsetse Fly Control

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ABSTRACT

The field trial of the Sterile Insect Technique conducted at Mkwaji Ranch in Tanzania in 1978-79 was initiated with 2 applications of endosulfan as an aerosol at an interval of 28 days. Following this, irradiated Glossina morsitans morsitans pupae were released throughout the 195 km² test site. Over the 14-month experimental period the release of 135 sterile males per km²/month provided an average of 81% control of the target species, whereas the check species G. pallidipes recovered to prespray levels within 5 months. Immigration of indigenous fertile flies from outside the 1 km perimeter barrier surrounding the experimental plot provided the nucleus of the 19% residual population.

Confirmation of the role of immigration in this research programme helps to resolve matters related to interpreting the quality and effectiveness of the released insects. This was accomplished by combined analysis of the indigenous fly population within the test site and the movements of marked flies released outside the perimeter barrier.

Some factors associated with cyclical transmission of Trypanosoma brucei brucei in Glossina morsitans morsitans with a note on separation of sub-populations of bloodstream from T. brucei

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ABSTRACT

The susceptibility of G.m. morsitans to T.b. brucei infection was shown to be age dependent, the youngest age group (1-8 hours after emergence) being more susceptible than the older ones. The susceptibility was enhanced by cooling the young flies to 0-5°C for 30 minutes. Male flies were found to be more susceptible than females and artificially inoculating the young flies with T.b. brucei resulted in higher infection rates than flies infected through the normal route. The number and morphology of trypanosomes ingested did not influence the subsequent salivary gland infection rates observed in G.m. morsitans, however, there was a relationship between the number ingested and subsequent T.b. brucei midgut infections in the flies. A technique for separating sub-populations of bloodstreams from T.T. brucei is described and preliminary results obtained outlined.

A review of recent work at the Tsetse Research Laboratory

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ABSTRACT

1. Production of Glossina for research

Research at the TRL is undertaken with insect produced by major colonies of Glossina morsitans morsitans and G. austeni fed on fresh defibrinated blood in a membrane feeding system. Surplus puparia from these colonies are also supplied to workers elsewhere. Smaller colonies of G. palpalis palpalis, G. pallidipes and other subspecies of G. morsitans are also maintained. The performance of the production colonies of G.m. morsitans and G. austeni in 1981 is shown in Table 1.

Table I: Performance of the Production colonies of
G.m. morsitans and G. austeni, 1981

	<u>G.m. morsitans</u>	<u>G. austeni</u>
Mean ♀ stock	9,271	1,989
Mean daily ♀ mortality (5)	0.85	1.18
Puparia/♀/week	0.50	0.51
Mean weight of puparia (mg)	33.3	25.7
Puparia produced	239,525	53,816
Puparia and adults distributed	113,075	24,932

2. Nutritional studies on G.m. morsitans

Studies on the nutritional requirements of G.m. morsitans are being undertaken with the objective of developing an artificial, defined diet for Glossina. A diet containing only commercially available constituents has been developed on which tsetse will survive and reproduce, but the puparia produced are well below optimum size.

3. Hormonal control of reproduction in *G.m. morsitans*

A water soluble lipid-mobilising factor has been isolated from the nervous system of *G.m. morsitans*. This factor is probably a neurohormone synthesised by the median neurosecretory cells of the brain which stimulates the fat body to synthesise proline for flight and/or release lipid for uptake by the uterine gland. Another neurohormone from the brain and an inhibitor present in the haemolymph are involved in the synthesis of larval milk by the uterine gland.

4. Contact sex recognition pheromones of *Glossina*

The contact sex recognition pheromone of *G.m. morsitans* was identified and synthesised some years ago and recent work has been concerned with evaluating combinations of pheromone and chemosterilant on decoys as an approach to control of *Glossina* in the field. Laboratory studies at the TRL and field work in Zimbabwe have shown that the responses of *G.m. morsitans* males to pheromone-baited decoys were of much shorter duration in the field than in the laboratory, but field responses were not affected by high concentrations of the chemosterilant, bisazir, on the decoy. Calculations indicate that this method might be used to induce permanent sterility in at least 2% of all male flies that visit a cylindrical black model bearing treated decoys.

5. Insect growth regulators

Topical application of 0.5 ug Diflubenzuron (DFB) to the thorax of female *G.m. morsitans* prevents the production of viable offspring throughout most of the reproductive life of the fly, some 50 days. Recent physiological work suggests that the surface of the cuticle is the main source of DFB, from where it is slowly released into the body of the fly. The route of entry for DFB into the larva is with the milk from the uterine gland. A trial of the effectiveness of DFB against a natural population of *G.m. centralis* was undertaken in Zambia.

6. Potential for insecticide resistance in *Glossina*

Laboratory assays, by Dr. F. Barlow, Centre for Overseas Pest Research, Porton, have shown that *G.m. morsitans* are capable of metabolising small amounts of DDT to DDE, from which it may be inferred that this species carries the gene for DDT-ase production. A computer model has been developed which suggests that insecticide resistance would be most likely to evolve where isolation of the treated population is not practicable and when there is time for several generations to exist between successive applications of insecticide. The possibility that a low level of resistance may have already evolved in the some areas is being examined.

7. Inheritance of susceptibility of *G.m. morsitans* to infection with *Trypanosoma congolense*

G.m. morsitans fed on *Trypanosoma congolense* through a membrane on blood containing procyclic culture forms of *Trypanosoma congolense* had an infection rate of 17.5%. By breeding experiments involving flies susceptible or refractory to infection, lines of flies have been selected which consistently have infection rates of over 80% and others with rates well below normal. Maternal effects are probably controlling susceptibility to infection in this *Glossina* sp.

8. Responses of G.m. morsitans to light of different wavelengths

This research was carried out under IAEA Research Contract No. 2407/R1/TC. Glossina spp. exhibit a strong positive phototaxis under certain conditions and this study is aimed at defining the responsiveness of tsetse to different wavelengths of light, with a view to improving methods of trapping tsetse in the field. A spectral response curve was produced from the phototactic choice behaviour of groups of flies between two sources of monochromatic light. Ultraviolet light, at about 350 nm, was most attractive. Electrophysiological studies showed that the compound eye of G.m. morsitans has peaks of sensitivity and the ultraviolet peak corresponds with that found during the behavioural studies. Two other smaller peaks of electrophysiological sensitivity were identified, which did not correspond with any maxima in the behaviour studied, and the significance of this finding is unknown.

RESEARCH COORDINATION MEETING

Joint FAO/IAEA Division of Isotope and Radiation Applications
of Atomic Energy for Food and Agricultural Development

Coordinated Research Programme on
Tsetse Fly Control or Eradication by the Sterile Insect Technique
Vienna, Austria
10-14 May 1982

AUTHOR (S) : ITARD (J.)^(**), CUISANCE (D.)^(**), POLITZAR (H.)^(***), BAUER (B.)^(***)

ORGANIZATION : ^(**) I.E.M.V.T. et G.T.Z. ^(***)

TITLE of Working Paper : Le programme de lutte intégrée contre les Glossines
du Centre I.E.M.V.T./G.T.Z. de Recherche sur les Trypanosomiasés animales
(C.R.T.A.) BOBO-DIOULASSO (Haute-Volta)

Le programme de lutte génétique contre *Glossina palpalis gambiensis*
par lâchers de mâles irradiés entrepris, sur financement franco-allemand, depuis
Juin 1975, à Bobo-Dioulasso (Haute-Volta), a pris fin en Août 1980.

Au cours de ces 6 années, près de 900.000 mâles ont été produits
et lâchés dans 5 gîtes à *G.p. gambiensis*, le long de 32 km de galeries fores-
tières bordant les sources de la Volta Noire, représentant une zone d'environ
100 km².

G.p. gambiensis a été éliminée de ces gîtes, alors que la den-
sité de cette espèce a fluctué, de façon normale et régulière, sur le gîte
témoin, et que *G. tachinoides*, espèce non visée par les lâchers, s'est main-
tenu normalement, d'une année à l'autre, dans les gîtes d'expérience.

L'intensification des recherches au laboratoire a permis la
mise au point d'un régime alimentaire pour *G. tachinoides* et *G.p. gam-
biensis*, ce qui autorise, d'une part une utilisation moindre des ani-
maux nourriciers, d'où un développement des colonies de mouches et,
d'autre part, une approche effective de l'industrialisation des dif-
férents stades de production. Cette alimentation mixte, 2 j. sur lapins

et 5 j. sur membrane artificielle avec du sang de bovins défibriné, est en voie d'être encore simplifiée avec usage du lapin un seul jour par semaine.

Des innovations techniques (stockage des glossines, collecte des pupes) ont permis de réduire les manipulations.

Il est à regretter que l'expansion des colonies ait dû être volontairement bloquée par manque de place, les nouveaux insectariums et les installations complémentaires prévus n'ayant pu être construits.

Sur le terrain, les activités entomologiques n'ont pas atteints le niveau escompté par manque de véhicules et de moyens. Les travaux ont été concentrés sur les points à haut risque d'invasion où un système de piégeage permanent a été mis en place en toutes saisons permettant de tester les barrières de pièges, d'estimer les densités, les fluctuations saisonnières et l'abondance relative des espèces de glossines, dont les mieux représentées sont *G. tachinoides* sur la rivière Panapra et *G.m. submorsitans* dans les savanes riveraines du fleuve Koba. 74 kilomètres de piste ont été construits sans assistance mécanique afin de permettre la surveillance des tronçons de galeries et de poursuivre l'inventaire entomologique.

Glossina pallidipes Aust and Glossina morsitans centralis (Machado 1970) colonised at UTRU Tororo, Uganda. The historical background and the present status of the colonies.

Peter Einyu.

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Summary.

The Uganda Trypanosomiasis Research Organisation has since 1967 and 1970 maintained Glossina pallidipes Aust. and Glossina morsitans centralis (Machado 1970) colonies respectively. Glossina pallidipes colony was originally fed on defibrinated cow blood, lop eared rabbits, cows and goats on experimental basis. G. morsitans centralis was fed on the rabbit ears when it was first established in June 1970 but the shaven flanks of oxen were soon introduced as the sole host for this colony in 1971. G. pallidipes was introduced to the live ox diet as late as 1976 when the original experimental colonies was at the verge of extinction.

Originally both colonies were reared on the basis of a "generations" system, which was discontinued in 1971 for G. morsitans and in 1976 for G. pallidipes. When all "generations" system was introduced.

There were 7000 females of G. morsitans centralis and 1200 G. pallidipes by the end of 1978. The "Liberation war" in 1979, reduced G. morsitans to about 3000 with an average productivity of 2.2. pupae per female per month and G. pallidipes to 800 females with an average monthly productivity of 1.8. During the year 1980/81 both G. morsitans and G. pallidipes recovered to 8000 and 1300 respectively. Presently, G. morsitans stands at about 10,000 while G. pallidipes is at 2,000 females with average monthly productivity of 1.7 and 1.4 respectively.

Analysis of a Defined Diet for Adult Stomoxys calcitrans

(Diptera: Muscidae)*

42

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ABSTRACT

The fecundity of stable flies (Stomoxys calcitrans) fed on nondialyzed, freeze-dried bovine hemoglobin plus bovine serum albumin was equal to the fecundity of flies fed on bovine whole blood. Neither freeze-dried hemoglobin nor bovine serum albumin alone were adequate diets for stable flies. An amino acid analysis of freeze-dried bovine hemoglobin hydrolysates revealed deficits in several essential amino acids. When compared with whole blood, bovine serum albumin also showed a number of amino acid deficits. Dialyzed, freeze-dried hemoglobin plus bovine serum albumin also proved to be an inadequate diet. Physiological or free amino acid analysis of different hemoglobin preparations showed levels of approximately 30% of those found in whole blood. Lipid analysis of commercial preparations of hemoglobin and of freeze-dried preparations of hemoglobin revealed substantial amounts of lipids, about one-third to one-half those in whole blood. The dietary requirements of S. calcitrans are similar to those of Glossina spp. in that both hemoglobin and albumin are required.

RESEARCH COORDINATION MEETING

Joint FAO/IAEA Division of Isotope and Radiation Applications
of Atomic Energy for Food and Agricultural Development

Coordinated Research Programme on
Tsetse Fly Control or Eradication by the Sterile Insect Technique
Vienna, Austria
10-14 May 1982

Use of fat body analyses for physiological examination on Tsetse flies

Udo Feldmann

Summary

Total lipid extractions of G.p.palpalis were conducted in order to evaluate different diets fed to Tsetse flies at Seibersdorf and to correlate the development of the lipid content with some incisive physiological changes in the flies.

In the first lipid extraction experiments diet effected differences in the total lipid content of virgin females and males from the day of eclosion to an age of 30 days and of copulated females during their third larval cycle were examined. The diets for feeding these flies were as follows:

- Guinea pig blood, fed in vivo
- bovine blood, defibrinated, freeze dried, reconstituted, supplemented with ATP and fed in vitro, through a silicone membrane;
- porcine blood, treated and fed like the bovine blood.

In females, the development of the lipid content indicated the time of first ovulation and during pregnancy the hatching processes from egg to second instar larva. In the late pregnancy and after larviposition of copulated G.p.palpalis females, that were fed on freeze dried bovine blood, the development of the lipid content was comparable to the results of experiments with G.morsitans fed on fresh bovine blood reported from Bristol.

Virgin males of all three diet groups reached a maximal amount of lipids 9 days after their eclosion, and they achieved a second LC-peak after 9 days again, what might have an influence on their competitiveness especially under natural conditions.

Another lipid extraction experiment with copulated G.p.palpalis females fed on two different mixtures of freeze dried bovine and freeze dried porcine blood indicates that the mixtures can overcome the nutritional deficiencies of FD-bovine blood. The sonication of mixed blood with a percentage of up to 50% FD-porcine blood had no positiv effect on survival, productivity and pupal weights of G.p.palpalis females.

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Cyclical maintenance of trypanosomes in the laboratory is a prerequisite to studies in natural conditions on host-parasite relationships, and chiefly on life cycle, immunity, chemotherapy, induced immune protection and trypanotolerance. Cyclical transmission, however, is still restricted to a limited number of laboratories.

Preliminary experiments have been carried out with Glossina morsitans morsitans and Trypanosome brucei brucei. Flies were received from the Langford laboratory as pupae and they were maintained at $26^{\circ}\pm 1^{\circ}\text{C}$ and $75\pm 5\%$ relative humidity.

Survival rates at day 30 under various nutritional conditions (Table 1) showed that in vivo feeding on rabbits was generally superior to the in vitro feeding. However, marked fluctuations amongst batches maintained in control conditions (rabbit, in vivo) indicated that some factors were still sub-optimal. Two detrimental possibilities are possible, (i) the despatching of pupae, (ii) a too high relative humidity during maintenance. No significant difference was observed in vitro between freeze-dried porcine (FD.P) blood with or without ATP and frozen bovine (F.B) blood.

Table 1: Survival rate at day 30

	<u>No. of flies*</u>	<u>Survival (%)</u>
<u>In vivo</u>		
rabbit	184	83 (68-93)
mice	158	18 (10-32)
<u>In vitro</u>		
FD.P	57	58
FD.P + ATP	33	61
F.B	28	61

* 9 different batches tested over six months

Best scores of metacyclic infection are obtained by giving teneral flies an infective feed within the first 32 hours following eclosion. Rabbits showed to be more attractive than membrane feeding (Table 2). No significant difference was observed among the bloods offered in vitro.

Table 2: First feed taken \leq 32 hours

	<u>No. of flies*</u>	<u>% flies feed</u>
rabbits	137	65.8
mice	93	45.0
FD.P	92	43.1
FD.P+ATP	64	47.6
FP	62	37.1
FB	28	46.4

* 5 different batches tested over two months

There is evidence that the sooner the infective feed is taken, the higher the mature infection rate is obtained. The periodic distribution of first feeds was recorded (Table 3). It was observed that 86% of the flies feeding before hour 32, did so between hours 8 and 24, whatever the source of blood was.

Table 3: Distribution of first feeds \leq 32 hours

<u>2-8 hours</u>	<u>8-16 hours</u>	<u>16-24 hours</u>	<u>24-32 hours</u>
6.45%	36.86%	49.76%	6.91%

In the future, environmental conditions of maintenance of pupae as well as of adult flies, will be improved. Present experiments will then be expanded and further parameters will be considered.

Vectorial capacity of gamma-irradiated sterile male Glossina morsitans morsitans for pathogenic Trypanosoma species

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SUMMARY

A study was conducted on the vectorial capacity of sterile male Glossina morsitans morsitans for Trypanosoma vivax, T. congolense and T. brucei. Tsetse used were rendered sterile by exposing them as pupae after the first flush of female emergences with 10 krad gamma radiation using a ¹³⁷ caesium source under ambient conditions. The emergent teneral males were fed on goats which were infected with the above three pathogenic trypanosome species. The non-irradiated males were infected simultaneously on the above goats to serve as controls. The infection rates of the irradiated groups of males which had been infected with T. vivax, T. congolense and T. brucei were 82.3, 21.1 and 17.3%; for the control groups, they were 75.7, 23.6 and 14.3%, respectively. There were no differences in the transmission characteristics to susceptible hosts between the sterile and normal vectors. These results indicate that release of sterile male tsetse in tsetse control programmes will increase the trypanosomiasis risk in the affected areas. The risk would not be reduced below that pertaining before the SIR control campaign, even if the target tsetse populations are initially reduced by non-residual insecticide applications.

Experiments concerning blood treatments, handling and evaluation procedures of practical importance for mass rearing of tsetse flies on membranes

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Abstract

The suitability of freeze-dried blood exposed to high doses of gamma radiation before reconstitution for feeding to *G. palpalis*: Gamma-irradiation of reconstituted freeze-dried and of fresh blood is used in some tsetse laboratories as a routine procedure to sterilize the diets fed to tsetse flies through membranes. An additional or alternative exposure of lyophilized blood before reconstitution could be necessary if either a later irradiation is impossible or there is a need to overcome bacterial contaminations of the dry blood, which could deteriorate it under suboptimal storage conditions. Freeze-dried porcine blood was exposed at a ^{60}Co -gamma source to 0, 1, 2 and 4 Mrad before and additionally to 0 and 100 krad after reconstitution. Each of these 8 blood samples was fed to 30 females of *G. palpalis* to perform the 25 day quality control test established in the IAEA-laboratory Seibersdorf. Feeding response, survival, production and dissection data show that irradiation of freeze-dried porcine blood in the dry stage does not deteriorate its suitability for feeding *G. palpalis*. A comparison of deep-frozen whole with reconstituted freeze-dried porcine and bovine blood mixed in various ratios as diets for *G. palpalis*: The limited capacity of the freeze-drying plant now in use in the IAEA-laboratory could result in a shortage of freeze-dried blood when the consumptions in BICOT will increase in the near future. If deep-frozen whole blood can be proven as suitable for membrane feeding of *G. palpalis* colonies at Seibersdorf, then the entire produce of lyophilized blood can be supplied to BICOT. Bovine and porcine blood were either only deep-frozen or additionally freeze-dried after collection. From the thawed deep-frozen whole blood and the reconstituted freeze-dried blood mixtures were prepared containing porcine and bovine blood in the percentage ratios 0/100, 25/75, 50/50, 75/25 and 100/0. These 10 samples were subjected to the 25 day quality control test using 30 *G. palpalis* females each. Additionally the 6 mixed diets were fed to 60 females for 50 days. During the first 25 days the flies do not perform on whole deep-frozen thawed mixtures worse than on reconstituted freeze-dried mixtures. But after 50 days the latter seem to be more suitable with respect to fly performance.

In the evaluation of the experiments efforts have been made to develop a simple numerical system which adequately summarizes and combines the various data obtained from survival, puparia production and dissection.

Biochemical investigations of the nutritional requirements of
tsetse flies and the development of an artificial diet

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SUMMARY

The nutritional importance of individual or combinations of mammalian blood fractions was evaluated in a series of diet-deletion and addition experiments in which the manifestation of deficiencies in nutritional requirement of experimental diets was indicated by the reproductive performance of deprived flies. Flies fed on serum-free diets (bovine erythrocytes suspended in saline) failed to reproduce and showed marked abnormalities in oocyte development. Attempts to correct this nutritional deficiency by the addition of individual serum protein fractions to washed bovine erythrocytes established that albumin (and to a lesser extent, lipoproteins) was associated with nutritional value. The results of further experiments, however, suggested that albumin-bound substances (mainly lipids) rather than albumin per se are of nutritional value. The significance of dietary lipids was further indicated by the discovery that the nutritional value normally associated with whole serum, albumin or serum lipoproteins was abolished by delipidation procedures. The difficulty of rendering lipid substances soluble in aqueous dietary media may be responsible for the sub-optimal nature of an artificial diet so far achieved for tsetse flies. The applications of an artificial diet for tsetse are discussed in terms of economic and logistic improvements in existing feeding methods and with reference to programmes of trypanosomiasis control.

RESEARCH COORDINATION MEETING

Joint FAO/IAEA Division of Isotope and Radiation Applications
of Atomic Energy for Food and Agricultural Development

Coordinated Research Programme on
Tsetse Fly Control or Eradication by the Sterile Insect Technique
Vienna, Austria
10-14 May 1982

MASS-REARING OF Glossina palpalis palpalis AT SEIBERSDORF: A Review of
research conducted in support of the BICOT Project in Nigeria.

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Summary

Investigations on the maintenance of G. p. palpalis have been in progress in this laboratory since 1975. Cooperative efforts and active exchange of information on biological, physiological and technical aspects between the Seibersdorf staff, consultants and scientists working at institutes participating in the Agency's Coordinated Programme, have enabled the accomplishment of the necessary breakthroughs for practical application of the SIT approach against this and other tsetse species under African conditions. Meanwhile, the staff of the Seibersdorf Laboratory has been closely involved in the design, development and transfer of in vivo and in vitro mass-rearing procedures and facilities supporting the BICOT Project.

Within the past two years, major emphasis has been given to standardization of the tsetse diet and development of new methods for extending the shelf life through freeze-drying of blood collected from a slaughterhouse. Searching questions have been asked and partially answered about the effect of freeze-drying on basic components in bovine and porcine blood, about the effect of additives and the possible usefulness of commercial products. Results of nutritional studies, refinement of blood preparation methods and implementation of quality control measures for testing the suitability of the blood before it is offered routinely to the flies, led to the establishment of highly self-sustaining in vitro fed colonies with a reproductive rate equal to 90% of that of colonies fed on living guinea pigs.

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Currently, the main membrane-fed colony of G. p. palpalis is kept on a diet containing equal volumes of reconstituted freeze-dried bovine and porcine blood, irradiated with 100 krad and supplemented with ATP before use. Between January 1981 and December 1981, without any external input, colony size increased from 2,000 to over 24,000 female flies. During the period January 1981 through March 1982, more than 350,000 puparia were produced of which 30,000 were transferred to the BICOT in vitro rearing plant in Nigeria.

The excellent performance in terms of survival, fecundity and offspring size of the colony fed on freeze-dried blood, has permitted the phasing-out of the original stock colony fed on guinea pigs.

It is the purpose of this report to summarize the major methods of rearing and quality control under consideration and to indicate future directions in which research may profitably proceed.

THE PERFORMANCE OF THE RECIPROCAL CROSSES OF G. PALPALIS PALPALIS
AND G. PALPALIS GAMBIENSIS

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ABSTRACT

The reciprocal cross breeding of Glossina palpalis palpalis (Gpp) and Glossina palpalis gambiensis (Gpg) was carried out thus:-

	<u>Female</u>		<u>male</u>	<u>hybrid</u>
Control	Gpp (60)	x	Gpg	Pf
	Gpg (60)	x	Gpp	FP
	(Gpp (60)	x	Gpp	PP
	{ Gpg (60)	x	Gpg	FF

Hybrids obtained from the crosses were also crossed.

	PF	40 x PF
	FP	40 x FP
	(PP	40 x PP
Control	{ FF	40 x FF

Records of the fecundity, mortality and average weight of pupae produced in 54 days for each cross was recorded.

G. palpalis palpalis and G. palpalis gambiensis mated without any structural difficulties, producing fertile progeny. Pupal production of the reciprocal crosses was lower than it was for the intra-subspecies crosses (controls).

For the cross of hybrids pupal production was again found to be lower than it was for the intra-subspecific crosses (controls).

Blocking of a river system against reinvasion by a series of
Challier-Laveissiere traps

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ABSTRACT

Every tsetse control or eradication programme faces as a major danger the reinvasion of previously cleared areas. Therefore a series of experiments was carried out to see if reinvasion of an area in West Upper Volta, selected for a biological control operation, could be prevented with simple and cheap methods, applicable by the local tsetse service and without any external input. This paper presents the results of a trapping arrangement over one year on the river Koba, main river system of the above mentioned area. Following the first results penetration studies with marked flies were carried out, specially during the month of February where in previous experiments the highest mobility of tsetse was found.

100 Challier-Laveissiere traps were placed directly on the banks of the river at intervals of 100 m starting in January 1981. Three times a week captures were recorded following the species and sexes. During the rainy season the traps were placed higher or lower to assure that with rising or falling water level they remained always at the water edge. No barrier clearing was done, only around the traps cutting down of bushes was practised where it was necessary to increase the visibility of the traps. To establish the initial true density two marking-release-recapture sessions were carried out before the traps were installed definitively.

A density per km of river of 38 G. tachinoides and of 65 G.p. gambiensis was found. The density of G. morsitans could not be reliably established due to the low recapture rate.

Following the results of the captures it was decided to release marked flies at both ends of the trapping area to see how quickly and how far they could penetrate. To avoid a too big attractivity of the first

traps flies were fed before the releases. A total of 11565 o and 2545 o of G.p. gambiensis and 692 o of G. tachinoides were released either 100 m upstream of tap 1 or 100 m downstream of traps 100. To distinguish the delay of recapture and the number of traps passed before being caught, different colours were applied for each release session and each release point. The flies were marked with acrylic paints on the thorax.

A comparison of the captures of Jan.-March 1981 to these in Jan.-March 1982 clearly show the influence of the trapping for G.p. gambiensis and G. tachinoides (Graph 8 and 9). As also no complete absence of the riverine species in the inner part of area could be achieved a massive release of marked flies was to yield information about the origin of these remaining flies. Among the 1180 marked o of G.p. gambiensis (=10,2%) recaptured, only 2 were able to cross more than 50 traps. The bulk of the released flies was caught within the first 20 traps of each side. From the 95 recovered o of G.p. gambiensis none had crossed trap 50 and of the 56 recaptured o of G. tachinoides nearly all were stopped by the first 10 traps; no crossing of more than 30 traps was found. Due to the radial dispersion and the subsequent reinvasion from all sides of G.m. submorsitans a drop in population density could not be achieved in spite of the high yields of the traps. Laveissiere and Couret found that traps can restrict that reinvasion (80-89% reduction) so that they can reduce the nuisance in the dry season but for our purpose of protecting an area entirely, the linear arrangement of traps along a river is not sufficient. It is hoped that further experiments with traps impregnated with insecticide will increase the efficiency against this species.

The results confirm, that the chosen system of blocking a reinvasion by riverine species of tsetse is efficient in the dry season.

The first part of the report deals with the general situation of the country and the progress of the work of the Commission. It is followed by a detailed account of the work of the Commission in the various fields of its activity.

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