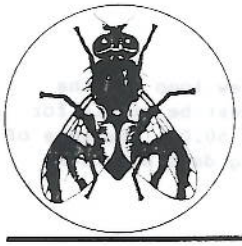

Insect and Pest Control Newsletter

JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE
INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA

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I. GENERAL INFORMATION

A. Logo Contest

The last Newsletter announced a contest for a new logo for the Insect and Pest Control Section. The drawing must be simple for easy reproduction. The winner will receive US$ 50.00. Because of the limited response to the contest, the closing date has been extended to 30 November 1989.

B. The Staff

Our staff, including both those in the Joint Division located in the Vienna International Centre and those in the IAEA's Seibersdorf Laboratory, are listed below with their nationality and the year they started working in Vienna.

Name	Duties
Joint FAO/IAEA Division	
Björn Sigurbjörnsson, Director	
L. LaChance, Deputy Director	
Insect & Pest Control Section	
D.A. Lindquist (USA, 1980)	Section Head, fruit flies
A. Van der Vloedt (Belgium, 1974)	Tsetse flies
B. Butt (USA, 1988)	F-1 sterility
Seibersdorf Laboratory	
R. Gingrich (USA, 1980)	Head, Entomology Unit, microbiology
U. Feldmann (FRG, 1988)	Head, tsetse programme, tsetse mass-rearing
R. Anouchinsky (Italy, 1987)	IAEA Junior Professional Officer, tsetse isozymes
M. Vreysen (Belgium, 1987)	FAO Associate Professional Officer, tsetse mass-rearing
A. Economopoulos (Greece 1985)	Head, medfly programme, medfly mass-rearing
E. Busch-Petersen (Denmark, 1982)	Medfly genetic sexing
P. Kerremans (Belgium, 1987)	FAO Associate Professional Officer, medfly genetics

Dr. E.N. Lambremont completed a successful sabbatical at Seibersdorf and in July returned to the position of Director of the Nuclear Science Center at Louisiana State University, Baton Rouge, Louisiana, USA. We enjoyed Ned's stay with us and wish him all the best upon his return to the USA.

A new position for a molecular biologist has been established at Seibersdorf. This position will be in the Plant Breeding Group, but the molecular biologist will be made available to all scientists at Seibersdorf. The new position is a step forward in our movement into biotechnology.

C. What we want from you

To make this Newsletter "News", we need your input. Please send us information on your SIT and genetic control programmes including plans and opportunities. We would also like each of you to send us slides, video tapes, reports or publications on your research. We would also welcome important citations or brief write-ups on action SIT programmes, as well as summaries of research activities and abstracts.

We are enclosing the standard form for submission of your contribution. Please use a separate form for each contribution and type your name and address, in capital letters, in the upper left block. The text should be no longer than one side of the standard form and double-spaced. We are unable to edit submitted contributions. The abstracts in this issue should not be published or referred to in articles for publication without first obtaining permission from the authors. Please direct contributions and request for information to:

Dr. D.A. Lindquist
Head, Insect and Pest Control Section
Joint FAO/IAEA Division of Nuclear
Techniques in Food and Agriculture
P.O. Box 100
A-1400 Vienna, AUSTRIA

D. The Programme

The overall programme of the Section, including Seibersdorf, is divided into three project areas: tsetse fly, medfly and newer methods of control which include F-1 sterility. We have active R&D programmes at Seibersdorf on tsetse fly and medfly. The limitation of space and resources prevents R&D on F-1 sterility at Seibersdorf.

Currently the Section has the following Co-ordinated Research Programmes:

1. "Laboratory and Field Evaluation of Genetically Altered Medflies for Use in Sterile Insect Technique Programmes".

   G. Gasperi (Italy), M. Loukas (Greece), D.O. McInnis (USA), T.K. Mukiama (Kenya), M.E. Riva Francos (Spain), A. Robinson (UK), Y. Rössler (Israel), J.A. Seawright (USA), J.M.M. Walder (Brazil), R.J. Wood (UK), M. Zapater (Argentina), A. Zacharopoulou (Greece).

2. "Standardization of Medfly Trapping for Use in Sterile Insect Technique Programmes".

   M.H. Blak (Libya), A.M.K. El-Sayed (Egypt), F. Hentze (Guatemala), B.I. Katsoyannos (Greece), A. Lekchiri (Morocco), J. Reyes (Mexico), P.J. Ros Amador (Spain), G.A. Zervas (Greece), A. Zümreoglu (Turkey).

3. "Development of Practices for Area-wide Tsetse Eradication or Control with Emphasis on the Sterile Insect Technique".

   W. Decleir (Belgium), G.O.C. Ekejindu (Nigeria), R.H. Gooding (Canada), P.A. Langley (UK), C.I. Mahama (Ghana), D. Molyneux (UK), L.M. Ogwal (Uganda), T. Soldan (Czechoslovakia), G. Tikubet (Ethiopia).

4. "Radiation-induced F-1 Sterility in Lepidoptera for Area-wide Control".

   A.I. Anisimov (USSR), V. Arthur (Brazil), A. Barbulescu (Romania), J.E. Carpenter (USA), M.S. Hoedaya (Indonesia), V. Mastro (USA), Z.A. Qureshi (Pakistan), H.A. Sallam (Egypt), Yang Rong-xing (China), Zhang He-qin (China).

5. "Genetic Engineering Technology for the Improvement of the Sterile Insect Technique".

   M. Ashburner (UK), A.L. Devonshire (UK), F. Graziani (Italy), F.C. Kafatos (Greece), K. Matsuzaki (Japan), I.C. McDonald (USA), J.G. Oakeshott (Australia).

A research co-ordination meeting on "Genetic Engineering Technology for the Improvement of the Sterile Insect Technique" will be held in Vienna from 16 - 20 October 1989.

A training course, "Use of Isotopes in Entomology", is planned for mid-1990 in Gainesville, Florida, USA.

A national workshop/training course on "Nuclear Techniques in Entomology" will be held in Malaysia in June 1990.

Screwworm

The New World Screwworm, Cochliomyia hominivorax, is established in North Africa. FAO has taken the lead in attacking the problem.

A "Preparatory Meeting on the Formulation of a Regional Strategy for the Control/Eradication of Screwworm in North Africa" was held in FAO, Rome, Italy, from 5 to 6 June 1989. It was attended by specialists on screwworm control and eradication from Mexico, the United Kingdom and the United States, by officials from veterinary services of Egypt, Libya and Tunisia, observers from international organizations, and interested countries in Africa.

The FAO programme for an effective screwworm control/eradication comprises four elements, namely, control of animal movement, treatment of wounds, surveillance, and the release of sterile flies.

The IAEA, through the Joint FAO/IAEA Division, will co-operate with FAO on an eradication programme using sterile flies.

The following are excerpts from FAO Screwworm Information, Issue Nos. 1 and 2:

New World Screwworm in North Africa (excerpt from FAO Screwworm Information, Issue No. 1):

A type of parasitic fly that causes widespread damage to livestock in the Western Hemisphere is now present in North Africa and could pose a similar threat to Africa, the Middle East and southern Europe.

After experts confirmed the presence of the insect known as New World Screwworm (Cochliomyia hominivorax) in North Africa, FAO immediately notified the Organization's Member States which could be affected by the spread of the infestation, saying the parasite "could have disastrous consequences to livestock, wildlife and

perhaps even human populations in Africa, the Middle East and southern Europe. It is feared that the insect can spread rapidly to areas with suitable tropical and sub-tropical climatic conditions in other countries causing damage to livestock industries".

FAO asked for urgent surveillance, identification of the parasite and information on confirmed cases and offered technical assistance.

The screwworm could be particularly damaging to wildlife where inspection and treatment of individual animals is not possible. Livestock can be treated and cured, but frequent inspection and treatment of wounds with insecticide is required to prevent losses. The insect can be eradicated using the Sterile Insect Technique (SIT) as has been successfully done in the USA and Mexico. However, such a control programme will take a long time to develop and is very expensive.

The screwworm is a parasite of warm-blooded animals. The female is attracted to wounds, even those as small as tick bites, where she deposits a batch of eggs which hatch into larvae within a few hours. These larvae penetrate the flesh, increasing the size of the wound. Other females are attracted to the wound, quickly aggravating the situation. Wounds become infected. Non-treatment of affected animals lead to debilitation or even death with consequences to newborn animals being particularly severe.

Any warm-blooded animal including man is susceptible to the screwworm. The insect is a strong flier and can move long distances. Transportation of infested animals was the most significant cause of the spread of screwworm during control programmes in the Western Hemisphere. FAO experts said it is not known how the fly reached North Africa.

Since this insect was previously present only in the Americas, the disease it causes is not familiar to Africans or Europeans.

There is reason to believe that screwworm is spreading and that many countries of Africa are at risk of being infested.

Breeding populations of the pest appear to have been established in North Africa and an immediate assessment of the extent of infestation in neighbouring countries now considered at risk must be made in order to consider measures for its control and possible eradication.

FAO's cable notifying the Organization's interested Member States was sent to Algeria, Chad, Djibouti, Egypt, Ethiopia, Libyan Arab Jamahiriya, Mali, Mauritania, Morocco, Niger, Somalia, Sudan, Tunisia, Yemen AR, Yemen PDR, United Arab Emirates, Qatar, Oman, Kuwait, Lebanon, Saudi Arabia, Jordan, Syria, Iraq, Iran, Albania, Bulgaria, Cyprus, France, Greece, Israel, Italy, Malta, Portugal, Spain, Turkey and Yugoslavia.

What is the screwworm?

The screwworm (Cochliomyia hominivorax) is an obligate parasite in which the female oviposits on any type of wound or abrasion in live warm-blooded animals, including man, causing myiasis. The eggs

hatch within 24 hours and the larvae eat into the tissues. They leave the wound after 5 to 7 days and burrow into the soil to form pupae. The adult flies emerge in 7 to 10 days. The females may mate at two days of age and begin to oviposit 4 days later. The usual life cycle is 3 to 4 weeks, with the adult flies living 10 to 14 days. The pupal stage may persist for as long as 2 months in cool weather. The adult males and females feed on nectar and pollen. The female obtains protein from animal wounds.

Screwworms are not found in cold-blooded animals or in the carcasses of dead animals, unless infestation originated prior to death. Maggots found in such locations are usually larvae of common blowflies.

Until its introduction into North Africa, the screwworm was found only in the Western Hemisphere, primarily in the southern United States, Central America, the Caribbean islands, and South America as far south as Argentina. Freezing winter weather eliminates the pest temporarily, but during warm weather the insect spreads in great numbers into more temperate climates.

The adults are strong fliers and have been recorded to fly 300 km during their lifetime. Transport of infected livestock can spread the pest long distances. In the USA, infestation sometimes occurred as far north as Minnesota and adjacent States, a distance of 1,500 to 2,000 km from where the insect overwintered.

The screwworm larvae chew, tear and eat the living flesh of live warm-blooded animals. As the larvae get larger, so does the wound. The screwworm-infested wound is attractive to adult female screwworm flies, which lay more eggs from which more larvae emerge, further enlarging the wound.

Wounds which attract initial screwworm infestations include navel, dehorning, castrating, shearing (sheep), branding, tick bites and wounds from barbed wire fences.

If not treated, screwworm infestation can cause death of the animal. Mortality rates of 20% or more of infected animals have been reported in the Americas. Death of humans also has been reported.

The screwworm fly does not cause any direct damage and in fact is rarely seen in nature, except around wounds.

Screwworm control

Screwworm control is targeted primarily against the larval stage. Individual wounds are treated with the insecticide coumaphos. Other insecticides may be used, however, they are not approved by all governments. If the wound is infested with screwworm, the treatment will kill the worms. If the wound is not infested, the treatment will, for several days, kill the screwworms which may hatch from eggs laid after treatment.

During livestock management activities such as dehorning, castrating, branding and shearing, all animals must be sprayed or dipped with the appropriate insecticide, if these activities take place during the time of year when the screwworm is active (temperature above about 19^{o}C).

Routine dipping of livestock for control of ticks or other ecto-parasites is effective in curing screwworm infected animals.

Movement of infected animals is the major cause of long distance spread of the screwworm. Thus, effective control of animal movement is mandatory to reduce the rate of screwworm spread. This requires dipping or spraying of animals with wounds. Dogs, a frequent host of the parasite, also must be subjected to inspection and treatment.

The screwworm fly can move considerable distances. Animals in non-infected areas, but close to infected areas, must be under stringent inspection/treatment schedules. Unfortunately, this will not completely stop the spread of the screwworm because of the movement of wild animals and feral dogs and the occasional untreated infected domestic livestock.

Thus, even with the most stringent control of animal movement and a very effective inspection/treatment programme, the screwworm infestation will spread.

The American experience shows that the only effective method to stop the spread of a screwworm infestation is to combine control of animal movement, an inspection treatment programme, and the release of sterile screwworm flies to form a barrier.

Screwworm eradication

Utilizing the SIT in combination with an effective inspection/ treatment programme and with stringent control of animal movement, the screwworm has been eradicated from Curacao, USA, Mexico, Puerto Rico and the Virgin Islands.

The SIT is based on mass-rearing, radiation sterilization and release of sufficient sterile insects to overwhelm the native population. Exposure of 6-day old screwworm pupae to 6,000 rads of gamma radiation sterilizes both sexes. When the wild female mates with a sterile male, no offsprings are produced.

The screwworm mass-rearing facilities in Mexico produced 500 million sterile flies per week from 1983 through 1986 for the Texas/Mexico programme. The facility is now producing about 250 million flies per week.

Screwworm in North Africa

An FAO mission, composed of Dr. V. Kouba and Dr. S.M. Touré, visited Libya from 15 - 22 April 1989 to study the situation related to a possible occurrence of the New World Screwworm fly in Libya. They concluded that:

1. There is evidence that myiases were prevalent in domestic livestock during the summer and autumn of 1988 in the north of Libya with the Tripoli area as the epicentre of occurrence.

2. Entomological determination of specimens given to the mission and subsequently investigated in the laboratory led to the clear conclusion that Cochliomyia hominivorax was effectively a myiasis causing agent in Libya. This reconfirmed previous findings by Libyan specialists.

3. From registered cases in surveyed veterinary clinics it was determined that the situation should be considered extremely serious since cases were reported within a territory ranging from 80 km east to 100 km west of Tripoli.

4. The disease pattern was that of dramatic myiases caused by Cochliomyia hominivorax in domestic animals, i.e. deep wounds including a great number of larvae. Observations were made on sheep (rams, ewes, lambs) in various parts of the body without any specificity (the fatty tail, face, abdomen, anus, vagina, navel, base of horn, mouth). Other animal species were affected in various parts of the body: cows, calves, camels, dogs, stallion. However, the main prevalence was on sheep.

Human myiasis cases were reported in a Tripoli hospital.

5. It cannot be ascertained how this species came to be introduced into Libya from the American continent.

Subsequently, FAO missions visited:

Tunisia: From 9 - 12 May 1989 (Dr. P. Finelle and Dr. R.E. Reichard).
Egypt: From 13 - 17 May 1989 (Dr. R.E. Reichard and Dr. D.A. Lindquist).
Algeria: From 27 - 31 May 1989 (Dr. D.A. Lindquist).

They concluded that, for the moment, there is no evidence that the fly has been introduced into countries neighbouring Libya. However, it is anticipated that without immediate control action, the fly will spread rapidly, particularly during the next few months, when weather conditions are most suitable for the fly.

The following are excerpts from FAO Screwworm Information, Issue No. 2:

"Preparatory Meeting on the Formulation of a Regional Strategy for the Control/Eradication of Screwworm in North Africa", FAO, Rome, 5 - 6 June 1989

The following recommendations for follow-up action by governments, FAO, and on international co-operation, were agreed upon:

1. Countries infested or at risk of infestation, should:

(a) establish and implement national prevention and control programmes, the target being to protect non-infested countries and zones and to eradicate the parasite from infested areas;

(b) establish committees for the control of screwworm at national, provincial and district levels. These committees should include representatives from all ministries concerned;

(c) implement the relevant control activities within the existing infrastructures of veterinary services in close co-operation with public health services, livestock/ agriculture services, farmers organizations, etc.;

(d) consider the possibility of using locust control programme infrastructures for screwworm emergency activities;

(e) establish a national system for screwworm surveillance and reporting, including daily inspection by farmers and treatment of animals, investigations by veterinary and public health services, specimen (larvae) collection, identification and a reporting system;

(f) report screwworm infestation to neighbouring countries, FAO and OIE; this should be followed by regular monthly reporting on the situation in infested countries to FAO's Animal Production and Health Division and OIE, using FAO's questionnaire for monitoring and reporting;

(g) strengthen measures to control movement of animals from infested countries and zones;

(h) establish veterinary inspection and treatment units which should individually inspect and treat all animals from countries or areas in which transhumance occurs;

(i) carry out preventive or curative treatment of individual wounds with a 5% wettable powder of coumaphos. If this product is not available, other organophosphates recommended for the control of ectoparasites should be effective if in a carrier which facilitates wound penetration;

(j) dip/spray whole herds/flocks with:

- coumaphos according to the manufacturer's recommended procedures and concentration for C. hominivorax; or

- a nationally approved organophosphate compound at the maximum concentration for ectoparasites recommended by the manufacturer;

(k) implement an appropriate nation-wide official information and communications campaign;

(l) organize training courses for staff of veterinary services and other staff involved in screwworm control;

(m) contact the respective FAO office or FAO headquarters directly in case of need for FAO technical assistance; and

(n) become familiar with the screwworm eradication programme in Mexico.

2. Countries importing livestock from screwworm-infested countries:

Until the OIE Zoo-Sanitary Code Commission determines import/export conditions, importing countries should request official certification from the country of origin that animals, the point of exportation, and transport route of export are not known or suspected to be infested with C. hominivorax.

Immediately before export, all animals should be individually inspected by an officially accredited veterinarian and all animals with visible wounds separated to be excluded from those which will be exported. If there are any visible fly eggs or larvae in the wounds of individual animals inspected, all animals should be rejected for export.

All unwounded animals for export should be dipped or sprayed under official supervision with:

- coumaphos according to the manufacturer's recommended procedures and concentration for C. hominivorax; or

- a nationally approved organophosphate compound at the maximum concentration for ectoparasites recommended by the manufacturer.

The importing country should inspect all animals at the point of entry and treat all wounds with an appropriate insecticide.

II. MEDFLIES AND OTHER FRUIT FLIES

A. Meetings

1. Past

The FAO/IAEA Regional Seminar entitled, "The Sterile Insect Technique for Fruit Fly Eradication or Control in Latin America" sponsored by FAO and IAEA, was very well attended with 75 participants from 20 countries. Thus the seminar was much larger than normal.

Throughout the seminar two main themes were repeatedly brought forward by the scientific presentations and discussions. These were the need to develop fruit exports and environmental concerns.

Many Latin American countries are working towards the development of exports of tropical fruit to major importing countries. One of the limiting factors in rapidly developing fruit export markets is the presence of fruit flies since many of the importing countries have restrictions (quarantines) on importing fruit from areas where certain fruit fly species exist. Thus many of the scientific presentations on fruit fly taxonomy, host range, trapping techniques, fruit infestation, control, and eradication methods all revolved around the question of "What can we do to remove the fruit fly as a limiting factor to develop export markets?". Since the importing countries write the regulations for importing fruit, the exporting country must follow these regulations. In many cases, eradication, at least in a limited area of a country, appears to be the only realistic approach to solving this problem. The SIT will probably play an increasing role in the development of this approach.

Environmental concerns are not the prerogative of developed countries. Developing countries are rapidly developing sensitivity to this problem and are actively implementing legislation, as well as research and development programmes, to reduce environmental problems. Some of these environmental problems relate to the use of pesticides and, in the case of fruit flies, to insecticides. The desire to develop export markets is partially responsible for concerns about insecticide residues on fruit since the importing countries determine the permissible residues. Considerable activity is being undertaken to use biological control of fruit flies as a means of reducing fruit fly populations and to increase the quality of the fruit produced. However, classical biological control (introduction and release of parasites or predators so that they will become established and effectively reduce the pest population) is not sufficiently effective against fruit flies. There is an increasing interest in the use of the SIT because it is environmentally friendly. The same is true for the use of inundative release of parasites and the development of insect pathogens, such as Bacillus thuringiensis, for use in fruit fly control.

The SIT is being developed for use against the Caribbean fruit fly, Mexican fruit fly, and a number of Anastrepha species.

In some cases, a "fruit fly free zone" will be established for producing fruit for exports. The use of the SIT in these cases offers great opportunities.

The eradication of accidentally introduced new fruit fly species was a topic of much discussion, particularly the recent introduction of the medfly into Belize and Dacus dorsalis into Surinam. The presence of the medfly in Belize immediately terminated their export of tropical fruit to the USA. This was a limited export market, however, it was expected to increase rapidly in the next few years. Thus, medfly eradication from Belize is essential to further develop this export market. The technology to eradicate this species will be the use of malathion bait sprays and possibly the use of sterile flies, however, the availability of sterile flies for the eradication programme is not assured. The Dacus dorsalis situation in Surinam is much more severe. No export of tropical fruit exists from Surinam, however, the pest will move into adjacent countries. When it gets into Brazil it will cause a severe restriction on the export of certain tropical fruit to developed countries. Technology to eradicate Dacus dorsalis is available and includes male annihilation as well as the SIT. Unfortunately, the availability of sterile flies is limited. Both of these examples point out the importance of the availability of sterile fruit flies for eradication of accidental introductions.

The 6th Meeting of the International Medfly Working Group and the 2nd Meeting of the International Anastrepha Working Group were held the week of 27 February in Sao Paulo, Brazil. The objective of both meetings is to bring together research scientists with officials responsible for control or eradication of fruit flies, primarily in the Western Hemisphere. Formal papers are not presented, but topics which have previously been identified as high priority by managers of control or eradication programmes are discussed and recent research findings described. A continuous revision of the high priority research needs takes place at these meetings. When solutions to specific research needs are found, these needs are then either eliminated from the priority list or downgraded from high priority to low priority. By this mechanism, a number of high priority research needs have been solved and eliminated from the research needs list.

During 1988, 23 researchable topics of major importance to large medfly eradication or control programmes were identified. At the IMWG meeting, 6 of these were ranked as high priority. These were:

(a) Improved detection and monitoring of adult wild flies.

(b) Reduce the non-target impact of chemical treatments for population suppression prior to the release of sterile medflies.

(c) New and improved biological control technologies as replacements for chemicals for population suppression prior to the release of sterile insects.

(d) Improved mass-rearing techniques to stabilize mass-production in factories, reduce handling effects on fly quality, and reduce rearing costs.

(e) Continue to improve quality control procedures with emphasis on field parameters.

(f) Development of genetic sexing strains for use in SIT projects.

Reports were made by many scientists working on various aspects of medfly control or eradication. Some of the highlights included experiments to determine efficacy of inundative release of parasites (Hawaii, Mexico, Costa Rica), genetic sexing (IAEA, Hawaii (USA)), the development of hot water treatment as a regulatory procedure for mangoes (many Latin American countries), the continued development of quality control procedures with emphasis on field cage and field assessment criteria (Guatemala, Hawaii (USA)), and the general recognition that biological control, including the SIT, must be developed for medfly control/eradication programmes.

Of the 8 Anastrepha species which had been identified as the most important in Latin America and the Caribbean, sufficient progress has been made on rearing and related studies to initiate SIT experimentation on a fairly large scale on two species. The Caribbean fruit fly will be the subject of a SIT experiment on some of the Florida Keys. The expanding Mexican fruit fly programme in south Texas utilizes the SIT, as it has for the past 15 to 20 years to protect citrus in the Rio Grande Valley from this pest. Sterile Mexican fruit flies are released along the Texas-Mexican border and have effectively prevented the pest from becoming established within the USA.

Considerable discussion was held with regard to the possibility of utilizing some of the Caribbean islands as test sites for the SIT against various Anastrepha species. Because of their relatively small size and in some cases intensive agriculture, eradication of one or more Anastrepha species would pay great dividends, both with regard to the availability of local fruit and also to increased exporting opportunities. It would be appropriate for the IAEA to investigate the possibility of encouraging one or two of these island nations to apply for assistance to initiate such programmes.

Dacus dorsalis is common in South-east Asia, but the infestation in Surinam is the only one in the New World. If Dacus dorsalis spreads into Brazil, which it will in the very near future unless an active programme to prevent spread is initiated, the results will be immediate and costly for Brazil. Also, the presence of Dacus dorsalis in Latin America will greatly increase the chance of this pest being introduced into the Mediterranean Basin. This would have a serious effect on fruit production and exports. A decision whether to attempt eradication of Dacus dorsalis from Surinam will be made in the near future and will be partially dependent on the extent of the area presently infested. Regardless of how

widespread it is in Surinam, an eradication programme is justified because of the extensive losses the pest will cause when it spreads to other Latin American countries.

In conclusion, it can be said that there is increasing interest in development of the SIT for fruit fly control/eradication in Latin America. Despite the frequent occurrence of more than one species of fruit flies in an area, technologies are required to eliminate these species at least in limited areas within the country so that they can develop export markets. The loss of ethylene dibromide (EDB) as a quarantine treatment continues to have an extremely serious negative effect on export of many tropical fruits because of the presence of fruit flies. The major importing countries, Japan and the USA, have very strict regulatory requirements for countries where fruit flies occur. It is very likely that Europe will tighten its regulatory requirements over the next several years as people become more aware of fruit fly related problems which presently do not occur in the Mediterranean Basin. An infestation of an exotic fruit fly species is likely to occur in one of the EEC countries within the next few years. If this happens, intensive discussions will take place regarding the feasibility of eradication as compared to the cost of living with the pest. The presence of one of the Anastrepha species or one of the Dacus species in one of the Mediterranean countries would have an immediate and probably very severe impact on exports of the affected fruits and vegetables from that country to other countries in Europe.

A research co-ordination meeting on "Standardization of Medfly Trapping for Use in Sterile Insect Technique Programmes" was held from 8 - 12 May 1989 in Madrid, Spain. Following is a summary of the meeting:

During 1988, 5 Mediterranean fruit fly trapping systems were compared in 8 different countries, namely, Egypt, Greece, Guatemala, Libya, Mexico, Morocco, Spain and Turkey. The experiments were under different climatic and host tree conditions. The trapping systems tested were:

(a) Jackson Trimedlure (male attractant) plug trap with a white sticky insert;

(b) as above but with a yellow insert;

(c) International Pheromones McPhail trap with Nulure protein and Borax water solution and a Trimedlure plug;

(d) as above plus a DDVP (insecticide) dispenser;

(e) International Pheromones McPhail trap with Nulure protein and Borax water solution; and

(f) as above plus ammonium sulphate.

Trap (b) was very suitable for humid climates (e.g. the rainy season in Guatemala and Mexico), while traps (c) and (e) were very effective in dry-hot areas (Mediterranean summer).

Following evaluation of data, it was decided to discontinue testing traps (d) and (f). For 1989, it was decided to continue the experiments with traps (a), (b), (c) and (e) and to add two new traps; one which is similar to trap (c) but with the Trimedlure plug hung in a different position, the other which will be a dry trap manufactured in Spain and provided with a Trimedlure plug and DDVP dispenser. The latter appears suitable for dry-dusty climates with high medfly populations (North Africa).

2. Future

The final research co-ordination meeting on "Standardization of Medfly Trapping for Use in Sterile Insect Technique Programmes" will be held in Morocco in November 1990.

The research co-ordination meeting on "Laboratory and Field Evaluation of Genetically Altered Medflies for Use in Sterile Insect Technique Programmes" will be held in Vienna in April 1990.

The Third International Symposium on Fruit Flies will be held in Antigua, Guatemala in October 1990.

3. Training Courses

The FAO/IAEA Regional Training Course on "The Sterile Insect Technique for Fruit Fly Control or Eradication" was held from 27 March - 14 April 1989 in Guatemala City, Guatemala. The course was well attended and the participants were very pleased with the presentations and training.

B. Fruit Flies at Seibersdorf

A computer model has been developed which simulates the progression of instability in genetic sexing strains in the presence of male recombination when this instability is influenced by a range of associated genetic and biological parameters. According to this model, male recombination alone was found to contribute relatively little to the rate of instability progression, whereas both a reduction in mutant viability and mutant competitiveness had a strong effect on this rate. Conversely, the viability of the Y-linked translocation was of relatively minor importance. The sex ratio and the ratio of "sexing" allele carriers to non-carriers were found to show patterns characteristic of the factors modelled. This kind of data is important when selecting potentially suitable strains for genetic sex-separation and when contemplating strain replacement under mass-rearing conditions. The validity of this model was shown during the present mass-rearing of the T:Y(wp^+)30C genetic sexing strain, when 5.62% brown female puparia were observed in generation 15 as compared to the 5.8% predicted by the model.

The induction and isolation of temperature-sensitive lethal (tsl) factors is now being phased out. A single tsl factor has been found which has remained stable for 3 generations. The temperature tolerance pattern and the sensitive stage of this mutant are now being investigated. Subsequently, this mutant will be linked to the Y-chromosome with the aim of separating the males and females

in the egg or early larval stages through the application of a discriminating temperature.

The stability of a genetic sexing strain of the medfly, based on dimorphic puparia is being studied under large-scale mass-rearing and selection procedures. After about 15 generations there is still reduced productivity (eggs collected, egg hatch, pupal recovery) but adult emergence and flight ability improved. Breakdown (females emerging from brown puparia) has increased very slowly and is currently about 6%.

Comparison of the mating behaviour of adults of the genetic sexing strain with that of wild flies under natural light in large cages in a greenhouse revealed that:

1. adults of the sexing strain recognized and concentrated their activities on the host trees and fruits;

2. males of the strain participated in mating or mating related activities with wild males and females less than would be expected from the ratios; and

3. when the laboratory-reared males were younger than 5 days or had been sterilized by gamma radiation, their participation in mating activities was reduced.

An experimental field release of only males of the genetic sexing strain is underway in Israel in co-operation with the Citrus Marketing Board. About 2 million male pupae, separated from female pupae by an electronic colour sorting machine, are sterilized and shipped to Israel weekly. After emergence in paper bags, they are released in an isolated, medfly-infested agricultural area containing about 6,000 citrus and 3,000 mango trees.

In co-operation with the Agrochemicals Unit, the heterogeneous nature of the Bacillus thuringiensis exotoxins is being examined by HPLC. By this technique it is intended to find exotoxins that are suitable for use in aerial bait sprays for suppressing wild populations of the medfly. The elution pattern of Thuringiensin, a known exotoxin, has been determined and is being compared to patterns of exotoxins from other strains of B. thuringiensis.

III. TSETSE FLIES

A. General Information

At the request of the Government of Zimbabwe, an IAEA expert, Dr. J.L. Gringorten (Canada) undertook a 4-week mission (5 - 31 March 1989) to the Tsetse and Trypanosomiasis Control Branch, Department of Veterinary Services, within the framework of a project entitled "Tsetse Fly Movement and Behaviour" (IAEA Project ZIM/5/004).

The purpose of the mission was to assist in the implementation of an experiment in the Zambezi Valley involving the use of radioisotopes to study reproduction in field-released tsetse flies.

The main objective of the experiment was to test an injection system for labelling flies with radioactive ^{59}Fe and observe whether bioelimination of the isotope in female flies could be correlated with reproduction under field conditions.

The expert, in collaboration with the project leader, Dr. John Hargrove, demonstrated that injecting tsetse flies with ^{59}Fe is a very useful technique for estimating the interlarval period and fecundity in female flies under natural conditions.

In April 1989, Dr. L.C. Madubunyi (Nigeria), completed a 12-month mission to the Tanzania Livestock Research Organization (TALIRO) within the framework of TC project URT/5/007 entitled "Tsetse Fly Eradication/Zanzibar". The expert's terms of reference were testing traps and targets, studying the ecology of G. austeni and assisting national staff in preparing a protocol and strategy for an eradication programme using the SIT.

B. Meetings

1. Past

The 20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) was held from 10 - 14 April 1989 and the 7th Organization of African Unity's (OAU) International Training Seminar on African Trypanosomiasis was held from 3 - 14 April 1989. Both the meeting and the training seminar were held in Mombasa, Kenya. Drs. Van der Vloedt, Feldmann and Vreysen attended these meetings.

Opening of 20th Meeting of the OAU International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) was by the Hon. Maina Wanjigi, Minister for Livestock Development. The meeting was attended by ca. 350 scientists from almost 40 countries, including representatives of governments and international organizations. More than 130 papers and posters were presented dealing with protozoology, immunology, biochemistry, human and animal trypanosomiasis, entomology, Glossina control as well as planning and management of control, country reports and contributions of international organizations, including the IAEA.

The joint OAU/FAO/WHO leadership training seminar was attended by 50 sponsored participants (medical doctors, veterinarians,

entomologist-vector controllers) from nearly all African countries affected by trypanosomiasis. All countries where the IAEA has on-going TC projects related to tsetse-trypanosomiasis control were represented by at least one scientist in a responsible co-directing and/or decision-making position. The organizers expressed appreciation for the fact that the IAEA, for the first time since the course began, sponsored two participants: Mr. H. Haffidh from the Zanzibar Tsetse Control Unit, Department of Livestock Development and Mr. S. Esiru, from the Uganda Tsetse Control Department.

Dr. Van der Vloedt's contribution to the training course as far as lectures and demonstrations are concerned, dealt with the practical aspects of reproductive biology, tsetse population structures and dynamics, population suppression techniques and basic principles of the SIT. The main objectives of his presentations were to highlight the importance of some biological parameters during entomological monitoring of natural tsetse fly populations, define criteria for evaluating the impact of control strategies (e.g. trapping, use of targets, insecticide-treated animals), explain the mechanism behind sexual sterilization of flies with ionizing radiation, and explain and illustrate the role of sterile males during an eradication campaign.

Lectures and presentations by colleagues were devoted to disease characteristics, diagnosis and treatment and implementation of control strategies based on community participation (case study: "Uganda Sleeping Sickness Epidemic"). An absolute highlight of the training programme was the ca. one and a half days devoted to area development, cost-benefit analysis and project formulation and management.

Also included in the programme was a field trip to the Muhaka field station (Coast Province of Kenya) where workers of the Veterinary Department are studying the efficacy of chemoprophylaxis for the control of trypanosomiasis on some 700 head of East African Zebu cattle maintained in village herds in a tsetse-infested area (G. austeni, G. brevipalpis and G. pallidipes). Diagnostic techniques were demonstrated to the trainees and the KETRI-ICIPE teams (assisted by Dr. Lancien, presently working on the WHO project in Uganda) made an excellent in situ exhibition of a variety of traps and targets and explained the relative merits of combinations of olfactory attractants for catching the East African tsetse fly species.

The seminar provided a unique forum for training, exchanging views and ideas and dissemination of recent information on country-related tsetse-trypanosomiasis problems.

2. Future

A regional training course on tsetse rearing and SIT is planned for Ghana in the fall of 1990.

C. Tsetse Flies at Seibersdorf

Colonies of 7 species of tsetse flies are maintained on in vitro systems in the laboratory (see Fig. 1). They provide material for in-house research and distribution to outside institutes.

The initial stages of a semi-automated feeding system for tsetse flies has been completed. This system should reduce the costs of rearing.

Differences are being sought to separate the sexes of third instar larvae or pupae so that only males are released in SIT projects. No correlation was found between the sex and length of the larval stage or specific gravity of G. palpalis palpalis pupae. However, up to 90% of G. tachinoides males could be identified in the early pupa stage by their distinctive size. The diurnal larviposition pattern of G. tachinoides was determined and efforts are directed to standardize the treatment and holding of immature stages in view of:

(i) facilitating the separation of sexes upon emergence; and
(ii) improving the transport of puparia from rearing facilities to field release sites.

In addition, semi-automatic methods are being developed to reduce the labour required to separate sexes after mating.

The negative effect of high temperature (27 - 31°C) incubation during pupal development was further investigated. The influence of high temperature during different stages of puparial development affects female fecundity.

In follow-up experiments on the hybridization of G. palpalis palpalis and G. palpalis gambiensis, laboratory cage tests showed that increased ratios of gamma sterilized G. p. gambiensis males released in a G. p. palpalis population reduced female G. p. palpalis fecundity.

Radiosensitivity studies of adult G. tachinoides and G. brevipalpis males were initiated. In addition, experiments were set up to assess the influence of different doses of gamma irradiation on G. tachinoides puparia. Increasing irradiation doses and treatments at younger pupal stages reduced emergence rate, male and female fecundity and male viability.

TSETSE FLY SPECIES COLONIZED AT THE ENTOMOLOGY UNIT, JOINT FAO / IAEA PROGRAMME AGENCY'S LABORATORIES, SEIBERSDORF

Group: Species:	country of origin	colony held at Seibersdorf since	Distribution of the groups
palpalis – group			
Glossina palpalis palpalis ROBINEAU–DESVOIDY	Nigeria	1974	
Glossina tachinoides WESTWOOD	Burkina Faso	1984	
Glossina fuscipes fuscipes NEWSTEAD	Central African Republic	1986	
morsitans – group			
Glossina austeni NEWSTEAD	Tanzania, Zanzibar	1986	
Glossina pallidipes AUSTEN	Uganda	1987	
	Zimbabwe	1989	
Glossina morsitans submorsitans NEWSTEAD	Burkina Faso	1988	
fusca – group			
Glossina brevipalpis NEWSTEAD	Kenya	1986	

Fig. 1

IV. NEW TECHNIQUES FOR INSECT CONTROL

A. General Information

Review papers were prepared on the diamondback moth, Plutella xylostella, and the Colorado potato beetle, Leptinotarsa decemlinata. These papers will not be published, but are available upon request.

B. Meetings

The second Research Co-ordination Meeting on "Radiation-induced F-1 Sterility in Lepidoptera for Area-wide Control" was held in Beijing, China, 22 - 26 May 1989. Six of the seven contractors and one of the three co-operators attended the meeting. Results were reported on European corn borer, Asian corn borer, Spodoptera litura, S. littoralis, sugar cane borer, codling moth and wild silkworm. Rearing is still a major problem with most insects. To reduce costs, locally bought products are being evaluated as replacements for imported materials.

In general, the insect should be treated with 100 GY in the late pupal stage. There was a high level of sterility in the F-1 generations, but less in the F-2. There was very little apparent effect on the F-3 generation. There were more males than females in the F-1 generation.

F-1 insects can be identified by examining the chromosomes using the squash technique.

In codling moths, and probably other insects, there are two types of sterility, one being completely sterile and the other partially sterile. The individual pairs should be crossed to separate these two types of sterility.

In the future, the main focus of the programme will be on the diamondback moth, Plutella xylostella and the pink bollworm, Pectinophora gossypiella.

THE SHEEP BLOWFLY GENETIC CONTROL PROGRAM IN AUSTRALIA

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INTRODUCTION

The blowfly *Lucilia cuprina* is the most important myiasis pest of sheep in Australia(3). Other species are associated with sheep myiasis, but *L. cuprina* is probably responsible for initiating more than 90% of infestations. Annual costs of production losses, prevention and treatment have been estimated at $149 millions in 1985(1). Prevention and treatment encompass both insecticidal applications to sheep and non-chemical management practices. In the absence of effective preventive measures, the sheep industry would be non-viable over much of Australia.

Insecticide usage against *L. cuprina* has been marked by the appearance of widespread resistance to cyclodienes in 1956, the organophosphates in 1965, and carbamates in 1966(12). Resistance has not yet been reported against the triazine compounds introduced for blowfly control in 1981.

The most effective non-chemical control measures are surgical (removal of skin from the breech in certain breeds of sheep, and tail-docking). They protect sheep by reducing favourable oviposition sites (dung and urine-stained wool).

The spectre of insecticide resistance and the early success of the sterile insect technique (SIT) against screwworm fly in the U.S.A., led this Division to consider SIT and other autocidal methods in the 1960s. The *L. cuprina* genetics research program was established in 1966 and subsequently expanded in 1971. More recently, lobbying by animal welfare groups against surgical blowfly control practices, as well as increasing consumer awareness of insecticide residues in animal products, have accelerated the search for alternatives to chemical control.

When SIT was first considered for *L. cuprina* control in 1960, little was known about the population dynamics of *L. cuprina*. There were insufficient ecological data to evaluate the prospects of alternative strategies such as suppression or containment. The number of flies which would have to be released in a SIT program was unknown, as were the costs. Assuming that the cost of SIT against *L. cuprina* in Australia would be similar to that reported for screwworm in Florida, the costs of a SIT campaign were estimated to be several times the potential annual benefits(17).

The discovery of meiotic drive (MD) in *Drosophila* rekindled interest in autocidal control and triggered establishment of the *L. cuprina* genetics research program in 1966. Subsequent research failed to detect MD systems suitable for genetic control, but by 1970 it was recognised that strains carrying chromosome rearrangements might be more cost-effective than SIT(6,18). This led in 1971 to expansion of the genetics and strain development program, and establishment of an ecological research program (quantification of population dynamics)(21). Both programs have been enormously successful. Not only have they enabled the development and successful field testing of a genetic method of suppressing sheep blowfly populations, but they have increased our knowledge of this pest to the stage where a simulation model of genetic control (GENCON), which incorporates both genetic and ecological data, is a valuable tool in strain design, selection of control strategies, and evaluation of field trial data.

Comparison using computer simulations, of SIT with female-killing (FK) systems based on sex-linked translocations, suggests that with a suppression and containment strategy, the latter would provide greater benefits for less cost than SIT(5). Moreover, our projections indicate that large-scale genetic control would be less costly and more effective than the current system based on insecticides and other control practices.

ECOLOGICAL OVERVIEW OF THE SHEEP BLOWFLY PROBLEM

L. cuprina probably breed for 6 to 10 generations per year in various parts of their range(14), and overwinter in the soil as mature larvae in cooler areas(2,3). Although *L. cuprina* are capable of moving distances in the order of 20 km during their life span, they are normally much less

mobile(8,11,13). They are probably not capable of natural migration across large bodies of water such as Bass Strait.

There is a complex interaction between weather, fly density, myiasis incidence and the response of farmers to rates of infestation(3). The latter are related both to fly density and to sheep susceptibility, which are in turn influenced by weather conditions(16). Mild wet weather both increases the availability of favourable oviposition sites and keeps soil temperatures cool enough to permit larval and pupal survival, both of which tend to cause higher fly densities. Hot dry weather reduces the number of oviposition sites and leads to lethal soil temperatures, resulting in lower densities.

Increasing rates of infestation of sheep cause increasing proportions of farmers to treat sheep with insecticides. This reduces survival of larvae and pupae, and leads to lower fly densities the next generation than would be the case without the treatments. The net effect of this is to create a form of density-dependent population regulation, i.e. an inverse relationship between fly population density and the rate of population increase(3).

STRAINS WITH POTENTIAL FOR GENETIC CONTROL

Of the types of chromosome changes considered for genetic control (homozygous translocations(18), compound autosomes(7), and sex-linked translocations(19,20)), sex-linked (Y-autosome) translocations have shown the most promise.

The results of several field trials indicate that female-killing (FK) systems constructed from sex-linked translocations and deleterious genes such as eye colour mutations, are sufficiently effective in the field to justify evaluation of their commercial potential. This type of strain causes genetic death partly from semisterility caused by the chromosome rearrangement, and partly from death due to homozygosis of the mutations, mainly in the female descendants of the released males(4,19,20). Genetic death from semisterility and homozygosis persists for many generations after cessation of releases, giving this control system a considerable advantage over SIT, in which suppression ceases when releases stop(5,9).

FIELD TRIALS

Two recent field trials have demonstrated the technical feasibility of the FK system of suppressing *L. cuprina* populations. A third larger trial, involving releases on the Furneaux Is group in Bass Strait, is scheduled to begin in October 1989.

The first of the recent trials was conducted in the southern Shoalhaven River valley of N.S.W. (240 km^2) during the 1984-85 season(10). A pre-release study had established that population trends in the release area and two nearby non-release areas were similar over the three years of the study(15). Weekly releases of 400 males/km^2 achieved a 9:1 average released:wild male ratio, which resulted in a genetic death rate of 50% per generation. Populations in the release area remained at or below springtime levels for the entire season, whereas peak-season populations were 24-fold and 31-fold higher than springtime levels in the two non-release areas.

Using GENCON as a predictive and analytical tool, ecological data from the non-release areas was combined with genetic data on matings by released males, to give predictions of the rates of genetic death and population suppression. This simulation predicted that genetic death levels should have averaged 90% and the population should have been suppressed to a level 20-fold lower than that observed. Thus, while the results of the Shoalhaven trial were highly encouraging, the rates of genetic death and degree of population suppression fell somewhat short of expected levels. Further simulations suggested that the differences between observed and expected genetic death and population levels, could be minimized by simulating immigration of wild flies into the release area. On average, 12% of the females in the release area were estimated to be mated immigrants.

The Shoalhaven trial findings suggested that suppression in the trial area was limited mainly by immigration. To test this finding, a trial was conducted on Flinders Is, 40 km^2 in area and 27 km off the coast of the Eyre Peninsula in South Australia, i.e. effectively in the absence of a source of natural immigrants(9). This island was run as a single sheep grazing property, and carried 6,000 merino sheep. As insecticides were not used on sheep on this property, very large *L. cuprina* populations were present.

Weekly releases of 34,000 males/km^2 on Flinders Is in 1985-86 resulted in a peak genetic death rate of 87% after six months, and a decline of the population from a peak of 345 females/ha in October 1985 to less than 1 female/ha (i.e. undetectable by trapping) in May 1986 (eight months), at which time releases were discontinued. Both the genetic death and population suppression results were in line with the theoretical predictions in the absence of immigration. The maximum genetic death rate observed was close to the maximum 94% possible with this strain.

Fly populations and (where trapped flies were available for testing) genetic death rates on Flinders Is, were monitored for a year following cessation of releases. The population remained undetectable until October 1986, and recovered very slowly because of the continuing genetic death from translocation semisterility and homozygosis of mutations. It did not exceed 4 females/ha until March 1987.

The next field trial, in the Furneaux Is group (1992 km^2 in Bass Strait, is planned as a joint exercise with the Tasmanian Department of Agriculture. A pre-release ecological and economic survey begun in 1987-88 has been intensified in 1988-89. Releases are scheduled to begin in September 1989. The target is to complete eradication from these islands within two years. The trial will be monitored using ecological, genetic and economic criteria. Associated rearing, transport and release costs will be evaluated with respect to the economic feasibility of expanding the technique to larger land areas such as Tasmania and the mainland of Australia.

TECHNICAL STATUS OF AUTOCIDAL CONTROL METHODS FOR LUCILIA CUPRINA

Because of the similarity of the life cycle of sheep blowflies and screw worm flies, some of the rearing and release methods used in the American screw worm program have proved to be adaptable to *L. cuprina*. However, it would seem preferable in Australian conditions to invest capital in one or more high-technology rearing plants and employ a relatively small number of skilled workers, than to install a labour-intensive rearing methodology as was done in the screw worm rearing plants in Texas and Mexico. This would result in savings in rearing costs, greater immunity from human error/industrial disputation, and reduction of workers' compensation liability for both physical injury and debilitating occupational diseases such as respiratory allergy to blowflies.

Systems for handling bulk materials and automation of the entire mass-rearing and packaging processes are currently being developed. Much of this can probably be accomplished by use of machinery such as that presently used in the food processing and grain-handling industries.

Larvae are reared on a diet comprising meat and bone meal, cotton linters and water, costing approximately $A50 per million larvae produced. Flies are reared to maturity and chilled for transport and release once adults have hardened their wings. Release of chilled adult flies from aircraft is likely to be the preferred release method over most of Australia.

Sex-linked translocations can be used to couple lethal mutations to sex, and thus have considerable potential for the elimination of unwanted female flies, with large savings in rearing and release costs. The system used to harvest newly emerged adult flies for chilled adult release has the potential to eliminate the majority of females as adult flies, from the current FK strains prior to release. This sex separation depends mainly on behavioural differences between mutant females and wild-type males. Development of early-acting female killing systems based on temperature-sensitive egg-stage lethal mutations, is currently a top priority.

IMPLEMENTATION - UPTAKE BY GOVERNMENT/PRIVATE SECTOR

Because of the mobility of *L. cuprina*, the choice of whether to employ autocidal control methods cannot be left to individual graziers except perhaps in isolated areas. Regional control programs involving large areas are the only conceivable means of effectively using such methods.

The question of uptake by the government versus private sector raises several questions, among them: (i) the relative efficiency of private enterprise vs government agencies; (ii) what private enterprise would have both resources of sufficient magnitude to undertake such a massive task and the willingness to risk them in such a venture; (iii) if private enterprise were to run an autocidal control program, who would assess performance and how would payment for results achieved be made.

These questions are especially relevant where state borders are involved, as in southeastern mainland Australia. Possibly state and federal legislation will be required, to establish authority for funding and quarantine procedures.

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PROGRESS AND PROBLEMS IN THE CYTOLOGY OF *Ceratitis capitata*

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Introduction

In recent years cytological work with *Ceratitis capitata* has expanded rapidly in concert with increased interest in the application of genetics and molecular biology in the control of this species (IAEA 1985a,b, 1987). Previous studies using mitotic chromosomes produced essential basic karyotypic data but resulted in a confused situation in which several chromosome nomenclature systems were in use. The need for better cytological methods and a standardisation of nomenclature was recognised by the IAEA (1985a) which recommended a system based on that used for *Drosophila melanogaster*. Polytene chromosome analysis was soon available with the development of techniques for preparing chromosome squashes from male pupal orbital trichogen cells (Bedo 1986, 1987) and larval salivary glands (Zacharopoulou 1987).

The application of chromosome banding techniques quickly provided detailed morphological information on mitotic chromosomes, particularly the sex chromosomes (Bedo 1986), and mapping of chromosome rearrangement break-points on newly prepared polytene chromosome maps commenced (Bedo 1987). This report describes recent unpublished results and offers comment on the still unresolved nomenclature problems in *C. capitata*.

Recent Advances in the Cytology of *C. capitata*

Quinacrine fluorescence studies revealed multiple bright bands in the X chromosome and a double band in the Y chromosome long arm (Bedo 1986). I have now applied Counterstain-enhanced fluorescence which highlights chromosome regions with particular DNA base composition. Distamycin-DAPI (DA-DAPI) staining reveals the X chromosome Q bands to be largely AT rich. The GC specific stain Chromomycin A3 (CMA) does not produce bright bands but elevates background fluorescence in the Y chromosome suggesting that it may have a somewhat higher GC content than other chromosomes. In the X chromosome a complementary fluorescence pattern between AT specific and GC specific stains is not clearly seen, indicating that this chromosome may have a complex structure. This is further emphasised by the correspondence of silver staining to the fluorescent bands and the lack of C banding in much of the X chromosome long arm where these bands occur.

The application of new fluorescent stains did not differentiate any of the autosomes. All autosomes have AT specific fluorescence at their centromeres with no other bright bands to serve as distinguishing features. The difficulty in recognising the smaller autosome pairs which have similar lengths and arm ratios (Bedo 1987) has therefore not been resolved.

In testis preparations, meiotic chromosomes show a marked reduction in the intensity of fluorescence and a loss of banding detail. The potential for using fluorescence banding for investigation of meiotic pairing is therefore limited. Nevertheless X chromosome fluorescence is consistently brighter than that of the Y and can be easily traced throughout meiosis and sperm development. Mature sperm can be scored as X or Y bearing by observing their fluorescence characteristics.

Nucleolar structure

I have used *in situ* hybridisation to examine the nucleolar structure in several tissues of *C. capitata*. A tritium labelled ribosomal gene probe originating from *D. melanogaster* gave good results. Mitotic karyotypes are clearly labelled in the short arms of the X and Y chromosomes. In polytene nuclei from trichogen cells,fat body and salivary glands labelling occurs within the nucleolus concentrated over a net-like core. Thus nucleolar structure in different polytene tissues does not vary as dramatically as autosomal banding patterns.

The problem of Chromosome Nomenclature

The goal of standardising chromosome nomenclature (IAEA 1985a) has been hampered, and the problem compounded, by the inability to distinguish the smaller members of the mitotic complement (Bedo 1987) and the differences in banding patterns of trichogen and salivary gland polytene chromosomes (Bedo and Zacharopoulou 1988). The maps now published for trichogen cell (Bedo 1987) and salivary gland (Zacharopoulou 1988) chromosomes were independently made so that homologous chromosomes are not aligned.

Several nomenclatural problems have arisen from these findings. The inability to reliably recognise the smaller autosomes in mitotic cells makes even the application of a basic *D. melanogaster* based numbering system difficult. Accurate correlation of diploid and polytene karyotypes is also not possible for all elements. In addition, although it is possible to define homologous chromosomes using translocation analysis (Zacharopoulou, unpublished), trichogen and salivary gland chromosome maps cannot be aligned due to banding differences between the tissues.

One question of nomenclature is whether to renumber one of the polytene chromosome maps so that homologues are in the correct order. Two arguments can be made against this action. First, because the section numbers vary between the maps, loci will still have different designations in trichogen and salivary chromosomes. Given the effort required in translocation analysis and *in situ* hybridisation for example, it is unlikely to expect a band for band correlation of the two maps to be achieved for a long time. It is likely that there will always be two polytene chromosome mapping systems. Second, because polytene chromosome elements cannot be accurately correlated with the smallest autosomes of the diploid set, the future discovery of a reliable means of recognition may necessitate yet another renumbering if the assignment proves to be wrong. At this stage it seems prudent not to alter the numbering of polytene maps to avoid the possibility of further confusion in the future.

The wish for a neat, logical system of nomenclature appears to be frustrated by the characteristics of the organism itself.

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Recombinant DNA Technology and Insect Control

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IN THE PAST, the most successful avenue for the use of genetics in insect control has been the employment of the sterile insect technique, in which huge numbers of a species are produced in a factory, sterilized by exposure to ionizing radiation and released into the native habitat. This method is suitable for some species, but for logistical, economical, and biological reasons this control technique is not suitable for many economically important species. Our ability to use genetic approaches to cope with the myriad of insect pests will improve in the near future because of progress in the biochemical manipulation of genes. Molecular geneticists have created bacteria, plants, animals, and fungi that have useful new properties, and many of these are being used or tested for commercial use. A reasonable forecast is that a virtual revolution will occur in the way that we currently practice and perceive the genetic control of insects. Using genetic engineering manipulations to develop control techniques for insects of agricultural and public health importance is an exciting prospect and a highly desirable goal.

The first review (Cockburn et al. 1984) of the application of molecular genetics to insect control appeared only five years ago. Even though the use of gene manipulation for insect control is still in the conceptual, formative stage, there are techniques available for cloning, sequencing, splicing, and otherwise characterizing and manipulating genes. Application of these scientific tools by insect control specialists is currently stymied because of the lack of widely applicable techniques for germline transformation (the stable insertion of DNA into a genome). Efficient transformation systems are available for Drosophila melanogaster and a few of its close relatives, but these systems have so far not been useful for economically important insects. Without the ability to insert a cloned gene (modified in vitro to suit the purpose at hand) into either a homologous or heterologous genome, the engineering of genetic control systems will not be practical, and molecular genetics will not have a great impact on entomology. After this barrier is surmounted, the future application of modern molecular genetics will largely be a matter of creatively using contemporary knowledge.

The subject of applying genetic engineering to insect control is too extensive for a thorough coverage in this brief format (see Cockburn et al. (1984) and Cockburn and Seawright (1989) for references and more detail). In this present paper, we will confine our comments to the present status of germline transformation, possible solutions for overcoming this problem and a few general ideas on using genetic engineering for insect control.

The P element transposition system of Rubin and Spradling (1982) is widely used in transformation of D. melanogaster and closely related species. The P element is a transposon (jumping gene) that is extremely active when clones are injected into preblastoderm embryos. Introduced P elements transpose from the injected DNA onto the chromosomes of the germline pole cells and are passed on to the progeny. Other genes can be introduced into the germline by inserting them into a modified P element in vitro and then injecting them along with an intact P element into embryos (Rubin and Spradling, 1982). Unfortunately, the P element vectors have been unsatisfactory for mediating transformation of Lucilia cuprina, Ceratita capitata, Anastrepha suspensa, Plodia interpunctella, and Musca domestica. Recently, O'Brochta and Handler (1988) have studied P element mobility in other dipterans and found that P element mobility (precise excision) occurred only in drosophilid flies. Accordingly, most research groups have abandoned attempts to transform other insects with the P element vectors now available.

The few reports of non-transposon mediated transformation in insects appear to be instances of insertion via non-homologous recombination. However, the rate of achieving transformants is clearly too low (on the order of one transformant per several thousand injected embryos at best) to be of widespread practical use. Even though microinjection of DNA is feasible for the embryos of most insects, this technique is so tedious that a single person can inject only a few hundred embryos per day.

An obvious, worthwhile strategy for development of transformation techniques for insect pests is to isolate transposons, with properties similar to P element, from the pests. In D. melanogaster most of the moderately repetitive DNA consists of transposons, so isolation of transposons simply requires screening clones for moderately repetitive DNA. However, of about 100 families of transposons, only a few (P, hobo, I) transpose frequently enough to be worth considering for development as transformation vectors (Spradling and Rubin, 1981; Kidwell, 1984). Other transposable elements in D. melanogaster, e.g., copia, transpose at a much lower rate and are not useful in promoting transformation. Suitable transposons would probably be specific in application to the particular pest in which they naturally occurred, so each species, or group of closely related species, would have to be considered separately.

Another possible strategy involves the use of physical methods for the mass injection of DNA into thousands of embryos in a simple one-step operation. Since integration of DNA clones via non-homologous recombination will occur at a very low frequency, this should not adversely affect viability. Because of the large numbers of individuals that could be processed, mass injection techniques would make transformation practical even if efficient vectors are not developed.

Our research group has tried several methods involving mild physical trauma to mass inject embryos. Insect embryos are surrounded by a vitelline membrane and a tough chorion (which can be removed from some species). The problems in introducing DNA (or other materials) into insect embryos are similar to those of introducing DNA into plant tissue, which is surrounded by a crosslinked cell wall.

Preliminary evaluation of microparticle bombardment (Carlson et al., 1989) and sonication suggested that those techniques had promise, but would require considerably more development to be practical. We are currently using a vortexer and silicon carbide "whiskers" to make small holes in insect eggs. The eggs are immersed in a DNA solution containing the whiskers and vibrated. In preliminary experiments, we have successfully injected a non-toxic dye into housefly, caribfly, and Drosophila embryos. Using a vortex time of 60-90 seconds, essentially all embryos took up dye, and 10-30% survived to hatching. Shorter vortex times gave better survival, with less efficient injection. Using the plasmid recovery assay of O'Brochta and Handler (1988), we found that the effective volume of DNA solution introduced into embryos was about 10% of the volume that could be microinjected, and that the DNA persisted into the larval stage. Currently transformation experiments are underway with Drosophila to demonstrate that DNA can be introduced into the germline using this technique.

In the remainder of this paper we will present the basic ideas involved in altering genes for insect control schemes. Genetic engineering of genetic control systems is a much more complicated venture than often envisioned, but the payoff from success in this area will certainly change the control of insect pests.

For the sake of simplicity, a typical gene can be depicted as in Figure 1. The gene consists of basically two discrete parts, viz. the DNA sequence which codes for the final protein product and the regulatory sequences (enhancers and promoters) that control gene expression. It is usually possible to make functional hybrid genes by joining a protein coding sequence from one species to a regulatory sequence from another species. The existence of conditional promoters, activated by environmental stimuli, e.g., heat shock (reviewed in Ashburner and Bonner 1979), enable the engineering of genes that would be expressed upon imposition of the environmental condition.

With some imagination, one can envision how hybrid genes controlled by conditional promoters create many novel possibilities for insect control. Perhaps a good example would be the improvement of the sterile male technique, since this methodology is currently used for control of several pests such as the screwworm and the mediterranean fruit fly. A colony that has been genetically modified by germline transformation with suitable gene constructs, e.g. a lethal gene conditionally expressed in females and a sterility gene conditionally expressed in males, could be used to kill females and sterilize males by imposition of the environmental condition. The mass-produced colony could be reared with normal fertility and sex ratio under a set of permissive conditions, but under selective conditions, such as heat shock, the females could be killed and the males sterilized. Genetic sterilization would eliminate the somatic damage usually associated with the use of chemicals or radiation. Tissue, sex, or developmental stage specific promoters can be isolated and used for constructing hybrid genes that would disrupt the physiological processes that are necessary for normal development. The issue of determining which promoter and which gene to use will be resolved only after sound methods are

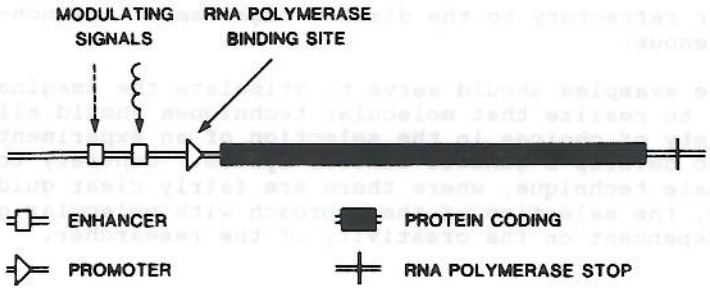

Figure 1. Modulating signals (hormones and intracellular factors) act at enhancer sites to turn genes on or off. When a gene is on, RNA polymerase can bind at the promoter and transcribe the gene. A downstream stop site causes termination of transcription.

developed for transformation, when systematic screening of hybrid gene constructs can be conducted in vivo.

When the necessary information is available for the synthesis of effective genetic control strains of pest insects, the actual process of using this knowledge can proceed principally along two lines, either management/eradication through increasing the genetic lethal load or replacement of the pest with an innocuous form. The choice of strategy will be limited by economic considerations, biological constraints, and environmental safety.

For increasing genetic lethal load, a genetic anomaly that will spread in a population has always been considered as the most desirable situation, because of the relatively inexpensive input of making only a small inoculative release of genetically-altered insects. Currently, there is much interest in transposons and meiotic drive, because they appear to have the desirable attributes for increasing the genetic load by spreading disadvantageous genes vertically in a pest population through the germ line. Hickey (1982) has shown that a transposon can spread in a population, in spite of causing deleterious mutations, chromosome breakage, inappropriate expression of genes, and cell death. This may explain the presence of the P element, which causes all of these deleterious effects (Rubin et al. 1982, Engels 1979). Meiotic drive systems are known from many species, in which the chromosome carrying the driving gene is preferentially passed on to the next generation (reviewed in Zimmering et al. 1970). The best characterized system is Segregation distorter (Sd) from D. melanogaster (reviewed by Ganetzky 1977), in which the presence of the Sd gene causes the homologous chromosome to break, resulting in inviable gametes.

Since eradication is not always a desirable objective, the replacement of a pest with a genetically modified, innocuous form will be an option for some situations. For example, in the case of a hematophagous mosquitoes, strains could be assembled that

are either refractory to the disease organism or are non-biting and autogenous.

These examples should serve to stimulate the imaginative scientist to realize that molecular techniques should allow for a wide variety of choices in the selection of an experimental program to develop a genetic control system. Contrary to the sterile male technique, where there are fairly clear guidelines to follow, the selection of the approach with molecular genetics will be dependent on the creativity of the researcher.

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VI. PUBLICATIONS AND ABSTRACTS

A. Publications from the Entomology Unit, 1988-1989

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B. Abstracts Received

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LABORATORY MAINTENANCE OF GLOSSINA AUSTENI (NEWSTEAD) BY THE IN VITRO TECHNIQUE

"Laboratory maintenance of large numbers of tsetse flies by the in vitro feeding technique is hampered, particularly so in the tropics, by bacterial contamination of the blood-diet. At the Tsetse and Trypanosomiasis Research Institute - Tanga, Tanzania, efforts to rear a large colony of G. austeni in vitro using cattle blood alone collected from the local abattoir, have been going on satisfactorily for the last three years. Flies which died with blood in their abdomens (blood mortality) were sent to the "Animal Diseases Research Institute", Dar es Salaam, for bacterial identification. The following bacteria, using Nutrient Broth technique, were identified as the main cause of mortalities: Escherichia Coli, Proteus Sp, Haemolytic and Non-haemolytic Stroptococci. Efforts taken to minimise blood-mortality include: Sterilization of feeding membranes and plates at 120°C over-night, Sterilization of the feeding-room with Ultra-Violet light over-night, Irradiation of the blood-diet at 150 Krad with gamma-rays from a Caesium - 137 source prior to feeding flies with blood which has attained temperatures between 36° - 37°C. All these measures have greatly reduced blood mortalities".

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AN APPRAISAL OF CERTAIN LARVAL BEHAVIOURAL ACTIVITIES AS AFFECTED BY GAMMA IRRADIATION OF SPODOPTERA LARVAE

J. Nuclear Agric. Biol. 16 : 183-186 (1987)

ABSTRACT

Gamma irradiation of Spodoptera litura larvae induced an abatement in their locomotor activity. The food perception range and feeding behaviour of the larvae were also impaired. Of various dosages tested, 4 Krad (the sterilizing dose for larvae) caused a decline in locomotor speed. The first instar larvae, for instance, showed decline in a speed of 3.74 cm/min to 2.96 cm/min. Similarly at this dose, the third instar larvae exhibited a reduction in speed to the extent of 38 to 45 per cent,

the normal speed being 8.02 cm/min. The reactive distances of the first and third instar normal larvae were 5.30 cm and 7.36 cm, respectively. This reactive distance was reduced by about 25 per cent after irradiation at 4 Krad, thus decreasing the food perception capacity significantly. The feeding behaviour too was significantly affected whereas the dose range less than 4 Krad did not induce such a marked impact.

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IMPACT OF GAMMA IRRADIATION ON LARVAE OF SPODOPTERA LITURA (F.) WITH REFERENCE TO ITS EFFECT ON GROWTH AND DEVELOPMENT

New Entomologist 36 : 1-11 (1987)

ABSTRACT

Studies to determine radiosensitivity of Spodoptera larvae in different instars showed the competency of larvae irradiated at various sublethal doses of γ - radiation to metamorphose and grow up to the pupal and adult stages. Newly hatched larvae of S. litura treated with different gamma doses (1-7krad) suffered a reduction in the percentage of pupa formation and adult emergence. Besides, they exhibited a retarded development and affected growth index. Also, these larvae gave rise to malformed pupae and adults. A preponderance of males over females was another deleterious effect among the adults emerged from such larvae. The adverse effects of radiation were also conspicuous on the larval weight and growth rate. Similar effects were noticeable when the larvae in the later instars (third and sixth) were treated with gamma rays though the intensity of the irradiation impact was lesser in the older larvae.

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CHANGE IN THE SUSCEPTIBILITY OF SPODOPTERA LITURA (FABR.) (LEPIDOPTERA, NOCTUIDAE) EGGS OF DIFFERENT AGE TO GAMMA RAYS IN RESPECT TO GROWTH, METAMORPHOSIS AND REPRODUCTIVE POTENTIAL

Pol.Pismo Ent. 58 : 689-701 (1988)

ABSTRACT

Irradiation effects on the eggs of Spodoptera litura, 1-, 2- and 3-day old, were evaluated in relation to growth and development and reproductive performance. When different age-eggs were treated with several sublethal doses of γ-radiation, per cent hatching, pupation and adult emergence decreased in relevance to the increase of dose. Larvae resulting from treated eggs suffered from procrastination of their development in the subsequent stages thus adversely affecting the growth index of these larvae. The sex ratio was distorted and skewed in favour of males. The third, fifth and sixth instar larvae showed reduction in their weight and growth rate as a consequence of γ-irradiation. Also, the pupal weight was reduced due to irradiation. The ensuing pupae and adults from treated eggs exhibited some morphological abnormalities. Fecundity, fertility and longevity of adults derived from irradiated eggs were reduced when P_1 adults were assayed. The wing expanse and body length of the adults were also affected at high sublethal doses, exhibiting the size reduction. The magnitude of the impact of gamma irradiation on the growth and fertility was more profound when the eggs of younger age were exposed, reflecting an excessive radio-sensitivity of the early embryonic stage.

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EFFICACY OF GAMMA-RAYS IN MODIFYING BEHAVIOURAL TRAITS OF SPODOPTERA LITURA (FABR.) AND THEIR EVALUATION IN F-1 GENERATION

Proc. XVIII Int. Congr. Entomology, Vancouver, Canada (1988)

ABSTRACT

A negative relationship between the age of pupae of Spodoptera litura at the time of irradiation and γ-radiosensitivity was exhibited when evaluated in terms of growth, development and reproduction.

Protracted growth with morphological deformities in the ensuing adults were quite evident at the dose range tested (2 to 20 krad). Gamma irradiation of pupae caused significant reduction in fecundity, egg-hatch, longevity and mating frequency in the crosses involving treated males.

Moths irradiated with different doses (4 to 25 krad) as 1 to 2 day old adults exhibited a decrease in egg-laying, egg viability and adult longevity. The sterilizing dose had a significant influence on these aspects. Irradiation had a little effect on the mating frequency. Specificity in the diel patterns of certain behavioural traits was studied in normal and treated adults at the sterilizing dose. Asynchrony in the rhythms of feeding, mating and ovipositional behaviours was apparent between treated and normal moths, hence lessening the competitiveness of irradiated insects. This incited us to assess the potential of sub-sterilizing doses, specially on F-1 progeny which exhibited protracted growth and distorted sex ratio. Two main attributes of using substerilizing doses for S. litura were ascertained: first, a higher degree of inheritance of sterility in F-1 progeny; second, higher competitiveness.

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ROLE OF AGE AFFECTING NUTRITIONAL BUDGET OF SPODOPTERA LITURA LARVAE IN RELATION TO GAMMA IRRADIATION

Proc. Natl. Symp. on Nuclear and Allied Techniques in Agriculture, Medicine and Environment Research, I.A.R.I, New Delhi, India (Sept. 6-9, 1988)

ABSTRACT

Computation of correlations between nutritional parameters of the sixth instar larvae of Spodoptera litura (Fabr.), exposed to gamma radiation doses ranging from 2 to 7 krad administered in the first, third and sixth instars, was done. This was carried out to understand the role of age of Spodoptera larvae on their nutritional indices. Phagoperiod (PP) showed a positive correlation with weight gained and food balance at 2-6 krad dose range. This relation was insignificant

at higher dose of 7 krad which, however, exhibited a positive relation between the faecal matter and consumption index (C.I.) unlike the lower doses. At lower doses growth rate (G.R.) had positive relation with C.I. and efficiency of conversion of ingested food (E.C.I.). A noticeable positive correlation existed between the food balance and mean weight of treated and unirradiated larvae whereas a positive relation of food balance with approximate digestibility (A.D.) was observed in the larvae treated up to 6 krad. As seen in the normal larvae, in the irradiated ones also, the weight gain was negatively correlated with C.I. up to 4 krad. No such correlation existed with the higher doses. Likewise, C.I. up to 4 krad had a negative correlation with E.C.I. and efficiency of conversion of digested food (E.C.D.) which did not continue at higher doses.

On the basis of such correlations between various nutritional parameters a right age in conjunction with suitable irradiation dose can be worked out for a successful eradication of this pest.

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ROLE OF AGE AFFECTING NUTRITIONAL BUDGET OF SPODOPTERA LITURA LARVAE IN RELATION TO GAMMA IRRADIATION

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between the faecal matter and consumption index (C.I.) unlike the lower doses. At lower doses growth rate (G.R.) had positive relation with C.I. and efficiency of conversion of ingested food (E.C.I.). A noticeable positive correlation existed between the food balance and mean weight of treated and unirradiated larvae whereas a positive relation of food balance with approximate digestibility (A.D.) was observed in the larvae treated up to 6 krad. As seen in the normal larvae, in the irradiated ones also, the weight gain was negatively correlated with C.I. up to 4 krad. No such correlation existed with the higher doses. Likewise, C.I. up to 4 krad had a negative correlation with E.C.I. and efficiency of conversion of digested food (E.C.D.) which did not continue at higher doses.

On the basis of such correlations between various nutritional parameters a right age in conjunction with suitable irradiation dose can be worked out for a successful eradication of this pest.

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Calling, mating and fecundity of the female spotted bollworm Earias vittella, in the absence and presence of okra fruit.

Calling, mating and fecundity of females of Earias vittella fabricius (Lepidoptera: Noctuidae) was studied in the presence and absence of okra fruit. Presence of okra fruit caused a significant increase in the duration of calling of virgin females. When host was accessible, females mated more frequently and there was a significant increase in the number of spermatophores received by them. Further, presence of okra fruit caused increased egg production and oviposition. In general, the number of eggs oviposited by a female increased with an increase in the number of spermatophores received by her and this increase was substantial when a female had an access to okra fruit. Most of the once-mated females initiated calling. Analysis of the data on once-mated and multi-mated females indicated that egg production potential of a female influenced its mating frequency.

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Electroantennogram responses of Phthorimaea operculella zeller males to trans-4, cis-7, tridecadienyl acetate.

Electroantennogram response amplitude and repolarization time of male antenna of P. operculella were dependent on the concentration of trans-4, cis-7, tridecadienyl acetate to which it was exposed and between 10^{-6} to 10 µg, both generally increased as the stimulus concentration increased. Between 1 to 4 days after emergence, age of male did not appear to significantly influence the response pattern. With progressive resection of the antennal segments, progressive reduction in antennal sensitivity occurred and repolarization time was also shortened. Analysis of dose-response data indicated that the EAGs were related to pheromone concentration by a power function.

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Effect of gamma-radiation on the different ages larvae of Tribolium anaphe Hinton.

The effect of gamma-radiation(1, 2, 4 and 6 Kr) were assessed on the different ages viz. 1, 8 and 14- day old larvae of Tribolium anaphe. 14-day old larvae was more resistant to all levels of teatments than that of 1 and 8 day old. The larval, pupal and adults weight were drastically reduced at 4 and 6 krad in all the age groups. The growth indices of T. anaphe on various age groups were in the order: control) 1 Kr) 2 Kr) 4 Kr) 6 Kr.

None of the doses could prevent pupation and adult emergence except at 6 Kr in 1- day old larvae but the frequency of deformed individuals i -ased as the dose increased. Lengthened larval and pupal perio-s were observed at higher doses (4 & 6 Kr) in all age groups. Gamma-radiation had no significant (P) 0.05) effects on the sex-ratios of T. anaphe.

Egg production and percentage of hatching gradually decreased [illegible] the increase in dose but at 6 K[illegible] no egg laid by the female in all the age groups.

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STUDIES ON DETERMINING THE LETHAL AND STERILIZING DOSES OF GAMMA RADIATION APPLIED TO VARIOUS BIOLOGICAL STAGES OF COWPEA WEEVIL (Callosobruchus maculatus F. Bruchidae, Col.)

SUMMARY

Eggs, larvae, pupae and adults of Cowpea weevil (Callosobruchus maculatus F.) were exposed to gamma-radiation from a radioactive cobalt (60 Co) source. All eggs (24-48 h.) died at doses higher than 1 Krad. Larvae emerged but could not reached to adult stages when the eggs (96-120 h.) were irradiated at doses higher than 2 Krad. However, a dose of 10 Krad was sufficient to ensure that none of the eggs(96-120 h.) hatched. 2 Krad dose gave 85.8 % sterility at the adult stage. Young larvae (3-7 days old) were irradiated at 1-5 Krad, only 3-5 Krad caused complete kill; 2 Krad dose gave 84 % sterility at the adult stage. Old larvae (10-14 days old) were irradiated and 4-5 Krad gave complete kill; 3 Krad caused 100 % sterility. When the pupal stage were irradiated at 7.5 and 10 Krad, adults emergencies were 83 % and 7.4 % respectively, but it occured up to 30 Krad. Exposure to 10 Krad and higher doses completely prevented egg laying. The eggs laid by the adults of which pupae were irradiated at 7.5 Krad did not hatched. The sterilising dose for adults both (0-1 and 4-5 days old) was 8 Krad. The adult life span was a little reduced up to the level of 10 Krad irradiations, but their activities were not suppressed. Irradiated males and females (0-1 day old) at 125 Krad lived for an avarage 6 and 7 days respectively while 4-5 days old ones both lived 6 days. But, they were completely inactive at this dose.

As a conclusion, 8 Krad could be recommended to prevent the reproductivity of Cowpea weevil.

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A TEST TO VERIFY THAT THE ACARID MITES (Acarida: Acaridae) HAVE BEEN IRRADIATED AND ARE INCAPABLE OF REPRODUCTION

Two methods using ionizing radiation have been proposed in controlling mite pests infesting agricultural commodities: (a) irradiation of the infested product with high levels of radiation to kill the pests, and (b) irradiation of the infested product with lower doses to produce sterility in the mites. The second method seems preferable because the use of high doses of radiation require more shielding and is more expensive. Sterility in the acarid mites (Acaridae) can be achieved following irradiation of adult males and females at much lower doses than needed to kill adults. Irradiation of a product infested by the mold mite, Tyrophagus putrescentiae (Schrank), or by the bulb mite, Rhizoglyphus echinopus (F. et R.), with a 0.25 kGy dose of gamma radiation could be the treatment required to produce an acceptable level of pest control. At this dosage, adult survivors of the mites will be present in the treated commodities for several weeks, but they will not give rise to offspring. However, the live mites present in the agricultural commodities will be of concern to quarantine personel. Thus, a simple test is needed to ensure that the mites were irradiated and they do not pose a risk.

Adults of the mold mite that emerged from irradiated eggs, larvae or nymphs (0.26-0.53 kGy) are unfecund. The mites irradiated as 0-48 hour old adults with a dose of 0.26 kGy or higher produce eggs during the first days after the treatment, but were unfecund thereafter.

Adults of the bulb mite emerged from inert deutonymphs irradiated with 0.2 kGy produced eggs during six weeks after the treatment. Those mites originating from deutonymphs given a 0.25 kGy or higher dose produced eggs during only 2 weeks after the treatment, being unfecund thereafter. However, mites irradiated as adults produced eggs longer than mites treated as inert deutonymphs. Males and females given 0.6 or 0.8 kGy laid eggs during the Ist, 2nd and 3rd week after the treatment, being unfecund thereafter.

Therefore, a test to verify that a pest has been irradiated and is incapable of reproduction based on this unfecundity of treated mites, could be applied as follows: (I) isolate alive females from a product; (2) place them into rearing cages containing food (wheat germ, yeast) (e.g., 5 females per cage); (3) keep the cultures at 25°C and 85% R.H. for 3 days; (4) record the number of eggs laid by the females.

When no eggs or only a few eggs are laid by females isolated from a product it means that this product has been irradiated. When females produce more than 20 eggs per female during the 3-day period, one may conclude that the product had not been treated.

Usually, varying periods of time may elapse between the radiation treatment and the quarantine control of the product. Therefore, the live mites were isolated from the product I, 2, and 3 weeks after its treatment with 0.0 (control), 0.25, 0.5, 0.75, and I.0 kGy of gamma radiation. For each treatment, at least 50-60 females were isolated and placed into I0-I2 rearing cages (5 females per cage) supplied with food. Number of eggs produced by these females were recorded at the Ist, 2nd, and 3rd day after isolation of mites into rearing cages.

Experiments with the mold mites. The results obtained show that the control mites (i.e., isolated from untreated wheat germ) produced I764 eggs during the 3-day period (35.3 eggs per female), whereas the mites isolated from the product I, 2, and 3 weeks after its treatment with 0.25 kGy of gamma radiation produced 57, 2, and I eggs, respectively. Mites isolated from wheat germ irradiated at higher dosages of gamma radiation occasionally produced a few eggs.

Experiments with the bulb mites. Fecundity of females isolated I week after irradiation with 0.25 and 0.5 kGy was sometimes high: there were groups of females producing more than 20 eggs during the 3-day period. Mites irradiated with higher doses laid a few eggs, always less than 20 eggs. Also, the females isolated 2 weeks after treatment with 0.25 kGy often produced more than 20 eggs per 5 females during the 3-day period. In other combinations, their fecundity was very low. The results obtained show that the test based on unfecundity of treated mites of R. echinopus may be used for the indentification of irradiated mites which were given 0.5 kGy or higher doses of irradiation.

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IRRADIATION OF ADULTS OF THE BULB MITE, Rhizoglyphus echinopus (F. et R.) (Acarida: Acaridae)

Roczn. Nauk Roln., Ser. E (submitted)

The fecundity of the bulb mite was greatly affected by gamma radiation at a dose of C.I kGy or higher. Adult mites treated with C.25 kGy produced 87% less eggs than the controls. The treated pairs exhibited a great variability in sensitivity to radiation.

Mites irradiated with I.O kGy laid a few eggs, but those treated with I.2 kGy only occasionally produced the eggs. All pairs given a I.5 kGy dose or higher were unfecund. Mites irradiated as adults produced eggs longer than mites treated as inert deutonymphs. Males and females given O.6 or C.8 kGy laid eggs during the Ist, 2nd and 3rd week after the treatment, being unfecund thereafter.

All eggs laid by mites irradiated with C.5 or higher dose were sterile. A single larva hatched from an egg produced by mite pairs treated with doses of O.25, O.3 and C.4 kGy. In these cases, however, lethality of eggs was higher than 99.8%. Viability of eggs laid by O.I- or O.2-kGy treated mites was low during the first days after irradiation. Later on, it reached a rather stable level. This indicates a quick post-radiation recovery in fertility by mites irradiated with low dosages of gamma radiation.

Larvae hatched from eggs laid by O.I- and O.2 kGy - treated mites were allowed to develop to the adult stage, and the sex ratio in progeny obtained was determined. A significant distortion of sex ratio (preponderance of males) in progeny that developed from eggs laid during the first 5 days after the treatment. It seems to be related with the lowered viability of eggs reported for the same period after irradiation.

When females were irradiated and mated to normal males (T♀ x U♂), the reduction in their fecundity was lower than for the pairs in which only males were treated (U♀ x T♂). At all treatment levels, the untreated females mated to the treated males laid significantly more eggs than the irradiated females mated to either treated or untreated males. The similar relationships were found for the mold mites treated with accelerated electrons.

Mortality of eggs produced by treated males and treated females (T♀ x T♂) was very high. Already, at O.3 kGy treatment, pairs produced eggs which all were sterile. However, females treated with O.3 kGy of gamma radiation laid about 30% viable eggs when mated to untreated males. In the opposite treatment combination (U♀ x T♂), I6.6% eggs hatched. When higher doses of gamma radiation were applied, hatchability of eggs produced by the T♀ x U♂ pairs was very low (2%). Still, I9.2% eggs hatched in the U♀ x T♂ combination. Females irradiated with O.5 kGy and mated to either treated or untreated males produced no viable eggs. When O.5 kGy-treated males were paired with normal females, only 5.3% eggs produced were hatched. At O.6 kGy dose of gamma radiation, both treated females mated to untreated males and untreated females mated to treated males produced no viable eggs.

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THE INFLUENCE OF THE TREATMENT AGE AND DOSAGE OF GAMMA RADIATION ON EGG MORTALITY OF THE BULB MITE, Rhizoglyphus echinopus (F. et R.) (Acarida)

Roczn. Nauk Roln., Ser. E (submitted)

The bulb mite, Rhizoglyphus echinopus (F. et R.), is an important pest of onion, bulbs of ornamental plants, and processed fruits and vegetables. This report deals with studies of the bulb mite eggs made to determine their sensitivity to gamma radiation from a Co^{60} source.

Table I shows the influence of treatment age and dosage of gamma radiation on egg mortality. It is seen that the age of the eggs at the time of irradiation had a profound effect on their hatchability. The 0-2-day-old eggs were very sensitive to radiation, and the sensitivity to radiation of 0- to I and I- to 2-day-old eggs was similar. A 0.I kGy dose caused 63.6-67% mortality of these eggs whereas irradiation at 0.2 kGy resulted in 74.2-74.8% embryonic deaths. A dose of 0.3 kGy or higher prevented hatching following irradiation of 0- to 2-day-old eggs. The older eggs (2- to 3-, and 3- to 4-day-old age groups) were resistant to radiation. None of the dose (0.I to 0.5 kGy) could induce mortality of these eggs. Because of moderate mortality rated in larval and nymphal stages, a significant part of these (> 2-day old eggs) eggs developed to the adult stage. However, the sex ratio in these adults was strongly male-biased: only I-5% females was recorded (Table 2). These males were sterile; untreated females paired with males that emerged from eggs (> 2 day old) treated with 0.5 kGy were found to produce no eggs.

Table I. Sensitivity of R. echinopus eggs to gamma radiation in dependance on their age at the treatment (Explanations: n - number of eggs observed; % - percent of larvae hatched).

Dose (kGy)	Age of eggs (days)							
	0-I		1-2		2-3		3-4	
	n	%	n	%	n	%	n	%
0.0	65	87.7	39	92.3	99	85.8	77	94.8
0.I	II5	33.0	88	36.4	I25	I00.0	234	I00.0
0.2	I47	25.2	93	25.8	I74	98.3	230	99.6
0.3	I87	0.0	I46	0.0	I08	I00.0	I68	I00.0
0.4	I32	0.0	49	0.0	I32	I00.0	282	98.9
0.5	93	0.0	64	0.0	I55	98.I	277	I00.0

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EFFECTS OF IRRADIATION ON INERT DEUTONYMPHS OF THE BULB MITE, Rhizoglyphus echinopus (F. et R.) (Acarida: Acaridae)

Roczn. Nauk Roln., Ser. E (submitted)

Unsexed, intermediate-aged inert deutonymphs were used, and the sex ratio was about I:I in the emerging untreated adults (control). No difference in sensitivity between the sexes was indicated in the adults that emerged from treated inert deutonymphs. The sex ratio was also about I:I.

Adults obtained from irradiated deutonymphs were paired, and production of eggs and their viability were recorded. The number of eggs laid by these mites decreased with an increase of the dosage ($r = -0.658$). All mites emerged from deutonymphs treated with I.O kGy were unfecund. As compared to the control, the fecundity of the bulb mites developed from irradiated deutonymphs was highly reduced. For example, adults emerged from deutonymphs treated with 0.35 kGy produced 93.6% eggs less than the control mites. A reduction in fecundity ranged from 90 to I00% when the inert deutonymphs were irradiated with a dose of 0.2 kGy or higher. Also, these mites produced significantly less eggs than the mites treated as adults.

Adults of the bulb mite emerged from inert deutonymphs irradiated with 0.2 kGy produced eggs during six weeks after the treatment. Those mites originating from deutonymphs given a 0.25 kGy or higher dose produced eggs during only 2 weeks after the treatment, being unfecund thereafter. Note here that adults of the mold mite, Tyrophagus putrescentiae (Schrank), emerged from irradiated nymphs (0.26 - 0.53 kGy) were unfecund.

Viability of eggs laid by mites emerged from treated deutonymphs was very low. At a 0.I kGy dose, ca. 37% eggs were dead, but at 0.2 kGy was 86.5% mortality of eggs. Eggs laid by mites irradiated with a 0.25 kGy or higher dose failed to hatch (only occasionally a few eggs hatched at 0.35 kGy dose).

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