Radio-Vaccines to Combat Human Parasitic Diseases

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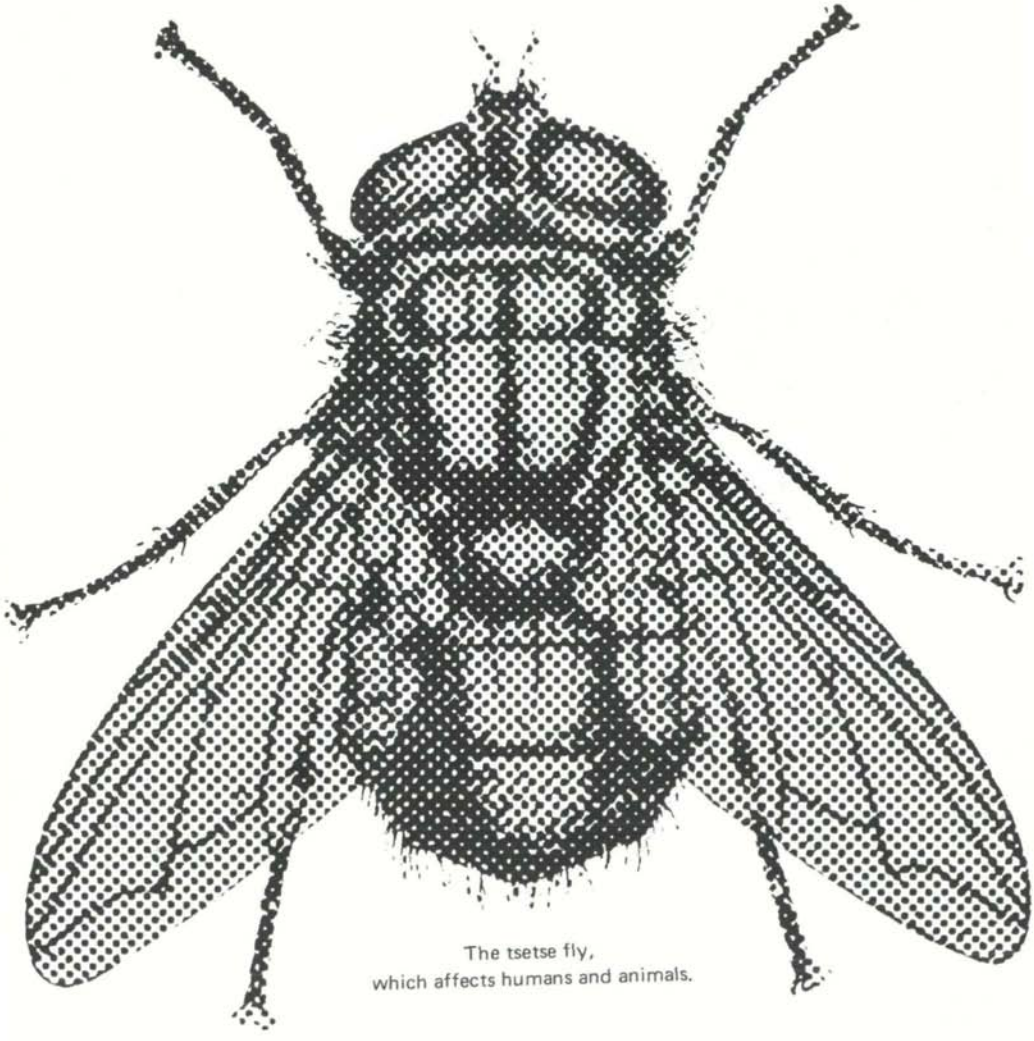


The tsetse fly,
which affects humans and animals.

Among the practical applications of radiobiological techniques that may be of considerable interest for human medicine and public health is the use of ionizing radiation and radioactive isotopes in the preparation of vaccines.

Attempts of vaccine production by exposing various kinds of micro-organisms including viruses, bacteria and fungi, as well as unicellular and multicellular animal parasites to ionizing radiation have been reported in scientific periodicals from time to time. Radiation attenuated vaccines have been demonstrated to be an effective means of controlling certain helminthic infections of domestic and farm animals and a few of them are already being applied on a commercial scale, such as vaccine against bovine parasitic bronchitis (DICTOL), vaccine against sheep lungworm disease (DIFIL), and a vaccine against the dog hookworm which will shortly be put on the American market. Although many helminth diseases of domestic animals are known to produce a high degree of acquired immunity, it has been impossible in the past to reproduce this immunity using artificially prepared vaccines – the striking exception being the radiation attenuated vaccines.

The successful use of irradiated helminth larvae as a vaccine depends on finding a radiation dose which will significantly reduce the pathogenic effect of the larvae without seriously impairing their immunogenic power.

Since 1966, the International Atomic Energy Agency, in collaboration with the Food and Agricultural Organization of the UN, has had a co-ordinated research programme on the application of nuclear techniques to veterinary parasitology. It soon became obvious that the similarity of certain parasitic infections in man and animals as well as the existence of cross-infections would make it extremely difficult to keep any strict and unnatural species barriers. The apparent similarity, for instance, between human and animal trematodes like *Trypanosoma* and *Plasmodium* clearly suggests the extension of these studies to human parasitic diseases.

The idea was given full support by the participants of two consecutive consultants' meetings jointly organized by the IAEA and WHO about a year ago. The recommendations of the meeting urged these two international organizations that a co-ordinated collaborative research programme be initiated on the use of nuclear techniques in preparation of vaccines against human parasitic diseases, with the highest priority being given to malaria and African trypanosomiasis (but not necessarily excluding other protozoan and helminth infections of importance to public health in many developing Member States).

Malaria was the most important disease of man in the world as a whole prior to the launching of the WHO's global eradication programme in 1957. More than one-third of the total world population were exposed to malaria infection and at least half the people who died from all causes were probably killed directly or indirectly by malaria. It has been suggested that the ancient Greek Empire was overthrown not by conquering peoples but by the small parasitic organisms.

The health implications of malaria in tropical Africa are reflected in the exceptionally high mortality and morbidity rates in rural areas. In West Africa at least 10% of death below five years of age are due to malaria, while the infant mortality rate from all causes, in which malaria plays a major role, may reach even 50% in unprotected rural communities. The highlands of tropical Africa are occasionally swept by severe malaria epidemics, which sometimes cause as high as 25% mortality.

Apart from tropical Africa, the disease is still endemic in large areas of the world including the Amazonas valley in South America, Indo-China, and some islands of South-East Asia and Oceania.

When the fast development of chemical industry after the World War II resulted in the large-scale production of several chlorinated hydrocarbons as the first synthetic and powerful insecticides, and in the manufacturing of effective chemotherapeutic agents including sulphonamides, antibiotics and antiparasitic drugs, the door opened to the development of global control programmes.

A new foundation was provided for massive efforts to eradicate malaria by interruption of transmission through the definitive host, the mosquito, which, in addition to the application of effective chemotherapeutic agents, led to some brilliant achievements.

However, both the mosquito and the parasite have, after a period of slumbering acquiescence, exhibited a new and alarming unwillingness to co-operate with plans to eradicate them. The potential danger of wide-spread resistance of the vector to insecticides, and of drug resistance among plasmodia which infect humans is steadily increasing and has recently been stressed repeatedly. These warnings have again directed attention to the study of alternative methods of protection against the disease, among them to the immunization of population at risk. Furthermore, because of the limitations of diagnostic procedures based upon the detection of the contributing parasites in the blood, investigations have been intensified in recent years to develop immunobiological tests and to evaluate them in individual cases as well as in epidemiological surveys, and in determining the effects of control and treatment.

Trypanosomiasis is continuing to be another world-wide problem of great medical and economical importance. African trypanosomiasis or sleeping-sickness, which is particularly lethal to man and domestic stock, is widespread throughout the African continent from the southern borders of the Sahara to approximately the 20° of south latitude (the frontiers between Rhodesia and South Africa). The disease affects several millions of people both directly and indirectly. In certain areas of Central and East Africa as many as 10% of the population examined have been found to be infected. It causes not only widespread suffering and poor health, but also results in loss of meat, milk, animal labour and manure. Making agriculture and stock-raising virtually impossible over more than 10 million square kilometers of fertile land, it is one of the major factors restricting economic development in Africa today.

The tiny flagellates, called trypanosomes, that cause the African sleeping-sickness are transmitted by the blood-sucking tsetse flies. Practically all wild game in Africa harbour trypanosomes in their blood, and when tsetse fly sucks the blood of an infected animal or man, the blood drawn into the intestine of the fly will contain these trypanosomes. From the intestine they invade the salivary glands and multiply. If such an infected fly bites a man, the trypanosomes will be injected into the blood of the victim with the saliva of the fly.

Medical treatment of African sleeping-sickness consists mainly of administration of various drugs, that are very effective especially in the early stages of the disease. However, if the dose injected is not adequate to kill the parasites, they may become drug-resistant and then are unaffected by doses large enough to kill the patient. As in the case of malaria, there has been a revival of interest in the possibility of controlling trypanosomiasis by immunological means.

The suggestion that active immunization may be of value in protection against some parasitic diseases, including malaria and trypanosomiasis, is not new, and is mainly based on consideration of the natural history of these diseases.

For many years it has been recognized that in individuals inhabiting areas with high malarial endemicity, who are exposed to frequent infections, the severity of malarial infections may be significantly mitigated, though a solid, sterilizing immunity usually does not develop. Recently, field studies, culminating in the successful passive transfer of immunity in man, have demonstrated the existence in the blood of immune persons of a humoral factor (or factors) capable of dramatically reducing parasitaemia. There is evidence to indicate that the naturally acquired immunity is effective against only the erythrocytic forms of plasmodia and that it has no detectable effect upon sporozoites.

Wild ungulates and certain breeds of cattle can exist under continuous heavy attack by infected tsetse fly without clinical evidence of trypanosomiasis. Their immunity, however, is not a sterile one (i.e. trypanosomes are not eliminated) but simply a state of balance in the host-parasite relationship. Some partial immunity of humans has also been postulated by several observers. The information from field examinations is inconclusive and confusing, probably because of the multiplicity of trypanosome antigenic types to which the various animals and man are exposed.

Laboratory evidence, however, makes abundantly clear that not only does immunity exist in malaria and trypanosomiasis, but that this immunity, at least in lower animals, can be induced artificially with vaccines prepared from killed or rather from attenuated organisms.

Convincing data were reported during the past few years that relatively small numbers of sprozoites *(P. berghei)* irradiated with X- or gamma-rays, provide an antigenic stimulus effective to induce a protective immune response in both mice and rats against subsequent sprozoite infection, but not against infection with blood forms of the parasite. For protection of the animals against an infecting dose of blood forms, a vaccination is necessary with parasitized red blood cells that have previously been exposed to ionizing radiation. According to preliminary studies carried out in monkeys, the results on rodent parasites cannot be extrapolated to primate plasmodia, though, in one of the experiments a definite protection has been obtained with four intravenous inocula of infected and irradiated red blood cells.

Further investigations with *P. knowlesi* have suggested that one of the reasons why irradiated parasites are better immunogens than killed ones is that, although non-infective, they are still metabolically active, as shown by continued protein and nucleic acid synthesis.

Studies on the effects of ionizing radiation on different species of trypanosoma have been published since the beginning of the century. It has become evident that normal multiplication and infectivity of trypanosomes can be suppressed by a small fraction of the dose required to kill the parasites. Thus irradiation may enable one to take advantage of the special immunological properties of living parasites. At the same time, the pathogenic effects of a vaccine prepared from parasites with an unimpaired reproductive capacity would be obviated. In spite of this promising hypothesis, the research on the possible employment of radiation attenuation for the control of trypanosomiasis has been resumed only recently.

A marked protection was induced in mice inoculated with irradiated trypanosomes and challenged with unirradiated ones. This acquired resistance could also be detected in rats, especially when the animals received two or three immunizing inoculations. Encouraging preliminary results were obtained in experiments with cattle and subhuman primates, while immunization of dogs proved to be ineffective.

Accordingly, the outlook of immunological protection against these two parasitic diseases using radiation attenuated vaccines is not unpromising, and certainly merits extensive investigation. Particularly, the potentials of an active immunization of man can only be evaluated if considerable additional research work is undertaken.

The main task of the new IAEA/WHO co-ordinated research programme is to promote research, and to establish a closer contact between scientists interested in the use of nuclear techniques for the preparation of vaccines against malaria and/or trypanosomiasis.

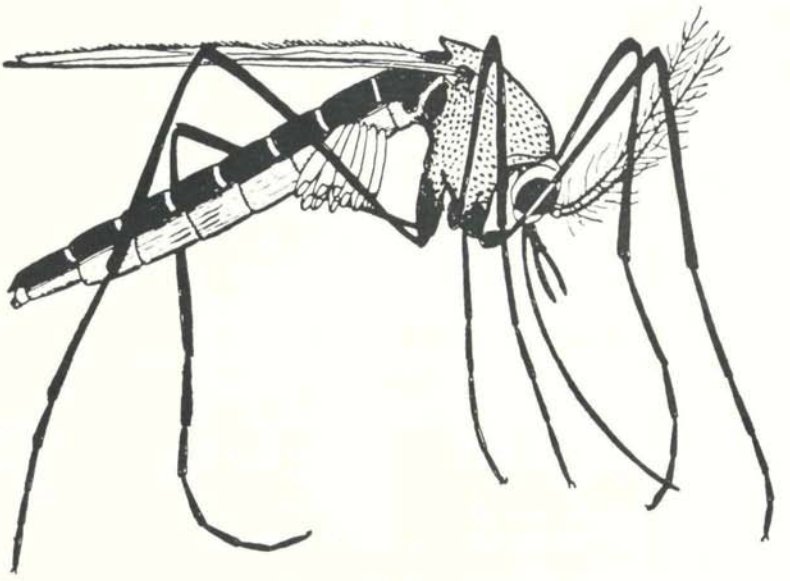
There are essentially two distinct ways in which nuclear techniques can be employed in research performed under this co-ordinated programme:

a) the use of ionizing radiation for attenuation of the parasitic organisms;
b) the use of radioisotopes as tracers in studies on the physiology of the parasites and on the host-parasite relationships.

Subjects recommended for research using a more empirical approach are: Further immunization trials with radiation attenuated vaccines on various host-parasite systems with both homologous and heterologous combinations of immunizing and challenging antigenic variants; determination of optimum dosage of irradiated parasites, number of boosters, spacing of vaccination; character, duration and species specificity of the protection; occurance of undesired side effects following vaccination; radiosensitivity of the parasites at their different stages of development; optimum range of radiation doses to be applied for attenuation. These studies should be carried out in parallel on subjects immunized with irradiated and with non-irradiated parasites, or with parasites attenuated by conventional means, like dehydration, formalinization, freezing and thawing, heating, etc.

Recommendations for basic studies that are to contribute to the general understanding of the host-parasite interactions in human parasitic infections and the physiology of the parasites include subjects such as: Methods for labelling of parasites with radioactive isotopes and for determining the nature and location of their antigens; morphological and physiological alterations in parasites induced by irradiation; survival and localisation of irradiated parasites in the inoculated host; existence of natural antibodies against parasites; the relative role of cell mediated versus antibody mediated immunity in host defense mechanism; passive transfer of immunity into sublethally irradiated recipient animals; induction and/or selection of genetic mutant parasites that are less pathogenic and thus more suitable for vaccination; metabolism of parasites and the essential requirements for their cultivation *in vitro.*

It is believed that a direct benefit which could be derived from this co-ordinated collaborative research programme might be the development of successful methods of vaccination, while an indirect benefit would be the acquisition of essential knowledge on several fundamental problems closely related to the principal aim of the project.



The mosquito, a dangerous carrier of diseases such as malaria.

For successful development of the co-ordinated programme, some selected laboratory centres that are already carrying out active research on the use of nuclear techniques in preparation of antiparasitic vaccines and others where such research might be initiated, have been invited to participate in the programme. Although the rather stringent financial situation does not at present make large-scale research possible, a limited number of projects, mainly in developing countries, can be supported. Institutes in industrialised countries are expected to join the programme under cost-free research agreements. The information provided by the participants in their research progress reports (once a year) will be made freely available throughout the world. In order to ensure a proper co-ordination of activities carried out by research teams in different countries on various aspects of the same problem, research co-ordination meetings are convened at appropriate intervals. In addition, exchange and dissemination of information will also be promoted by arrangement of scientific panels and other meetings, research fellowships for both established investigators and post-graduate students, training courses, and by publication of research contract reports and panel proceedings.

Prototypes of similar international collaborative programmes of the IAEA and WHO prove that such a joint effort can make more efficient use of limited resources and of the limited pool of scientific talent available for the solution of these vital problems.

Entomology at the IAEA Laboratory, Seibersdorf

The function of the Entomology Unit at the IAEA's Seibersdorf Laboratory is to support research for the Joint FAO/IAEA Division's Entomology programmes and to train personnel from developing nations who are interested in these programmes.

The entomology programmes are centered around development of the sterile male technique for insect control in developing nations. This is a relatively new method of insect control in which large numbers of insects are produced in laboratories or insect factories, sexually sterilized with gamma irradiation and released into the field. These sterile individuals mate with native insects and the resulting eggs are sterile. The greater the ratio of sterile to native insects, the greater the population suppression.

The sterile male technique was first successful in eliminating the screwworm, *Cochliomyia hominivorax* (Coquerell) from Curacao in 1954 and then south eastern USA in 1959. The eradication programme in south eastern USA cost $10 million and has saved the livestock industry $20 million per year ever since with little additional costs.

Since the beginning of the screwworm programme, several other insect species have been experimentally or practically controlled by the sterile male technique.

The sterile male technique is a non-chemical method of pest control. Therefore environmental pollution, pesticide residues and problems of insect resistance to insecticides are reduced. The technique can be used to reduce the insect populations to very low levels and in some cases even eliminate the pest from isolated areas. The sterile male technique often fits into integrated control programmes and may be cheaper than conventional methods of insect control. In many developing nations insecticides are imported and payment made in foreign currency. In some cases, the sterile male technique can be used without significant imports because the insects required can be reared on locally available products.

The Seibersdorf laboratory conducts research on a number of problems related to the sterile male technique.

Insect production

The first problem with any sterile male release programme is to rear the insect in large numbers. Many insects fail to mate or lay eggs when held in cages or deprived of their natural foods. When concentrated in small areas the insects often succumb to viral, bacterial or fungal infections. Once these problems are solved and proper diets have been developed, the production costs must be reduced to a practical level. Rearing costs are often reduced several thousand-fold from the first laboratory production to the first commercial production.

Sexual sterilization

Insects are usually sexually sterilized with ^{60}Co or ^{137}Cs. The dose for complete sterilization ranges from less than 4 Kr to more than 50 Kr, depending on the insect species. Such high dosages would be fatal to higher animals, but insects can tolerate these levels because they are more simple and because the damage is

limited to the areas of cell division. In adult insects cell division is usually limited to the reproductive organs, salivary gland, digestive tract, etc.

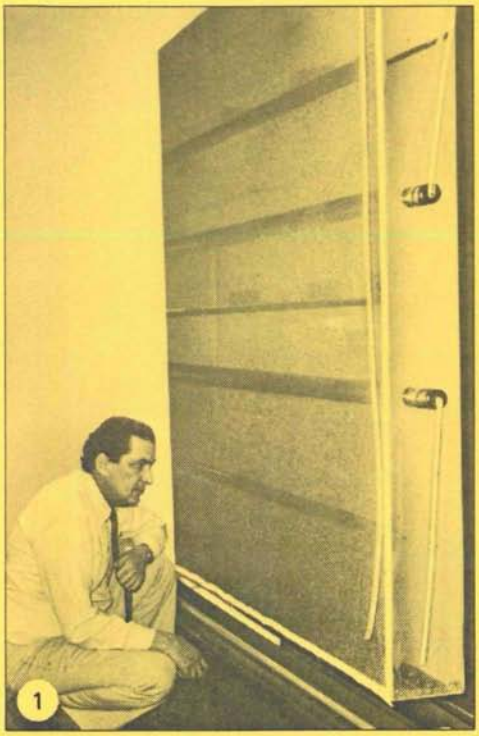
Damage by irradiation is not easily assessed. In the laboratory an irradiated male may mate a normal number of times and live as long if not longer than an untreated male; however, he may not be active in the field. The Seibersdorf laboratory has developed a number of methods to measure sexual aggressiveness or competitiveness of sterilized male insects.

Insect manipulation

In the process of rearing, sterilizing and releasing insects, they must be transferred from one container to another, and sometimes sexed, stored or marked. This may involve immobilizing the insects one or more times with cold, carbon dioxide or nitrogen. Methods of manipulation, marking, storing etc. of insects with minimal damage are developed at Seibersdorf.

Release strategy

The Seibersdorf laboratory has not only provided insects for sterile male release programmes, but the staff also aids in planning, conducting and evaluting these programmes. The strategy of sterile male releases is often complicated. These releases may be part of an integrated programme to control a complex of insects affecting one or more crops or animal hosts.



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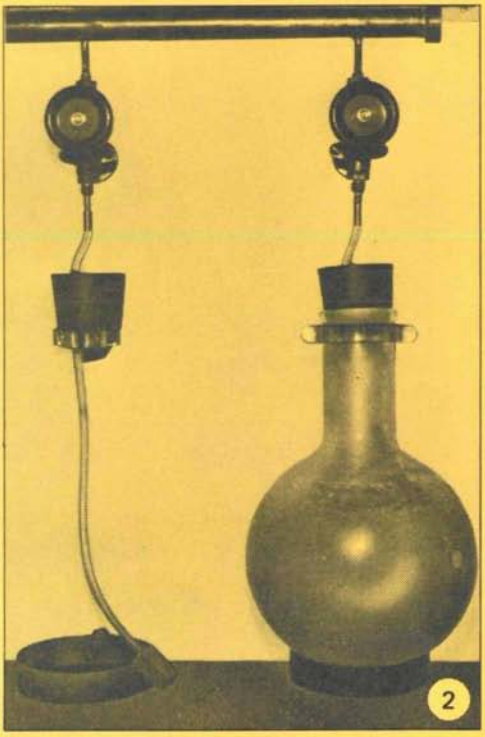

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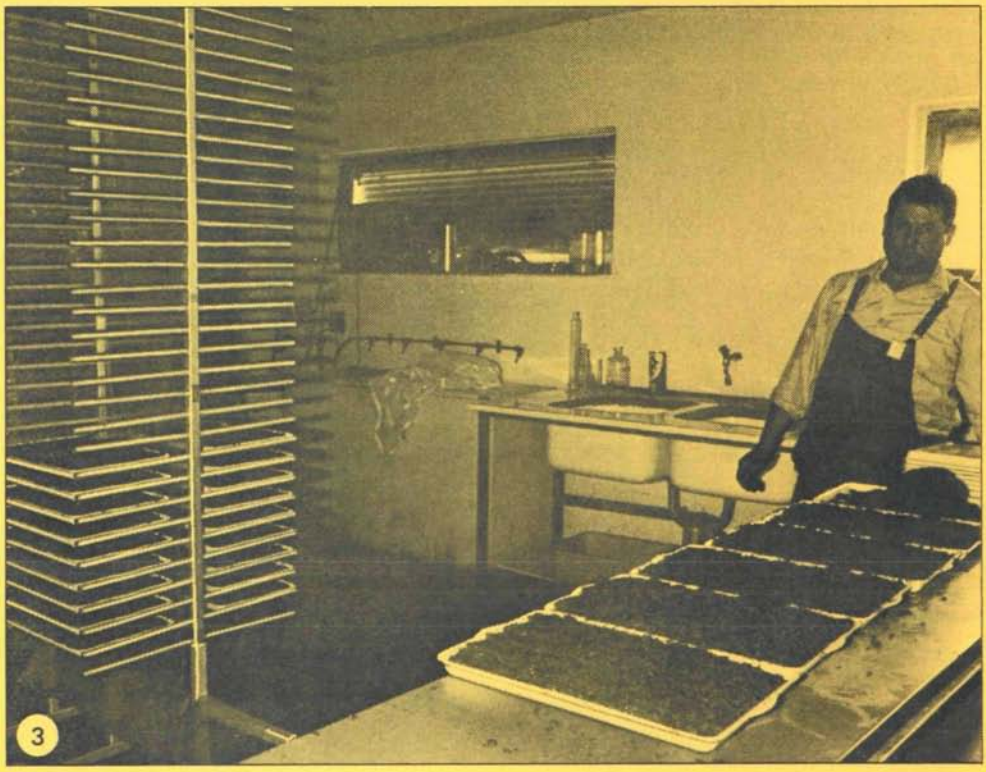

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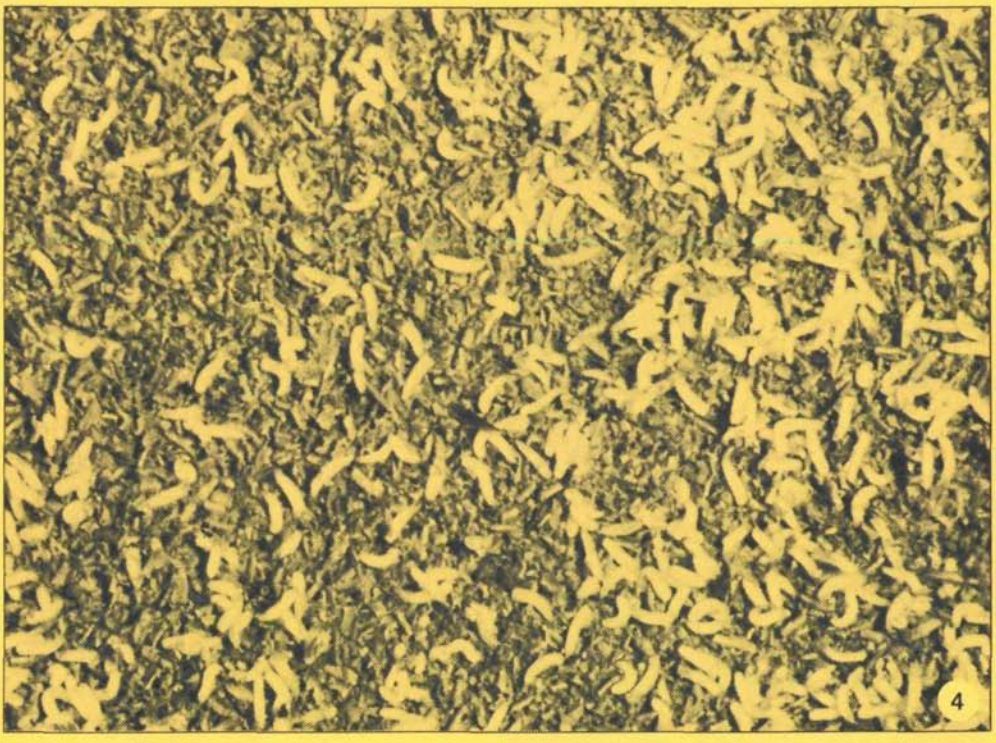


(1)

Medfly oviposition cage. Eggs are laid through the cloth screen and fall into a trough of water.

(2)

Egg incubator. Air is bubbled through a flask of eggs suspended in water.

(3)

Trays of medfly medium. Eggs or newly hatched larvae are put on medium — trays are then held in racks in background.

(4)

Full grown larvae in medium. At this stage, the larvae leave the medium and pupate.

(5)

Olive fly oviposition cage. Eggs are laid through wax cone, but must be washed from the cone.

The target insect and other pests, the hosts, and the various control methods are all interrelated. Some of the questions that must be answered include:

1. How and where will the insects be reared?
2. How many insects are required?
3. Design of mass rearing plant.
4. At what stage should the insect be irradiated and what dose should be used?
5. How many wild insects are present?
6. How to reduce the wild insect population to a level that makes the SMT economical.
7. When and what numbers of insects should be released?
8. How should the insects be released, i.e. how often, by air or ground, what distance between release points?
9. What quality-control methods will be used to determine if competitive insects are being released?
10. How will the programme be evaluated, i.e. is the target insect controlled, has the reduction of insecticide sprays allowed other pests to be controlled by natural enemies, what was the cost of the programme?

Among the various insects bred at Seibersdorf, the **Mediterranean fruit fly** or **medfly,** *Ceratitis capitata* Wied., is the longest resident. This insect is a serious pest in the Mediterranean Basin, South and

(6)
Counting olive fly eggs. Detailed records of each life stage must be made.

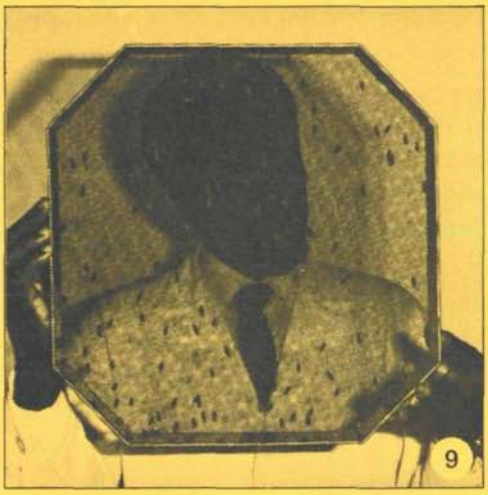
(7)
Tsetse flies feeding on rabbits. Goats, chickens and guinea pigs are also used as live hosts.

(8)
Tsetse flies feeding through membrane. The flies are fed bovine, horse or swine blood through a membrane. Hopefully this method will replace live animals.

Central America and Hawaii in the USA. The medfly can now be reared easily; in fact the laboratory personnel talk not only in terms of liters of pupae and millions of flies, but also liters of eggs. This insect has been controlled by the sterile male technique in Israel, Italy, and South and Central America. It is sterilized by 9 Kr in the late pupal or adult stage, immobilized by cold and has been released from the ground and the air.

The Agency now has contracts on medfly control by the sterile male technique with institutions in Israel, Peru and Egypt.

Another fruit fly reared at Seibersdorf is the **olive fruit fly,** *Dacus oleae* (Gmelin). In nature, this pest breeds in olives only and is the most serious pest of this fruit in the Mediterranean Basin. The olive fruit fly is not as simple as the medfly to rear. Workers are still searching for diets adequate for larval development and, to a much lesser degree, alternate diets for the adults. They are also concerned about diseases which weaken or destroy the various stages of the insect. Thus far olive fly production is in the thousands rather than millions; however, more effective rearing methods are being developed. The olive fly is also immobilized by cold, and sterilized with about 10 Kr.



(9)
New tsetse fly cage. Several sizes and designs of cages for feeding tsetse flies through membranes are evaluated.

(10)
Feeding adult stable flies. The adult of the stable fly feeds on blood-soaked cotton wool placed on top of the cages.

(11)
Preparing experimental diet for white top borer. To compare new diets, individual borers are placed in small vials of medium.

The Agency has contracts on olive fruit fly research in Yugoslavia and Greece.

The Seibersdorf laboratory rears three species of blood sucking flies. Two species are **tsetse flies** *(Glossina morsitans* Westwood and *G. tachinoides* Westwood). The third species is the **stable fly,** *Stomoxys calcitrans (L.).*

The tsetse flies occur only in a belt across Central Africa and cause sleeping sickness among humans and nagana in domestic and wild animals. The tsetse flies are preventing development of large areas of Africa, thus causing economic losses as well as human suffering.

At Seibersdorf, tsetse flies were first reared on rabbits. They are still being reared on those animals and, to a lesser extent, on goats and chickens; however, the main emphasis is being placed on feeding the flies through artificial membranes. Flies are now being fed on horse, cow and swine blood through membranes. The goal of the laboratory is to rear the flies without living hosts and later replace fresh blood by freeze-dried blood or a synthetic diet. The tsetse flies are immobilized by cold or nitrogen and are sterilized with 12 to 15 Kr.

The Agency has co-operative agreements on tsetse fly work with the UK, Belgium, Israel and Canada.

The stable fly occurs over large areas of the world, feeding on animals and also biting humans. The fly not only molests animals, but it also can take large quantities of blood, resulting in weight loss, reduction in milk production, etc.

At Seibersdorf the stable fly larvae are reared on a simple diet of straw, wheat bran and yeast while the adults are fed blood absorbed on cotton wool pads. Since this insect is a pest in Austria, the laboratory is conducting ecological studies in a village near Seibersdorf. The stable fly is immobilized by cold and sterilized with 4 Kr.

The Agency has a contract for studying stable flies with Korea and is co-operating with an FAO project on Mauritius.

In addition to flies, three species of moths are also being reared at Seibersdorf: they are the **gypsy moth** *(Porthetria dispar* L.), the **codling moth** (*Laspeyresia pomonella* L.) and the **white top borer** (*Scirpophaga nivella* F.).

The gypsy moth, a pest in Europe and Northeastern USA, often defoliates large areas of forests. The most serious problem in rearing this insect in the laboratory is a virus disease affecting the larvae and an obligate 120-day diapause (suspended development period) in the egg stage. Several artificial diets have been developed for this insect. The gypsy moth is normally sterilized with 30 Kr.

The Agency has a contract on gypsy moth research with Yugoslavia.

The codling moth is one of the most serious apple pests in much of the world. In addition to apples, this pest also attacks other fruits and some nuts. The codling moth has been reared at Seibersdorf on apples and several artificial diets. This insect has been immobilized with cold, nitrogen and carbon dioxide. It has been released from the ground and the air after receiving a range of 25 to 50 Kr.

The Agency now has contracts with Austria, Hungary, Czechoslovakia and Poland to try to control this insect.

The white top borer is a pest of sugar cane in Asia. A trainee from Indonesia is currently exploring artificial diets and methods for rearing this insect so that he can conduct sterile male technique studies in Indonesia.

The photographs illustrating the work at Seibersdorf were taken by Mr. B. Butt of the Entomology Unit at the laboratory.