

NUCLEAR STERILE INSECT TECHNIQUE

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INTRODUCTION

The United Nations' International Atomic Energy Agency (IAEA) at its Seibersdorf laboratory in Austria is harnessing nuclear technology to try to eradicate the *Anopheles* mosquito strain whose bite transmits malaria, a deadly disease devastating the African continent. The disease kills millions of Africans a year, most of them children, and costs the continent at least \$12 billion in lost gross domestic product.

Malaria is re-emerging as the World's number one killer infection. Once nearly-eradicated using insecticides such as DDT, the disease now affects more than 300 million humans and kills 1.5 to 3 million people every year. The dreaded disease is recognized as difficult to eradicate and its control is possible only with coordinated efforts of the general public, healthcare personnel and government and international agencies. An African child dies of the mosquito-borne disease every 20 seconds, according to some estimates, making it the number one killer of infants less than five years of age on the continent. There is one malarial death every 12 seconds somewhere in the world.

Ninety percent of the 1.5 to 3 million people who die from malaria per year are in sub-Saharan Africa, and it is still a problem that did not attract as much attention as the Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS). People in poor, remote villages are usually unable to get treatment.



Fig.1: Anopheles mosquito, the malaria vector.

The yearly mortality from malaria can be 9 times that of HIV/AIDS. Each year, if 1/3 of a million people die of HIV/AIDS, 3 million people die of malaria. Malaria ranks third among the major infectious diseases in causing deaths, after

pneumococcal acute respiratory infections collectively designated as pneumonia, and tuberculosis.

It is expected that by the turn of the century malaria would be the number one infectious killer disease in the world. It accounts for 2.6 percent of the total disease burden of the world. It is responsible for the loss of more than 35 million disability-adjusted life-years each year. Every year about 30,000 visitors to endemic areas develop malaria and 1 percent of them may die. Estimated global annual cost in 1995 for malaria was 2 billion dollars in direct and indirect costs, including loss of labor.

The estimated worldwide expenditure on malaria research is relatively limited at \$ 58 million, about one thousandth of the \$ 56 billion spent globally on health research annually. The estimated annual expenditure on malaria research, prevention and treatment is \$ 84 million. Estimated worldwide expenditure per malaria fatality is \$ 65; as compared to \$ 3,274 for HIV/AIDS and \$ 789 for asthma. One HIV/AIDS death is accordingly equivalent to about 50 malaria deaths.

The Plasmodium genus of protozoal parasites, mainly *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, have a life cycle which is split between a vertebrate host and an insect vector. The Plasmodium species, with the exception of *P. malariae*, which may affect the higher primates, are exclusively parasites of man. The mosquito is always the vector, and is always an Anopheline mosquito, although, out of the 380 species of Anopheline mosquito, only 60 can transmit malaria. Only female mosquitoes are involved as the males do not feed on blood. In fact they feed on flowers nectar, and may have a role in plant pollination.

This highly specialized life cycle requires specialized biology on the part of the Plasmodium species. The reason that not all mosquitoes are vectors for Plasmodium parasites is that refractory mosquitoes possess substances toxic to Plasmodium within their cells. A higher trypsin-like activity was also found in the midgut of resistant species, possibly inhibiting ookinete development. Plasmodium parasites seem capable of adapting to any suitable Anopheline mosquito, given sufficient time and contact. Sporogony within the mosquito are governed by environmental temperature as Anopheline mosquitoes are poikilotherms.

A possible solution to the malaria problem is to use the nuclear Sterile Insect Technique (SIT) to nip the problem in the bud by destroying the Anopheles mosquito strain shown in Fig. 1 that transmits the malaria parasite. The technique was successful in wiping out the dreaded tsetse fly, *Glossina* spp. shown in Fig. 2, which can transmit fatal sleeping sickness, from the island of Zanzibar. Efforts are also directed at the Mediterranean Fruit Fly or Medfly, *Ceratitis capitata* shown in Fig. 3, and some pest moth species.

As a result of requests from the Member States, in 1993, 1996 and 2001, the IAEA sought expert advice on the possible use of the Nuclear Sterile Insect Technique (SIT) for the control of malaria mosquitoes. Two consultants meetings recognized the potential of use of Sterile Insect Technique for area-wide control of mosquitoes, but noted that the technology for field application did not exist and substantial research and development was needed to develop the methods required.



Fig. 2: The Tsetse fly, *Glossina* spp.



Fig. 3: Mediterranean Fruit Fly, Medfly, *Ceratitidis capitata*.

THE NUCLEAR STERILE INSECT TECHNIQUE (SIT) FOR MALARIA

Malaria is the most important insect-transmitted disease. It causes approximately 1.5 to 3 million deaths a year and there are about 300-500 million cases of clinical malaria annually. The causative agents for malaria are parasites of the genus *Plasmodium* that are transmitted by female mosquitoes of the genus *Anopheles*. Most species of malaria parasites have now developed resistance to malaria drugs. Attempts to develop a vaccine have not yet succeeded. Traditional control of *Anopheles* mosquitoes through insecticides such as DDT has not solved the problem.

The Sterile Insect Technique (SIT) is an alternative for the control of Anopheles mosquitoes. Sterile Insect Technique relies on mass rearing and release of insects sterilized by gamma radiation. The released males mate with wild female mosquitoes, preventing production of offspring. Repeated releases lead to population control and eventual eradication. The proportion of infertile males to fertile wild males must be at least 10:1. In the eradication program of tsetse flies in the island of Zanzibar, the proportion used was as high as 50:1.

Insects are bred and then the males are exposed to enough gamma radiation to render them sterile, but not to incapacitate or kill them. The males are then released into the environment to breed with the females, whose eggs are unfertilized and never hatch. The whole concept is that the population would actually start to crash and eventually may actually lead to eradication of the insect, and therefore eradication of the disease

At the IAEA the project was still in its infancy. Over five years, there is a need to reach a point where a million sterile male insects a day must be produced per day. The males breed must be robust enough to survive when released in paper bags from planes into the environment and tough enough to compete with fertile males during mating. The females, the ones which bite humans, only mate once in their two-week lives. There is no alternative to irradiation for the sterile insect technique. It is a very clean technique with no risk of contamination and the insects are not radioactive when they are released.

Methods development is needed to assess the feasibility for mass rearing, sterilization and distribution of the male Anopheles mosquitoes. This involves:

a) Developing methods of mass rearing: Research will be conducted on critical aspects of large-scale Anopheles rearing including adult holding, egg handling, larval rearing and pupa collection. This will include improving membrane feeding of blood to adult females and improvements to blood diet or substitutes.

b) Improving sterilization, handling and release methodology: Radiation procedures will be evaluated to enable pupae to be efficiently sterilized and thus reduce the need for excessive handling of the more sensitive adults. Techniques for aerial release will also have to be investigated.

c) Devising genetic and molecular methods for the production of males: Any Sterile Insect Technique program for mosquitoes will require the release of males only. Genetic sexing methods must be developed.

d) Improving field evaluation of release mosquitoes: This will entail developing methods for assessing male competitiveness, migration and monitoring of the released mosquitoes.

NUCLEAR GENETIC BASIS OF STERILE INSECT TECHNIQUE

A method to induce sexual sterility has been devised during the 1920s by Herman J. Muller at the University of Texas. Muller used a dentist's x-ray machine to induce mutations in the genes and chromosomes of the vinegar fly: *Drosophila melanogaster*.

Muller discovered that x-rays when applied in sufficiently high doses break chromosomes in the cells nuclei and that two broken ends of chromosomes that contact each other, being sticky, tend to adhere and heal together as shown in Fig. 4.

Two broken ends, each with a site of attachment to a spindle fiber, may heal together to form a dicentric chromosome with two sites of attachment to spindle fibers.

When the cell begins to divide, the dicentric chromosome may be pulled toward opposite poles of the cell. Often, if the dicentric chromosome fails to break, then the cell cannot divide, and it dies. If the dicentric chromosome breaks at a new place, then one cell will receive a deficiency of genetic information, and the other a duplication of information. In addition, each chromosome piece from the original break that has no site of attachment for a spindle fiber cannot be pulled into a daughter nucleus during cell division. Thus, the daughter cells may not receive all of the genetic information needed to function. These and other chromosomal events culminate in the death of any embryo formed by the fertilization of a normal egg cell with irradiated sperm.

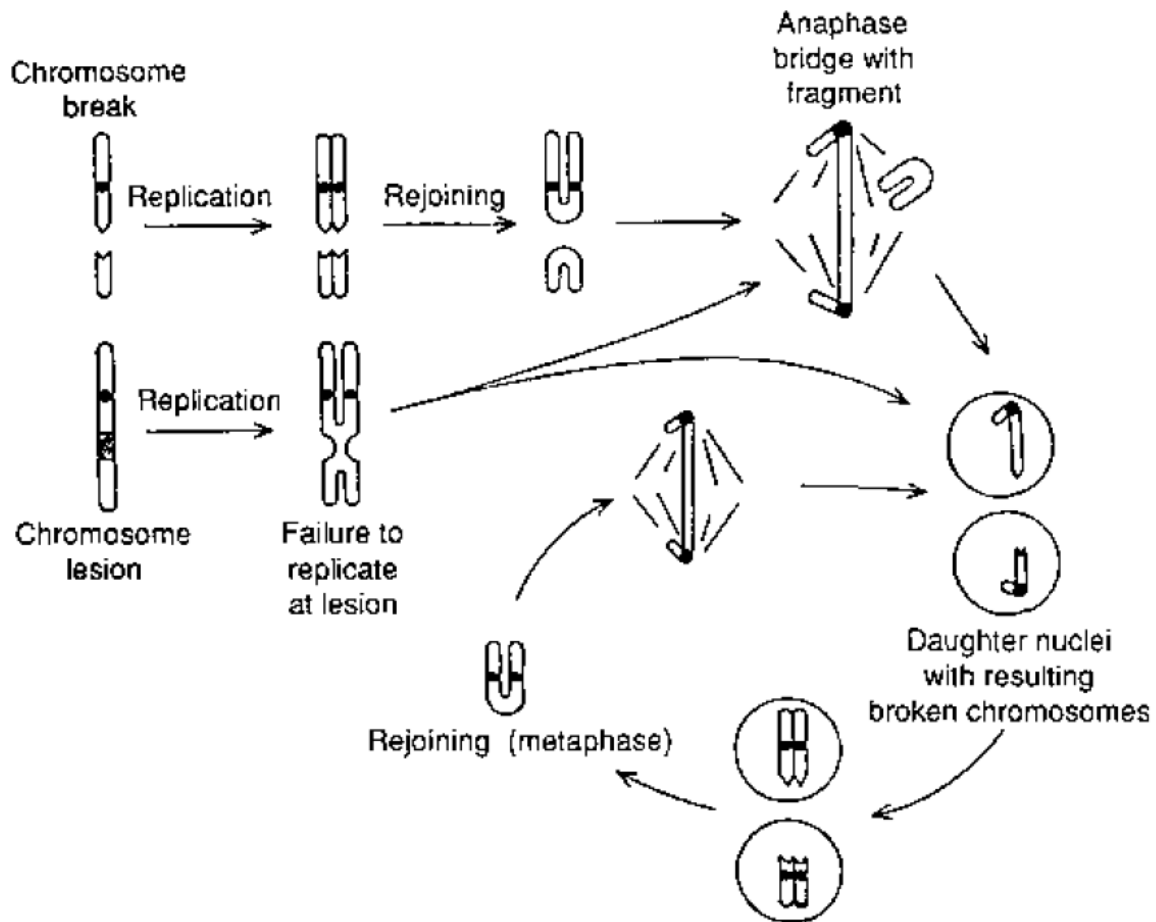


Fig. 4: The cytogenetic consequences of radiation-induced chromosome damage.

THEORETICAL BASIS OF STERILE INSECT TECHNIQUE

The Sterile Insect Technique is the first involving insect genetics for population control, and it can be applied only to pest species that reproduce by sexual means. It is effective only if the sexually sterile males are aggressive and successfully compete with wild males in searching for and mating with indigenous females.

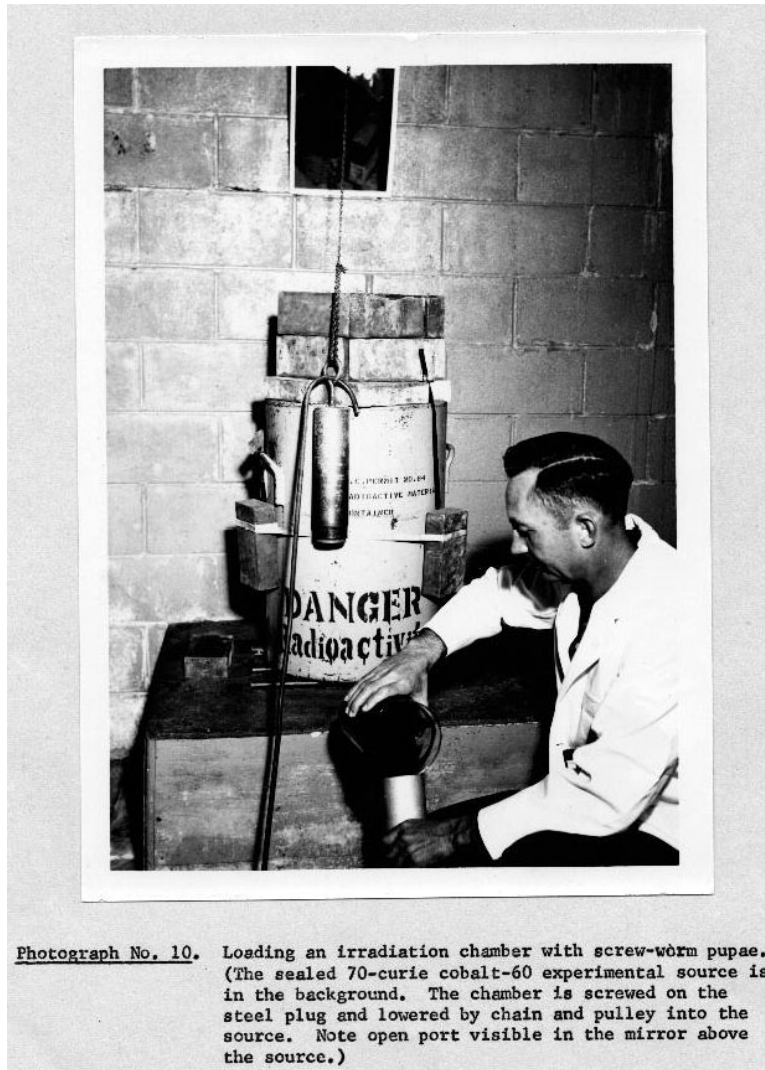


Fig. 5: "Loading an Irradiation Chamber with Screw-Worm Pupae" by Entomology Research Branch, Insects Affecting Man and Animals Section, Agricultural Research Service, United States Department of Agriculture. 1957. O.H. Graham Collection, Screwworm Eradication Collection, Special Collections, National Agricultural Library.

In its application, the target pest species is mass-reared, radiation sterilized then distributed over the range of the pest population being targeted. If the goal is eradication of the pest population, sexually sterilized males must be released within reasonable proximity of all indigenous females. Thus, release of sterile males should not be limited to selected zones; otherwise these zones would soon be reinvaded by individuals from adjacent untreated areas. To achieve eradication, the total pest population in a region must be targeted. Alternatively, segments of populations in large areas can be targeted, provided that the avenues of reinvasion are guarded first by means of quarantine check-points to prevent reinfestation through human activity and second by the release of sterile males to create a barrier zone that is wider than the flight range of the insect pest.

Theoretically, sexual sterility can be induced in target pest species by means of chemical and physical agents including alkylating agents, antimetabolites, x-rays, gamma rays and neutrons. To date, no chemical sterilants have been discovered that can be used without at least presenting some hazard to workers in mass-rearing factories, nor are chemicals yet available that can be applied to the indigenous pest population without risk to non-target species. Consequently, sexual sterility is induced with radiation emitted from radioisotopes such as cesium¹³⁷ and cobalt⁶⁰. The dosage of radiation applied must have no significant adverse effect on the males' longevity, searching behavior and mating ability. A historical photograph of the irradiation of insect pupae in a 70 curies cobalt⁶⁰ irradiator is shown in Fig. 5.

Matings of sterile males with wild females do not yield offspring. If sufficient numbers of sterile males are released, so that most indigenous females are mated by them, the total number of individuals in the wild population will be reduced in the next generation. Thus, continued releases of high-quality sterile males in overwhelming numbers over several consecutive generations will progressively reduce the wild population and eventually result in extinction.

DEVELOPMENT OF THE STERILE INSECT TECHNIQUE

The idea that populations of economically important insect species might be controlled, managed or eradicated through genetic manipulation was conceived in the late 1930s by an American entomologist, Edward F. Knipling. A similar concept was published independently by the Soviet geneticist Serebrovsky in 1940.

Knipling derived his concept while working on the screwworm fly problem as it afflicted livestock in the United States. He and his colleague, Bushland, assessed various ways of coping with the entire screwworm population in the United States. They recognized that the strategy whereby every livestock producer had to check all animals for infested wounds at least twice per week would greatly retard the development of efficient livestock production in the United States. In particular, this approach was ineffective and costly on large ranches where many cattle were not encountered routinely. Knipling and Bushland, aware that the screwworm population in the United States represented only a minute part on the northern border zone of a vast infested area that extended south into Argentina, began to consider ways of suppressing the entire northern fringe of this population.

In 1936, Bushland developed laboratory techniques for culturing the screwworm on ground meat. This was a major breakthrough, since previously no one had been able to rear an obligate parasite except on a live host animal. For the first time, substantial numbers of screwworms could be made available for research.

Knipling in 1937 observed the extreme sexual aggressiveness of the male screwworm, as well as the usual refusal of the female to mate more than once during her lifetime, a characteristic shared with the female mosquito, offering hope for the possibility of eradicating malaria. He realized that if sexual sterility could be induced in the males and if a means could be developed to rear, sterilize and release vast numbers of screwworm flies, the screwworm population in the United States could be reduced to insignificant numbers. To affect this, he devised a simple mathematical model of this revolutionary concept for insect control.

EXPERIENCE WITH THE STERILE INSECT TECHNIQUE

Feasibility studies for the control of the mosquito *Anopheles albimanus* and *A. stephensi* using Sterile Insect Technique were conducted respectively in El Salvador and in India in the 1970s, but not completed and the potential of the method is therefore not known.

El Salvador successfully used the Sterile Insect Technique to eradicate the malaria mosquito from a part of the country. In a small area of about 15 square kilometers, 100 percent sterility in the population was induced. They started a much larger project in which they were producing a million male insects a day. When civil war broke out the project collapsed.

The Sterile Insect Technique has also been successfully applied for fruit flies in the USA and Latin America, screwworm in the USA and Libya, and tsetse fly in Africa at the Zanzibar Island off the south eastern coast of Africa.

The Sterile Insect Technique was first applied against the screwworm fly, a serious pest of cattle. The female flies lay their eggs in sores or other open wounds on the animals. After hatching, the larvae eat the tissues of their host. As they do so, they expose a still larger area to egg laying, often finally killing the host. Prior to its eradication from the southeastern United States, the screwworm was causing huge annual livestock losses.

Starting in early 1958, up to 50 million sterilized both male and female flies were released each week from aircraft flying over Florida and parts of the adjoining states. Each time a fertile female in the natural population mated with a sterile male, the female laid sterile eggs. Since the females mate only once, her reproductive career was at an end. By early 1959, the pest was totally eliminated east of the Mississippi River.

Success depended only on the sterile males. In fact, the presence of sterile females was a drawback, because they competed with the intended target, but it was difficult to separate the sexes.

The southwestern states presented a harder problem because the fly winters in Mexico and with each new season could move across the border. Even so, by expanding the program to include Mexico as well, the screwworm fly was finally eliminated from both countries by 1991.

The Sterile Insect Technique has also been used with success against several other insect pests, including

1. Tsetse Fly (*Glossina* spp):

Tsetse flies (Fig.2) are vectors of trypanosomes which cause the African sleeping sickness in humans and nagana in cattle. They infest 10 million square kilometers in sub Saharan Africa and are a major cause of agricultural stagnation in the continent.

2. Medfly (*Ceratitis capitata*):

The Medfly (Fig. 3) or Mediterranean Fruit Fly is a destructive fruit fly, not *Drosophila*, in California and other fruit growing regions of the world. It is a major pest of fruit and

vegetables in sub-tropical countries and a key quarantine pest for the movement of agricultural products. There are many Sterile Insect Technique programs being carried out throughout the world.

ERADICATION OF TSETSE FLY IN THE ISLAND OF ZANZIBAR

A four-year campaign on the island of Zanzibar has achieved a historic breakthrough in the battle against the tsetse fly, an insect pest that causes hundreds of millions of dollars of damage every year and has forced farmers and herdspeople to abandon wide areas of land across Africa. Using the Sterile Insect Technique technique, the campaign succeeded in completely ridding the island of the flies that carry the parasitic cattle disease trypanosomiasis.

Trypanosomiasis was considered the most serious livestock disease affecting farmers on Zanzibar. The tsetse fly also transmits the sleeping sickness, a potentially fatal disease affecting people. Twenty-two species of the fly infest an area of 10 million square kilometres in 36 countries in sub-Saharan Africa. Direct losses from bovine trypanosomiasis in Africa are estimated to range from 600 million dollars to 1.2 billion dollars every year.

The campaign, which started in 1994, and ended in 1997, was the last step in a ten-year battle to rid the island of the tsetse fly. It was made feasible through control activities which, by using conventional methods, such as treating cattle with pesticides, first brought tsetse populations down to levels that made eradication using the Sterile Insect Technique a viable option.

For the Zanzibar campaign, tsetse mass-breeding technology and procedures developed at the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria, were transferred to the Tsetse and Trypanosomiasis Research Institute (TTRI) in Tanga, Tanzania. TTRI held the largest tsetse facility in the world - with a female fly colony of nearly 1 million insects that produced an average of 70 000 sterile males every week. Almost 8 million sterile male flies were released during the eradication campaign.

At the end of 1997, an independent expert group confirmed that, since September 1996, not a single wild fly had been captured in the once heavily infested areas of Zanzibar. In addition to monitoring the presence of the fly, which is small and difficult to find, routine blood samples are being taken from cattle in once-infested areas to be tested for trypanosomiasis. If no cases of the disease are identified over an extended period of time, this will confirm that the tsetse fly has indeed been eradicated. The fact that only one species of tsetse flies: *Glossina austeni*, was present on the island, and the isolated location of Zanzibar led to sustainable results.

The 50 km journey across to the mainland is too long for the tsetse fly to travel, even with a strong favoring wind. So the island will remain tsetse-free if precautions are taken to ensure that the flies are not reintroduced as stowaways on shipments from the mainland.

ERADICATION OF SCREWWORM IN THE USA, MEXICO AND LYBIA

The New World Screwworm (NWS): *Cochliomyia hominivorax*, has been a serious enemy of warm-blooded animals, including humans, in an area of the Americas extending from the southern United States southward into Argentina. Originally native peoples in infested areas devised and relied upon herbal remedies to treat screwworm-infested wounds. Subsequently, settlers from Europe and their descendants progressively introduced more effective treatments for infested wounds. Only in recent decades has this pest been eradicated from the United States and Mexico, through the use of the sterile insect technique (SIT).

To apply this technique, screwworms were mass reared on an artificial medium. Sexual sterility is induced by exposing the pupae to gamma rays, which damage the chromosomes in the sperm. When the eggs of wild females are fertilized with sperm from irradiated males, cell division is disrupted and the embryo dies. If sufficient numbers of sterile males are released into the wild over several generations, the reproductive success of the wild population can be progressively reduced and extinguished. In order to achieve eradication, a ratio of not less than 10:1 sterile to indigenous screwworms must be maintained throughout the ecosystem.

The introduction of NWS into the Libya in 1988, and its successful colonization of this new environment far from its original habitat in the Americas, has compelled the governments of the North African countries at risk to initiate emergency control measures of intensive surveillance of the deadly parasite, rigid control of animal movement and treatment of animals at quarantine checkpoints. This action is being undertaken in collaboration with FAO, the International Fund for Agricultural Development (IFAD), the United Nations Development Program (UNDP) and the International Atomic Energy Agency (IAEA), as well as with bilateral assistance.

A campaign to eradicate this parasite from the 20,000 km² of infestation in Libya has been launched. The principal method in the eradication strategy is based on the use of Sterile Insect Technique. The efficiency of this technique in eradicating screwworm populations while having no adverse impact on other non-target species has been well proven in the Americas. The use of the technique has consequently been recommended and support has been given by authorities in various countries in the international community.

The Sterile Insect Technique was previously applied to eradicate the New World Screwworm (NWS) from North Africa in the early 1990s. Before 1988, the NWS was confined to the tropical and subtropical Americas. Its discovery in the Libya posed a threat to the rest of Africa, Asia, the Near East and southern Europe. During a year long eradication campaign - headed by FAO, cardboard boxes of sterile male NWS were dropped from aircraft over an area of 40,000 square kilometers.

At the peak of the North African operation, 40 million flies were being released every week. These flies were transported by air in specially commissioned aircraft from the only available source in Mexico. The number of confirmed cases of screwworm in Libya had reached almost 3 000 in September 1990. By March 1991, there were no new cases.

CONCLUSIONS

The Sterile Insect Technique was applied over a period of 20 years to eradicate NWS from North America and Mexico, and efforts to eradicate it from all of Central America are now under way.

Chile and Mexico are trying to eradicate the Mediterranean fruit fly using the Sterile Insect Technique method, thereby overcoming barriers to the export of fruit to profitable markets in the USA and Japan. Argentina, Guatemala and several countries in the Near East are trying to eradicate this pest or establish pest free zones.

Malaria is an infectious disease caused by the parasite called Plasmodia. There are four identified species of this parasite causing human malaria, namely, *Plasmodium vivax*, *P. falciparum*, *P. ovale* and *P. malariae*. It is transmitted by the female *Anopheles* mosquito. It is a disease that can be treated in just 48 hours, yet it can cause fatal complications if the diagnosis and treatment are delayed. It is re-emerging as the highest infectious killer, and it is the First Priority Tropical Disease of the World Health Organization (WHO).

Malaria affects more than 2.4 billion people, over 40% of the world's population, in more than 100 countries in the tropics from South America to the Indian peninsula. The tropics provide ideal breeding and living conditions for the *Anopheles* mosquito, and hence this distribution. Every year 300 million to 500 million people suffer from this disease with 90% of them in sub-Saharan Africa, two thirds of the remaining cases occur in six countries: India, Brazil, Sri Lanka, Vietnam, Colombia and the Solomon Islands.

The WHO forecasts a 16 percent growth in malaria cases annually. About 1.5 million to 3 million people die of malaria every year with 85% of these occurring in Africa, accounting for about 4-5 percent of all fatalities in the world. One child dies of malaria somewhere in Africa every 20 sec., and there is one malarial death every 12 sec somewhere in the world. It is hoped that the experience gained in applying the Sterile Insect Technique can be successfully applied to the control and eventual eradication of malaria.

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