Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.37, 2015

A Review on Sterile Insect Technique (SIT) and its Potential towards Tsetse Eradication in Ethiopia

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Summary
Tsetse transmitted trypanosomosis constitute the greatest single constraint to livestock and crop production in sub-Saharan Africa. There were a number of attempts for the past few decades in order to control tsetse and trypanosomosis in the continent. Control strategies based on chemical approaches targeting on the parasite and vector were extensively applied but in many cases control has not been sustainable in the long term. This might be due to the fact that insecticide resistance, re-invasion, environmental damage and poor control program implementation were the attributing factors. Thus, an alternative approach namely sterile insect technique (SIT) was developed and has been widely used in the control of tsetse flies as well as other agricultural pests. SIT involves production of target tsetse species in mass-rearing facilities, sterilization of the male and the release in sustained numbers in the natural habitat large enough to outnumber the wild male tsetse flies. It is species-specific and has no effect on other 'non-target' species. The SIT project in Ethiopia was initiated and designed in 1997 in the southern rift valley area with the collection and evaluation of entomological, veterinary, environmental and socio-economic baseline data. Consequently, the project confirmed the presence of only one species of tsetse fly, i.e. *Glossina pallidipes* Austen. The ultimate objective of the project is to create a tsetse-free zone in a 25, 000 square kilometer area suitable for agricultural development. In the long term, the project aims to develop adequate national capacity for applying the concept of Area-Wide Integrated Pest management (AW-IPM) with a SIT component to the other parts of the country affected by the tsetse and trypanosomosis problem. Mass production of tsetse flies should be achieved in order to meet the demands for the regular release of sterile males. Currently, there is a coordinated effort to produce adequate amount of *Glossina pallidipes* Austen to implement the actual SIT in the southern rift valley areas of Ethiopia. It is expected that once tsetse eradication is achieved in the southern rift valley, the area-wide strategy would eventually be expanded to all other tsetse-infested regions in the country, bringing enormous benefits to agricultural development in Ethiopia.
Keywords: Ethiopia, Eradication, SIT, Tsetse flies

1. INTRODUCTION

Tsetse transmitted trypanosomosis in man and domestic animals pose a serious threat to the lives and livelihood of entire communities and constitute the greatest single constraint to livestock and crop production in sub-Saharan Africa (Hoare, 1972). The limitations imposed by tsetse and trypanosomosis remain to frustrate efforts and hampers progress in crop and livestock production there by contributing to hunger, poverty and the suffering of entire communities in Africa (PATTEC, 2001).

Tsetse flies (*Glossina* spp) can be ranked among the world's most destructive pests and are the vectors of the causative agents for sleeping sickness in humans and African Animal Trypanosomosis (AAT) or Nagana in livestock (Vreysen, 2001). Based on both their morphological and ecological specifics, there are about 31 tsetse species and subspecies divided into three subgenera or groups, namely the subgenus Austenina (fusca group), the subgenus Nemorhina (palpalis group) and the subgenus *Glossina* (morsitans group) (Jordan, 1993).

Current vector control interventions involve the use of insecticides either through sequential aerosol spraying technique (SAT); ground spraying; insecticide-treated targets or insecticide treated animals - live baits; the use of traps, and the sterile insect technique (SIT) (WHO, 2011). Insect control strategies based on chemical and biological control have had some notable successes but in many cases control has not been sustainable in the long term. This can be attributed to many reasons, including insecticide resistance, re-invasion, environmental damage and poor control program implementation (Wilke *et al*., 2009). Moreover, some of the interventions conducted in the past such as bush clearing (tsetse habitat destruction) or elimination of wild animals (tsetse reservoir hosts) have been discarded for ecological and environmental concerns.

For this reason, a species-specific, effective and environmentally friendly technique of insect control has been developed around for decades and has been widely used in the control of agricultural pests (Bushland *et al*., 1955).

The sterile insect technique as a method of pest control integrates well into area-wide integrated pest management (AW-IPM) programmes (Dyck *et al*., 2005). The sterile insect technique is one area-wide insect

pest management method where the insect pest is controlled or eradicated by affecting its reproductive capacity. The technique relies on the production in large numbers of the target insect in mass-rearing facilities, sterilization of one (i.e. in tsetse) or both sexes (i.e. screw worm) and the release in sustained numbers in the natural habitat large enough to outnumber the wild pest population (Knipling, 1955; 1959). The Sterile Insect Technique, best known by its acronym SIT and also identified as the Sterile Insect Release Method (SIRM), is a biologically-based method for the management of key insect pests of agricultural and medical/veterinary importance (FAO/IAEA/USDA, 2003-2010). It is environmentally-friendly and sustainable methods to control major insect pests of crops and veterinary and human importance. This an area-wide integrated pest management approach, by the use of the sterile insect technique, enhances food security, introduce sustainable agricultural systems, reduce losses and pesticide use, preserve biological diversity, and facilitate international trade in food and agricultural commodities by promoting the development and application of Sanitary and Phytosanitary (FAO/IAEA, 2004). SIT is species-specific and has no effect on other 'non-target' species. The technique has been effectively used for eradication of the new world screwworm (NWS) (*Cochliomyia hominivorax*) from north and then Central America to panama (Richard, 1997) and tsetse (*G. austeni*) from Unguja Island in Zanzibar (WHO, 2011).

Accordingly, the sterile insect technique seems to be promising towards the eradication of tsetse flies in Africa in general and in particular in Ethiopia. Therefore, the objective of this seminar paper includes:

- To review the sterile insect technique and to specifically assess its potential in tsetse eradication under Ethiopia context.

2. BIOLOGY OF TSETSE FLIES

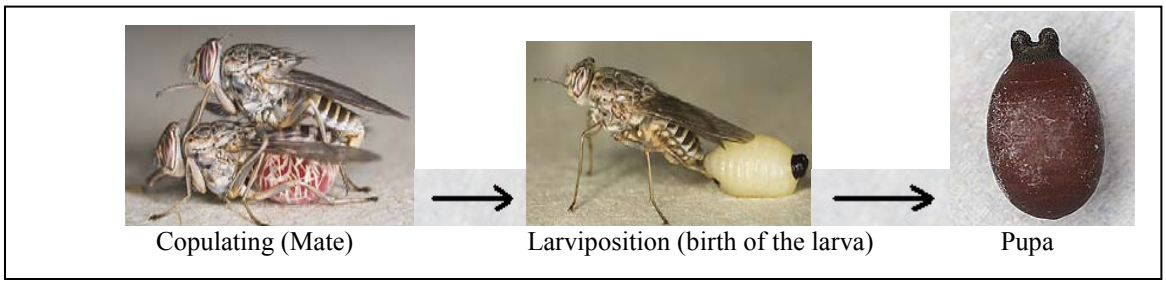
Adult *Glossina* species are dull in appearance, varying in color from light yellowish brown to dark blackish brown (Leak, 1999). In some species the abdomen may have alternate darker and lighter bands. The smallest species is 6-8 mm long and the largest 10-14 mm (Jordan, 1986). Tsetse flies can be distinguished from other biting flies by their forward-pointing mouthparts (proboscis) and characteristic wing venation.

Life cycle: the adult female produces a single egg, which hatches to first stage larva in the uterus. After a period of development and moulting, a third stage larva is deposited on the ground (Figure 1). Females produce one full grown larva every 8-10 days which pupates in light clay or sandy soil. The adult fly will emerge after a puparial period that varies according to temperature but may be around 30 days at 24^0 C. Consequently, tsetse flies have a very low rate of reproduction, closer to that of a small mammal than to most insects. This reproductive method of tsetse flies is known as adenotropic viviparity (Leak, 1999).

Egg stage: Tsetse flies are unusual insects in which their egg and larval developmental stages are takes place within the female (IAEA/FAO, 1997). The egg is fertilized immediately as it enters the uterus by sperm from the spermathecae coming into contact with and penetrating the anterior part of the egg. The fertilized egg remains lying in the uterus for about four days, while development of the first instars larva takes place inside. The egg is about 1.6 mm long (*Glossina morsitans*) (Kettle, 1984).

Larval stages: as with other flies, the larva in *Glossina* passes through several stages or instars, as it grows. There are three larval instars in *Glossina* up to the time when the fully grown larva is dropped by the female fly: the first, second and third in-stars.

The larva has a mouth at the anterior end, and two posterior spiracles. The unusual feature of the *Glossina* life history is that the larva spends practically all its time, and does all its feeding; within the body of the female fly (Leak, 1998).). The fly gives birth every 9-10 days to a full-grown larva, which immediately burrows into the soil and forms a pupa. Female tsetse produces at most nine larvae, and therefore has the lowest reproduction potential of any insect (IAEA/FAO, 1997).



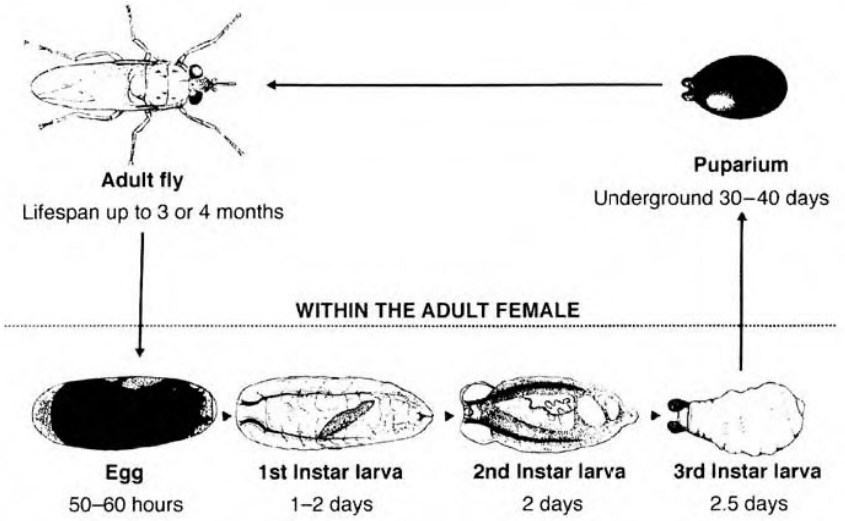


Figure 1: Life cycle of tsetse flies.
Source: (Leak, 1998).

First instars larva: this is the stage that emerges from the egg. It breaks out of the chorion using a sharp egg tooth. The first in-star grows to 1.8 mm in length (*G. morsitans*) before changing to the next stage by getting rid of its old skin. The first instars lasts for about 1 day (Vreysen, 2001)

Second instars larva: this is a stage of rapid growth and development. To either side of the posterior spiracles are swellings, and between the spiracles is an area of small spines. The second instars lasts two days, and the larva grows to a length of 4.5 mm (*G. morsitans*) (Leak, 1998).

Third instars larva: this is also a stage of rapid growth and development. The fully grown larva has a pair of large black swellings at the posterior end. These are the polypneustic lobes, which carry many small holes through which the larva breathes. The polypneustic lobes are at first white, becoming black later (Vreysen, 2001). The rest of the larva is white in color. Most of the weight and volume of the third instars larva is due to the gut which contains large amounts of unassimilated food. The third instars lasts just over two days and the larva grows to a length of 6–7 mm (*G. morsitans*).

Feeding by the larva: Apart from the food already in the egg, all the food of the three larval in-stars comes from the milk gland of the mother fly. The milky secretion of this gland is poured out of the duct of the gland, at the head end of the larva. The larva sucks up this secretion and passes it straight to the mid-gut (Leak, 1998). Here it is slowly digested and assimilated.

Breathing by the larva: for its air supply the larva depends on air entering the vulva of the female and then passing into its posterior spiracles or polypneustic lobes.

Abortion sometimes: a larva fails to reach its full size and is expelled from the uterus before the usual time. This is called an abortion. The aborted larva dies (Vreysen, 2001).

Abortions can be caused by the mother fly not obtaining sufficient food, and may also occur when the fly is carelessly handled, or when it comes into contact with insecticide. The egg may also be aborted for the same reasons (Roberts, 1993).

Larviposition (birth of the larva): When the larva in the uterus is fully grown, the female *Glossina* flies around looking for a suitable area in which to drop it. This will usually be a place where there is a patch of loose sandy soil, sheltered by an overhanging rock, branch or twig. The female tsetse settles down either on the ground or on the overhanging object. The larva then works itself backward cut of the vulva of the female, helped by pushing movements of the female's legs, and drops to the ground (Roberts, 1993). The larva burrows into the ground and out of sight the female flies' away within an hour or two the larva becomes barrel-shaped, darkens

and may then be called a pupa. There is no feeding by the larva after it is dropped by the female.

Pupa: the pupa is a dark brown rounded object; at the posterior end are the polypneustic lobes the shape of which helps to distinguish the tsetse pupa from the pupae of other flies (Kettle, 1984) (Figure 2) .

Figure 2: Picture showing polypneustic lobes of pupae.
Source: (Leak, 1998).

The pupa is slightly shorter than the larva that produces it. Inside the pupa two main processes take place: the food still remaining in the midgut is digested and assimilated, the organs of the adult fly begin to form. The pupal stage usually lasts about four to five weeks, depending on the temperature. Higher temperatures shorten the pupal period; lower temperatures lengthen the pupal period (to more than 50 days in some climates) (Jordan, 1993).

Too high or too low a temperature will cause the death of the pupa. At the end of this period, the adult fly is ready to emerge (Leak *et al*., 1993).

Adult fly: emergence of the adult fly when ready to emerge the young adult fly expands its ptilinum to burst open the end of the puparium. The body works its way out of the hole so made, and also gets through the surrounding soil by using the ptilinum. In this way the young fly struggles to the top of the soil and out into the open air (Leak *et al*., 1993). At this stage, the body is very soft and the wings are small and crumpled. After a few urinates the wings begin to expand to reach their proper size.

Teneral fly: from the time the fly emerges to the taking of its first meal, the young fly is called a teneral fly. The underside of the abdomen appears whitish and semitransparent, the ptilinum can sometimes be everted when the sides of the head are squeezed between the fingers, and the body has a soft feel to it.

Non-teneral fly: after the first blood meal has been taken, the underside of the abdomen appears more creamy yellow, and when held up to the light the dark shape of the last meal can be seen (Vreysen, 2001). The thorax feels firmer and harder, because of the greater development of muscles in it the ptilinum cannot easily be everted. The fly is then termed a non-teneral fly.

Rate of reproduction: once mated a female fly can produce larvae for the rest of her life. At a temperature of about 25°C a female fly will produce a mature larva every 9–10 days, except for the first one which may take 18–20 days from the time of emergence of the fly from the puparium. Lower temperatures give a lower rate of breeding; higher temperatures increase the rate of breeding. However, temperatures that are too high or too low will cause breeding to stop altogether (Teesdale, 1990).

As with all insects, the tsetse fly has three main body segments: the head, thorax and abdomen (Figure 3). The wings and legs are attached to the grayish-brown colored thorax.

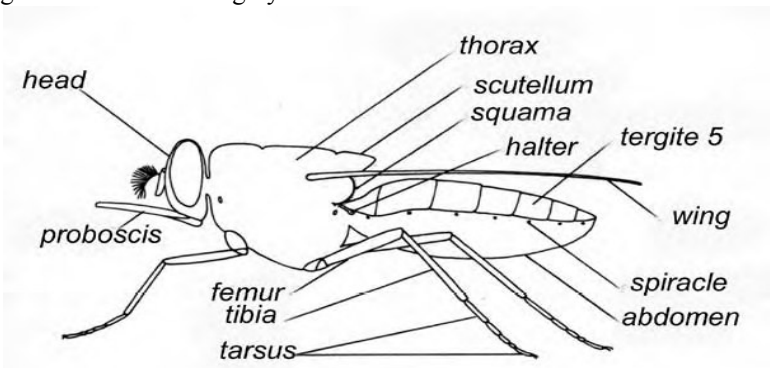
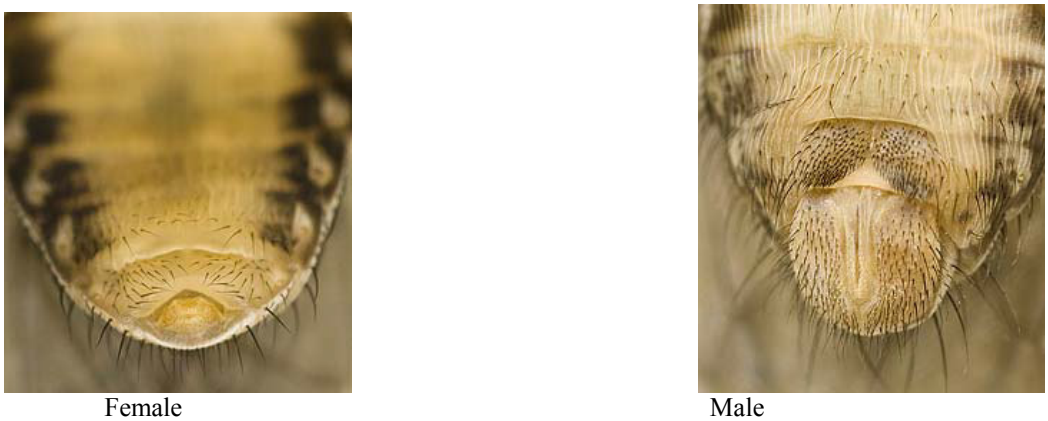


Figure 3: Diagrammatic side view of a tsetse fly: showing the three segments of fly body. Source: (FAO, 1982).
The abdomen has seven visible segments and the male fly has a structure at the posterior tip, folded underneath the last two segments called the hypopygium (Figure 4), forming part of the external genitalia. The male has a pointed abdomen and superior claspers or external genital which are heavily pigmented. On the other hand, the female has an abdomen which is truncated and pale in its appearance and used for sexing (Tadese, 2010). Each segment of the back of the abdomen has a harder cuticle forming a plate or tergite, unlike the elastic ventral surface. The coloring and markings of the tergites are sometimes useful for species identification. There are seven pairs of spiracles, one pair for each segment, along the sides of the abdomen, and an anus at the posterior end (Pollock, 1992).



Female Male

Figure 4: Comparison of external genetalia of male (Right) and female (Left) savannah tsetse flies (*Glossina morsitans morsitans*), showing the presence of hypopygium in males.
Source: (FAO, 2004)

The genitalia, particularly of the male, are useful features for species identification. It is easy to distinguish the sexes of tsetse by the presence of the folded hypopygium at the posterior tip of the abdomen, compared to the female in which there are no equivalent obvious structures, simply a small hole surrounded by a variable number of small flat chitinous plates (Figure 5) (Pollock,1992).

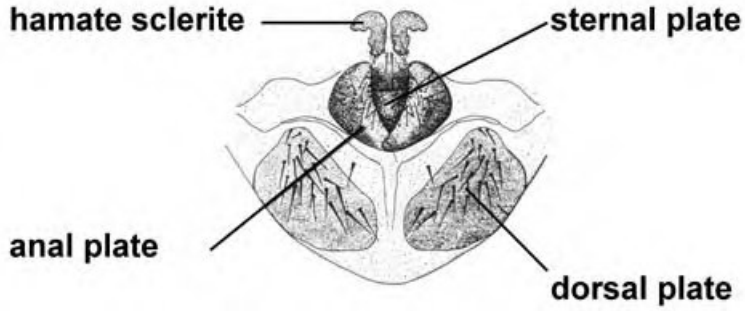


Figure 5: The external genital armature of female tsetse indicating the dorsal plates, the anal plates, the sternal plate and the hamate sclerite
Source: (Mulligan, 1970).

3. STERILE INSECT TECHNIQUE (SIT)

Definition: Defined as a method of pest control using area wide inundative release of sterile insects to reduce reproduction in a field population of the same species (http://www-naweb.iaea.org/).

3.1. History and principles of SIT

3.1.1. History of SIT

The infestation of livestock by *Cochilomia hominivorax* (Figure 6) was a major economic problem and in Texas alone, in the epidemic year of 1935, there were approximately 230,000 infestations of livestock and 55 in humans (Richard and David, 2001). As a result of the economic cost of this pest, large-scale screwworm fly control was initiated in the south-eastern states of the USA in 1957-1959. This was achieved by the release of large numbers of male *C. hominivorax* which had been sterilized by radiation. Sterilized males mate with wild females which are in turn rendered infertile. Subsequent control operations spread the area of sterile male release and in 1966 effective control of the worm in the USA was declared. In 1988, the worms were discovered in area of 10 kms south of Tripoli in Libya and causes a wide spread of myiasis and hence great economic loss. This leads to the implementation of a major international control programme which has successfully eradicated the fly from this area, again using the release of sterile males.

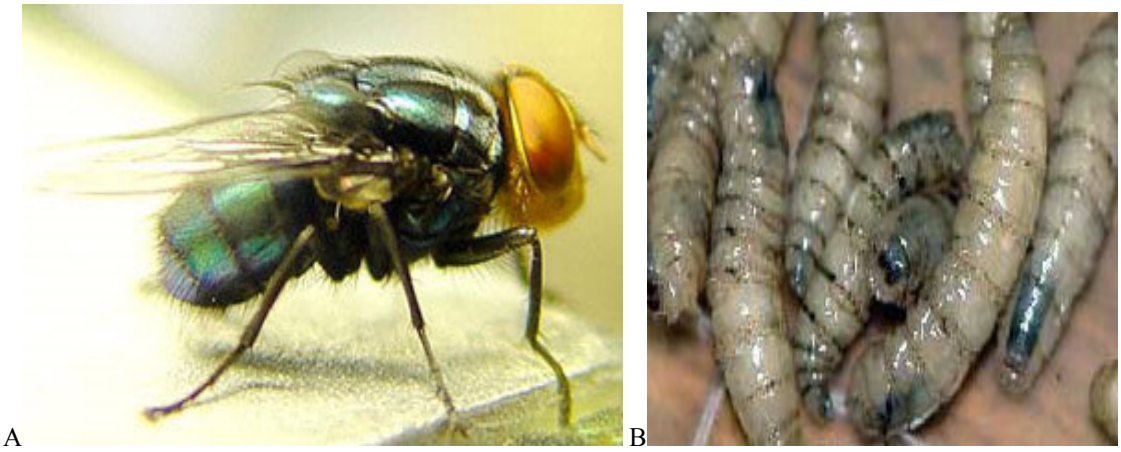


Figure 6: A. Screw worm (*C. hominivorax*) adult fly
B. Larvae of *C. hominivorax*
Source: (FAO/IAEA, 2003-2010)

In the island of Zanzibar, the introduction of the SIT helped in the eradication of tsetse fly in 1996 campaign that had been commenced two years earlier (Reichard, 2002). In Zanzibar, a sterile insect plant producing 70,000 irradiated pupae weekly was constructed that made the release of over 7.8 million sterile male flies possible. Dispersal of the irradiated males over time was done to achieve an estimated ratio of 50 sterile males for every 1 wild male in order to overwhelm the residual wild tsetse population (Reichard, 2002).

3.1.2. Principles of SIT

The SIT is non-intrusive to the environment, has no adverse effects on non-target organisms and it is species-specific and can easily be integrated with biological control methods such as parasitoids, predators and pathogens (Leak, 1999). The sterile insect technique (SIT) includes the mass production, sex separation, sterilization and release of sterile males. Contemporary methods available to induce sterility in the released insects are ionizing radiation or chemo-sterilization (Dame *et al*., 1981; Dame, 1985). As a prerequisite, tsetse density has to be suppressed through the widespread application of insecticide treated SADs (stationary attractive devices), live baits or fly trapping to a point where the SIT is considered feasible. The concept of sterile male release technique is based on the biology of the flies. It is known that tsetse females copulate only once, and if the male of a copulating pair is sterile the female will not produce offspring during her lifetime. It is also estimated that ten sterile males are required per female. In order to reduce the number of sterile males required it is necessary to carry out two to three insecticide sprays and then to release 12000 sterile males per square kilometer, even in areas where the tsetse density is low. Female tsetse produces at most nine larvae, and therefore has the lowest reproduction potential of any insect. A single mating provides sufficient sperm for fertilization through the female´s 90-100 day life-span. Since females usually mate only once, if they are mated by a sterile male they will not produce any offspring. These features of the life cycle make the tsetse fly a good target for SIT (IAEA, 1997). However, the irradiated tsetse flies are fully capable of developing and transmitting trypanosomes to livestock (Moloo and Kutuza, 1984). In order to avoid the risk of disease transmission, the sterilized tsetse flies are fed on either uninfected blood meals or blood-meals are medicated with trypanocidal drugs before the sterilized insects are fed.

3.1.3. Mass rearing of flies

Successful rearing of large number of insects for their continuous availability in the laboratory depends on the knowledge of insect biology, behavior, habitat and nutrition. An understanding of the mating habits, pre-oviposition and ovipositor periods, fecundity, longevity, sex ratio, environmental requirements, and food and feeding preferences of the insect is necessary in developing rearing techniques (Singh, 1984 and Orozco-Davila *et al*., 2007). Conservation of space is also a major consideration, especially when several days are required from the time of eggs in rearing containers until the desired life stage has been harvested. During the process of establishing a strain, along the rearing process, and during treatment before release, the insects are subjected to highly artificial conditions, including extreme population densities, a sterilization process and sometimes genetic manipulation. These all factors highly affect the biological manes of the treated insects and their performance during SIT operation (Lux *et al*., 2002). The difficulties of mass rearing of an insect vary depending up on the nature of reproduction of an insect. For example, as compared to screwworm flies, moths mass; rearing of tsetse flies have more advantageous. The screwworm requires special resources and rearing conditions at all stages of its development but in the case of tsetse fly, only the pupal and adult stages have to be considered because the egg and larval stages remain within the pregnant female fly. In nature, the larvae of the screwworm fly grow with in living mammalian flesh. Therefore for mass rearing purpose, a very complex larval medium, simulating

this living tissue, had to be developed and deployed on a very large scale. For the adult stage, screwworm flies normally feed on liquids in animal wound for protein requirements, and nectar from flowers for carbohydrates and water. The diet provided to an adult screwworm colony must take into account all three requirements (Feldmann and Hendrichs, 2001). The ingredients of screwworm diet, most of which have to be imported using hard currency. But in tsetse flies, larvae do not have to be fed as they develop within the female fly. Originally, living animals had to be used to provide adult tsetse flies with a movement. Adult tsetse flies do not required water or carbohydrates, only high-quality blood (Feldmann and Hendrichs, 2001).

3.1.4. Irradiation (Sterilization)

When biological material is irradiated, molecular bonds are broken, ions created, and free radicals formed. The free radicals attack further molecular bonds, and when DNA is damaged it can lead to the formation of dominant lethal mutations in the germ cells (LaChance, 1967; Curtis, 1971). Damage to somatic cells also occurs, especially in cells undergoing mitosis. In general, damage to the germ and somatic cells increases with dose and somatic damage decreases when irradiated later in development of the insect as the number of cells undergoing division decreases. As field competitiveness is a crucial parameter, it is important to minimize the adverse effects of irradiation. Although it is generally believed that the released males need to be fully sterile, it has been suggested that more sterility can be introduced into the field population using lower radiation doses but with more competitive insects (Parker and Mehta, 2007; Robinson, 2002). Moreover, reduced competitiveness can be partly overcome by increasing the ratio of sterile-to-wild insects (Knipling, 1955).

3.1.4.1. Types of radiation

3.1.4.1.1. Ionizing radiation technique

The term radiation includes radio waves, radar, infrared, microwaves, ultraviolet, electrons, X-rays and Y-rays. Radiation may be used as direct treatment against stored-product pests as an alternative to fumigants and other control methods or by genetic control involving the release of irradiated sterile males so that most of the females will lay sterile eggs (Hasan and Khan, 1998). Irradiation of male insect may result in dominant lethal mutations in the sperm, killing spermatogonial cells, inactivation of sperm and weakening of males (Coleman and Alphey, 2004). Depending on the dose and age or physiological condition of the insect, irradiation of the actively growing stages of an insect can have several effects. Most of the genetic sterility can be caused by irradiation that may result in failure of sperm bundles to separate, lack of motility in the spermatozoa, failure of the sperm to move to the spermatheca, or other malfunctions that can prevent reproduction and can be induced in ether sex by selecting the appropriate dose and developmental stage. Different types of ionizing radiations produce damage in significantly the same way and the effects are therefore equivalent if the dosage is measured in the same way (Tiltone and Brower, 1982).

3.1.4.1.2. Radiation source and dosimetry

For the irradiation of insects, gamma rays are usually used due to their high energy and penetration. The most common sources of gamma rays are the radioisotopes 60Co and 137Cs as both have a long half-life and emit high-energy gamma rays. 60Co is more easily manufactured and is therefore more often used. In conventional self-shielded irradiators (e.g. the Gammacell 220®, Figure 7), the sample chamber is surrounded by several rods or "pencils" of the isotope. The dose rate of the cell is determined by the activity of the source and the absorbed dose delivered to the insects is controlled by adjusting the exposure time The sample chamber is a vertical cylinder, approximately 150 mm diameter by 200 mm tall (volume 3.7 L) and has a typical dose uniformity ratio of about 1.7. Such self-shielded isotopic irradiators (60Co or less commonly 137Cs) are the main means of insect sterilization for SIT programmes worldwide. (Bakri *et al.,* 2005). The dose rate distribution in the chamber is not uniform and accordingly, insects receive different dose rates when placed at different positions in the chamber with the dose rate being most uniform towards the centre of the chamber. Besides gamma rays, x- rays and accelerated electron beams can also be used to irradiate insects. X rays of appropriate energy have similar penetration as gamma rays, and they have been used in a number of studies on Anopheles irradiation (Shoukry , 1980), but the use of electron beams has not been reported.

Figure 7: Cobalt^{60} irradiator. The Gamma cell 220 ®, a conventional self-shielded irradiator.
Source: (http://www.radsource.com/)

Dosimetry is used to quantify the dose received by the irradiated insects. The selection of a suitable dosimetry system depends on several considerations including: dose range of interest, ease of handling, expertise available, cost, and uncertainty that is inherent in the system. For SIT programmes, a radiochromic film dosimetry system has been proposed (FAO/ IAEA/ USDA, 2003).

The radiation dose absorbed by an insect to induce sterility is the main importance in sterile insect release program. Insects those receive too low dose may not be sterile efficiently and those received high dose may be uncompetitive (Parker and Mehta, 2007). The International System (SI) unit of radiation is Gray (GY) which equals to 100 rad (radiation-absorbed dose). There is no standard radiation dose for all organisms to undergo sterility. Radiation doses that result in sterility in insects and related arthropods range widely between and within order and vary also according to the species (Bakri *et al*., 2005). Apart from Lepidoptera, the radiation doses required to sterilize males of most pest specie commonly (20-150Gy) which result in dominant lethal mutations (Curtis, 1985).

3.1.4.1.3. Radiation sensitivity

Radiation-induced dominant lethal mutations arise as a result of chromosomal damage in the treated cells (LaChance, 1967). An excellent overview on the induction of dominant lethal mutations by irradiation or chemosterilization is provided by La Chance, 1967. A dominant lethal mutation occurring in a germ cell does not affect the maturation of the cell into a gamete or the participation of the gamete to form the zygote but causes the death of the developing embryo (LaChance, 1967). According to Bakri, *et al*., (2005), cells with a high mitotic rate, with a long mitotic future (that will undergo many divisions under normal circumstances) and that of the germ cell type and most radiosensitive. This implies that cells are most sensitive to radiation when they are dividing. It may be because the cellular damage is not expressed in adult insects but some tissues such as the gonads that normally undergo cell division in the adult are radiosensitive (Tiltone and Brower, 1982).

In general, the earlier stages of spermatogenesis (spermatocytes and spermatogonia) are more radiosensitive than later stages (spermatids and spermatozoa) in terms of irreversible damage, and radiation can result in the death of the developing cell (Proverbs, 1969; Anwar *et al*., 1971; Bakri *et al*., 2005). Irradiation of the later stages results in dominant lethal mutations in spermatozoa that lead to embryonic mortality after fertilization (Sobels, 1969; Anwar *et al*., 1971; Lecis *et al*., 1975). Irradiation also damages somatic cells, with those undergoing mitosis being the most sensitive. Reduced longevity is one of the most commonly observed results of somatic damage (Proverbs, 1969). Other effects of irradiation can be more subtle. A study in the male house fly Musca domestica showed that irradiation induced considerable changes in the fine structure of the fibrillar flight muscle and caused damage to the flight muscle mitochondria; the damage persisted longer in flies irradiated with higher doses (Bhakthan and Nair, 1972).

3.1.4.1.4. Developmental stage

To reduce somatic damage, insects should be irradiated at, or near to, the completion of their development the late pupal and adult stages. In general, somatic damage is less pronounced in adults compared to pupae (Curtis, 1976) although much depends on the dose and pupal age. However, handling and irradiation of pupae is considered easier due to their relative robustness compared to the adults (Clements, 1992).

3.1.4.1.5. Handling

In experimental settings, pupae can be irradiated in small wells or Petri dishes, lined with wet cotton wool

covered with filter paper (Abdel-Malek *et al*.,1967; Andreasen and Curtis, 2005) allowing for the irradiation of relatively large numbers of pupae (~500). Adults, however, are much more fragile and require careful handling. Prior to irradiation, adults can be inactivated by chilling (Smittle and Patterson, 1974) which allows them to be confined in a small space within the irradiator so that dose variation can be reduced and mechanical damage to the insects minimized. In small-scale studies, adults can usually tolerate the chilling and stacking for the irradiation but in operational campaigns, very large numbers of insects will have to be irradiated and new protocols will be required. One system has been developed (Curtis, 1976; Smittle and Patterson, 1974) that allows relatively large numbers of adults to be irradiated simultaneously (~7,000-14,000).

3.1.4.1.6. Finding the optimal dose

To determine the optimal dose for released insects, a wide range of doses is used to generate dose response data. Initially, it is important to confine the insects in a small volume in the centre of the irradiation chamber to ensure a dose uniformity ratio of < 1.1 (where dose uniformity = highest dose/lowest dose). In operational programmes, this precision in dose distribution cannot be obtained as very large numbers of insects will need to be irradiated and the programme managers will need to define the range of acceptable doses. When determining the optimal dose, effects on sterility, longevity, and importantly, competitiveness need to be taken into account (Parker and Mehta, 2007).

3.1.4.1.7. Radiation-induced sterility

The level of sterility induced in irradiated males is measured by mating the males with un-irradiated virgin females. Eggs are then collected from females individually or en masse and checked for hatching (mosquitoes) but in tsetse the laying of larvae is checked. Unhatched eggs are presumed to have died due to a dominant lethal mutation (after correcting for the control sterility naturally present in the colony (Abbott, 1971). When the residual fertility is plotted on a logarithmic scale against dose, an insight into the number of dominant lethal mutations in a cell is provided (LaChance *et al*., 1967; Curtis, 1971). A linear response indicates a "one-hit" relationship whereas departures from linearity indicate a "multi-hit" relationship (i.e. two or more independent events in the same cell produce a single dominant lethal event (Curtis, 1971).

3.1.4.1.8. Ionizing sources

Although gamma rays are the most common source of ionizing irradiation used for insect sterilization over the last decades, high-energy (5 to 10 MeV) electrons generated by accelerators and X rays can also be used (Parker and Mehta, 2007; Bakri *et al*., 2005). High energy photons, both x rays and gamma rays, are gradually absorbed by the material they pass through so that the absorbed dose decays exponentially with depth into the material. The rate of the decay depends on the photon energy; at the energy of 60Co gamma rays it declines to half after about 23 cm in water. In contrast, electrons penetrate only a short distance before the beam is completely stopped. At energy of 5 MeV, the penetration of an electron beam is around 4 cm in water. Even if a sample is exposed from both sides, the use of high-energy electrons, therefore, places important restrictions on the size of the irradiation canister used. Electron beams may also be converted to X rays by directing the electron beam at a high-atomic number material, such as tungsten, but the conversion efficiency, which depends on the electron beam energy is low, yielding only a few percent of the electron beam energy at 5 MeV. The recent approval of 7.5 MeV X rays from accelerators in the USA will increase the conversion efficiency available (FDA, 2004). X rays can also be produced by orthovoltage tubes producing X rays with energies in the 100-500 keV range. Penetration is lower than from gamma rays or X rays produced by electron beam machines but adequate dose uniformity can be achieved by rotating the samples.

X ray irradiators have suffered from low dose rates caused by the difficulty of removing the waste heat produced by the tubes, but recent advances in tube design have increased the maximum tube power substantially and dose rates of 10-15 Gy min-1 are now possible in self-shielded cabinet irradiators (Figure 8) with a working volume of about 20 L (RS2400, Rad Source Technologies Inc, Alpharetta, Georgia, USA; http://www.radsource.com/). These self-shielded irradiators require no special provisions for radiation security.

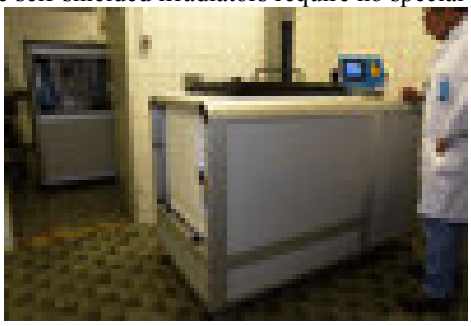


Figure 8: Prototype X ray machine
Source: (http://www.radsource.com/).
*An RS2400 self-shielded cabinet 150 kV X ray irradiator (Rad Source Technologies Inc, Alpharetta, Georgia,

USA), with a working volume of about 18 L divided into 5 horizontal cylinders 176 mm diameter by 150 mm long (3.6 L each) with a dose uniformity ratio of 1.3. This unit requires a 10 kW, three phase electrical supply and is cooled by chilled water or a self contained water-to-air heat exchanger (seen in the background). High power X ray units have only recently become available and are not in general use yet.

Isotopic irradiators have the advantage that they have a long half-life and that their dose rate is high, but the problems associated with transportation and disposal of radioactive materials are becoming increasingly difficult. Furthermore, dose uniformity is poor, forcing the utilized volume to be further restricted. Panoramic irradiators are therefore more suitable as several containers can be placed around a radiation source in a large irradiation room. The containers are then rotated around their axis to achieve adequate dose uniformity (Bakri *et al*., 2005) but dose rates tend to be lower than in self-contained irradiators.

3.1.4.1.9. Optimizing sterilization

Many factors influence the competitiveness of irradiated insects. Several strategies to reduce somatic damage during the irradiation process are discussed below.

Low oxygen environment

An important factor during radiation is the oxygen level as oxygen molecules form free radicals that induce biological damage (Fisher, 1996). Irradiation in a low oxygen environment reduces genetic and somatic damage and consequently, higher doses are needed to induce sterility levels comparable to those induced in air, although it is often observed that competitiveness and longevity are improved despite the higher dose required. Two strategies are commonly used to reduce oxygen levels. Irradiation under hypoxia brought about by respiration of pupae kept in sealed bags is routinely performed with Mediterranean fruit fly pupae. Nitrogen has been used experimentally in tsetse (Mutika and Parker, 2006) and routinely in Western Australia for Mediterranean fruit fly (Fisher, 1997). Prior to irradiation, the container that holds the insects is flushed with nitrogen for some time after which irradiation follows. Beneficial effects, i.e. long-term survival, of irradiation in a nitrogen environment were demonstrated in Mediterranean fruit fly (Fisher, 1996) and tsetse (Dean and Clements, 1969; Vreysen and Vloedt, 1995; Mutika and Parker, 2006).

Radioprotectors

Radioprotectors are substances which when present during irradiation diminish its effects. A wide range of radioprotectors is available with various modes of action (Weiss and Landauer, 2003). A range of protectors including amino-acids, cysteamine (aminothiol), diaminoethanetetraacetic acid (EDTA) and 2-aminoethyl isothiuronium bromide (AET) were tested on Cx. quinquefasciatus (El-Gazzar and Smittle, 1984). Pupae were soaked for a number of hours in the compounds, pre- and post-irradiation. None of the tested radioprotectors seemed to have a beneficial effect on the competitiveness or sterility of the irradiated males.

However, little absorption of the radioprotector is expected in the non-ingesting pupal stage. Another protector, dimethyl sulphoxide (DMSO), ingested in the adult stage before irradiation, decreased the induction of dominant lethal mutations by X rays in Anopheles atroparvus (Lecis and Orru, 1974). However, DMSO is toxic and even at low concentrations a reduced life span was observed.

Another potential class of radioprotectors are anti-oxidants which neutralize free radicals and thus prevent damage. One of these is nordihydroguaiaretic acid (NDGA), a reducing agent that replaces the naturally present reducing agent glutathione, whose amount decreases with the age of an organism (Richie *et al*.,1987). NDGA administered to the larval diet of *Ae. aegypti* increased the longevity of both sexes over un-treated controls by 42-64% (Richie *et al*., 1986).

3.1.4.1.10. Longevity

Reduced longevity is often a result of radiation-induced somatic damage (Proverbs MD., 1969), and this must be measured, ideally, under conditions that induce stress to emphasize any differences. Specifically, male survival during the first days of adult life is important as this is the period when mating is expected to occur after release.

3.1.4.1.11. Competitiveness

The ability of irradiated males to locate, compete for, and successfully couple with and inseminate the wild females is as important as their level of induced sterility (Calkins and Parker, 2005). Mating competition experiments are performed to study how well males are able to compete against un-irradiated males for females. Initially, competition experiments are carried out in the laboratory, but field or large outdoor cage tests must also be conducted to reveal those effects that are not evident under laboratory conditions (Knols *et al*., 2002). Ideally, irradiated males are competed against wild males for wild females in a semi-field setting as wild males are likely to perform poorly under laboratory conditions.

To perform competition experiments, un-irradiated males and virgin females are introduced into a cage in a 1:1 ratio and irradiated males are introduced at equal and higher ratios. In mosquito, Males will compete for the females and hatching data are collected from eggs laid en masse or from egg batches collected from individual females that are separated after mating.

When eggs are collected en masse, a method has been developed (Haisch, 1970) for determining a point estimate of competitiveness for sterilized insects. This value, usually called the Fried index, can be determined

provided egg hatch data are known for control (Ha) (i.e. un-irradiated females mated with un-irradiated males) and sterile (Hs) matings (i.e. un-irradiated females mated with irradiated males). The competitiveness index (C) is then estimated as C = ((Ha-Ee)/(Ee-Hs))*(N/S); where Ee is observed hatch, N = number of un-irradiated males, and S = number of irradiated males (Fried, 1971). Moreover, procedures have been developed (Hooper and Horton, 1981) to calculate an estimate of the variance of the C value where a number of replicates have been run, which permits detection of significant differences between values. The Fried index is independent of the ratio of un-irradiated to irradiated males but the variance depends strongly on the ratio and has the lowest value when half the observed matings are by irradiated males.

SIT technique may be applied as part of an area-wide control (integrated pest management) approach of insects of medical, veterinary, and agricultural importance. It was in 1937 when Edward Knipling proposed using sterilization to control or eradicate insect pests after observation that screwworm fly males mate repeatedly while females mate only once. He then made the hypothesis that if large numbers of sterile males could repeatedly be released into wild populations, it would eventually eliminate population reproduction and lead to eradication (Knipling, 1955; 1979).

3.1.4.1.2. Chemo-sterilization technique

Chemosterilants are chemical compounds, which can reduce or destroy the reproductive ability of an organism to which they are applied (Borkovec *et al*., 1968; Campion, 1971). Chemosterilants substituted for radiation believing that the chemicals should be easier to use and less costly than gamma radiation. Most importantly, chemosterilants have also possibility that could be used to sterilize the natural population without resorting to rearing and release of sterile specimens (Campion, 1971). The chemicals should sterilize both sexes of the pest population, or separation of male and female sterility must be developed and used simultaneously, highly selective against the target pest, and provide permanent sterilization without serious deleterious effects on the mating behavior and mating competitiveness of the organisms sterilized. The required amount of chemosterilant doses actually administered. The dose that administered must not kill the insect or not change its mating behavior (Borkovec *el al*., 1968). Chemosterilants used to sterilize insects are not as hazardous as chemicals applied in the environment to control insects because small quantity is needed to sterilize insects and the release of treated insects can be delayed until most of the chemosterilant on the insects has degraded. Alikylating agents containing best known, cheap and commercially available compounds such as aziridines tepa, metepa and apholate, and have been used in much of the laboratory and field work. These compounds have the ability to sterilize at lower doses and males are more susceptible than females (Campion, 1971). Alkylating agents are capable of replacing hydrogen in an organic molecule with an alkyl group and result in the induction of dominant lethal mutations but antimetabolites cause the insects to fail to produce ova or sperm, and may cause the death of sperm or ova after they have been produced. Antimetabolites include such compounds like 5-fluorouracil and methotrexate that interfere with nucleic acid synthesis and only have been used in the laboratory. These compounds lack specificity, have high cost, relatively scarce and generally sterilize only female insects. Miscellaneous group contains the phosphoramides, triazines, organotins, boron compounds, urea derivatives and others (Campion, 1971).

3.1.5. Aerial release operations

The release planes used are twin-engine turbine class aircraft, with a two-person crew (pilot and fly disperser). Most of the flies are released at an altitude of 2000 feet above the ground. In some cases the altitude can be modified as directed by Federal Air Traffic Controllers in release areas close to the major airports. The aircraft fly an average of 10-12 flights per week (35 hours/week).The release box is mounted inside the cargo area of the plane. There is an air conditioning compressor mounted in front of the box to keep the temperature around 38 degrees Fahrenheit which keeps the flies immobile during flight and release. Chilled flies are released through the bottom of the aircraft at a rate of at least 125,000 flies/ sq. mile or PRP (Release rate is increased to 400,000 flies/sq mile during eradication programs) (http:/www.freshfromflorida.com/).

All SIT aircraft use Global Information System/GIS-generated data to record a variety of information about each flight as it occurs. This data can be reviewed and overlaid onto mapping software to chart exactly where the plane flew and details about the fly release (http://www.freshfromflorida.com/).

3.1.6. Attributes of SIT

The SIT has special attributes, which make it a unique insect pest management tool (http://tc.iaea.org/tcweb).

Species specificity: SIT represents a biologically based tool directed at controlling only the target pest population without any adverse impact on non-target organisms.

Inverse density-dependence: SIT has the unique attribute of increased efficiency with decreasing target population density - the sterile males have the ability to find the last wild females in the whole area.

Compatibility for integration: SIT can be effectively integrated with other methods including biological methods, such as parasitoids, predators and insect pathogens giving a totally biological system for managing some of the world's most important insect pests.

3.1.7. Feasibility considerations

3.1.7.1. Economic considerations

The most persistent argument made against the use of the SIT to eradicate the tsetse fly from strategic areas in Africa is the claim that it is not "cost effective." A group of experts assigned by the FAO in 1988 agreed that the technique might be cost effective provided that the scale of the operation was at least 20,000 km^2. However the implementation of an SIT component for tsetse eradication needs an investment over 18 months equals to 3.2 to 8 years recurrent expenditures for tsetse control. The cost of the SIT package will further reduce the economies of scale through the commercialization of various aspects of the fly production (for example the local collection and processing of abattoir blood as standard diet for tsetse), the ongoing development and refinement of methods that are partially already in use and economizing the aerial dispersal of sterile males according to habitats and actually required sterile male densities or ratios in relation to wild flies (http://tc.iaea.org/tcweb) .

3.1.7.2. Political considerations

The trans-boundary nature of tsetse fly infestations and past cases of re-infestation make the success of any such undertaking, involving different countries calls for an efficient mechanism of co-ordination and co-operation between and among the affected countries. The development of one centrally coordinated continental programme, such as is envisaged and advocated by PATTEC provides an historical opportunity to facilitate this possibility and enhance the management of area-wide approaches (http://tc.iaea.org/tcweb).

3.2. Application of SIT on tsetse flies

Area-wide integrated pest management (AW-IPM) programs using SIT depends on a reliable supply of large numbers of high quality sterile insects for release. The insects are reared in special large rearing facilities or factories under defined condition (FAO/IAEA, 2006). Similar to other pests, the sterile insect technique (SIT) of tsetse flies includes the mass rearing of tsetse flies, sex separation, sterilization and release of sterile males.

Mass rearing of tsetse flies is simplified in the laboratory because only two developmental stages need to be considered, i.e. the adult and pupal stages. New colonies of tsetse flies are established using field collected pupae after quarantine to avoid introduction of any parasitoids in to the main insectary (FAO/IAEA, 2006), collection of adult females with efficient trapping systems that attracts wild flies for collection (Bandah, 1994, Kuzoe and Schofield, 2004), collection of pupae of known age from an existing tsetse fly (FAO/IAEA, 2006). During mass rearing, adult flies are kept in production cages placed on shelves on trolleys. In the conventional holding system, adults are held in cages kept on trolleys with wheels where larvae and pupae are collected (FAO/IAEA, 2006).

Colony feeding: larvae of tsetse fly do not have to be fed as they develop within the female fly. Adult tsetse flies do not required water or carbohydrates, only high-quality warm vertebrate blood. Originally, living animals had to be used to provide tsetse flies with a movement. With the development of membrane feeding system, which flies accept as host skin and through which they ingest the blood, living animals are no longer required as hosts. Animal blood for tsetse rearing can be collected at a local abattoir and then treated with gamma radiation to eliminate any micro organisms (Feldmann and Hendrichs, 2001).

The membrane or vitro feeding with a reliable source of quality-tested blood is recommended for tsetse (Feldmann, 1994; Gooding *et al*., 1997; Opiyo *et al*., 2000). The membrane for the invitro feeding system is made of silicon (Bauer and Wetzel, 1976). A silicon membrane is reinforced with netting; the size of the netting determines the thickness of the membrane. Small tsetse species are fed using a thin membrane and large species a thick membrane. Bovine or porcine blood or a mixture of both has been used for colony maintenance but the choice depends on the dietary requirements of particular tsetse species. Tests using only bovine blood (at the FAO/IAEA Laboratories, Seibersdorf, Australia) demonstrated that most tsetse species can be maintain on bovine blood alone(FAO/IAEA, 2006). Colonies of tsetse flies can be maintaining on sterile, fresh frozen, defibrinated blood or blood to which anticoagulants have been added. The procedure for feeding flies using the membrane system aims to ensure that flies are given sterile blood in a suitable state. Before feeding, the blood is heated to the body temperature of mammals.

A quantity of 100 ml of blood is sufficient to cover a surface area of 2000-2300 cm^2 and to feed 1500 flies. In the trolley membrane feeding system, cages of flies are removed from the trolley and placed on membranes lying flat on a tray containing warm blood; after feeding the cages are returned to the trolley (FAO/IAEA, 2006).

In the tsetse production unit (TPU) 3 feeding system, the opposite occurs-blood is moved to the flies and the cage-holding system is stationary. The mobile blood system is moved on trails that are fixed to the floor. Two rows of cages are fed at the same time. It is vital that the temperature of the feeding membrane is correct and therefore must be checked carefully, both before and after pouring the blood on to the tray. Lower trays are filled with blood and the blood smoothed out under the membrane by a person standing on the floor bit upper trays are filled from a working platform (FAO/IAEA, 2006).

Sterilization: Sterility is induced in the male by exposing puparia or young adults to a source of gamma

radiation such that the sterilizing dose does not adversely affect their mating and inseminating ability (http://www.iaea.org/). Sterilization of males is done by exposing the tsetse flies to a specific dose of gamma radiation emitted from radioisotopes (Cobalt 60 or Caesium 137) (http://tc.iaea.org/tcweb/) or Chemical compounds which can reduce or destroy the reproductive ability of male tsetse flies to which they are applied (Borkovec *et al*., 1968; Campion, 1971). Chemosterilants substituted for radiation believing that the chemicals should be easier to use and less costly than gamma radiation (Campion, 1971). Irradiation of male insect may result in dominant lethal mutations in the sperm, killing spermatogonial cells, inactivation of sperm and weakening of males (Coleman and Alphey, 2004).

Depending on the dose and age or physiological condition of the insect, irradiation of the actively growing stages of an insect can have several effects. Most of the genetic sterility can be caused by irradiation that may result in failure of sperm bundles to separate, lack of motility in the spermatozoa, failure of the sperm to move to the spermatheca, or other malfunctions that can prevent reproduction and can be induced in either sex by selecting the appropriate dose and developmental stage (Tiltone and Brower, 1982). According to Bakri *et al.*, (2005), cells with a high mitotic rate, with a long mitotic future (that will undergo many divisions under normal circumstances) and that of the germ cell type and most radiosensitive. So only cells continually dividing in an adult male fly are its sperm, the fly is unharmed - but the radiation breaks the chromosomes in the sperm, making it genetically sterile and unable to reproduce. However, once a female fly has mated, she assumes she is fertile and will not mate again for the rest of her lifespan. As a result, the population of flies can potentially fall dramatically.

Principles of aerial release: Sustained, systematic release of Gamma-sterilized flies, packed in special paper boxes are released by air craft twice a week among the indigenous target population. For maximum effectiveness, the sterile males released must outnumber the fertile, native male flies by a considerable margin. In order to reduce populations when conditions are highly favourable for fly reproduction, the ratio of released sterile males to native males should be at least 2 to 1 (Knipling, 1955) and may, in certain circumstances, have to be as high 15 to 1.Over time, the fertile population and the reproductive capacity are progressively reduced until fertile matings do not occur and the population is eliminated (http://tc.iaea.org/tcweb).

The SIT is used if the objective is tsetse eradication. As was the case in the island of Zanzibar, the introduction of the SIT helped eradicate the fly from this island in 1996 in a campaign that had been commenced two years earlier (Reichard, 2002). As a prerequisite, tsetse density has to be suppressed through the widespread application of insecticide treated SADs, live baits or fly trapping to a point where the SIT is considered feasible. In Zanzibar, a sterile insect plant producing 70,000 irradiated pupae weekly was constructed that made the release of over 7.8 million sterile male flies possible. Dispersal of the irradiated males over time was done to achieve an estimated ratio of 50 sterile males for every 1 wild male in order to overwhelm the residual wild tsetse population (Reichard, 2002). The released sterile males in the target area do out-compete the wild male population for wild females (Vreysen, 2005). Mating of the sterile males with virgin, native females result in no offspring. With each generation, the ratio of sterile to wild insects will increase, making this technique more and more efficient with lower wild female population densities (inversely-density dependent).

The SIT is non-intrusive to the environment, has no adverse effects on non-target organisms, is species-specific and can easily be integrated with biological control methods such as parasitoids, predators and pathogens (Leak, 1999). There is no threat of resistance development to the effects of sterile males, provided that adequate quality assurance is assured during the production process and that the sterile insects cannot get established in released areas as is the case with other biological control programmes (Vreysen, 2001). In addition, the SIT necessitates efficient release and monitoring methods, which have to be applied on an area-wide basis (Vreysen, 2005). Since the irradiated tsetse are fully capable of developing and transmitting mature trypanosomes of all three main species pathogenic to cattle (Moloo and Kutuza, 1984), the sterilized tsetse are fed on either uninfected blood meals or blood-meals are medicated with trypanocidal drugs before the sterilized insects are fed. Sterilized tsetse are less likely to become infected (at least with Nannomonas and Trypanozoon parasites) after they have taken an uninfected blood meal and trypanocidal drugs in medicated meals helps reduce the establishment of infections in subsequent meals. 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 herds people to abandon wide areas of land across Africa. Using the Sterile Insect Technique (SIT), the campaign succeeded in completely ridding the island of the flies that carry the parasitic cattle disease (FAO, 1998).

The SIT is an ideal mechanism to be combined with other methods of control and it fits well within the concept of IPM. One of the major advantages of the SIT is demonstrated by the theoretical model of Knipling i.e. the control effort becomes more economical and efficient as the natural population declines and increasing ratios of sterile to wild males are achieved (DAME, 1970). This is in contrast with conventional methods of killing insects, e.g. with insecticides. Here, the continued use of the same treatment will result in the same percentage effect regardless of population density (Lachance *et al*., 1967) and the technique will become therefore less

efficient in terms of numbers killed as the natural population declines. The complementary use of both conventional techniques and SIT in a phased approach would therefore result in maximum efficiency throughout the intervention phase (Figure 9).

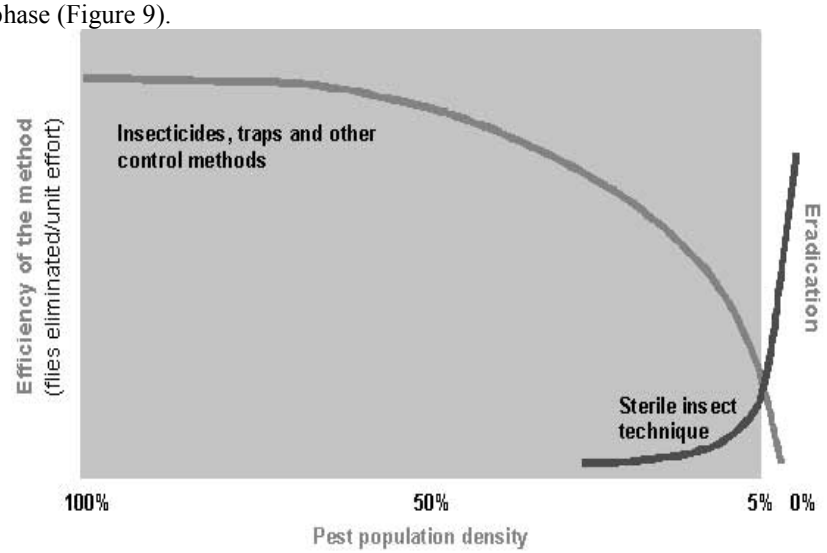


Figure 9: Optimizing the efficiency of an insect pest intervention campaign by using conventional control and SIT in an integrated, phased approach.
Source: Vreysen (2001).

The SIT has also its limitations: i.e. it is not cost- efficient with high population densities and requires therefore prior population suppression with limited use of insecticides in those cases and it has a delayed effect on the numbers of wild insects present. Specifically in the case of tsetse flies, the low reproductive capacity of tsetse makes the rearing in large numbers cumbersome and there is a potential danger of disease transmission in case the sterile male insects are released as pupae (DAME, 1970). Furthermore, the initial cost is high due to the need to provide adequate facilities for mass rearing and irradiation of the fly the use of special planes for release of sterile flies and for maintenance of equipment and personnel engaged in field and laboratory operations (http://www.iaea.org/).

4. STERILE INSECT TECHNIQUE (SIT) IN ETHIOPIA

4.1. Tsetse and Trypanosomosis in Ethiopia

According to Langridge (1976) and FLDP (1989), tsetse flies in Ethiopia are confined to the western and southern regions between longitude 33 º and 38º E and latitude 5º and 12º N. The total area infested by tsetse flies in 1976 and 1988 was 98,000 km^2 and 120,000 km^2, respectively. Tsetse infested areas lie in the lowlands and also in the river valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo (Amare, 1995).

The infested area extends from the southern part of the Rift Valley, around the southwestern corner of the country and along the western lowlands and escarpments to the Blue Nile. Restricting a further eastward spread is the cold limit imposed by highlands that rise to the height above which tsetse cannot survive, or the semi-desert condition along the southern border east of the Rift Valley. Elsewhere there have been advances of tsetse, including extension of the upper altitude limit of the fly from about 1,600 to 2,000 meters above seal level (Abebe *et al*., 2004). Tsetse fronts in many places are unstable and tsetse-animal interface is constantly moving. Consequently new areas are being invaded and settled communities are being continually evicted by the advancing tsetse. Such hot spots include the areas in Upper Didessa Valley, the northern and north eastern edges of Lake Abaya in the Rift Valley, the upper reaches of the Omo-Ghibe and its tributaries (Amare, 1995; NTTICC, 1996 and SRVL, 2000). According to survey result conducted by Langridge (1976) five species of Glossina (*G. m. submorsitans*, *G. pallidipes*, *G. tachinoides*, *G. f. fuscipes* and *G. longipennis*) have been recorded from Ethiopia but only four are widespread and significant economic importance. These are *G. m. submorsitans* and *G. tachinoides*, which have a west to east distribution across Africa south of the Sahara desert, and *G. pallidipes* and *G. f. fuscipes* which often occur together in East Africa, although the former extends far to the south whereas the latter has essentially central African distribution. The highest catches of G. pallidipes were in bushes and wooden grass land in the Southern Rift Valley of Ethiopia (Veryesen *et al*., 1999). Out of the nine regions of Ethiopia five (Amhara, Beneshangul- Gumus, Gambella, Oromiya and SNNPR) are infested with more than one species of tsetse flies (Abebe *et al*., 2004).

Adult Glossina species are dull in appearance, varying in color from a light yellowish brown to a dark blackish brown. In some species the abdomen may have alternate darker and lighter bands. The smallest species (*G. tachinoides*) is 6-8mm long and the largest (*G. pallidipes*) 10-14 mm (Vreysen, 2001). The adult female

produces a single egg, which hatches to a first stage larva in the uterus. After a period of development and molting a third stage larva is deposited on the ground. An adult female produces one full grown larva every 9-10 days which then pupates in light or sandy soil. The adult fly will emerge after a pupal arial period that varies according to temperature but may be around 30 days at 24 ^{o}C (Leak, 1998). Consequently, tsetse flies have a very low rate of reproduction, closer to that of a small mammal than to most insects. The reproductive method of tsetse flies is known as adenotropic viviparity (Vreysen, 2001). When a tsetse fly emerges from its pupal case it is free from trypanosomes. Until its first blood meal, it is called a teneral fly and after its first meal it is called nonteneral. It acquires a trypanosomal infection when it feeds on a parasitaemic mammalian host. The trypanosomes undergo a cycle of development and multiplication in the digestive tract of the fly until the infective metacyclic trypanosomes are produced (Stevens and Brisse, 2004). Different trypanosomes develop in different regions of the digestive tract of the fly and the infective metatrypanosomes occur either in the biting mouthparts or the salivary gland of the fly (Leak, 1998). The period from ingesting infected blood to the appearance of the infective forms varies from one to three weeks and once infected trypomastigote are present the fly remains infective for the remainder of its life (Seifert, 1996). During the act of feeding the fly penetrates the skin with its proboscis. By the rupture of small blood vessels a pool of blood is formed in the tissue and the fly injects saliva to prevent coagulation. Infection of the host takes place at this stage, with infective metacyclic trypanosomes in the saliva (Hargrove *et al*., 2003 and Hargrove, 2004). Tsetse flies once infected with trypanosomes are likely to transmit the parasite for the remainder of their lives.

4.2. Status of SIT in Ethiopia

In 1997, the Ethiopian government-assisted by the International Atomic Energy Agency (IAEA)-initiated a project in the Southern Rift Valley called the Southern Tsetse Eradication project (STEP).

Its long-term objectives are:

(1) To create a tsetse-free zone in a 25, 000 square kilometer area under agricultural development

(2) To develop adequate national capacity for applying the concept of Area-Wide Integrated Pest management (AW-IPM) with a Sterile Insect Technique (SIT) component to the other parts of the country affected by the tsetse and trypanosomosis problem (Alemu *et al.,* 2007).

The project was initiated with the collection and evaluation of entomological, veterinary, environmental and socio-economic baseline data which reconfirmed the presence of only one species, i.e. *Glossina pallidipes* Austen, in the main valley and the positive socio-economic and agro-economical impact anticipated. This situation generated international acceptance of the Southern Rift Valley as a high priority area for the control of tsetse and trypanosomosis and for related sustainable agriculture and rural development (Alemu *et al.,* 2007). A colony of *G. pallidipes* Austen originating from the Southern Rift Valley was also initiated in 2002; community-based tsetse suppression was initiated in localized areas using insecticides on cattle and on blue-black blue fabric targets that attract tsetse flies. These localized tsetse suppression activities have been expanded to all operational grids of the 10500 square kilometers STEP block-1 area limited entomological and veterinary monitoring in 15 sites suggests that the apparent density of *G. pallidipes* in these localized control sites may have been reduced by 92% while the prevalence of trypanosomes in live stock in those areas decreased by 58% (Alemu *et al.,* 2007). An analysis using geographic information system (GIS) has indicated that the community-based tsetse suppression does not cover all of the tsetse-infested areas in the STEP block-1 and it is therefore assumed that some cattle herds remain with high disease prevalence in areas that were not adequately covered by the community fly control measures. The operational programme will include the introduction of a set of implementation of rules and regulations conducive to the special needs of an operational AW-IPM campaign, i.e. an efficient management structure and the provision of adequate financial flexibility (Alemu *et al.,* 2007).

Tsetse fly infestation in the southern Rift Valley of Ethiopia has limited the expansion of mixed farming in the technical cooperation project ETH/5/012-Integrating Sterile Insect Technique for tsetse Eradication.

The project aims to create a zone free of tsetse and trypanosomosis in an area of 25 000 km^2 in the Southern Rift Valley (SRV), Ethiopia. This will free up the area to enable the introduction of mixed farming according to a land use plan being developed by the Government (IAEA, 1957-2007). The STEP Kaliti Tsetse Rearing and irradiation centre (Kaliti centre) was inaugurated on 3 February 2007. When completely equipped and operational, the new facility will have a colony capacity of approximately 7 million female flies and will be able to produce over 700 000 sterile male flies per week enough to treat approximately 7000 km^2 at a time (IAEA, 1957-2007).

The STEP is currently preparing for large-scale field operations, including the sterile insect technique. The creation of the tsetse-free zone in the entire target area is expected to be completed by 2017, including a four year verification phase. The project is currently focusing on an area of 10,500 km^2 which has considerable potential for agricultural development. Operational activities to clear this area will depend on the built-up of a sufficiently large colony of target tsetse fly species in captivity and other essential preparatory work, particularly in the field. It is likely that related field intervention work will need to continue through at least 2013(IAEA,

1957-2007).

4.2.1. *The model project*

The International Atomic Energy Agency is providing support to the Ethiopian authorities for tsetse control/eradication in an area, initially of 5,000 km^2, in the Southern Rift Valley. Over the ten-year life of the project, it is planned to extend the target area to about 25,000 km^2. Following an assessment of entomological, veterinary and socioeconomic factors which will influence subsequent project operations, a phase of tsetse suppression by appropriate conventional means will be introduced. The project area will be divided into eradication zones, each chosen to take as much advantage as possible of natural isolating features such as high or arid land. Six to eight months prior to aerial releases of sterile flies, locally made, odour-impregnated traps will be placed at a density of four per km^2 by specially trained local teams to start reducing the fly population in the target area. When fly numbers have been sufficiently suppressed, the traps will be removed and aerial release of sterile males initiated (http://tc.iaea.org/tcweb/).

As the project develops, a pattern of suppression will be followed by an airborne operation during which sterile male tsetse flies will be released over the targeted area. The flies will be produced and reproductively sterilized by irradiation at a mass rearing factory Kaliti. In order to begin mass rearing, a suitable strain of tsetse fly has been collected from the project area and adapted to the artificial conditions of factory production. The Agency's laboratories at Seibersdorf in Austria, and the Tsetse and Trypanosomosis Research Institute at Tanga, Tanzania, are involved in this work and will maintain a back-up colony of this strain respectively. Initially, the mass rearing facility in Ethiopia will supply 250,000 sterile males per week. Production will eventually be doubled in order to meet the project's needs. The flies will be chilled to immobility, avoiding the need to box them for aerial release (http://tc.iaea.org/tcweb/).

The project area has been chosen because it has high agricultural potential and is well confined from neighboring tsetse-infested areas by high escarpments and arid land. Furthermore, tsetse infestation is high and local population pressure is putting a severe strain on available fly free highlands (http://tc.iaea.org/tcweb/).

Fly releases: Unlike conventional insect control methods that are preferably applied to a high population of the target insect pest, SIT is most effective when fly populations are low. The ratio of released sterile male flies to wild fertile male flies should be as high as possible to minimize the odds that wild flies mate (http://tc.iaea.org/tcweb/).

Flies will be released over the targeted eradication zone at a rate of approximately 100 sterile males per km^2 per week over almost two years. These numbers will be adjusted as necessary in order to achieve the desired ratio of released sterile to wild fertile males. Aircraft will fly regular sorties over the area, ejecting chilled flies at a computer controlled, and specified rate. The flies warm up and become active as they reach ground level (http://tc.iaea.org/tcweb/).

4.2.2. The benefits

According to Langridge (1976) and FLDP (1989), tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33º and 38º E and latitude 5º and 12º N. The total area infested by tsetse flies in 1976 and 1988 was 98,000 km^2 and 120,000 km^2, respectively. This area will be free after the successful implementation of SIT and the fertile land will be used for investments and for the subsistence agricultural production of the local farmers (http://tc.iaea.org/tcweb/).

So far about 10, 000 km² of land with good opportunities for sustainable agricultural and rural development have been covered by the STEP tsetse suppression activities. The experienced substantial reduction of the tsetse and trypanosomosis problem already permitted an increase of productive livestock in the area. For the first time the rural communities can make use of horses and donkeys in the southern rift valley, where previously they were unable to be used, because they are very susceptible to tsetse-transmitted trypanosomosis.

Once free of the risk of trypanosomosis, farmers in Ethiopia should feel confident in the benefits of investing in better livestock breeds and, therefore, being able to get better productivity from a given number of animals. Foreign currency spending on treatment of trypanosomosis will be reduced. Ecological pressure on the fragile highlands will be lessened through a slight expansion of the area under crop cultivation particularly in the previously abandoned fertile land. The use of better, not necessarily more, livestock including draught animals will allow intensified agricultural practices (http://tc.iaea.org/tcweb/).

Initially, the mass rearing facility in Ethiopia will supply 250,000 sterile males per week. Production will eventually be doubled in order to meet the project's needs. The flies will be chilled to immobility, avoiding the need to box them for aerial release. Ethiopian authorities hope that once eradication is achieved in the Southern Rift Valley, the area-wide strategy would eventually be expanded to all other tsetse-infested regions in the country, bringing enormous benefits to agricultural development in Ethiopia (http://tc.iaea.org/tcweb/).

The project intends to expand the tsetse suppression operations to some 25 000 km² in the next 1-2 years. It is anticipated that, once developed for large scale application in Ethiopia, the sterile insect technique (SIT) will complement the area-wide and integrated pest management efforts, aiming at a complete elimination of the tsetse and trypanosomosis problem. There are, however, some critical issues to be addressed with a sense

of urgency, before the SIT component will be available to STEP (http://tc.iaea.org/tcweb/).

5. CONCLUSION AND RECOMMENDATIONS

In tropical and subtropical rural areas of Africa several tsetse fly species, while sucking blood on humans and livestock, transmit unicellular blood parasites that eventually affect the central nervous system, causing sleeping sickness among humans and a similar disease among livestock, called nagana. The diseases particularly affect poor rural communities and their livestock, which is why the tsetse fly vector is often referred to as the 'poverty insect'. This leads to high population pressure in the nearby highlands of the country. A lot of control options have been attempted for the last decades but not yet economically visible results were attained. As a result a new alternative control option namely SIT was proposed to implement the eradication of this the devastating vector, tsetse flies. Since its inception in 1997, STEP managed to train and involve more than 220, 000 farmers in methods for suppressing tsetse fly populations and the disease they transmit, African animal trypanosomosis (AAT). The project applies pour-on formulations of insecticides onto livestock and, in addition, positions into the fly habitats insecticide-impregnated blue / black fabric targets, which attract tsetse flies and kill them. So far about 10 000 km^2 of land with good opportunities for sustainable agricultural and rural development have been covered by the STEP tsetse suppression activities. The experienced substantial reduction of the tsetse and trypanosomosis problem already permitted an increase of productive livestock in the area. For the first time the rural communities can make use of horses and donkeys in the southern rift valley, where previously they were unable to be used, because they are very susceptible to tsetse-transmitted trypanosomosis. However, beginning from the establishment of SIT in Ethiopia up to now the actual SIT has not been implemented for the execution of tsetse eradication in the in the southern rift valley area. All the necessary efforts and commitments should be provided to commence mass rearing of sterile male *G.pellidipes* Austen for effective insect release.

Based on the above conclusion, the following recommendations are forwarded:

- Ethiopian government and all concerned authorities should work hand in hand to promote SIT in Ethiopia for the ultimate tsetse eradication program.
- Pre-suppression of the wild tsetse flies should be exercised strictly before the actual implementation of the SIT. Moreover, any program dealing with SIT should actively involve the participation of the local farmers.
- Over all SIT technologies are too expensive, so less expensive technology should be developed.

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