Joint FAO/IAEA Centre
Nuclear Techniques in Food and Agriculture

Thematic Plan for the Development and Application of the Sterile Insect Technique for Tsetse Area-Wide Integrated Pest Management Programmes

Food and Agriculture Organization of the United Nations
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SUMMARY

In February 2001, an expert meeting was convened to seek advice from a panel of external experts to align the IAEA's Sterile Insect Technique (SIT) Programme and related activities with PATTEC's (Pan African Tsetse and Trypanosomosis Eradication Campaign) plan of action and set priorities for its effective integration into area-wide campaigns aiming to create tsetse-free zones in sub-Saharan Africa. The experts were also asked to deliberate and provide recommendations on the most effective and efficient way for the International Atomic Energy Agency (IAEA) to support its Member States in addressing the tsetse and trypanosomosis challenge. All the compiled information contributed for the development of a *"Thematic Plan for establishing tsetse-free zones through area-wide tsetse control interventions involving the Sterile Insect Technique-SIT"*. The thematic plan was developed based on discussions, observations and conclusions reached during the experts meeting aiming to provide strategic guidance and direction on how and where the SIT technology could be most efficiently and effectively applied to control and ultimately eradicate the tsetse fly.

Following the recommendations after the meeting of experts and the publication of the thematic plan, the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, particularly its Insect Pest Control Subprogramme, in collaboration with its Member States, improved the SIT package as a tool for managing tsetse populations as a component of Area-wide Integrated Pest Management (AW-IPM) during the last two decades. Extrabudgetary contributions have been instrumental to support research and developments activities and transferring the SIT technology from the laboratory to the field.

During the last twenty years, the technology has been evaluated in the field and success has been demonstrated on Unguja Island, United Republic of Tanzania and in Senegal with the eradication of *Glossina austeni* and *Glossina palpalis gambiensis*, respectively from highly productive agricultural regions using an AW-IPM approach with an SIT component. The creation of "free areas" of tsetse flies and the disease trypanosomosis in both countries has resulted in significant increase in milk yields, more productive cattle with a very positive overall return on investment. The increased livestock and crop productivity and the use of animals for transport and traction significantly contributed to an increase in the quality of people's lives. Therefore, these successful results in field operational programmes and the new technological challenges regarding efficient and economical delivery of SIT package for tsetse species in view of the continuous request of the African Member States were the main drivers for reviewing and updating the existing Thematic Plan to support the creation of sustainable tsetse and trypanosomosis free areas in sub-Sahara Africa.

The discussions during this meeting resulted in the development of an updated version of the Thematic Plan which states: 1) the magnitude of the tsetse and trypanosomosis (T&T) problem in the region; 2) conventional tsetse control tools; 3) the need for an area-wide integrated pest management approach; 4) the advantages, constraints, gaps, and challenges for the implementation of field operational programmes with the SIT component targeting tsetse species; 5) the socio economic impact of African Animal Trypanosomosis; 6) the recent developments of the FAO/IAEA and ongoing projects and collaborations; 7) research and development (R&D) needs for fine-tuning components of the SIT for tsetse flies and identification of knowledge gaps and potential future role of the IAEA and the Joint FAO/IAEA Subprogramme; 8) priorities on capacity building and other needs; 9) partnerships and collaborators and 10) recommendations from the Member States to the FAO/IAEA to continue supporting the control of tsetse and trypanosomosis following the Phased Conditional Approach (PCA) in an AW-IPM strategy.

1. STATEMENT OF THE PROBLEM

1.1 Importance of tsetse fly as livestock disease vector in Africa

Scientists, the African Union and producers in the endemic areas have described African animal trypanosomosis (AAT) as one of the major health constraints to livestock farming in sub-Saharan Africa (J. Bouyer et al. 2015). Tsetse flies and the trypanosomosis (T&T) problem which they cause constitute one of the greatest constraints to socio-economic development on the African continent, affecting the health of humans and of livestock, limiting sustainable rural development, and thus causing increased poverty and food insecurity.

Tsetse flies infest an estimated 8.5 million km² in 37 countries in Africa (Figure 1-3), where 46 million cattle are exposed to AAT, directly affecting livestock health, agricultural capacity and land use (Swallow 1999; Alsan 2015). On a continental scale, annual livestock losses are estimated at US $1.3 billion and agricultural production losses are estimated at US $4.5 billion, excluding indirect losses such as manure and animal traction (Zoma, B.L. 2014). These losses are all the greater because the tsetse fly's range is located in the most fertile areas (Alsan 2015). The importance of the presence of T&T in the continent is epitomized by the fact that 32 of the 39 poorest countries world-wide are found in Africa.

Substantial literature exists documenting the extent and magnitude of the T&T problem in agricultural terms. Clinical AAT in livestock is a major impediment to livestock production in most of the 37 tsetse-infested countries in sub-Saharan Africa but this is only part of the problem. The uneven distribution of livestock restricted by the tsetse belts, the inability of farmers to use draught animals for crop production, the distorted land use and settlement patterns in high tsetse-infested areas, all severely constrain agricultural and rural development. However, potential benefits can be obtained with an appropriate T&T control programme. The expected direct benefits are an increase in productivity for a stable breeding system: reduction in morbidity, mortality and treatment costs, and an increase in the number of animals and in milk and meat production. Indirect benefits mainly concern access to new pastures or the cultivation of new land (Kamuanga, Hamadou & Kaboré, I 2006). Kristjanson et al (1999) estimated that in terms of milk and meat productivity, the benefits of improved trypanosomosis control would amount to more than US $700 million per year.

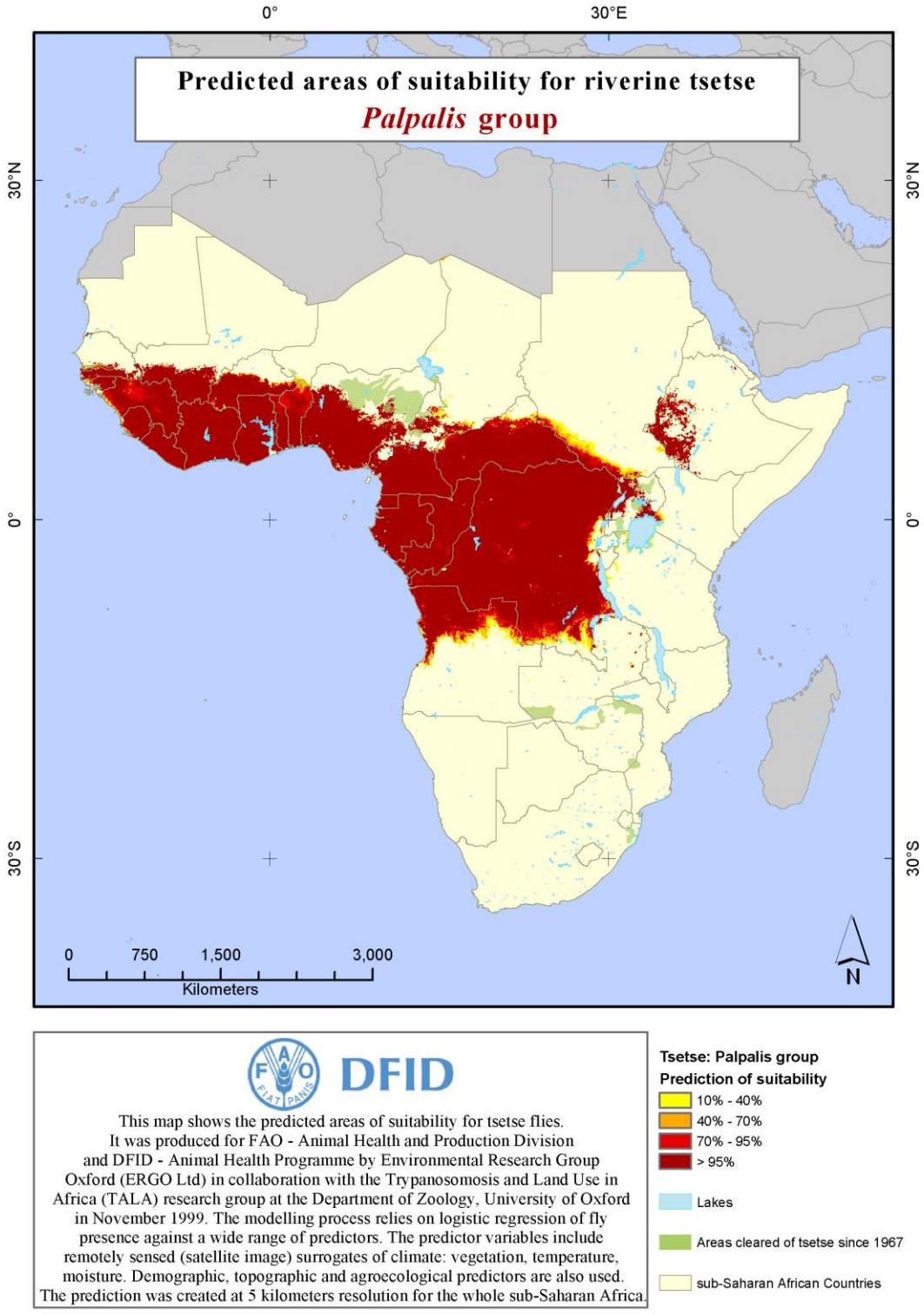

Figure 1: Predicted suitability for the palpalis groupe tsetse species (Source: http://ergodd.zoo.ox.ac.uk/tseweb/distributions.htm).

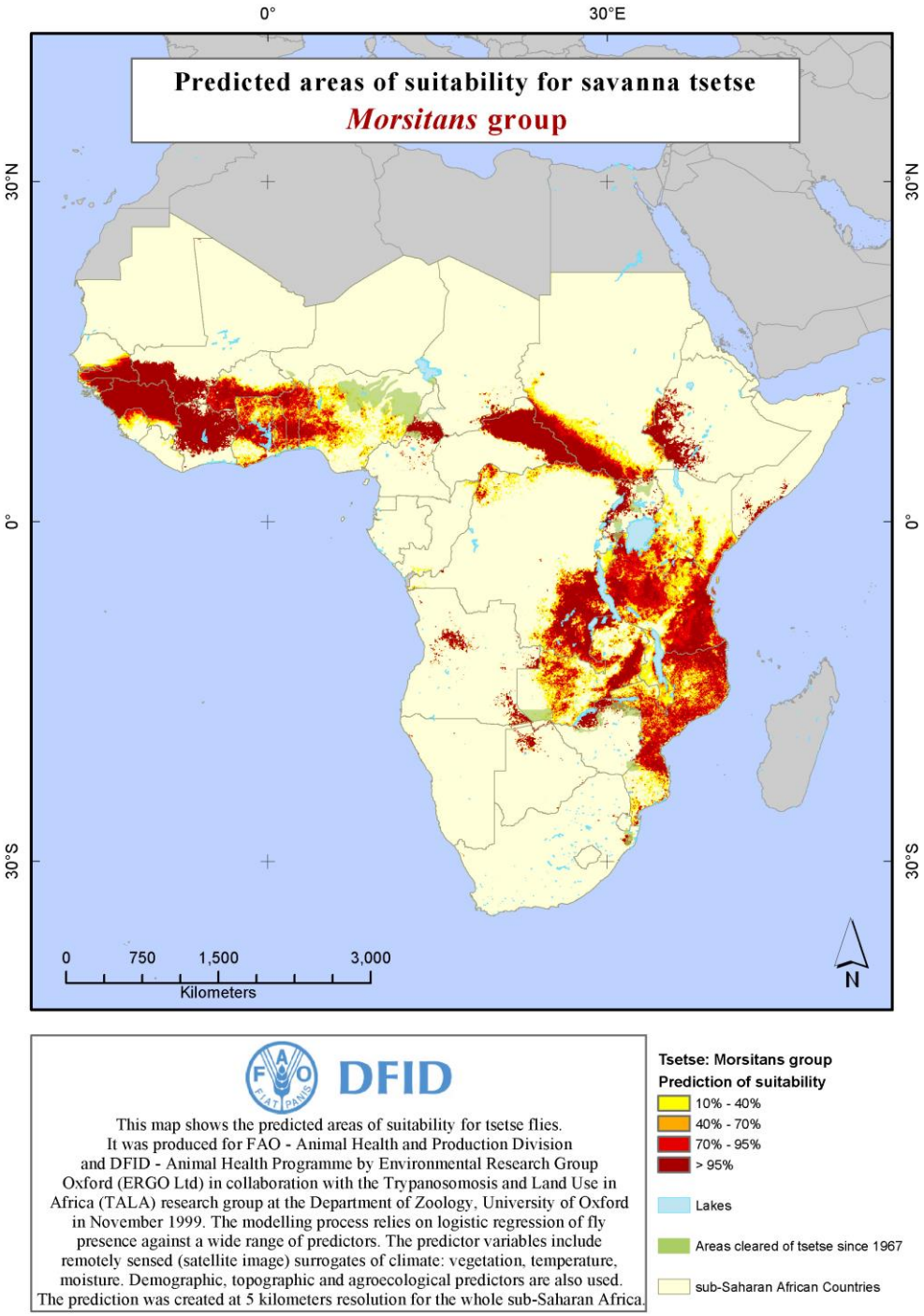

Figure 2: Predicted suitability for the morsitans groupe tsetse species (Source: http://ergodd.zoo.ox.ac.uk/tseweb/distributions.htm).

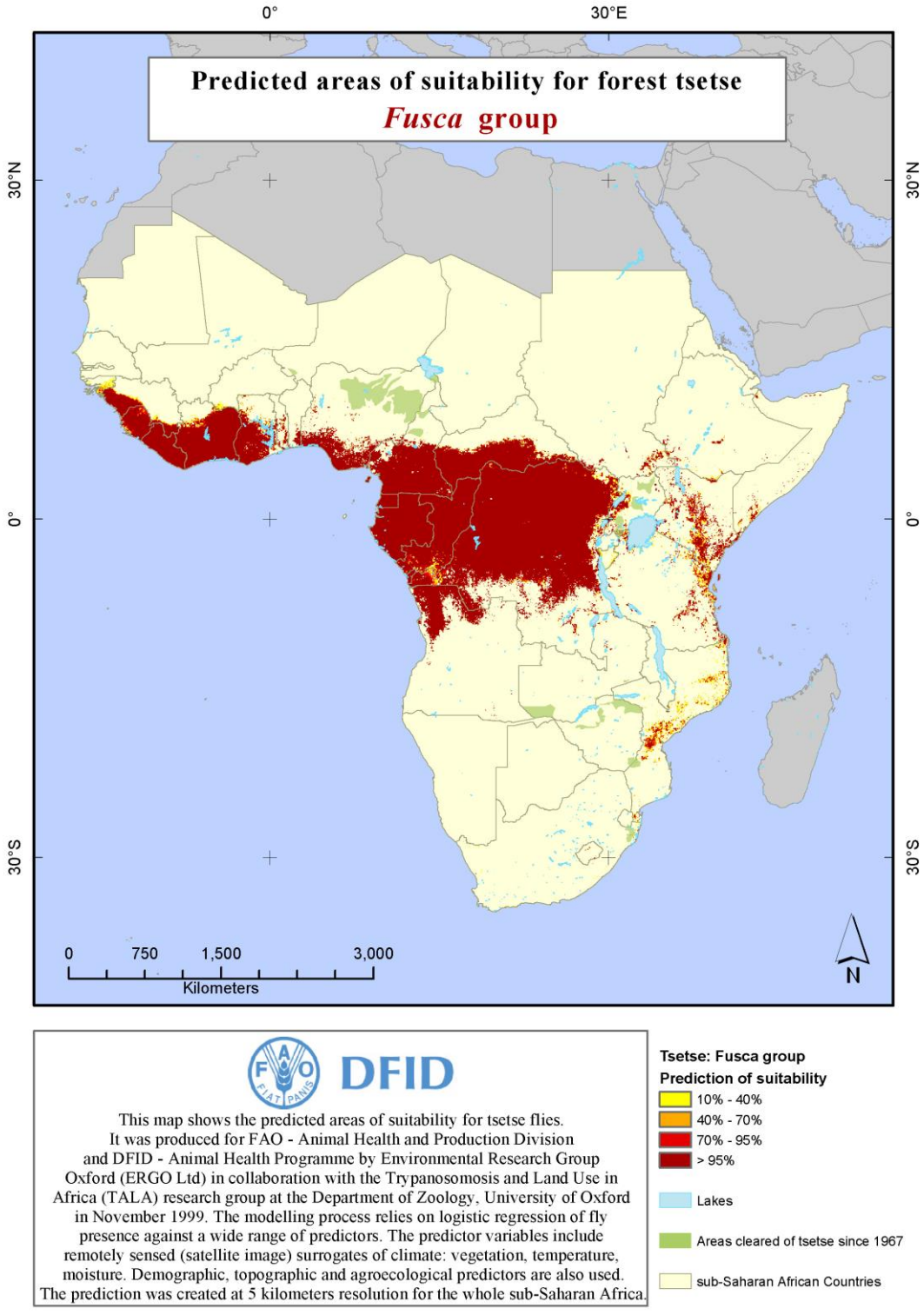

Figure 3: Predicted suitability for the fusca groupe tsetse specie (Source: http://ergodd.zoo.ox.ac.uk/tseweb/distributions.htm).

2. CONVENTIONAL TSETSE CONTROL TOOLS

2.1 Overview, advantages, and limitations

Tsetse control methods have been available for more than 50 years and most of them rely on the use of insecticides for the suppression or eradication of tsetse fly populations. Recent advances have improved the efficiency of some of these control tactics. Table 1 summarizes the tsetse control tools:

Table 1: Available control tools used for managing tsetse populations.

Tsetse control tool	**Overview**	**Advantages**	**Limitations**
Traps	• Devices for trapping and killing tsetse flies. They could be impregnated with insecticides or fungal compounds and growth hormones. • They are made of blue and black cloth in a shape that attracts the flies and then funnels them upwards into a non-return collector (Malele 2011).	▪ Can also be used for entomological surveys and control. ▪ More acceptable means of controlling tsetse flies in terms of less direct ecological and environmental impact compared to insecticide spraying.	- Not species specific. - Efficiency depends on the length of time the devices remain operational. - High trap densities required against certain species and in certain dense habitats make the use of these devices over large areas uneconomic. - Prone to theft and vandalism.

Insecticide-treated target	Screens of blue and black cloth impregnated with insecticides such as deltamethrin(Gimonneau, Rayaisse & Bouyer 2018).	Conventional: Developed for savannah species (1 × 1.7 metres).	▪ Less environmental impact compared with insecticide spraying. ▪ Low-cost technology. ▪ Less logistical cost ▪ Easy to deploy.	- Efficiency depends on the length of time the devices remain operational. - Needs regular maintenance. - Theft and vandalism.
		Tiny target: Developed for riverine species. East African targets (0.25 × 0.5 metres) and West African targets (0.5 × 0.75 metres)		- Efficiency depends on the length of time the devices remain operational. - Needs regular maintenance. - Theft and vandalism.
Insecticide-treated cattle	Treatment of cattle with insecticide formulations (mainly based on pyrethroids) using a wide range of techniques (pour-on, spraying, whole body dips/baths and sprays/showers) (Kuzoe & Schofield 2005).		▪ Easily adopted by farmers to control tsetse. ▪ Spill-over benefits on other livestock and human health problems. ▪ Can encounter tsetse flies in wider areas as cattle act as mobile targets. ▪ Less prone to theft. ▪ Minimal maintenance required.	- Re-invasion will only be prevented if large numbers of treated cattle are evenly distributed over the whole area. - Success of the method depends on a relatively large proportion of feeds being taken from domestic animals and a sufficient proportion of the livestock population being treated. - High treatment frequency. - High cost of the insecticides. - Insecticide residues in cattle dung might have an impact on dung beetles. - Potential development of resistance to the insecticides in both tsetse and ticks.

Targeted residual spraying (ground and drone)	Application of residual insecticides using vehicle-mounted equipment or on foot or by a drone.	Ground	Cost effective due to targeted application.	- Potential high ecological impact. - Difficult to use in rugged terrains and thickets.
		Drones	▪ Cost effective due to targeted application. ▪ Can access rugged terrains and thickets.	- Cannot be used for large areas due to payload and battery limitations.
Sequential Aerosol Technique (SAT)	Involves aerial application of non-residual insecticides applied in serial low doses as tiny drops. The application of low dosage aerosols is traditionally done with fixed wing aircraft and use of drones is also being considered (Cooper & Dobson, H. 1993).		▪ Rapid and efficient method for tsetse elimination. ▪ Does not have any serious lasting negative environmental impact. ▪ Ideal for inaccessible and remote infested areas.	- Most effective in savannah vegetation. - High cost of implementation. - High level of technical skills.

3. NEED FOR AN AREA-WIDE INTEGRATED PEST MANAGEMENT (AW-IPM) APPROACH

Since ancient times, humankind has suffered the effects of insects and other arthropods that compete for our food and fibre or transmit diseases. Various methods or strategies to suppress insect populations and/or reduce damage they cause have been developed and used, and the history of pest control could be divided into the pre- and post-insecticide eras. From the 1940s to the 1960s, insect pest control focused on the use of chemical insecticides; during this time, relatively cheap and effective products were available. The negative effects on the environment, on beneficial organisms, the accumulation of toxic waste, and the emergence of resistance and of secondary pests were phenomena that were initially ignored. The abuse of insecticides has in some cases caused irreparable damage to nature and even the loss of human lives. The exclusive reliance on insecticides has resulted in the search of more effective chemicals with less negative impacts on human health and the environment but at a much-increased cost.

As a result, the concept of Integrated Pest Management (IPM) emerged about 60 years ago, with the general idea being to combine different control methods to reduce the use of insecticides. IPM has been the dominant paradigm of insect pest control in the last 6 decades. Although some satisfactory results have been achieved, damage caused by insect pests remains very high and both resistance to the insecticides used and secondary pests have emerged, begging the question whether we should continue doing the same thing or seek more efficient and sustainable alternatives. Pest management over large areas is not a new concept. This approach was used before the era of insecticides to address the most important pests (e.g. Bubonic plague, locusts, livestock ticks, etc.), or for the application of biological control. However, it was not until the early 1990's when Edward F. Knipling emphasized its importance and potential.

The area-wide idea is to manage the total population of a pest, rather than limit control actions to areas where the pest causes damage. Unlike traditional IPM, the AW-IPM approach requires coordinated actions at an ecosystem level in a preventive way rather than a reactive strategy when the pest populations reach damaging or economically unacceptable thresholds.

The application of environment-friendly control methods, such as the SIT, require an AW-IPM approach to be effective. Among the difficulties or limitations for the application of the AW-IPM approach, two requirements stand out: 1) a greater understanding of the biology and ecology of the pest species, particularly its population dynamics in time and space; and 2) major community organization and engagement due to the complex social dynamics essential for application. In any case, control methods considered for AW-IPM should ideally be environmentally acceptable, for example avoiding application of insecticides in natural protected areas or human settlements. This second constraint requires an assessment of direct and indirect socio-economic costs and benefits in the short and long term. If the AW-IPM approach is found to be feasible and worthwhile, research on public information strategies to facilitate its implementation will be the next step.

In some cases, the AW-IPM approach has been equated with pest eradication programmes and this was opposed to the accepted view of IPM. Fortunately, progress has been achieved in understanding that these two concepts are not opposed but that they are in fact complementary.

Depending on specific conditions, AW-IPM can be used to prevent, contain, suppress, or eradicate pests. Some examples of successful contemporary applications of the AW-IPM approach are the eradication of the New World screwworm from North and Central America and Libya, eradication of the tsetse fly *Glossina austeni* from Unguja Island, Zanzibar, Tanzania, eradication of the Khapra beetle from Northern Mexico and South-Western USA, fruit fly prevention, suppression or eradication programmes in Argentina, Chile, Dominican Republic, Guatemala, Israel, Mexico, Spain and the USA, management of cotton pests in the USA, and suppression of the cassava mealy bug in sub-Saharan Africa. Insecticide resistance management strategies also involve an AW-IPM approach.

An AW-IPM approach can only be effective when the following questions are addressed:

- Where are individuals of the pest species located when they are not attacking or causing damage?
- How are pest populations naturally regulated?
- How do they survive from season to season?
- What are the populations' abilities to grow and spread?
- What are the natural boundaries of the pest population?

The current difficulty in sustaining continuous tsetse fly control with insecticides highlights the need for smarter sustainable strategies. A more successful pest control strategy will likely involve an AW-IPM approach that integrates modern and novel control methods, such as the SIT. The most desirable way of containing the disease trypanosomosis is undoubtedly the elimination of entire populations of the vector from delimited geographical areas using an integration of various control tactics, i.e. using an AW-IPM approach. The area-wide approach aims at the sustainable removal of an entire tsetse fly population within a delimited geographical area (Vreysen 2006).

4. STERILE INSECT TECHNIQUE (SIT)

Since the 1950's it is known that insect pests can be controlled or eradicated through a "birth control" method based on genetic manipulation known as autocidal pest control or the SIT. The technique involves the colonization and mass-rearing of the target pest species, sterilization of the males by exposing the insects to a specific dose of gamma radiation emitted from radioisotopes (Cobalt 60 or Caesium 137) or X rays, and then, the sustained, systematic releases of sterile males among the indigenous target population causing infertility in the mated females. Over time, the fertile population and the reproductive capacity are progressively reduced until fertile matings do not occur, and the population is eliminated. The validity of this method has been demonstrated for many insect pests including New World screwworms in the Caribbean, the Southern USA and Central America, the Mediterranean Fruit fly in the USA and Central and South America and tsetse flies in Zanzibar.

The SIT has special attributes, which makes it a unique insect pest management tool:

- Species specificity: the SIT represents a biologically based control tactic directed exclusively at the target species, thereby affecting only the targeted pest populations without any adverse impact on non-target organisms.
- Inverse density-dependence: the 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 entire area.
- Compatibility for integration: the SIT can be effectively integrated with other control tactics including biological methods, such as parasitoids, predators, and insect pathogens.
- The implementation of the SIT following a Phased Conditional Approach (PCA) (Figure 4) will reduce the risk of failure. In a PCA, project implementation follows distinct phases in which support to the next phase is conditional upon completion of all (or at least the majority of) activities in the previous phase (Feldmann, Leak & Hendrichs 2018). Whereas the diverse phases of the PCA might differ with the target pest species, or if a suppression rather than an eradication strategy is selected (eds. Hendrichs, Pereira & Vreysen 2021). The PCA consisted of 4 phases for the tsetse project in Senegal: (1) commitment of all stakeholders and training, (2) baseline data collection and feasibility studies, (3) pre-operational activities and (4) operational activities.

The SIT also has limitations:

- Requires detailed knowledge on the biology and ecology of the target pest;
- The SIT is not a stand-alone technique. To be effective it should be integrated with other techniques as part of an integrated pest management strategy such as the use of odour-baited traps and targets, pour-ons, ground and aerial spraying etc;
- Not efficient against high population densities and requires in most cases suppression, often by means of insecticides;
- Low reproductive potential of the tsetse fly makes current systems of mass-production cumbersome and expensive;
- Delayed effect on the numbers of vectors present – the effect of the SIT is only visible in the next generation;
- Necessitates efficient release and monitoring methods;
- The SIT is not suitable for all insects and can only be used for those insects where the development phase that is being released (i.e. the adults), does not contribute to the damage.

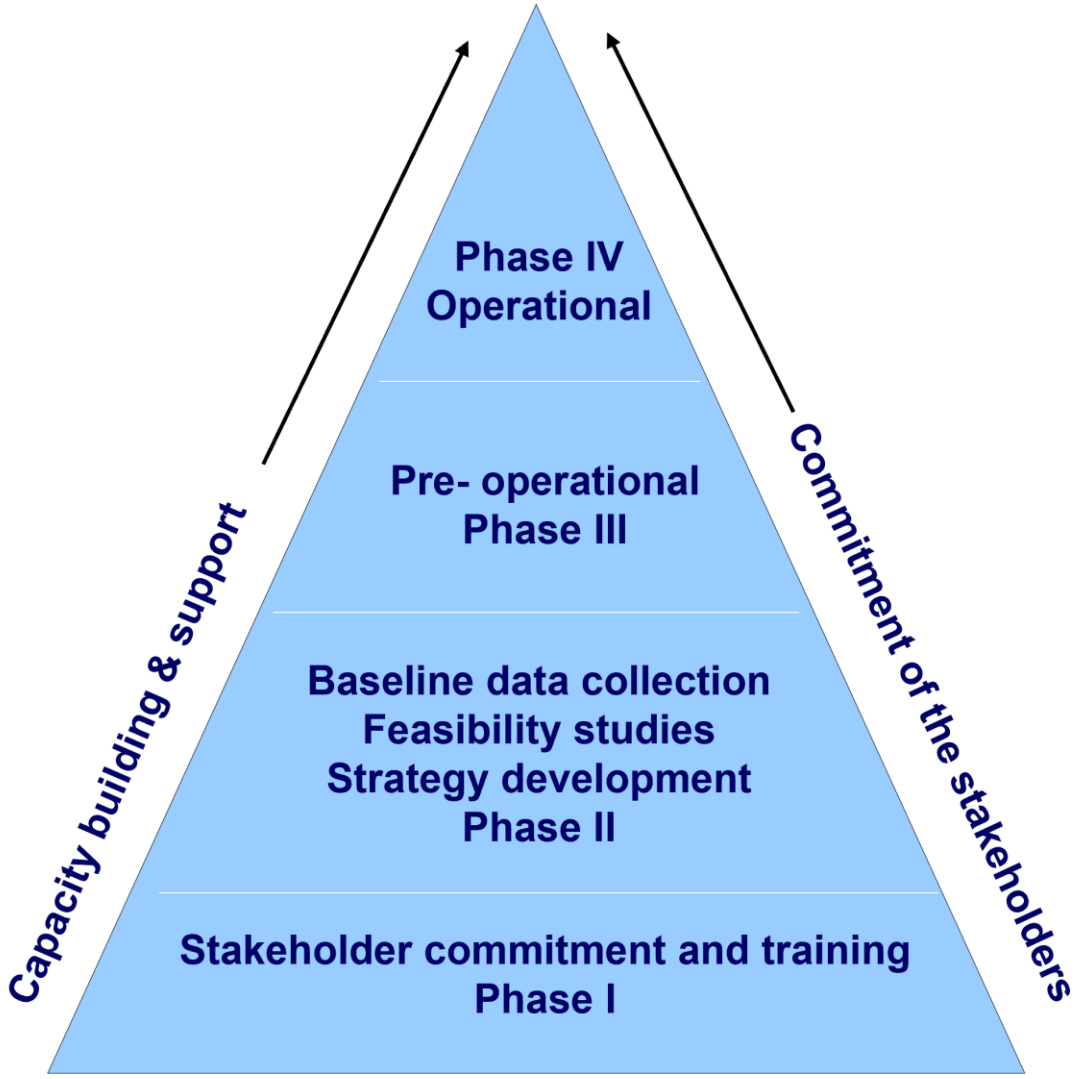

Figure 4: The general outline of the proposed phased-conditional approach.

4.1 Operational SIT programmes

Early pilot studies

Early pilot studies that were carried out in Burkina Faso, demonstrated already the benefits of prior suppression by application of a non-residual insecticide on the outcome of a SIT programme (Cuisance et al. 1980). Sterile male *G. p. gambiensis* were released in optimal ratios (7 to 10) in a native population with and without prior application of a non-residual insecticide. In the area with prior suppression, the fly was eradicated after 19 months, whereas an additional 5 months were needed to achieve the same in the area without prior insecticide application (Cuisance et al. 1980). The prior application of insecticides did reduce considerably the numbers of sterile males and the time required to achieve eradication and this implied reduction in costs. Further economic

savings can be obtained by optimizing releases in terms of time spacing (7 days versus 10 days) and release sites (2 km versus 200 m apart) (Politzar & Cuisance 1982).

Another small release trial was carried out in the Volta Noire (Mouhoun) source tributaries in Burkina Faso (Van der Vloedt et al. 1980). During the dry season of 1977/78, two spraying operations of a synthetic pyrethroid (deltamethrin), separated by 14 days, were applied from a helicopter and followed by the release of ± 5 500 sterile male *G. p. gambiensis* in 10 release sessions. A 95% reduction of the original native *G. p. gambiensis* population was obtained following the insecticide application. Some interesting aspects of the performance of the released male flies were revealed during the relatively short monitoring period of 33 days: (1) dispersal rates up to 2 000 m after 48 hours, (2) good survival with maximum periods between release and recapture of 20-44 days, (3) good response to the monitoring device (30 % recapture rate with the biconical trap). During the entire trial period, on average 8.7 sterile males were trapped for each wild male (Van der Vloedt et al. 1980).

4.1.1 Tanzania

The first SIT programme of any magnitude was carried out in Tanzania from 1972 to 1979 against *Glossina morsitans morsitans* at Mkwaja Ranch. In this programme, major contributions were made in the development of procedures for sterilisation, handling, packaging and releasing of the sterile males. During the 15-month trial period of active releases, a colony of 60 000 female flies was maintained, the first of its kind in Africa. The main objective of the programme was to evaluate the SIT methodology against a tsetse species and to test the concept in the field (Williamson, Baumgartner, Mtuya, Warner, et al. 1983). The option of releasing pupae rather than adult flies was taken, as the experimental release area was free of human sleeping sickness. At the test site, the pupae were placed in field emergence cages and buried under sand mixed with a fluorescent day glo powder, which automatically marked the flies during emergence. Losses due to storage (2.9%), radiation (1.4%) and transport (4.2%) were minimal (Williamson, Baumgartner, Mtuya, Gates, et al. 1983). To prevent re-invasion, a 1 km wide fly barrier was cut surrounding the entire experimental area (195 km^2). The fly density of *G. m. morsitans* was estimated at 630/km^2 in the test site (Gates et al. 1983). Prior to the release of sterile males, the *G. m. morsitans* native fly population was reduced by two aerial applications of a non-residual insecticide (endosulfan) used as a 20% ULV aerosol formulation with an interval of 28 days (Williamson, Dame, Lee, Gates, et al. 1983). Sterile males were released as pupae at a rate of 135/km^2 resulting in an average ratio of 1.12 sterile to 1 wild male. This low ratio was however enough to keep the indigenous population at the 80 - 95% reduction level obtained after the insecticide applications. Migration of wild flies from outside the experimental area prevented the eradication of the test species (Williamson, Dame, Lee, Gates, et al. 1983).

This emphasizes the need for an area-wide approach when planning for tsetse control, and to take on board sympatric species present in the target control area. The control of *G. m. morsitans* without control of *G. pallidipes*, shifted the balance and *G. pallidipes* is now the dominant species in the area.

4.1.2 Nigeria

In Nigeria, the use of insecticide impregnated targets, traps and the release of sterile males eradicated *Glossina palpalis palpalis* from a total area of 1 500 km^2 in Southern Plateau State. The flies were reared at a production centre in Vom, situated on the tsetse-free Jos plateau. Both, *in vivo* and *in vitro* feeding techniques, were used to maintain colonies through the entire project period. Feeding of the *in vivo* colony was done on guinea pigs (Oladunmade et al. 1990), and this colony reached its maximum size of 60 000 flies in 1986. On average, 4 700 sterile males were released per week from the *in vivo* colony. These were supplemented on a weekly basis with approximately 3 500 sterile males from the *in vitro* colony. The maximum *in vitro* colony size of 139 000 female flies was reached in mid-87. Fly reduction in the project area was achieved by means of insecticide impregnated targets (with Deltamethrin, leaving a deposit of 150 mg/m^2) (Oladunmade et al. 1985) in the boundary areas or by removal trapping with the biconical trap. Both devices proved equally efficient in reducing native fly numbers, i.e. between 90 and 99%. In both cases however, extending the period of control with traps and targets did not achieve eradication. A major concern was the loss of, on average, 30% of the targets due to theft, flooding and fire (Takken et al. 1986). The programme demonstrated that a ratio of 10 sterile to 1 wild male was a prerequisite to achieve eradication (a ratio of 3:1 only achieved control). It also showed that different habitats (more humid and more extensive riverine forest galleries as in Burkina Faso) and different species (*G. p. gambiensis*) demand different release rates and different time frames. Weekly releases for 18 months resulted in eradication in the pilot phase of 4 isolated forest patches (Takken et al. 1986). The programme was extended over the entire 1 500 km^2 block and in 1988 the fly was declared eradicated (Oladunmade et al. 1990).

4.1.3 Burkina Faso

The project in Burkina Faso sought to control tsetse in an agro-pastoral area of more than 3000 km^2 in Sidéradougou, targeting *Glossina tachinoides* and *Glossina palpalis gambiensis*. Prior suppression of the native fly population was achieved by placing 6 500 insecticide impregnated targets along 650 km of linear gallery forest for 4 months during the dry season (Politzar & Cuisance 1984); an approach which avoided the spraying of a non-persistent insecticide for population reduction (Van der Vloedt et al. 1980). The targets were treated with 200 mg of deltamethrin and placed at intervals of 100 m. These operations were followed by releases of sterile males in the rainy season. To supply the required number of sterile male flies, a colony of 150 000 *G. p. gambiensis* females and 85 000 *G. tachinoides* females was maintained in the mass-rearing facility using the *in vitro* feeding technique. The use of the targets reduced the native *G. p. gambiensis* and *G. tachinoides* populations with 91 and 94%, respectively. This reduction in the native fly population reduced the requirements of sterile males to only 20-35 sterile males per linear km to obtain the desired ratios of 10:1. The main river systems were free of tsetse flies 6 months after the release of sterile male flies (Politzar & Cuisance 1984). The programme was however not implemented following area-wide principles, and suffered from re-invasion from areas nearby (Cuisance et al. 1984; Politzar & Cuisance 1984).

4.1.4 Unguja Island, Zanzibar

In 1994, the Government of the United Republic of Tanzania in collaboration with the FAO/IAEA implemented an AW-IPM strategy with an SIT component aiming the eradication of *Glossina austeni* on Unguja island, Zanzibar. Suppression of the tsetse population was initiated in 1988 by applying residual pyrethroids as a pour-on formulation to livestock and by the deployment of insecticide impregnated screens in some of the forested areas. This was followed by sequential releases of gamma-sterilized male flies by light aircraft. The flies, packaged in carton release containers were dispersed twice a week by light aircraft (the first time that tsetse flies were released by air) along specific flight lines separated by a distance of 1–2 km. More than 8.5 million sterile male flies were released by air from August 1994 to December 1997. A sterile to indigenous male ratio of more than 50:1 was obtained in mid-1995 and it increased to more than 100:1 by the end of 1995. As a consequence, the proportion of sampled young females (1 - 2 ovulations), with an egg *in utero* in embryonic arrest or an uterus empty due to expulsion of a dead embryo, increased from less than 25% in the 1st quarter to more than 70% in the last quarter of 1995. In addition, the age structure of the female population became significantly distorted in favour of old flies (≥ 4 ovulations) by the end of 1995. The apparent density of the indigenous fly population declined rapidly in the last quarter of 1995, followed by a population crash in the beginning of 1996. The last trapped indigenous male and female flies were found in week 32 and 36, 1996, respectively. Time for 6 fly generations elapsed between the last catch of an indigenous fly and the end of the sterile male releases in December 1997.

The eradication of the tsetse fly from Unguja Island in 1997 was followed by the disappearance of AAT, and this enabled farmers to integrate livestock keeping with cropping in areas where this had been impossible before. The increased livestock and crop productivity and the use of animals for transport and traction significantly contributed to an increase in the quality of people's lives.

The success of the eradication programme on Unguja Island was possible due to many technical and managerial prerequisites that were in place during the planning and implementation of the programme. The project was implemented within the Ministry of Agriculture, Livestock and Environment of Zanzibar, who gave full autonomy and independence to the senior project managers by to implement the project and this contributed significantly to its success. Beyond the complete isolation of the target population, the programme on Unguja Island benefitted from an extensive planning phase that allowed a good strategic choice of control tactics that are very effective at high population densities and very effective at low population densities, such as the SIT; sterile flies of adequate quality were available in adequate numbers and were released throughout the campaign without interruption; routine quality control procedures in place; an extensive monitoring component with permanent feedback between field teams and managers, allowing the making of adequate strategic choices based on sound scientific principles rather than on process-oriented bureaucracies or political wishes; adequate national and international expertise; transparency of project operations to all project stakeholders; sufficient financial resources and adequate logistics and regular independent programme reviews.

4.1.5 Ethiopia

In 1997/98, the Government of Ethiopia, with the support of the FAO/IAEA, initiated a project in the Southern Rift Valley called the Southern Tsetse Eradication Project (STEP) (Alemu et al. 2007). The projects long-term objectives were: (1) to create a tsetse-free zone in a 25 000 km^2 area under agricultural development, and (2) to develop adequate national capacity for applying the concept of AW-IPM with an SIT component. The project was initiated with the collection and evaluation of entomological and veterinary baseline data that started in 1999/2000. These were later complemented with an environmental impact study and a socio-economic study.

The entomological surveys (4 in one year) revealed the presence of *G. pallidipes* in the main valley and of *Glossina fuscipes fuscipes* in the Deme basin, the latter having an area of 750 km^2 representing about 7% of the block-1 of the STEP project. In 2002, community-based tsetse suppression was initiated in localized areas using insecticides on cattle and on blue-black-blue fabric targets. These localized tsetse suppression activities were expanded to all operational grids of block 1 (10 500 km^2) of the STEP area. A geographic information systems (GIS) analysis indicated however, that the community-based tsetse suppression was not implemented area-wide, and some cattle herds continued to show a high disease prevalence in those areas that were not adequately covered by the community fly control measures. Therefore, it was clear that the operational programme will need to include the introduction of a set of implementation rules and regulations conducive to the special needs of an operational AW-IPM campaign.

To address the SIT component, the Government of Ethiopia established a small field insectary in Arba Minch, where a seed colony of *G. pallidipes* was initiated with biological material collected in the target area. In addition, a modular mass-rearing facility was constructed in Kaliti near Addis Ababa, where colonies of *G. pallidipes* and *G. f. fuscipes* were maintained. This mass-rearing facility has the capacity to maintain a colony of 10 million producing female tsetse. This could potentially ensure a weekly output of one million sterile males, which, at a dispersal rate of 100 sterile males per km^2, could cover a total area of 10 000 km^2. However, with the outbreak of the salivary gland hypertrophy virus (GpSGHV) in the *G. pallidipes* colony, the capacity of 10 million producing females was never reached.

Activities in the Southern Tsetse Eradication Project are still ongoing and the eradication of *G. pallidipes* and *G. f. fuscipes* was not achieved.

4.1.6 Senegal

In 2005, the Senegal's government initiated a field project with the aim to eradicate *Glossina palpalis gambiensis* from the highly productive agricultural region of the Niayes in the north-east of Dakar, using an AW-IPM approach with an SIT component (Vreysen et al. 2021). This project received technical and financial support from different international organizations such as the IAEA, the FAO, the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) and the US Department of State through the Peaceful Uses Initiative (PUI). The project was implemented following the PCA and comprised the following 4 phases: 1) commitment of all stakeholders and training; 2) baseline data collection, feasibility studies and strategy development; 3) pre-operational activities and 4) operational activities. With the successful implementation of this project, it is believed that the tsetse fly populations have been

eradicated and transmission of trypanosomosis has stopped. At the time of writing (2023), no wild flies have been trapped in the area since March 2021, with the exception of two virgin females caught in January 2022 which could not be considered as a self-sustaining population as no further catches were obtained despite increased trapping intensity (Suckling et al. 2016). The parasitological and serological results from 2021 and 2022 showed that transmission of trypanosomes by the biological vector has stopped in all the blocks (Gueye Fall, 2023, submitted). Consequently, 3 335 exotic cattle were introduced in the area by the Government between 2017 and 2021. This represents a third of the exotic breeds introduced in the area by private investors. This fact has led to an increase in milk production of 14.5 million litres and the birth of 3 094 calves.

Among the relevant lessons learned from this project particular mention can be made to 1) The project adopted an "adaptive management" approach, and it was not implemented by an independent organization. It is believed that this approach was critical to the project's success. This management approach involved all the stakeholders, including researchers, ensuring transparency and decision-making by consensus. The important decisions in the project were based on scientific principles (never political, personal, or emotional) and were guided by analysed field or other data; 2) The stability in project staffing, with basically no turn-over experienced in 12 years, both at the management and at the technical (insectary/field staff) level is considered another important factor for the project's success. This created a personnel culture of reliability, transparency and trust, and ensured the necessary institutional memory.

4.2 Lessons learned

Long distance shipping and production of sterile flies: After the baseline data collection in the Niayes area that indicated the complete isolation of the fly populations from the remainder of the tsetse belt in the Sine Saloum, it was decided to embark on an AW-IPM programme with an SIT component. The project area was considered too small (1 000 km^2) to justify the funds to construct a rearing facility in Senegal, and therefore the project relied on other partners to produce and deliver the required number of sterile insects.

> *Lesson learned:* long distance shipping and production of sterile flies by other partners has the drawback that the quality of the insects is reduced as compared with insects produced in a local production unit. This needs to be compensated with the release of a higher number of sterile insects.

Regional vs. national rearing facilities: The supply of sterile male pupae of high biological quality is a crucial component of an SIT programme. Relying on a regional rearing facility has the drawback that various parameters remain beyond the control of the project managers, i.e. low quality of the produced insects, delays during transport, detrimental transport conditions, etc. This will delay the project with strong financial impact.

> *Lesson learned:* National projects that target large scale areas should ideally develop a local mass-rearing facility to minimize risk linked to external factors (i.e. COVID-19 lockdown, colony crash).

Adherence to the PCA: Rigorously following the PCA will significantly increase the chances of success. Especially the collection of relevant base-line data is crucial to allow the development of an appropriate AW-IPM strategy.

> *Lesson learned:* projects need to be implemented following the PCA to increase the chances of success. All key stakeholders must be aware of the different phases and ensure that all activities of each phase are implemented before embarking on the next phase.

Area-wide approach: A vector control programme that includes an SIT component, must be implemented area-wide, targeting an entire population and not only the areas that have suitable habitat or that are of interest to the farmer.

> *Lesson learned:* The AW-IPM programmes on Unguja Island and in the Niayes area of Senegal were implemented following an AW-IPM approach, and no pockets of tsetse flies were omitted. On the contrary, all other SIT projects did not follow the AW-IPM approach, although they were successful in eradicating the target populations, suffered from immigration and the eradication status could not be maintained.

Adaptive management approach: Effective and transparent coordination is a key component of project success.

> *Lesson learned:* A strong project management team should be established at the onset of the project. The management team must follow an adaptive management approach leading to fast decision making, having consensus of all senior managers in the programme, and implementation that are crucial in SIT programmes.

Stability in project staffing: SIT projects need to rely on personnel that have the necessary qualifications, and staff turn-over should be limited as much as possible.

> *Lesson learned:* Young and motivated staff should be favoured to ensure long term stability. Anticipate staff departures (retirement/personal mobility) to ensure an efficient transition.

Relevant monitoring system: An extensive and permanent entomological monitoring system must be set up for the entire project area to obtain reliable feedback that will allow immediate corrective measures if required.

> *Lesson learned:* The data provided by entomological monitoring (weekly/bimonthly) are essential to ensure decision-making that is based on scientific data.

Sufficient long-term funds: AW-IPM projects are usually long-term projects that last for several years if not decades. They require the availability of sufficient funding covering the entire project duration.

> *Lesson learned:* The lack or cessation of adequate funding can affect and/or destroy all the control efforts acquired previously and lead to the failure or unnecessary extension of the project.

Transparency: All data obtained from the monitoring activities (entomological, veterinary, environmental) must be shared openly with all staff involved in the project, including important donors.

> *Lesson learned:* Lack of transparency will lead to a lack of trust and increase suspicion that irregularities are occurring which are not communicated. Especially problematic data or activities that were not implemented as required should be communicated to all. This will create an atmosphere of trust in the senior project managers.

External review: Projects not subject to regular external evaluations may fail to achieve objectives.

> *Lesson learned:* External and independent project reviews are helpful to identify shortcomings and to emphasize positive elements of the project at all stages of implementation.

Publish data: Project outcomes and impact are of major interest to the donor, recipient, and all other stakeholders. Positive and negative results of the project must be shared.

> *Lesson learned:* As far as possible all new scientific and development data should be published to inform about the progress of the project.

Government commitment: countries following the PCA must comply with the different stages whereby implementation of the next phase is conditional to completion of all or most of the activities in the previous phase. Government commitment and leadership is crucial to adhere to the PCA.

> *Lesson learned:* Only a total commitment from the government at all levels (political, human resources, infrastructure, financial, etc.) can ensure the success of the project.

4.3 Challenges in operational SIT programmes

Funding to support upcoming SIT projects: Member States (MSs) should play an active role in securing and mobilizing funds for targeted AW-IPM programmes planned in their countries. MSs, the FAO/IAEA, and other relevant partners should develop bankable project proposals to mobilize resources from other bilateral and multilateral sources to support T&T field programmes. MSs and other funding partners are encouraged to allocate more financial resources to support T&T programmes.

SIT projects should be implemented at a regional scale: A regional project proposal under the AU could be developed and be based on the strategic principles of AW-IPM with an SIT component. The project activities must be implemented at a regional or sub-regional scale to ensure joint and simultaneous actions with neighbouring countries affected by the same problem. There is a good example between Senegal and the Gambia regarding the tsetse control project that is initiated in the Sine Saloum area. In this context, technical collaboration agreements should be developed, to implement joint vector control actions.

Security issues in the region: In recent years, the African continent has been faced with civil unrest, acts of terrorism and other security issues in several areas, which has been a constraint to

T&T control. Access to these areas to collect the base-line data needed to develop a control programme is severely restricted, and in areas where actions are or were underway, it is a challenge to sustain the lasting impact due to re-invasion. In addition, these security issues reduce the possibility of mobilizing financial resources to support field activities.

Convince decision-makers to follow the PCA (baseline data collection, feasibility studies): All field projects should be implemented following the PCA. The implementation of SIT projects is challenging and management intensive, therefore, following this PCA will minimize any potential risk of failure. As mentioned in the previous section, the PCA for tsetse projects comprise 4 phases, starting with a preparatory phase and ending with operational deployment, with some milestones that include go/no-go criteria.

Big programmes have to be implemented by independent organizations: To manage AAT efficiently, the African Trypanosomosis Control Programme (ATCP), that was established in 1997 at the 29ème session of the FAO Conference, has set the goal of reducing and eventually eliminating the burden of tsetse-transmitted trypanosomosis in humans and animals. As resources are limited, it is necessary to pool efforts through structured interventions, focusing on areas where the impact of the disease is most severe and where control provides the greatest benefits for health and for sustainable agricultural and rural development. The ultimate aim is to have a positive impact on the beneficiaries, which means that all the stakeholders need to be involved in co-constructing innovation. Small programmes can be managed at national level, but larger programmes, such as trypanosomosis, which transcends borders, need to be tackled at regional level for greater effectiveness, hence the need for an independent organization to implement the programmes. Furthermore, independent organisations have more autonomy, and this can be beneficial for the efficient programme implementation.

Lack of expertise in tsetse ecology, taxonomy, control, IPM, SIT: It is noted with concern that seasoned personnel and experts are retiring and most institutions are left with inexperienced staff who at times are not conversant with even the basics in the field. It is noticed that most fellowship applications requested from the IAEA, deal with genetics, molecular biology or GIS and remote sensing. There is a severe lack of interest in the MSs with respect to ecology, tsetse behaviour or any other related field work. It is key that efforts be made to ensure that there is continuity in MSs whereby mentoring and training of junior staff is promoted.

Sufficient numbers of sterile males available of high quality: Most of the insect pest species that have been the subject of SIT programmes (such as mosquitos, fruit flies, screwworms, lepidoptera) have a high reproduction rate, which facilitates the rearing of the millions of individuals required. Rearing of tsetse flies, and especially colonizing a new population, is time consuming, labour intensive, and challenging in view of the extreme low female productivity, i.e. one offspring every 10 days.

Opposition of environmental groups: Concern from conservation authorities in some regions, e.g. South Africa, related to the use of area-wide insecticide spraying and the adverse effect this may have on biodiversity and ecosystems, especially within protected areas (Armstrong & Blackmore 2017) present implementation challenges. Stakeholder engagement and advocacy is critical at high levels.

AU commitment, political and financial commitment, tsetse problem should be prioritized: The African Heads of State and Government, at the 36th Ordinary Session of the AU summit meeting in Lomé, Togo, in July 2000, recognized the seriousness of the T & T problem and made an historic decision ((OAU) Organization of African Unity 2000). The year 2001 marked the beginning of renewed efforts to suppress tsetse flies and trypanosomosis and the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) was born (African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) 2001; Pesticide Outlook 2002). The PATTEC Plan of Action to be implemented was approved at AU's Lusaka meeting in 2001 ((OAU) Organization of African Unity 2001). This decision was received with great enthusiasm by the T- and T-affected countries as it created expectations that this African-owned and African-led initiative would result in a substantial reduction in rural hunger and poverty. Despite the initial euphoria and high expectations, the reality after more than 20 years is more sobering and, with a few exceptions, little overall progress can be reported (Feldmann et al. 2021). It is important to emphasize that political commitment alone is not sufficient and needs to be accompanied by financial commitments.

Lack of socio-economic studies: In the past, several SIT studies have been carried out, but they have not always been accompanied by socio-economic studies. The collection of ex-ante socio-economic baseline data is part of the PCA and provides information about the benefits and costs of an operational programme. It provides data that can convince donor partners to provide adequate funding for a programme. In addition, such studies ensure that local realities are taken into account and ensure better ownership leading to proper sustainability of the proposed actions. (Rushton & Gilbert 2016) have therefore made recommendations to the World Organisation for Animal Health (WOAH) along the lines of starting a pilot project designed to generate data series on the global burden of animal diseases, with data on production losses, the costs of control operations and the impact of animal diseases on trade and the wider economy, but also starting a programme designed to collect regular data on investments made in veterinary medicine education, research, infrastructure and crucial coordination activities. This will make it possible to set priorities in real time among animal diseases and to objectively assess the productivity of the Veterinary Services geographically, by animal species and by area of expertise.

4.4 Support to enhance tsetse eradication

Priorities should be given to projects with a high socio-economic impact: Of the total area that is infested with tsetse flies in Africa, not all areas have the same socio-economic value. The Island of Mafia for instance, is infested with *Glossina brevipalpis*, but livestock plays a minor role in the economy of the island. Implementing an AW-IPM programme with an SIT component to eradicate this tsetse population from the island is therefore difficult to justify. The focus should therefore be on those areas with a high density of livestock, where livestock is important for the local and national economy, and where the impact of the removal of the fly is greatest.

Promoting tsetse schools in the region: A school that provided training on tsetse biology and control used to be operated in Zambia mainly with FAO funding. Due to lack of funding, the school is no longer operational. The school enabled students from across the continent to obtain training and qualification in tsetse biology and control. This was important in that a standardized module existed and was shared across participants from the continent. It is highly recommended that a similar arrangement be revived or initiated. There are institutions in existence which

conduct some training on these aspects, including the International Centre of Insect Physiology and Ecology (ICIPE) - Kenya, the Centre International de Recherche Développement sur L'Elevage en Zone Sub-humide (CIRDES) – Burkina Faso and the Ecole de Lutte Anti Tsétsé (ELAT) - Burkina Faso.

Enhancing capacity building through development of training programmes and local trainers: The FAO/IAEA has in the past decades continuously supported capacity building in most of the African MSs affected by tsetse flies. For those MSs that will assess the feasibility of implementing an AW-IPM approach with an SIT component, the IAEA through its TC department should develop and facilitate a "training programme", in collaboration with FAO and other international organizations to support building and enhancing technical expertise at the regional level on all key aspects of the SIT package as well as new technological advancements aiming at improved tsetse management.

The IAEA should also support the improvement and expansion of research infrastructure and conditions in the MSs through upgrading of entomology laboratories and facilities through national or regional TC projects. During the last 20 years the FAO/IAEA has promoted networking and sharing expertise among MSs at the regional level through Coordinated Research Projects (CRPs) and TC projects. However, additional efforts should be made, and increased financial support is crucial to continue enhancing regional capacities and organizing technical events to address high priority topics.

5. SOCIO ECONOMIC IMPACT OF AFRICAN ANIMAL TRYPANOSOMOSIS

5.1 Necessity of socio-economic data

As long as humankind has domesticated animals, livestock farming has been linked to the need to control animal diseases. Animal diseases have always represented a threat to animal species, individuals (livestock farmers and their households), pastoral communities and nations. The correlation between the prevalence of animal diseases, their impact and the capacity of veterinary services to control them has been widely demonstrated (Le Gall 2006). In particular, the rapidity of intervention and the type of control measures adopted are a guarantee of success in the fight against animal diseases.

After more than a century of efforts to control trypanosomosis, the subject of socio-technical controversy has not been exhausted (Maudlin 2006). Despite the success of the various control campaigns, there is still the problem of sustainability of what has been achieved (Shaw et al. 2015). This requires the acquisition of evidence (data) to enable the achievements of governments to be monitored, so that the various stakeholders can redirect their actions or renew their areas of intervention. It is for all these reasons that socio-economic data on animal diseases and their impact are needed to convince public authorities to invest in the prevention and control of these diseases. Therefore, in order to set economic priorities for the allocation of resources to improve the health and welfare of animals under human responsibility, accurate data are needed on both production losses and the costs of prevention and intervention, but also of the obtained benefits. (Rushton & Gilbert 2016).

5.2 Socio economic impact at the national, regional, and local level

Trypanosomosis presents a high degree of complexity and variability, and quantifying and assessing the costs of the disease and its associated benefits if the disease is managed, is relatively straightforward in an experimental situation but becomes complex when undertaken for populations and production systems. The burden of AAT and the potential benefits of eliminating it are not uniformly distributed in the countries affected, hence the need to study them on different scales. Indeed, the AAT is often present in relatively rich areas in African countries and has a direct impact on the incomes of populations who are mainly invested in agro-pastoral activities. At the national level, livestock is a major part of the economy in Sahelian countries. It represents 10-15% of their GDP, except in Senegal (4%) (Corniaux 2014) and the cross-border cattle trade is a major factor in the trade balance of Sahelian countries. This shows that there is a need for technical and economic information to facilitate national policy planning. In order to plan policies, technical and economic monitoring must be carried out at regional level to facilitate the coordination of actions and to liaise with agropastoralists to take better account of needs and identify constraints. The Niayes project in Senegal is an illustration of such approach, where the evaluation began in a restricted area of the Niayes, where the coordination of the partners' actions led to the announced eradication of the tsetse fly. The next step is to set up an eradication programme in the Sine Saloum area, capitalizing on all the achievements of this first step, where socio-economic studies (baseline study, ex-ante evaluation and a forthcoming ex-post evaluation) have been carried out in tandem with the technical process to achieve the tsetse fly eradication objectives.

5.3 Ex ante socio economic studies should be linked ex post

Ex ante validation is important for any AAT programme, as it enables the effects of the programme or changes to the programme structure to be simulated, and therefore forecasts to be made during the programme design phase. Ex-post evaluations analyze the impact of a programme on the behavior of beneficiaries using data from a survey carried out after it has been implemented. The two evaluation methods appear to be largely complementary in verifying that the programme has achieved the expected results. It is in this context that the two types of evaluation will be carried out for the tsetse fly eradication project in the Niayes. The ex-ante evaluation, carried out by Bouyer (2015; 2015), identified various scenarios for assessing the cost effectiveness of the programme. The cost of the programme was estimated at 6 400 euros/km², which is relatively high compared with the estimates (726-998 euros/km²) given by Shaw, al. (2007) in Uganda for an area of 10 000 km², which might be attributed to the relatively small target area which prevents economies of scale. The results show that except for scenario 1 (2% annual replacement rate and 10% discounting rate), the project has a positive NPV (Net Present Value) and an IRR (Internal Rate of Return) higher than average interest rates for financing the project. The payback period would be 18 years for the first scenario and 13 years for the more realistic scenario 2 (10% replacement rate followed by an initial period of 5 years with a replacement rate of 2%). The benefit-cost ratios ranged from 0.98 to 4.26 depending on the discount rates and scenarios. To assess the final impact of the programme, an ex-post evaluation of the Niayes project is currently being carried out, to confirm or refute the results of the ex-ante evaluation and identify new development priorities.

6. RECENT ADVANCES IN RESEARCH AND DEVELOPMENT RELEVANT TO SIT

6.1 Recent advances in R&D for tsetse control

6.1.1 Tsetse Distribution

Mapping tsetse distribution, suitable vegetation and ecological niche

In the past twenty years there has been a widespread adoption of the Global Positioning System (GPS) for geo-referencing tsetse fly field data. This includes both dedicated GPS devices as well as the GPS within mobile phones. Another major technological advance has been the progressive improvement and wide adoption of GIS software, and in particular of Open Source Freeware (e.g. QGIS, https://qgis.org). As we write, Open-Source Freeware is sufficiently advanced that the purchase of commercial GIS software is no longer needed for most applications related to tsetse fly mapping.

In the past ten years, major advances were also made with the management of tsetse fly field data, and in the development of continental and national level information systems (i.e. atlases). An atlas of tsetse distribution and AAT infection in Africa is being developed, based on published data of 1990–2020 (Cecchi et al. 2015) (Cecchi et al. 2014). The tsetse distribution component is expected to be published by FAO in 2024 within the PAAT Technical and Scientific Series. In parallel, with FAO and IAEA support, national level atlases/information systems were developed in six countries, including both published and unpublished data, i.e. Sudan (Ahmed et al. 2016), Mali (Diarra et al. 2019), Kenya (Ngari et al. 2020) (Figure 5), Zimbabwe (Shereni et al. 2021) (Figure 6), Burkina Faso (Percoma et al. 2022), and Ethiopia (Gebre et al. 2022). Eight additional national atlases are under development within the framework of the EU-funded COMBAT (Controlling and progressively Minimizing the Burden of Animal Trypanosomosis) project (Côte d'Ivoire, Chad, Cameroon, Mozambique, Senegal, South Africa, United republic of Tanzania, Zambia) (Boulangé, A. et al. 2022). The development of national atlases also allowed the establishment of a pool of experts in the Africa Region (e.g. Kenya, Zimbabwe, Burkina Faso), who are supporting other countries in the region through expert visits and training.

In addition to tsetse mapping, progress was also made with mapping suitable vegetation for tsetse flies using remote sensing (Bouyer et al. 2010; De Beer et al. 2021). These maps are very valuable to target entomological data collection and tsetse fly control activities, including the SIT. Similarly, species distribution models such as 'Maximum Entropy' (Phillips, Anderson & Schapire 2006) can be used to target tsetse surveillance and control, and substantial progress was made in their development and application for tsetse flies (J. Bouyer et al. 2015; De Beer et al. 2021) (Figure 7).

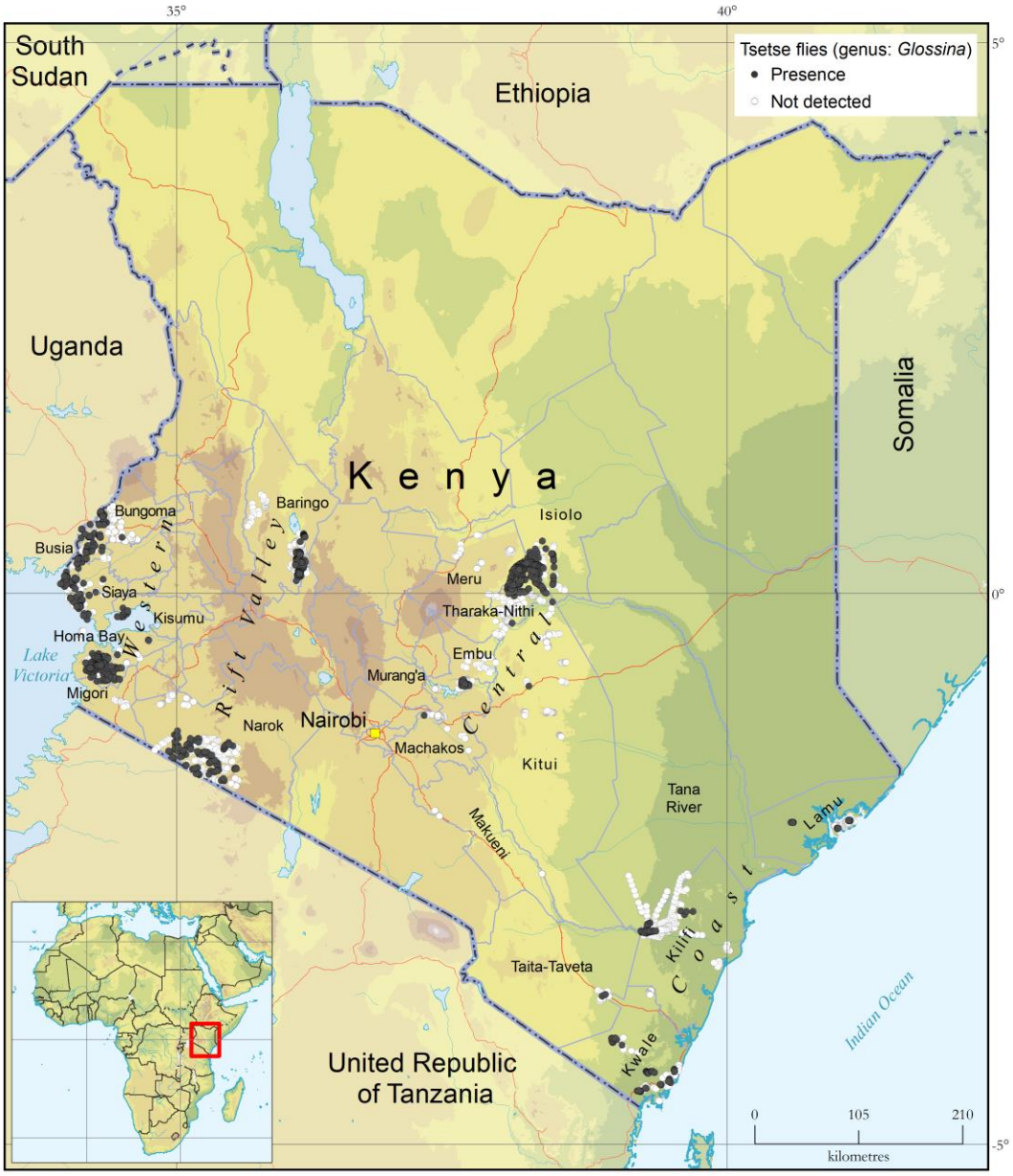

Figure 5: The national atlas of tsetse flies in Kenya (Source: Adapted from Ngari et al. 2020).

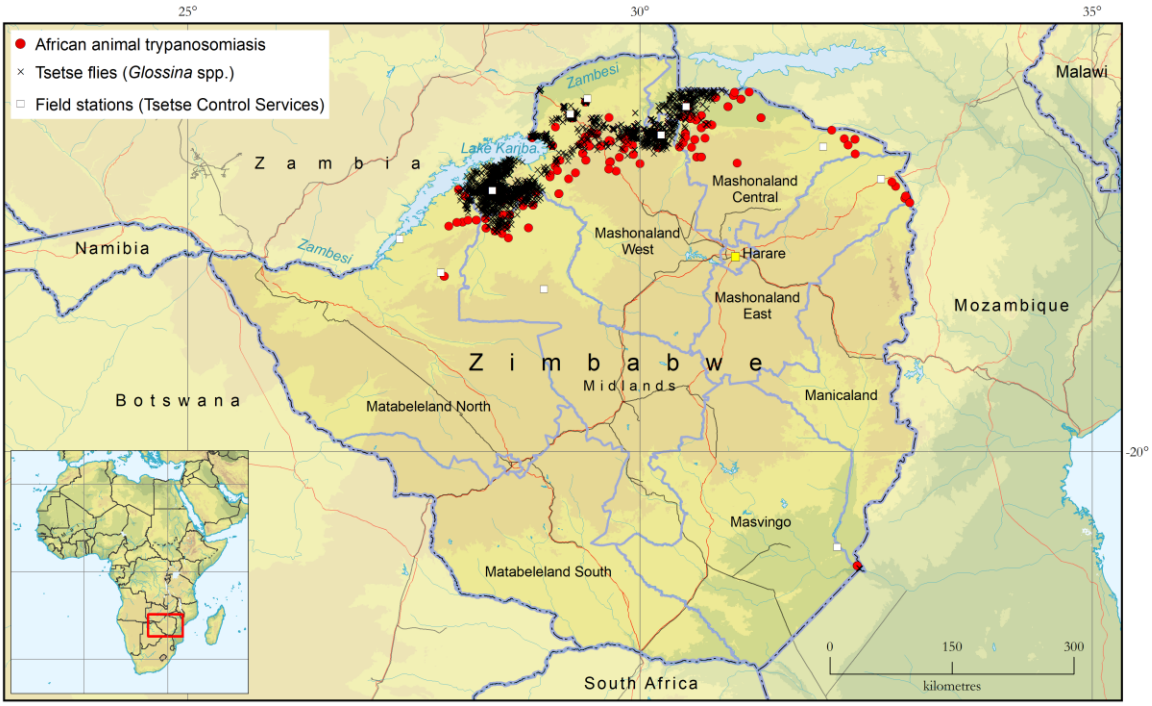

Figure 6: The national atlas of tsetse flies and African animal trypanosomosis in Zimbabwe (Source: Shereni et al. 2021).

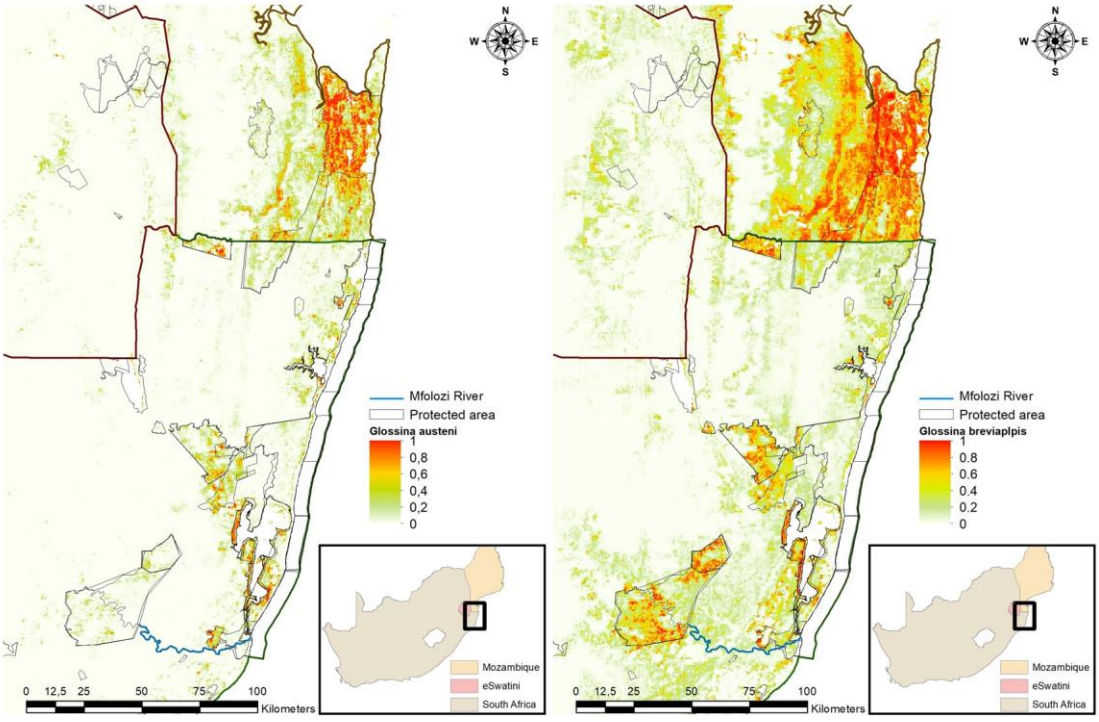

Figure 7: Transboundary distribution models for tsetse species (Source: de Beer et al. 2021).

Distribution and friction models

Species distribution modelling and landscape genetics have become a very useful tool for planning and optimizing tsetse fly control programmes, especially when sustainable eradication is the selected strategy (Bakhoum, Vreysen & Bouyer 2021). Potentially isolated clusters of tsetse fly habitats were identified based on species distribution models and ranked according to their predicted genetic distance to the main tsetse population, to locate potential target populations for eradication. Furthermore, distribution models can help improve the efficiency of control activities, leading to reduced costs.

Tsetse distribution models are very useful to optimize tsetse control operations. These models can be used for selecting priority intervention areas and guiding the management of the vector control operations. For example, these models were applied in pilot studies of *G. p. gambiensis* in the Niayes area (Dicko et al. 2014), and two savannah species, *G. m. morsitans* Westwood and *G. pallidipes* Austin in the Masoka area, mid-Zambezi valley in Zimbabwe (Chikowore et al. 2017).

Using a regularized logistic regression and Maxent, Dicko et al. (2014) compared the probability of presence of *G. p. gambiensis* and habitat suitability, respectively. Maxent predicted very well suitable areas considered the most important for an eradication objective, based on an expert-based landscape classification, as some suitable patches can be unoccupied at a certain time and colonized later (Peck 2012), but must nevertheless be included in the target area when applying an AW-IPM strategy (Cecilia et al. 2021).

Maxent predictions were used throughout the successful eradication campaign in the Niayes area of Senegal to make the entire operation more efficient in terms of deployment of insecticide-treated targets, release density of sterile males, and the selection of sites to deploy the monitoring traps used for programme evaluation. Thereby, Maxent predictions allowed optimizing efficiency and reducing the cost of the eradication campaign.

Recently, landscape genetics has established itself as an important area of research/investigation in the field of tsetse fly control (J. Bouyer et al. 2015; Bouyer & Lancelot 2018; Saarman et al. 2018). This has allowed the identification of potentially isolated tsetse populations, which offers the opportunity of: 1) selecting the most appropriate intervention strategies, 2) planning an integrated management approach and 3) the choice of suppression and elimination activities. Bouyer et al. (2015) developed a friction map (Figure 8) of 37 populations of *G. p. gambiensis* in different areas of West Africa by iterating linear regression models of genetic distance between the populations and environmental data as predictors and by determining least-cost dispersal paths.

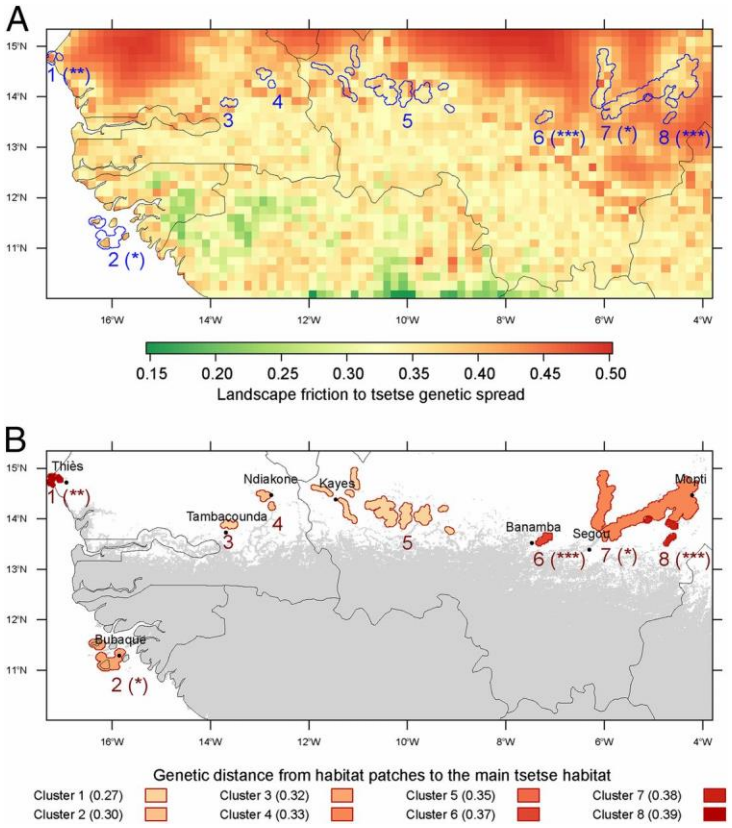

Figure 8: Isolated patches of suitable habitat for G. p. gambiensis. *(A) Landscape friction is the coloured background, and habitat patches are delimited with blue contours. (B) The main tsetse belt predicted by MaxEnt for a sensitivity of 0.90 is in grey and habitat patches are shown as filled, red shapes (Source: Bouyer et al. 2015).*

The effect of environmental factors on genetic distance was studied using a linear regression model to estimate the relationship between genetic distance and a set of environmental factors. The main variables influencing genetic distance were: 1) the geographic distance, 2) being located within the same river basin or not, and 3) three metrics of habitat fragmentation, namely the patch density, the surface of suitable area, and the maximum distance between the habitat patches. Eight potentially isolated clusters of suitable habitats containing tsetse populations that were located at least 10 km away from the main tsetse belt were identified. The population with the highest predicted genetic distance from the main tsetse belt was located in the Niayes area of Senegal and is the target of an ongoing eradication campaign.

Saarman et al. (2018) developed a similar approach to create a connectivity surface to identify isolated habitat areas reflecting the genetic and ecological connectivity at a spatial scale of interest. By integrating genetic data from 38 samples, remotely sensed environmental data, and hundreds of field-survey observations from northern Uganda, the approach of Saarman et al. allowed the identification of isolated habitat areas of *G. f. fuscipes*. To identify isolated habitat areas, the methodological framework (1) first identifies environmental parameters in correlation with genetic differentiation, (2) predicts spatial connectivity using field-survey observations and the most predictive important environmental parameter(s), and (3) overlays the connectivity surface onto a habitat suitability map.

6.1.2 Molecular and Genetic tools

Microsatellites

There are currently several molecular markers available that can be used to get an insight in the biology of wild tsetse fly populations and to assess the level of gene flow between adjacent populations. Among those, microsatellite markers are advantageous, because they do not require sequencing nor a big amount of DNA and are relatively cheap and simple to use (Solano, Duvallet, et al. 2010).

Microsatellite markers have been developed for tsetse species such as *G. f. fuscipes* (Abila et al. 2008; Brown et al. 2008; Ravel 2020), *G. p. palpalis* (Luna et al. 2001), *G. pallidipes* (Ouma et al. 2003; Ouma, Marquez & Krafsur 2006; Ravel 2020), *G. m. morsitans* (Baker & Krafsur 2001; Ravel 2020) and *G. p. gambiensis* (Solano et al. 1997; Ravel 2020). Attempts to use microsatellite markers developed for *G. pallidipes* gave only very limited insight for *G. brevipalpis* (Ouma et al. 2006). Microsatellites for *G. brevipalpis* were recently developed and evaluated, and 9 microsatellites were found to qualify for investigating the genetics of field populations. The development of new microsatellites for *G. austeni* is ongoing in the IPCL.

Population genetic studies to assess gene flow

Understanding how geographic and environmental features, structure genetic variation of tsetse populations, can greatly facilitate the development of intervention strategies of these cyclical vectors of HAT and AAT in sub-Saharan Africa. Evidence of restricted or absence of gene flow allows genetically isolated islands to be identified (Solano et al. 2009), or isolated ecological population islands (Solano, Kaba, et al. 2010), from where the tsetse populations present could be eradicated without risk of reinvasion. Even in the absence of isolation, an area-wide approach can be applied, providing connections between the target and surrounding populations are known and quantified. Temporary barriers can be installed to prevent re-invasion of the cleared areas and neighboring populations targeted sequentially in a rolling carpet approach (Hendrichs et al. 2021). However, in Africa, this rolling carpet approach may be challenged by political instability and security risks.

Genetic tools to distinguish between wild and sterile males

In AW-IPM tsetse programmes, monitoring the efficacy of the sterile male releases requires the discrimination between wild and sterile males that are sampled in monitoring traps (Vreysen 2021). Traditionally, the discrimination between sterile and wild males relied on the marking of sterile laboratory-reared males with a fluorescent dye powder (DayGlo®) mixed with sand, followed by their identification using a fluorescence camera and /or a fluorescence microscope. However, marking sterile males with fluorescent dye has some limitations. The marking is not infallible with some sterile flies only slightly marked or some wild flies contaminated with a few dye particles in the monitoring traps. Trapped flies can also be damaged due to predation by ants, making it difficult to discriminate between wild and sterile males using a fluorescence camera and / or a fluorescence microscope. In the frame of the *G. p. gambiensis* eradication programme in Senegal, a molecular technique based on the cytochrome oxidase gene has been developed that efficiently discriminates between wild and sterile males (Pagabeleguem et al. 2016).

Molecular tools for tsetse species and subspecies identification

Tsetse species identification using morphological keys is feasible for some species, but it requires expertise that in many cases is very challenging to transfer to the young generations. Therefore, the availability of molecular tools to support taxonomic keys would be very useful and easy to transfer to new scientists. PCR tools were used to identify tsetse species with a combined approach including mitochondrial markers, nuclear markers (including internal transcribed spacer 1 (ITS1), microsatellites, and bacterial symbiotic markers (*Wolbachia* infection status) (Augustinos et al. 2018). A standard operating procedure based on this method was developed (FAO/IAEA 2018). In addition, to increase the resolution between tsetse subspecies and different strains, a high-resolution melting curve technique based on the use of heterogeneous regions in the mitochondrial DNA was recently developed (Attardo et al. 2019). The use of this technique to identify more tsetse subspecies is in progress.

6.1.3 Tsetse Control Tools

New targets (tiny target, flyscreen)

Recent research has been carried out on cloth materials that could be used to develop trapping systems for biting flies. A synthetic polyester fabric commercially available was identified (Onju et al. 2020) and, more promising, a multilayer polyethylen (PE) film was developed (Teulé, C. 2019) with appropriate optical characteristics. Based on this new product, two pyrethroid-incorporated attracting screens have been developed. A blue and white PE screen able to attract and kill *Stomoxys* that is active for 6-7 months in the field (Desquesnes et al. 2021) and a full blue screen able to attract and kill tsetse flies up to 12 months in the field (Gimonneau in prep; (Salou et al. 2019). Field validation through Latin Square comparisons of these new targets is underway through the COMBAT project in several countries in Africa.

Tiny targets are a recent development in an attempt to improve cost-effectiveness of suppressing riverine tsetse fly species. Two models exist, the east African version (Esterhuizen et al. 2011) and the west African version (Rayaisse 2011). These have been used to suppress tsetse fly populations in several countries, including Uganda (Bessell et al. 2021), Democratic Republic of the Congo, Guinea, Cote d'Ivoire, and Burkina Faso.

Insecticide bioassay protocols for targets

Many tsetse control programmes rely on traps and targets that are impregnated with insecticides. Attempts have been made to assess their effectiveness during programme implementation by developing several bio-assays such as a flight tunnel (Torr 1985) or piston (Kernaghan & Johnston 1962) bio-assays, but these are time consuming and the flies' exposure time lacks homogeneity. The protocol of tarsal contact exposure of flies (Laveissiere, Couret & Traore 1985) was recently reviewed and updated (Gimonneau, in prep). This cheap and homemade method uses caged flies that are anesthetized by CO2 and transferred in a cup connected to the CO2. The flies are then taken with flexible forceps and brought into contact with the insecticide impregnated film/screen. Tarsi should come into contact with the impregnated surface for a duration of 3 seconds. After exposure, the flies are placed in a single-use paper resting cage and kept under the same conditions as in the insectary. As a general rule, flies wake up around 15-30

seconds after exposure. The number of knocked-down flies is counted after 1 hour and fly mortality assessed after 24 hours.

Novel attractants and repellents blends

All tsetse species have differential attraction/feeding preferences for wildlife, domestic animals, and/or humans. Long-range tsetse olfactory responses to natural cues emitted by preferred hosts and blends of synthetic versions that mimic these cues, have successfully been applied in odour attractant-based approaches to "pull or attract" some tsetse species to traps. The application of novel repellents (e.g. those derived from the waterbuck *Kobus ellipsiprymnus defassa)* on preferred host animals can offer protection for these animals against the tsetse burden (Saini et al. 2017).

The olfactory attributes associated with tsetse-refractory animals has been exploited in the design of repellent-odour-based protection for livestock. This repellent protection can then be used in combination with the available and newly developed attractants that are regularly used in tsetse trapping systems, to strategically develop odour based "push-pull" approaches.

The possibility of developing blends with enhanced attraction and repellence compared with those associated with savannah tsetse fly hosts and non-hosts, was explored by subtle changes of structure activity and blends of different components as demonstrated below, to generate two novel blends.

One blend was based on attractive constituents of the buffalo (*Syncerus caffer*) which include: +-nonalactone, nonanoic acid, 2-nonanone (in 1:3:2 proportion) delivered together with acetone and showed significant better attractancy for savannah tsetse flies than the standard blend of 3-propylphenol, octenol, p-cresol, and acetone (POCA).

The second blend was comprised of δ-octalactone, heptanoic acid, 4-methylguaiacol and geranylacetone (in 6:4:2:1 proportion) and showed significant better repelling properties than the previously characterized blend derived from waterbuck chemicals (d-octalactone, pentanoic acid, guaiacol and geranylacetone (Mireji et al. 2022).

Advances in sexual attractants

Pheromones are successfully used in the control of many insects (Reddy & Guerrero 2010). Recent advances have been made with the identification of a volatile sex attractant in *G. m. morsitans*. Ebrahim et al. (2023) identified methyl palmitoleate (MPO), methyl oleate, and methyl palmitate as compounds that are produced by the tsetse fly *G. morsitans* and elicit strong behavioural responses in the laboratory. This recent finding offers new options for the behavioural manipulation of these vectors and for the development of new vector control tools.

Boosted SIT

The SIT has proven to be effective in controlling tsetse flies when applied to isolated populations but necessitates the production of large numbers of sterile males, which can be challenging. A new approach, called boosted SIT, combines the SIT with the use of biocides that are coated on the released sterile males, and that will contaminate the wild females during mating (Bouyer & Lefrançãois 2014). The boosted SIT was tested in the laboratory on the riverine species *G. p. gambiensis* using pyriproxyfen. The contamination dose and persistence of pyriproxyfen on

sterile males, the impact of pyriproxyfen on male survival, and the dynamics of pyriproxyfen transfer from a sterile male to a female during mating, as well as the impact of pyriproxyfen on pupal production and adult emergence, were evaluated. For this purpose, a protocol to impregnate sterile males with a powder containing 40% pyriproxyfen was developed (Laroche et al. 2020). The results showed that pyriproxyfen had no impact on the survival of sterile males. Pyriproxyfen persisted on sterile males for up to 10 days at a dose of 100ng per fly. In addition, the horizontal transfer of pyriproxyfen from a treated sterile male to a female during mating could be measured with an average of 50ng of pyriproxyfen transferred. After contacts without mating, the average quantity transferred was more than 10ng. Finally, the pyriproxyfen powder was very effective on *G. p. gambiensis* leading to 0% emergence of the pupae produced by contaminated females. These promising results must be confirmed in the field.

Although this method may reduce the overall cost of the SIT for tsetse fly control and could be considered for suppression (rather than eradication) purposes, the combination of the SIT with biocides will make it a less environment-friendly technique and will require biocide authorizations for use in some countries. It may also be applied using biopesticides like fungi or viruses, as it is currently the case in fruit flies and mosquitoes (Ayasse et al. 1995; Noushini et al. 2020).

6.1.4 Operational control programmes

Colonization of wild tsetse strains

The *G. p. gambiensis* Burkina Faso (BKF) strain that has been maintained at the CIRDES for almost 50 years, was selected for the SIT component of the eradication programme in Senegal (Pagabeleguem et al. 2015). In spite of data that indicated adequate compatibility and competitiveness of the BKF strain with the local Senegal populations, two new strains were developed to serve as alternatives in case the BKF strain would not perform optimally in certain ecosystems of the Niayes region. These two strains were a *G. p. gambiensis* Senegal (SEN) strain that originated from Pout/Sebikotane in the Niayes and an introgressed (SEN BKF) strain, obtained from crossing BKF females with SEN males.

During the SIT control campaign in the 1980's in Sidéradougou, Burkina Faso, the BKF strain was used effectively (Politzar & Cuisance 1984), but 30 years later it showed signs of reduced competitiveness in the field (Sow et al. 2012). In view that the Government of Burkina has been involved in a campaign aimed at eradicating a population of *G. p. gambiensis* from the Mouhoun river under the auspices of the PATTEC since 2006, there was concern that the decline in the competitiveness of the BKF strain could possibly hinder the implementation of this objective. In that respect, the Insectarium of Bobo-Dioulasso (IBD) domesticated a new strain of *G. p. gambiensis* (IBD strain) from wild flies in 2016. The IBD has also established a hybrid strain, resulting from the crossing of the old BKF strain with the new IBD strain. The hybrid strain would use the productivity traits of the BKF strain and the hardiness traits from the wild IBD strain (Toé et al. 2021).

Use of bio-indicators to assess impact of tsetse control

The SIT is an environment-friendly control tactic and is species specific. However, it is not a stand-alone technique and has been used mostly in combination with other control tactics within

an AW-IPM strategy. That is why environmental monitoring is recommended to assess the impact of the integrated campaigns on the ecosystem. For a period of eight years, the direct impact of the campaign to eradicate a population of *G. p. gambiensis* in Senegal was monitored using a set of fruit-feeding insect species (Cetoniinae and Nymphalidae) (Ciss et al. 2019). These species were previously demonstrated to be good ecological indicators of the health of an ecosystem (Bouyer et al. 2007). These insects are very sensitive and reliable indicator of any perturbation of the ecosystems and their sampling is cheap and standardized. In the Senegal project, they demonstrated that during the pre-release phase using insecticides, the campaign had a measurable but small impact on the apparent densities of the most prevailing non-target species, as well as on species diversity. However, the indicator populations reverted to pre-intervention levels as soon as the release of the sterile male insects started. These results greatly expand our understanding of the impact of vector eradication campaigns on non-target species. The methodology used in Senegal can be applied in any future tsetse eradication projects.

Field database for the Niayes project

Data collection and analysis are key components in an SIT programme since they provide guidance to make informed decisions. In the tsetse fly eradication project in the Niayes, data were routinely collected in the field and at the insectary, which were entered into a relational database. The database could be accessed online at any time by all project staff and partners, whatever their level of involvement, with specific editing rights according to their authorization access by the administrators. The advantages of such a tool were numerous and includes entering and saving data collected routinely and in a safe way, proper analysis of spatio-temporal data and displaying them in various formats for easy interpretation through automatic queries. This allowed rapid feedback to the programme management and field teams, to the mass-rearing facilities on the quality of the flies, as well as to the release centre and release teams. A graphic interface available on a website also allowed access to synthetic data to the public and donor organizations.

6.2 Current Role of the IAEA and the Joint FAO/IAEA Centre

In the last decade, MSs have supported an IAEA General Conference resolution in support of the African Union's Pan African Tsetse and Trypanosomosis Eradication Campaign (AU-PATTEC). The resolution recognizes that tsetse flies and the trypanosomosis problem which they cause constitute one of the greatest constraints to the socio-economic development on the African continent, affecting the health of humans and of livestock, limiting sustainable rural development, and thus causing increased poverty and food insecurity.

The General Conference resolution requested the IAEA and other partners to strengthen capacity building in MSs for informed decision-making regarding the choice of tsetse and trypanosomosis strategies and the cost-effective integration of the SIT operations in AW-IPM campaigns. The General Conference resolution also requested the Secretariat, in cooperation with MSs and other partners, to maintain funding through the Regular Budget and the Technical Cooperation Fund for consistent assistance to operational SIT field projects and to strengthen its support for research and development and technology transfer to African Member States to complement their efforts to create and expand tsetse-free zones.

In response to the GC resolution 65/RES/11/A.2, the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Centre strengthened its research programme towards the development of the SIT package for tsetse fly. The goal was to develop more effective and cost-efficient protocols for mass-rearing, colony management, sterilization, sorting systems to separate males from females, quality control and assessment of the field competitiveness, long-distance transport / shipment to the field as well as for the release and the monitoring of sterile flies in the field. The main R&D achievements of the last decade can be summarized as follow:

6.2.1 Mass-rearing

Mass-production of the target insect is a primary component of any pest or vector control programme that requires the release of large numbers of sterile insects. As part of efforts to develop an area-wide programme involving the SIT for the control of tsetse flies, the IPCL has developed mass-production tools.

Tsetse production unit

The manual rearing of tsetse flies for the SIT is a labour-intensive undertaking and several systems have been developed to automate the mass-rearing that would simplify the rearing, reduce the rearing cost and standardize production. Four distinct systems were developed and tested in sequence.

The first tsetse production unit (TPU 1) was a fully automated system, but the fly survival and fecundity were unacceptably low. From this, a simpler TPU 2 was developed and tested, where 63 large cages were held on a frame that could be moved as a single unit to the feeding location. TPU 2 was tested in various rearing units in MSs and satisfied the basic rearing requirements. The adoption of a Plexiglas® pupal collection slope improved the light distribution that overall increased fly survival and fecundity. However, the cage holding frame was heavy and difficult to position on the feeding frame and the movement disturbed the flies. TPU 2 was superseded by the TPU 3, in which the cages remained stationary, and the blood was brought to the flies (unlike in the TPU 2 where the blood was stationary and the flies were moved). The blood feeding system is mounted on rails to make it easier to move, and with a simple locating system it is quick and easy to move the unit to the next set of cages. This system has proved satisfactory for all tsetse species tested and was used for large-scale rearing in Burkina Faso and Ethiopia. Recently, the TPU 3 was superseded by TPU 4 (Figure 9), in which the feeding unit with 7 levels remains stationary, and the cage-holding system is moved. This change achieved two main objectives. The first was to promote clean feeding by starting with the younger flies to avoid the virus contamination from older flies to younger ones. The second was the possibility of organizing or moving trolleys within the module to clean the room and avoid the accumulation of dirt that could be a source of fly contamination. The TPU 4 system (Figure 10) has been deployed in fly rearing facilities in Ethiopia, Burkina Faso and Bratislava.

Figure 9: TPU 4 system (left feeding unit, middle holding trolley front view, and right holding trolley front back view) in the fly rearing facility of the Insect Pest Control laboratory (IPCL/ IAEA), Seibersdorf: (Source: Chantel de Beer, IPCL).

Figure 10: TPU 4 system in the fly rearing facility of the "Insectarium de Bobo-Dioulasso", Burkina Faso (Source: Soumaila Pagabeleguem, IBD).

Virus management in colonies of Glossina pallidipes

Tsetse fly mass-rearing is a very challenging process due to the nature of tsetse biology (viviparous with K-strategy) with a low number of offspring produced by females. This makes colony expansion a very slow and tedious process. Likewise, the establishment of new colonies from wild collected materials is complicated, as the insects are not adapted to the *in vitro* membrane feeding system, and the high mortality and low productivity results in extended time to reach a reasonable colony size. Any problem that increases mortality of the flies reduces productivity of the females in a colony should therefore be avoided.

An important discovery was the impact of a virus (the salivary gland hypertrophy virus SGHV) infection on *G. pallidipes* colonies at the IPCL and in the rearing facility in Kaliti, Ethiopia.

Initial research investigated the dynamics of the virus infection (Abd-Alla et al. 2010) and showed that the SGHV was responsible for the poor performance of the *G. pallidipes* colony and proved that the *in vitro* blood feeding system favoured the horizontal transmission of the virus from infected flies to uninfected adults. Consequently, attempts were made to mitigate the propagation of the virus infection using antiviral drugs (i.e. Valacyclovir) (Abd-Alla et al. 2012). Moreover, a change in the tsetse feeding regime and the adoption of a "clean feeding system" (each membrane and blood was used only once for feeding) avoided horizontal virus transmission through the blood, resulting in a drastic reduction of the SGHV prevalence in the colony (Abd-Alla et al. 2013). Combining the clean feeding system and the antiviral drug eliminated the SGHV problem from the colony (Abd-Alla et al. 2014). Based on the above results, a standard operation procedure to manage the SGHV infection colonies was developed and implemented in Ethiopia which solved the SGHV problem.

In addition to the SGHV infection, two RNA viruses were recently discovered, *G. m. morsitans* Iflavirus and *G. m. morsitans* Negevirus. Research is continuing at the IPCL to elucidate the impact of the presence on these viruses on the performance of tsetse colonies (Meki et al. 2021).

Interaction between symbionts, parasites, and viruses

Tsetse flies harbour a unique bacterial community mainly consisting of the obligate *Wigglesworthia glossinidia*, the commensal *Sodalis glossinidius*, and the widespread symbiont *Wolbachia pipientis* (hereafter *Wolbachia*) in addition to the microbial communities in the mid-gut. Again, some tsetse species can be infected with the salivary gland hypertrophy virus (SGHV) or other RNA viruses (i.e. Iflavirus and negevirus). Tsetse mass-rearing is crucial for SIT programme implementation, however the presence of some of these symbionts have shown to have an effect on the fecundity of tsetse. Due to the low productivity of tsetse flies which limits a quick expansion of colonies or the initiation of new colonies from wild material, it is important to have stable production of these colonies. It is therefore important to provide optimal conditions for tsetse production through avoiding infections with pathogenic viruses and through enhancing the useful microbiota important for tsetse productivity (i.e. *Wigglesworthia*). The interactions between tsetse symbionts and the SGHV was recently studied with laboratory-reared flies (Demirbas-Uzel et al. 2021), or in field-collected flies (Dieng, Dera, et al. 2022; Dieng, Augustinos, et al. 2022). The results indicate that these interactions are complex. The presence of some tsetse symbionts increases tsetse refractoriness to trypanosome infection, and therefore the presence of these symbionts might lead to the production of sterile males being refractory to trypanosome infection, using either paratransgenesis with modified *Sodalis* or though *Spiroplasma* infection. This topic needs further studies.

6.2.2 Sex separation

Near Infrared Pupae Sex Sorter (NIRPSS)

The ability to determine the sex of tsetse pupae with the objective to separate the sexes before adult emergence, has been a major goal for decades for tsetse management programmes with a SIT component. Tsetse fly females need to be retained in the colony to enable stable production of sterile males, and, in addition, the release of sterile males only will increase the efficiency of SIT programmes. In past tsetse SIT programmes (e.g. on Unguja Island), adult males were

manually separated from adult females in a chiller that immobilized the insects. This was very tedious and labor-intensive and it had negative consequences for the quality of the insects.

Tsetse females develop faster than males and adult female pharates inside the pupae melanise 1-2 days before the males (Moran & Parker 2016). This earlier melanization can be detected by infrared cameras through the pupa shell, and the newly developed Near Infrared Pupae Sex Sorter (NIRPSS) (Figure 11) takes advantage of this (Argilés-Herrero et al. 2023). The melanization process is not homogeneous for all fly organs and the pupa needs to be examined ventrally, dorsally, and laterally to ensure accurate classification by an image analysis algorithm. When the pupae are maturing at a constant temperature and relative humidity of 25°C and 80% respectively and sorted at the appropriate age, i.e. 24 days post-larviposition for *G. p. gambiensis*, the sorting machine can efficiently separate the sexes. The recovered male pupae can then be sterilized for field releases of the males while the rest of the pupae can be used to maintain the laboratory colony. The sorting process with the new NIRPSS had no negative impact on adult emergence and flight ability.

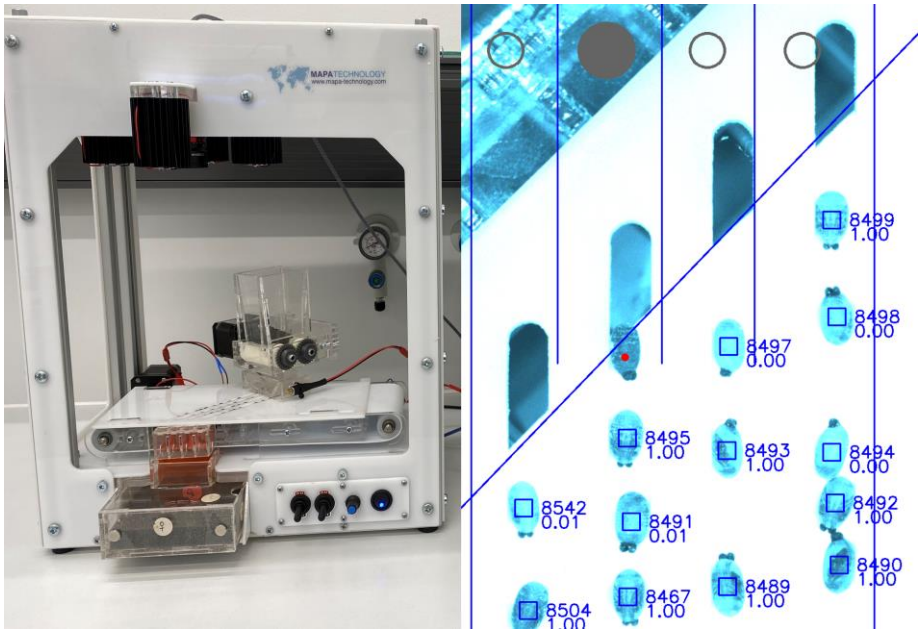

Figure 11: Near Infrared Pupal Sex Sorter (NIRPSS) (left), and image of rows of pupae along the channels under NIR light with the melanization index calculated in real time (right). (Source: Argilés-Herrero et al. 2023).

6.2.3 Irradiation

The SIT requires the release in the affected area of large quantities of the target insects, that have been sterilized by irradiation. Radiation sensitivity studies that produce dose response curves are required to ensure the production of sterile male flies that are competitive with their wild counterparts. Recent studies on radiation sensitivity of tsetse pupae and adults have been carried out for the following tsetse species, *G. p. gambiensis* (Ilboudo et al. 2022; Pagabeleguem et al. 2023), *G. pallidipes*, *G. f. fuscipes* (Debela 2019) *G. brevipalpis* (de Beer et al. 2017) and *G. austeni* (De Beer et al. 2020). This sterilization by irradiation was mainly done using gamma ionizing radiation until these sources began to encounter constraints related to the acquisition of

self-shielded irradiators with an appropriate dose rate for insect sterilization and the shipment of radioisotopes. Alternative sources such as X-ray irradiators have been tested for their effectiveness for several species of insect pests including tsetse flies. The tests showed that X-ray and gamma irradiators induced similar biological effects and confirms that the available X-ray irradiators are suitable for insect management programmes (Kaboré et al. 2023; Yamada et al. 2023).

6.2.4 Handling, transport and release

In AW-IPM programmes that include an SIT component, a large number of male tsetse flies are produced in mass-rearing facilities. Before being released into the target area, they need to be transported to the release site and this operation often requires the establishment of a dispersal/release centre where males can be emerged (in case pupae are transported), and the adult males offered blood meals before being released.

Long-distance protocols for pupae shipment

For projects that target small areas and do not have a mass-rearing facility, it is more cost effective to import the sterile flies from a mass-rearing facility that is located in another country. In such situations sterile male are ideally shipped at the pupal stage, following the long distance transport protocol described by (Pagabeleguem et al. 2015). This protocol has been developed based on chilled pupae using insulated transport boxes and phase change material packs (S8) to keep the temperature at around 10°C which prevents male fly emergence during transport. Recent developments in pupae sex sorting allows the shipping of un-chilled pupae and should be preferred if available (Argilés-Herrero et al. 2023). It significantly improves the sterile male quality, reduces the rate of female contamination in shipments and allows for more flexibility in the transport time as flies are irradiated at 24-25 days.

Chilled adult release systems

In most cases, area-wide releases of sterile males are done by air. The aerial release of sterile insects has many advantages as compared with ground releases such as it is fast, reaches areas that are inaccessible for ground release and provides a uniform distribution of sterile insects over the target areas.

To do so, adult sterile males could be packaged in bio-degradable cartons that are manually dropped from fixed-wing aircraft (Vreysen, Zhu, et al. 1999; Vreysen, Saleh, et al. 1999) or gyrocopters (Vreysen et al. 2021). However, it has several constraints such as the limited number of boxes embarked per flight, and as a consequence, an increase in flight rotation and ultimately the overall increase in cost of release (bio-degradable boxes are expensive). Therefore, to circumvent some of these constraints, automated chilled-adult release systems have been developed that not only reduce the implementation cost, but also increase the efficiency of the programme through improved sterile male fly distribution (Vreysen, Saleh, et al. 1999; Hendrichs et al. 2021). The first automated device to release chilled male tsetse flies were developed by the company Mubarqui, i.e. the Smart Release Machine that was adapted from a machine used to release fruit flies and based on a vibrating system to control the release rate of the flies (Mubarqui

et al. 2014). Initially, the system gave promising results but was abandoned due to inadequate consistency of the release rates (Mirieri et al. 2020).

A new automated chilled adult release system (BSI™, Bruno Spreader Innovation (BSI™)) was recently developed and is based on a cylinder rotating against a brush as an ejection mechanism (Bouyer, Seck & Gimonneau 2017). This BSI™ machine presents significant improvements but has also some drawbacks (Mirieri et al. 2020). It is a tablet computer driven machine that includes an automated guidance and navigation system, control for temperature and humidity, and a real time adaptive release system depending on the number of flies to be released, the gyrocopter speed, and the area to be covered.

The BSI was evaluated under laboratory conditions and was used during the last months of aerial release in the Niayes project. However, the BSI™ machine needs to be carefully evaluated under field conditions.

6.2.5 Product quality control

Laboratory testing

The successful implementation of SIT depends on several requirements, of which the biological quality and sexual competitiveness of the sterile males are amongst the most important ones. The sterile males must be able to compete with wild males for mating opportunities with the local virgin females, and hence, quality management assessments are an important aspect. A flight test has been developed and tested to monitor the quality of sterile male *G. p. gambiensis* throughout the production and transport processes to support the tsetse eradication programme in Senegal (Seck et al. 2015; Diallo et al. 2019).

Semi-field testing

Mating compatibility and competitiveness studies are essential to support SIT projects. These studies are challenging, expensive, and their results can be influenced by several environmental, climatic and ecological parameters, which cannot be controlled. For this purpose, walk-in field cages have been successfully used as a tool to carry out mating compatibility, mating competitiveness and other behavioural studies involving different tsetse fly species such as G. *p. gambiensis, G. brevipalpis* and *G. austeni* (Mutika et al. 2013).

A study was conducted in West Africa to assess mating compatibility of three strains of *G. p. gambiensis.* The first strain was maintained at the CIRDES, Burkina Faso since 1975 and established from seed material collected in Mare aux Hippopotames (Burkina Faso). This BKF strain was tested against strains from Senegal and Mali to assess whether this BKF strain could be used for SIT in target areas of Mali and Senegal. Mating performance assessments of the three strains showed that mating barriers were absent and therefore, the BKF males could be used for releases in Mali and Senegal.

In other studies, the optimal mating age for *G. brevipalpis* and *G. austeni* was determined in behavioural studies carried out in walk-in field cages. Age was identified as a factor that can influence mating competitiveness, and it was recommended that nine day-old or older males be used in the implementation of the SIT (de Beer, Venter & Vreysen 2015).

Field testing

Some quality control (QC) parameters such as survival, recapture rate, dispersal, and sexual competitiveness must be measured at regular intervals in the field and there is a need for better defining these parameters and standard protocols to measure them. A field evaluation of the mating competitiveness of a 40-year-old *G. p. gambiensis* colony indicated that the sterile males were able to induce nearly complete sterility in the wild female population when a sterile to wild male ratio of 10:1 was obtained (Sow et al. 2012). Furthermore, a study on the performance of the BKF and Senegal (SEN) strains in an urban area of Senegal showed lower daily mortality rates for the SEN strain, but the BKF strain was more competitive.

6.3 Coordinated Research Projects (CRPs) and Technical Cooperation Projects (TCPs)

6.3.1 Past, Current and Future Coordinated Research Projects (CRPs)

Four CRPs on tsetse flies have been implemented between 2003 and 2023, one of these CRP is still ongoing, and a new one will be initiated in 2025 (Table 2). Those are chronologically presented below with the objectives and achievements listed.

Table 2: Coordinated Research Projects (CRPs) on tsetse flies finalized, ongoing and planned to be initiated in 2025.

Project Number	**Finalized CRP**
G4.20.10	Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application (2003-2010)
D4.20.12	Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens (2007-2012)
D4.20.15	Enhancing Vector Refractoriness to Trypanosome Infection (2013-2017)
	Ongoing CRPs
D4.20.17	Improvement of Colony Management in Insect Mass-rearing for SIT Applications" (2018-2024)
	New CRP
D4.20.18	Tsetse Population Genetics (2025-2030)

CRP D4.20.10 on "Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application" (completed: 2003-2010).

The development of large-scale rearing highlighted the need for improved quality control procedures and the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture established a CRP in 2003 entitled "Improved and Harmonized Quality Control for Expanded

Tsetse Production, Sterilization and Field Application" with the objective of improving and expanding the quality control sections of the "FAO/IAEA Standard Operating Procedures for Mass rearing Tsetse Flies". Sixteen institutes from thirteen countries in Africa, Europe and Central America participated in this CRP. In the area of blood diet for tsetse colony maintenance, three groups looked at collection procedures, factors influencing diet quality and bacterial decontamination. Two groups worked on rearing issues, including emergence, and holding conditions, handling of adults, and feeding regime. Extensive and highly detailed studies of the courtship and copulation of tsetse flies revealed a wealth of information previously not seen. Other aspects that were investigated were tsetse vectorial capacity, development of the flight muscles, rhythm of larviposition and oxygen consumption as a measure of fitness. In summary, progress was made in all areas and resulted in modification or expansion of the existing quality control protocols in the FAO/IAEA Standard Operating Procedures for Mass rearing Tsetse Flies or proposals for new protocols. Nine papers were published in peer-reviewed scientific journals emanating from this CRP, and 24 new or revised quality control protocols were proposed. At the final Research Coordination Meeting (RCM) held in Addis Ababa, Ethiopia, 13–17 October 2008, some recommendations for future research were made by the participants. It was noted that, in general, some existing tsetse rearing protocols should be improved.

CRP D4.20.12 on "Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens" (completed: 2007-2012).

Sleeping sickness or HAT is caused by *Trypanosoma brucei gambiense* (T.b.g.) or *Trypanosoma brucei rhodesiense* (T.b.r.) and the disease occurs in 36 countries in sub-Saharan Africa. The former is responsible for about 95% of the chronic cases in central and western Africa, whereas T.b.r. causes the acute form of the disease in eastern Africa. Other *Glossina*-transmitted trypanosomes also infect cattle and cause a disease called nagana, a Zulu word meaning ''to be depressed''. Nagana results in millions of dollars of economic losses to countries that can ill afford such losses. The management of nagana based on the recurrent treatment of livestock with trypanocidal drugs is costly and not sustainable in view of increasing resistance of the parasites. In attempts to develop more sustainable approaches to the management of the disease in mainland Africa, several Governments adopted the SIT. This technique, when integrated with other control tactics, has been previously successful in eradicating tsetse flies in the Island of Unguja (Zanzibar), United Republic of Tanzania. It relies on limiting the reproductive capacity of the flies by releasing large numbers of reproductively sterilized, colony reared males. To initiate an SIT strategy against *G. pallidipes* in Ethiopia, tsetse fly colonies were established in Seibersdorf, Austria and in Addis Ababa, Ethiopia. However, two colonies in Seibersdorf collapsed due to infection by the *G. pallidipes* salivary gland hypertrophy virus (GpSGHV). The virus also caused low production and poor stability of the colonies in Ethiopia. The question of how to limit the spread of the virus in the colonies to produce sufficient flies for an SIT strategy became paramount. Approximately seven years ago a coordinated research project (CRP) entitled ''Improving SIT for Tsetse Flies through Research on Their Symbionts and Pathogens'' was initiated under the auspices of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture. The CRP included 23 scientists from 18 countries, representing a broad range of expertise to investigate the problem and to develop solutions. These scientists had expertise in insect viruses and especially in a closely related virus that causes similar symptoms in the common house fly, *Musca domestica* (MdSGHV), as well as in tsetse symbionts, parasites, and

fungal pathogens. The individual studies in the programme involved detailed investigations into the biology of the insect in relationship to the causative trypanosomes, parasites, and symbionts, as well as epidemiological investigations of the disease in various parts of Africa and practical procedures to manage the virus that have been transferred to tsetse mass-rearing facilities. The scientists convened at about 18 months intervals to report their findings and to coordinate their research; the last in Vienna in March 2012, when the CRP was completed.

CRP D4.20.15 on "Enhancing Vector Refractoriness to Trypanosome Infection" (completed: 2013-2017).

In 2013, a CRP entitled "Enhancing Vector Refractoriness to Trypanosome Infection" was initiated under the auspices of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture. The project included 23 scientists from 19 countries, representing a broad range of expertise, to gain a deeper knowledge of the tripartite interactions between the tsetse fly vectors, their symbionts, and trypanosome parasites and to acquire a better understanding of mechanisms that limit the development of trypanosome infections in tsetse and how these may be enhanced. The studies involved detailed investigations into the biology of the insect in relationship to the causative trypanosomes, parasites, and symbionts, as well as epidemiological investigations of the disease in various parts of Africa.

CRP D4.20.17 on "Improvement of Colony Management in Insect Mass-rearing for SIT Applications" (ongoing: 2018-2024)

This CRP aims at developing best practices for insect colony management for the cost-effective production of high-quality sterile males for SIT applications against major insect pests and disease vectors through a multidisciplinary approach involving entomologists, geneticists, ecologists, microbiologists, pathologists, virologists, and mass-rearing experts**.**

CRP D4.20.18 on "Tsetse population genetics and distribution models to support SIT application" (new: 2025-2030)

Tsetse population genetics that assess gene flow between populations provide important information for the planning and selection of appropriate intervention strategies that includes an SIT component. Adopting a population genomic approach based on a systematic sampling of a tsetse population can be costly. Developing models that uses known genetic distance and remotely sensed environmental data to identify natural barriers to tsetse dispersal will be a valuable tool in planning the implementation of a tsetse elimination strategy. The main objective of this CRP will be to develop and evaluate modelling and genetic analysis tools to identify isolated tsetse populations that can be suitable candidates for SIT implementation in Africa.

6.3.2 Current support to Technical Cooperation Projects

Seven Technical Cooperation projects dealing with livestock pests are ongoing (6 national and 1 regional) (Table 3). The new projects to be initiated in 2024 are listed in Table 4.

Table 3: IAEA Technical Cooperation Projects on Livestock pests that are ongoing in 2019.

Country	Project Number	Ongoing National Projects
Burkina Faso	BKF5023	Implementing the Sterile Insect Technique to Reduce Wild Populations of *Aedes aegypti* and Tsetse
Chad	CHD5011	Implementing the Sterile Insect Technique to Control *Glossina fuscipes fuscipes* — Phase II
Ethiopia	ETH5023	Enhancing Livestock and Crop Production through Consolidated and Sustainable Control of Tsetse and Trypanosomosis to Contribute to Food Security
Senegal	SEN5040	Strengthening National Capacities to Create a Tsetse-Free Zone Using the Sterile Insect Technique
South Africa	SAF5015	Supporting the Control of Nagana in South Africa Using an Area-wide Integrated Pest Management Approach with a Sterile Insect Technique Component - Phase I
United Republic of Tanzania	URT5034	Implementing Pre-Operational Activities for the Elimination of *Glossina swynnertoni* through Area-wide Integrated Pest Management with a Sterile Insect Technique Component
		Ongoing Regional Projects
Regional Africa	RAF5087	Enhancing Regional Capacity for the Implementation of the Sterile Insect Technique as a Component for Area-Wide Tsetse and Trypanosomosis Management (AFRA)

Table 4: IAEA Technical Cooperation Projects on Livestock pests that are approved to be initiated in 2024.

Country	Project Number	New National Projects to Start in 2024
Ethiopia	ETH5024	Enhancing Livestock and Crop Production through Control of Tsetse and Trypanosomiasis to Contribute to Food Security
Senegal	SEN5044	Developing National Capacity for Implementing the Sterile Insect Technique against Tsetse Flies in the Sine-Saloum for 2024–2027
South Africa	SAF5020	Radiation Biology and Population Genetics of *Glossina brevipalpis* in Preparation of a Sterile Insect Technique (SIT) in Affected Communal Areas of North Eastern KwaZulu-Natal Province, South Africa

7. POTENTIAL FUTURE ROLE OF THE IAEA AND THE JOINT FAO/IAEA CENTRE

7.1 Priority Target Tsetse Species for SIT Applications

Tsetse species where SIT will have a competitive advantage should be prioritised and species that should take priority are:

- In the Subgenus Nemorhina (palpalis) group: *Glossina palpalis gambiensis, Glossina palpalis palpalis, Glossina fuscipes fuscipes, Glossina fuscipes quanzensis, Glossina tachinoides*
- In the Subgenus Austenina (fusca) group: *Glossina brevipalpis*
- In the Subgenus Glossina (morsitans) group: *Glossina austeni, Glossina pallidipes, Glossina morsitans submorsitans*

7.2 R&D Priorities to Address Bottlenecks

7.2.1 Improve cost-effectiveness of mass-rearing

During the last years, significant progress was made in improving and optimizing the rearing process aiming to reduce cost and improve the flies' quality as mentioned in section 6.2.1. However, improvements are still required to produce equipment at lower cost, minimize the labor required for cleaning, and ensure that the equipment is suitable to produce high quality males, measured by key biological characteristics. The following studies are recommended:

1. Studies on blood feeding to improve the recently developed tools.
2. Studies on alternative and cheaper membranes for blood feeding.
3. The use of blood alternatives and or nutritional supplements to increase blood quality.
4. Inexpensive rapid tests to identify antibiotics and trypanocide contaminants in blood meals.
5. Alternative blood sterilization methods such as the use of UV.
6. Increased automation to reduce workload and increase efficiency.
7. Incorporation of the newly developed tsetse pupal sex sorter into regular tsetse management colonies under operational conditions.

Tsetse males and females are obligate hematophagous and so far, tsetse fly colonies are fed with cow blood collected from slaughterhouse, defibrinated and sterilized by irradiation treatment with a radiation dose of 1.5-2 kGy. The blood is then tested for bacterial contamination and flies are fed for 25 days to assess the quality factor based on the fly's mortality and productivity. Blood is one of the most expensive components in tsetse fly mass-rearing and its quality severely affects colony sustainability and productivity. Attempts to find alternatives, i.e. using artificial diets was carried out in the past, but so far failed to be used as a practical solution. However, the

advancement made in the last two decades might open new doors that justify revisiting the topic again with the goal to find practical solutions.

One new approach to improve tsetse mass-rearing could be the use of supplements in the blood used to feed tsetse colonies to:

1. control pathogens (e.g. the use of valacyclovir to manage the SGHV in *G. pallidipes* colonies).
2. nutritional chemicals that might increase tsetse productivity or the flies' quality.
3. substances that can affect tsetse symbionts and pathogens (i.e. the use of silver nanoparticles).

Recently, a study that supplemented the blood with some amino acids resulted in an increase in the size of the produced pupae. More investigation to explore this point is in progress. In addition, the effect of silver nanoparticles (NBs) (a potent antibacterial and antiviral agent) on tsetse mortality and productivity was assessed and a concentration of 300 ng/ml did not affect tsetse performance. The impact of these NBs on tsetse symbionts and pathogens is ongoing. Complementing these investigations is highly recommended as it might improve tsetse mass-rearing.

7.2.2 Development and/or improvement of sex-separation methods

Genetic sexing strains using morphological markers

Despite the recent development of the NIRPSS, and its potential adoption in mass-rearing facilities, it is still crucial to improve the sorting process by reducing the influence of temperature on the accuracy of the current pupae sorting system. This could further reduce the number of residual females and improve the recovery rate of males.

In addition, genetic sexing strains should be developed either through classical genetics and/or genetic engineering approaches to be able to reliably eliminate females before any tsetse release in the field projects.

Other sex-separation methods

With the newly developed NIRPSS, accurate sex separation of tsetse pupae is now possible. The pupae can now be sorted five days before emergence of the adults and can be shipped long distance without using low temperature conditions to prevent emergence. The sorting protocol is available for *G. p. gambiensis* and sorting protocols will be needed for the other relevant SIT tsetse species such as *G. pallidipes*, *G. f. fuscipes*, *G. brevipalpis* and *G. austeni*. Further improvement on this sorting system can still be done by introducing machine learning into the customisation of the melanisation thresholds that is currently being used by the NIRPSS software to select the melanised pupae.

7.2.3 Refine irradiation procedures for target tsetse species

Exposure to ionizing radiation is currently the standard method to sterilize tsetse flies in SIT projects. The response to irradiation dose is species-specific and it can also vary in relation to

biological factors (such as the developmental stage and insect age) and/or external factors (such as rearing conditions, handling, irradiation source, dose rate, irradiation device, and protocols). Therefore, it is essential to establish dose response curves for each SIT target species and rearing facility. There is a natural trade-off between sterility level and performance of sterile males in the field. The optimal dose is defined based on the highest sterility level detected without compromising the performance of sterile insects, and thus the highest capacity to induce sterility in the wild target population. Despite many significant biological and physical factors affecting dose-response during tsetse irradiation have been identified, further studies are needed to investigate for example the possible advantages of pupae sterilization over adults. Energy independence of dose-rate also needs further attention aiming to determine the possible advantages of a given radiation source over another leading to improved sterile male quality.

7.2.4 Tsetse handling, transport and release

Optimize transport protocols for tsetse pupae and adults

Ten years of long-distance shipments have indicated that the quality protocol described by Pagabeleguem et al. (2015) works very well. As flies for the Senegal project were transported as chilled pupae and the main constraint was to maintain the pupae in the right temperature range and to ensure a fast delivery to avoid fly emergence during transport (although the project was totally dependent on airlines for this last point). NIRPSS development offers the opportunity to review the transport protocol as pupae are no longer chilled, improving considerably the sterile male quality.

Whether pupae are transported under chilled conditions or not, they are exposed almost permanently to vibrations and/or shocks. This topic should be carefully studied, and technical solutions developed to limit as far as possible vibration/shocks experienced by pupae during transport.

The protocols that have been developed previously for the transport of adult flies from the insectary to the field release sites are outdated and needs to be reviewed. Studies should be performed on this specific topic to identify the best practices procedures and guidelines should be developed for ground and aerial flies release with or without an automatic machine.

Aerial release systems

Aerial release in SIT programmes often involves the use of chilled sterile insects, which can improve dispersal, survival and competitiveness of sterile males. Currently available means of aerially releasing chilled tsetse are however limited.

A first design was proposed by the Mubarqui Company, and the system was based on the use of vibrating conveyors (Mubarqui et al. 2014). The machine was controlled through Bluetooth by a tablet with an Android Operating System including a completely automatic guidance and navigation system (MaxNav software). The tablet was also connected to an online relational database facilitating the preparation of flight schedules and automatic storage of flight reports. The recapture rates of the sterile males released with the machine and with carton release containers was similar in Senegal. The use of a gyrocopter reduced the cost of release and

allowed an accurate release pattern. This technology limited damages to insects and allowed a release rate of 10 tsetse flies/km^2.

A new prototype of an automated chilled adult release system (Bruno Spreader Innovation, (BSI™)) for tsetse flies was tested for its accuracy (in counting) and release rate consistency under laboratory conditions (Mirieri et al. 2020). Its impact on the quality of the released sterile males was evaluated on performance indicators, including flight propensity, mating competitiveness, premating and mating duration, insemination rate of mated females and survival of male flies. The BSI release system accurately counted and homogeneously released flies at the lowest motor speed set (0.6 rpm), at a consistent rate of 60±9.58 males/min. Also, the release process, chilling (6 ± 1°C) and passing of flies through the machine) had no significant negative impact on the flight propensity, mating competitiveness, premating and mating durations and the insemination rates. Only the survival of flies was reduced irrespective if the flies were fed or starved. The results of this study showed that the BSI™ release system is promising for future use in tsetse SIT programmes. However, the negative impact of the release process on survival of flies needs to be addressed and the results of the laboratory study needs to be confirmed under operational field conditions in Africa. This machine is currently being tested in the tsetse eradication programme in Senegal.

Similar systems have been developed to release sterile male mosquitoes in the field (Bouyer et al. 2020; Marina et al. 2022) and might be tested with tsetse flies should releases with drones ever be considered in tsetse fly SIT programmes.

7.2.5 Colonization and domestication of tsetse strains and species

There are 31 species and subspecies of *Glossina* of which about 13 species have been domesticated: *G. m. morsitans, G. m. submorsitans, G. m. centralis*, *Glossina swynnertoni*, *G. austeni, G. pallidipes, G. tachinoides, G. p. gambiensis, G. p. palpalis, G. f. fuscipes*, *Glossina fuscipes quanzensis, Glossina medicorum*, and *G. brevipalpis*. Some of these original established tsetse colonies have been maintained to this day, however, some colonies have been lost such as the *G. tachinoides* colony. This has consequences for scientific research and vector control activities.

The domestication of new tsetse strains and species

Since 2006, the Governments of Burkina Faso, Mali and Ghana have been involved in the PATTEC campaign aimed at eradicating a tsetse population from the common areas. The tsetse population in the target areas are a mixture of *G. p. gambiensis*, *G. m. submorsitans* and *G. tachinoides* and the governments have opted for an eradication strategy because a sustainable tsetse-free zone can be created. For most efficiency, the SIT programme should include the release of sterile males of all three species. The tsetse mass-rearing facility at IBD has a large colony of *G. p. gambiensis*, a small colony of *G. m. submorsitans* but the *G. tachinoides* colony needs to be established from females collected in the field to adapt and optimize its mass production.

Investigations into the effect of long-term domestication on tsetse flies

The impact of long domestication of tsetse flies on their biology, microbiota and vectorial capacity is an important aspect for SIT implementation. In an ongoing study, the results demonstrate that domestication resulted in a major change in the prevalence of tsetse symbionts such as *Sodalis*. Whereas its prevalence in wild flies is extremely low, after 2-3 generations in the mass-rearing facility the *Sodalis* prevalence reached 100%.

The domestication of *G. p. gambiensis* for more than 50 years has not affected the genetic structure and the genetic diversity of the colony. The changes in the gut microbiota, the mating competitiveness, and the vectorial capacity in response to the domestication process is ongoing.

7.2.6 Mating behaviour, compatibility and competitive studies

Rearing of tsetse flies, and especially colonizing a new population, is time consuming, labour intensive, and challenging. Using flies from an existing laboratory-adapted colony to develop new colonies or using flies from a regional mass-rearing facility for release in another country would be a more cost-effective approach. Although pooling of regional resources will significantly reduce the financial resources required to implement a control programme, the mating compatibility and competitiveness between the released sterile flies and the target wild strain is of prime importance in such a regional or international approach. Indeed, the quality of the released sterile males remains one of the most crucial prerequisites for success of an AW-IPM programme that has an SIT component, as flies of low quality (i.e. low survival rate and/or deformed wings) can't compete with wild males in the field to mate with females and induce sterility in the native population (Parker et al. 2021).

7.2.7 Symbionts to improve mass-rearing and sterile male performance

Pathogen, symbiont, parasite and host interactions

Due to the nature of tsetse biology (low productivity and therefore not easy to scale the colony up or initiate colonies from wild materials), it is important to keep tsetse colonies stable. To this end, providing the optimal conditions for tsetse production through avoiding pathogenic infections (fungi, bacteria and virus infections) and enhancing the useful microbiota important for tsetse productivities (i.e. *Wigglesworthia*) is required. The interactions between tsetse fly symbionts and pathogens might affect the productivity of tsetse fly colonies, fly mortality, and other performance indicators. Although several studies were carried out to explore these interactions, the results remain inconclusive and indicate that these interactions are complex as evidenced from field collected samples (Dieng, Dera, et al. 2022; Dieng, Dera, et al. 2022) or from laboratory-reared flies (Demirbas-Uzel et al. 2021). Due to the complexity of this topic, more investigations are required to uncover the molecular dialogue of these interactions to identify the best practices to rear tsetse flies. Moreover, the interaction of tsetse pathogens (viruses), symbionts (*Wigglesworthia*, *Sodalis*, *Wolbachia* and *Spiroplasma*) in the tsetse fly host might be affected with the irradiation treatment either in air or under hypoxia conditions that might be used in SIT programme.

Enhancing paratransgenesis for developing trypanosome refractory tsetse trains

The implementation of the SIT in areas with HAT would require additional measures to eliminate the potential risk of the sterile males contributing to disease transmission. Paratransgenesis offers the potential to develop tsetse flies that are refractory to trypanosome infection by modifying their associated bacteria such as *Sodalis*. *Sodalis* has been genetically engineered to express and release significant amounts of functional anti-trypanosome nanobodies in different tissues of the tsetse fly (De Vooght et al. 2012; De Vooght et al. 2022). The feasibility of combining the paratransgenesis approach with SIT by analysing the impact of ionizing radiation on the copy number of *Sodalis* and the vectorial capacity of sterilized tsetse males was recently investigated. The results showed that irradiating tsetse pupae on day 22 post-larviposition did not significantly affect *Sodalis* density and therefore this combination is feasible (Demirbas-Uzel et al. 2018). Moreover, report on the interaction between *Sodalis* and trypanosomes indicated that the presence of *Sodalis* might increase tsetse flies' refractoriness to trypanosome infection (Dieng, Dera, et al. 2022; Dieng, Dera, et al. 2022). Moreover, long domestication of tsetse flies might select for refractoriness to trypanosome infection (Marc Vreysen, personal communication) to ultimately establish a refractory strain (Maudlin & Welburn 1992). The observation of some tsetse strains showing a higher level of refractoriness to trypanosome infection, open the door to explore the molecular mechanism governing this phenomenon and to identify the key factor(s) which play a major role in this process. Transcriptomic and proteomic analysis of the trypanosome susceptible and refractory tsetse stain will be required to explore this mechanism.

7.2.8 Standard quality control protocols

Most economically important tsetse species have been colonized, domesticated, and are maintained in the IPCL and some other facilities in the world. Most of these colonies are maintained using artificial *in vitro* feeding systems. However, a comprehensive quality control protocol to assess the quality of the produced flies is not available. This is however a key tool to support programme managers in the decision-making process in field projects. Therefore, it will be useful to develop “Standard Quality Control Protocols” to evaluate the performance of various colonies in a comparative way under mass-rearing and field conditions.

The ability of sterile males to disperse and compete with wild males for mating with wild females is vital for the success of the SIT. The accurate estimation of the number of sterile males that are able to fly in a batch of pupae delivered to a release facility, would assist with the planning of field releases. Thus, an appropriate tsetse flight quality control test is required. Flight quality control protocols were developed and validated for *G. p. gambiensis*, but these existing protocols need to be validated for other relevant tsetse species such as *G. brevipalpis, G. pallidipes, G. fuscipes*. Furthermore, the relationship between the flight quality control tests and other QC tests such as the mating competitiveness test in semi-field cages need to be investigated and defined.

7.2.9 Mapping tsetse distribution, suitable vegetation and ecological niche

The promotion of the development of tsetse distribution maps can be facilitated by the FAO/IAEA. This support includes the provision of equipment/hardware (e.g. GPS, PC/laptops/workstations), training on free Open-Source GIS software and relational database management systems (DBMS). Further support, in collaboration with FAO, can be given to

countries with the development of national atlases of tsetse flies. This should include the enhancement and updating of existing atlases and development of new atlases in the countries that have not been developed them yet. To this end, expert missions to countries for training national authorities/counterparts should be supported, and priority should be given to experts from the recently established pool in the Africa region. Efforts should also be made to further expand this pool of experts. In addition to expert missions, training courses on the development of national atlases should also be organized.

With respect to mapping of suitable vegetation for tsetse flies and their ecological niches, the opportunities provided by the atlases in terms of comprehensive training data should be exploited. There is also a need to broaden the limited pool of experts with the necessary skills to develop tsetse vegetation maps and ecological niche models. More attention should also be paid to the validation of already developed models, with a view to assessing and further improving their quality. Finally, work and training should go into prompting the use of the model outputs to support decision making (e.g. to target entomological data collection/surveillance and to target control operation, including SIT).

7.2.10 Enhancing Molecular and genetic tools

Extended population genetic analyses

Assessments of the gene flow between adjacent field populations provides important knowledge that can facilitate the development of optimal strategies to implement the SIT. In that respect, more population genetics studies should be carried out using adequate numbers of field samples from different locations. For those tsetse species where efficient, specific, and polymorphic microsatellites are available, arrangements to collect the needed tsetse samples from the targeted field areas should be organized with the relevant counterparts. For the species where microsatellites are not available, e.g. *G. austeni* the development and evaluation of new microsatellites should be supported. More local and regional training courses should be organized to enhance the capacity of FAO/IAEA MSs on this topic.

Molecular identification tools for tsetse fly species and subspecies

The reduced availability of experts on tsetse taxonomy presents a challenge for accurate tsetse identification. However, the availability of molecular tools that can be used to identify tsetse species/subspecies is a useful development. The availability of PCR identification tools (FAO/IAEA 2018) used in combination with classical morphological identification (Leak, Ejigu & Vreysen 1998) can be further developed. However, from various discussions with many counterparts, it seems that this approach requires more attention and more dissemination through training to explain this approach. In addition, more investigation to analyse and validate the use of the high-resolution melting technique to accurately identify more tsetse species and subspecies (including non-vector species) is required.

7.2.11 Studies on the biology and ecology of the targeted tsetse species

Mark-release-recapture studies have traditionally been used to study the ecology and biology of tsetse flies. The data that emanate from these studies are however limited to population densities, spatial occupation of the habitat, preferred vegetation sites etc. These studies do not provide

information on the actual behaviour of the insects, be it wild or sterile insects. One of the essential prerequisites for the successful implementation of the SIT, is that the factory-produced sterile male insects must be competitive with their wild counterparts and must exhibit a similar behaviour in a natural environment. Gathering information on the behaviour of wild and sterile insects in the natural habitat and assessing whether there are similarities or discrepancies between the two groups of insects is important for action programmes. The ability to track sterile tsetse males can lead to better understanding of aspects such as survival, dispersal, dispersion and mobility at the individual level. However, there are methodological constraints to identify and track individuals of most insect species within complex ecosystems. Harmonic radars using micro transmitters fitted onto individual tsetse flies might offer opportunities to overcome this constraint.

7.2.12 Mathematical models to estimate the probability of tsetse fly eradication

International guidelines have been developed by WHO for the eradication of HAT, and by WOAH for AAT (Terrestrial Animal Health Code, on the following website https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/). There are however, no international guidelines for demonstrating tsetse absence or eradication. This is a limitation for AW-IPM projects and also for the PCP for AAT, and therefore it should be a priority area for IAEA and FAO. This topic is also relevant for WHO in the context of the eradication of HAT (FAO & WHO 2022).

Mathematical models exist that can be tested, improved and used to this purpose, and work is ongoing with the COMBAT project and in the framework of the progressive control pathway (PCP). However, further work is needed to test these theoretical mathematical models on a variety of field datasets to assess their performance and suitability for possible future guidelines. There is also a need to promote consultation with experts, with a view towards deriving general guidelines from specific case studies.

8. PRIORITIES FOR CAPACITY BUILDING AND OTHER NEEDS

8.1 IPCL personnel for tsetse research should be increased

The tsetse laboratory of the IPCL is an important independent laboratory for new scientific developments and the focal point of international groups developing tsetse control strategies. Current personnel are not sufficient to address the future needs of tsetse SIT and should therefore be expanded at the professional but also the technical personnel level.

8.2 Networking and sharing expertise among Member States

The IAEA has addressed this need through Coordinated Research Projects and regional TC projects. Additional efforts should be made, and financial support given for the organization of technical panels and workshops on high priority topics.

8.3 Training of Member State staff

The IAEA has already supported capacity building in some Member States. For those States that will assess the feasibility of an AW-IPM approach with an SIT component, the IAEA may facilitate onsite training courses or develop, in alliance with other Agencies of the UN or the Member States parties themselves, other models for the education of permanent staff on new technologies in the field of T&T control. A global, standardized training package covering all aspects of the SIT for mosquitoes has been initiated and needs further development as new technological advancements become available.

8.4 Research infrastructure needs to be improved

The IAEA could support better research infrastructure and conditions in the Member States by supporting the improvement of tsetse laboratories through national or regional projects.

8.5 Peaceful Uses Initiative

In addition to on-going IAEA-TC projects, the IAEA Peaceful Uses Initiative (PUI) and other sources should be explored even further for potential extra-budgetary support in support of IPCL activities in the peaceful uses of nuclear technology. In the past few years, the Insect Pest Control subprogramme received substantial support through the PUI and other mechanisms, thanks to the pledge of France and USA.

9. PARTNERSHIPS AND COLLABORATIONS

9.1 UN-Agencies and the Programme Against African Trypanosomosis (PAAT)

Alongside IAEA that focuses on tsetse/pest management with an SIT component, the main UN-Agencies mandated to work on African trypanosomosis are FAO and WHO, the former focusing on AAT and the related food security and poverty impacts, and the latter focusing on HAT and related public health impacts. In 1997 the 29th FAO Conference, through the Resolution 5/1997, established the Programme Against African Trypanosomosis (PAAT) as an FAO Statutory Body (Hursey 2001; FAO 2016). In addition to being an FAO programme, PAAT is also a platform for interagency collaboration, and alongside FAO as the focal point, the PAAT Secretariat includes WHO, IAEA and the African Union-Interafrican bureau for animal resources (AU-IBAR). Areas of UN collaboration and joint work within PAAT include policy development, training, capacity development for endemic countries, data management and risk mapping for strategic and operational decision-making, development of guidelines, socio-economic burden of the disease and One-health. PAAT also provides a platform for collaboration and coordination between UN agencies and AU.

In the area of development of harmonized policies, the progressive control pathway for AAT was developed by FAO within PAAT jointly with IAEA and AU, and with scientific advice from CIRAD (Diall et al. 2017) (Figure 11). The PCP for AAT is consistent and harmonized with the AW-IPM, and in particular with the phased-conditional approach (PCA) for SIT application. PAAT also provides a platform for supporting harmonization of vector control policies with the

WHO strategies for HAT elimination. Vector control is a tool that, in combination with medical interventions, contributes to the elimination of HAT (WHO 2013) (Figure 12), and expert meetings are organized within PAAT to discuss the opportunities and challenges of the different approaches, including all relevant international organizations (i.e. WHO, FAO, IAEA and AU) (FAO & WHO 2022).

9.2 African Union

The PATTEC is a structure of the Commission of the African Union with the objective to develop coordinated strategies to create sustainable tsetse and trypanosomosis free areas. PATTEC was established on 12 July 2000, in Lomé (Togo), by the 36th ordinary session of the Assembly of Heads of State and Government of the Organization of African Unity (OAU) - now African Union Kabayo 2002)(Kabayo 2002). Yearly resolutions from the IAEA General Conference have been approved in support of the PATTEC initiative, although the lack of a PATTEC coordinator in the past few years has constrained the ability of the IAEA to support PATTEC.

IAEA is also a partner of the AU-IBAR, and in particular it is a member of the Executive Committee of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), a statutory council of the African Union with the secretariat at AU-IBAR. IAEA regularly attends the General Conferences of ISCTRC and the related meetings of the ISCTRC Executive Committee.

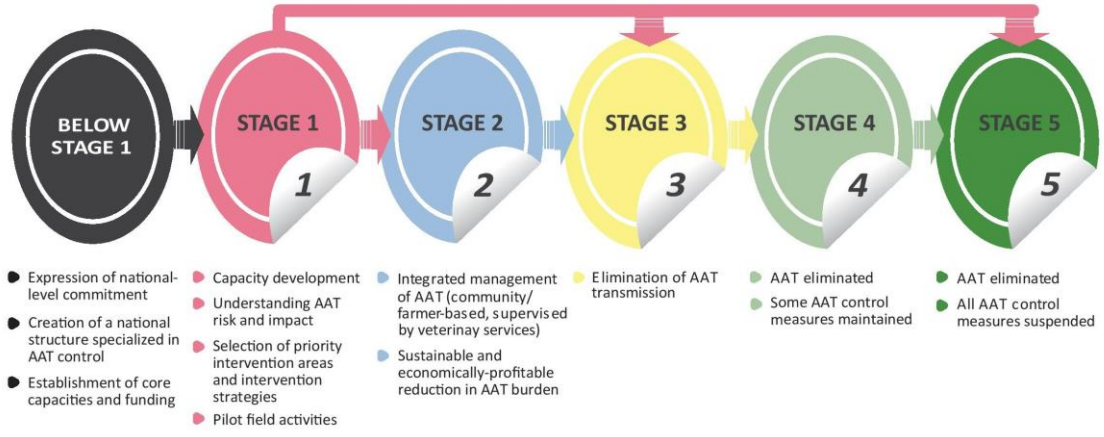

Figure 11: The progressive control pathway for African animal trypanosomosis (Source: Diall et al., 2017).

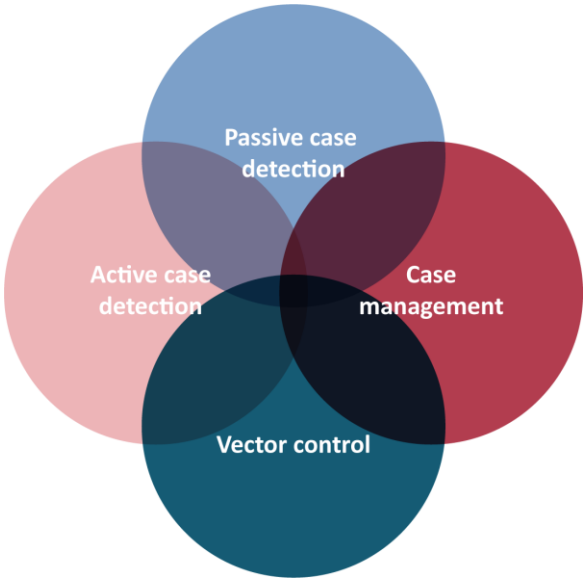

Figure 12: Pillars of the strategy for human African trypanosomiasis elimination (Source: FAO and WHO, 2022).

10. GENERAL RECOMMENDATIONS TO THE FAO/IAEA

- We recommend that the Agency should continue investing in supporting the control of tsetse and trypanosomosis (T&T) through increased funding of the development of the sterile insect technique (SIT) and other environment-friendly methods. Pilot population suppression projects and operational programmes should be supported and applied following the Phased Conditional approach (PCA) through an Area-Wide Integrated Pest Management (AW-IPM) strategy.

- Control of T&T requires significant mobilization of resources. We therefore recommend that the Agency, in cooperation with the Food and Agriculture Organization of the United Nations (FAO), the African Union (AU), Member States, the World Health Organization (WHO) and other international agencies, continues to seek strategic partnerships, advocacy and mobilization of funds and supports the development of bankable documents to support AW-IPM approaches with an SIT component for tsetse management.

- We recommend the Agency to further strengthen the UN-inter agency collaboration and coordination in the control of tsetse flies and African trypanosomosis, within the framework of the Programme against African Trypanosomosis (PAAT) and promote harmonization of strategies and policies at the FAO, and WHO level (e.g. the PCP for AAT).

- We recommend the Agency to continue engaging with AU to ensure political commitment in the fight against tsetse and trypanosomosis at the continental level.

- The Agency should continue to facilitate and support/assist/encourage neighbouring Member States to develop technical collaboration agreements, in order to implement joint vector control actions.

- The Agency has a unique role unmatched by any other institution in developing methods, evaluation and standards for producing, releasing and monitoring insects used in SIT. We therefore recommend the Agency to continue and expand these Research and Development and technology transfer activities in response to requests by Member States.

- Recent progress of the IPCL includes the development of tools and protocols for colony management, sex-separation, sterilization, handling, release methods and quality control. We therefore recommend that the IPCL continues these activities for their further improvement and dissemination to Member States; to accomplish this and maintain the momentum will require secured long-term support in human and associated resources.

- Nuclear technology is an essential tool for the development and application of SIT. However, significant challenges still exist in respect to the shipment of gamma cells to end-users or the efficient and robust use of X-rays. We therefore recommend the Agency to investigate efficient, environment-friendly and economically affordable irradiation-induced sterility methods for SIT and blood meal sterilization.

- Enhance capacities in African countries for data management and mapping to guide tsetse control and eradication efforts.

- We recommend the Agency to support improving knowledge on the socio-economic burden of tsetse flies and trypanosomosis in Africa for advocacy, resource mobilization and strategic decision-making.

11. SPECIFIC RECOMMENDATIONS TO THE FAO/IAEA

11.1 Research and development aspects

To ensure the successful implementation of pilot projects and AW-IPM operational campaigns with an SIT component to control tsetse, applied research is required so that the latest technologies can be incorporated and thus improve the cost-effectiveness of the programme, we therefore recommend:

- The Agency has a unique role unmatched by any other institution to be the custodian of tsetse colonies and genetic material. The colonization and domestication process of tsetse is very challenging and requires time and technical expertise. Currently, 9 species are colonized, and the IPCL maintains 7 of them. In the past twenty years 4 tsetse (i.e. *G. swynnertoni, G. tachinoides, G. f. quanzensis and G. medicorum*) that were colonized were lost and need to be recolonized.
- The Agency should continue and expand its effort as a "custodian institution" of seed colonies as a support to SIT projects and ecological studies.
- The Agency should enhance its efforts to establish a network and play a central role as a tsetse fly genetic material (DNA and RNA) repository.
- The Agency should continue its efforts to secure extra budgetary resources to support R&D activities on the development and improvement of the SIT package at the IPCL. Therefore, it is recommended to organize donor meetings to mobilize additional funds.
- The Agency should continue its effort to improve tsetse mass-rearing, sterilization, shipping and release procedures to ensure the quality of the sterile flies and reducing cost.
- The Agency is strongly encouraged to improve the rearing protocol to ensure a more stable and predictable tsetse production including studies on fluctuating environmental conditions.
- The Agency should facilitate collaborations and promote discussion between research groups on sourcing sustainable trapping and target materials.
- The Agency should support research aimed for enhancing tsetse refractoriness to trypanosome infection to reduce potential risk of increasing trypanosome transmission with sterile tsetse male releases.
- The Agency should enhance selecting target areas accounting for the distribution and genetic relationship between the target and surrounding tsetse populations (area-wide strategy), accounting for the regional political and security context.

11.2 Operational Programmes

Current SIT developments allow the implementation of pilot projects and operational programmes. However, for successful implementation, the IAEA should assess the technical, economic, social, and ecological feasibility and requirements, and should provide expert advice to the MSs. These pilot projects and operational programmes will serve to scale up and further develop the AW-IPM strategy, including the SIT to control tsetse, and will contribute to analyse the feasibility of its use at the national and regional level. We therefore recommend:

- Enhance capacity building in IAEA MSs on tsetse management and stimulate exchanges between field projects through south-south collaboration.
- Develop and facilitate a "training programme", in collaboration with FAO, training centres in the region, and other international organizations to support building and enhancing technical expertise at the regional level on all key aspects of SIT technology as well as new technological advancements. The IAEA should also consider supporting the revival of tsetse training academy/school dedicated to train tsetse personnel. Investment in this training ensures that key competencies continue to be streamlined to staff in Member States hence minimizing/ eliminating the challenge of knowledge gaps.
- Provide technical assistance on following the phased conditional approach to reduce the risk and minimize the cost of field projects with special need on baseline data collection including mating compatibility and competitiveness studies.
- Provide technical assistance to design mass-rearing facilities which includes the site selection, radiation sources, and key equipment to support SIT field projects.
- Develop a follow-up TC regional project with sufficient resources for upcoming cycles to continue supporting capacity building in the region.
- Support R&D activities that focus on tsetse species and geographical areas where SIT has a competitive advantage supported with population genetics studies.
- Provide technical assistance to ensure a proper monitoring of the efficiency of control activities and timely reporting to the management team to apply adaptive management.
- Encourage collaboration between implementing teams and national research centres in order to receive inputs from operational research.
- Encourage and facilitate socio-economic studies to measure the cost-benefit of AW-IPM against tsetse with an SIT component.

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13. ANNEXES

Meeting agenda

Technical Cooperation Project RAF5087

Enhancing Regional Capacity for the Implementation of the Sterile Insect Technique as a Component for Area-Wide Tsetse and Trypanosomosis Management

Updating the Thematic Plan for establishing tsetse-free zones through area-wide integrated tsetse management programmes that includes the sterile insect technique

29 May to 2 June 2023, VIC, Vienna, Austria

Vienna International Centre (IAEA Headquarters), Room M0E03 – M Building

Monday 29 May 2023

08:00 – 09:00 Identification and registration at VIC Gate 1

09:00 – 09:20 Welcome and opening of the meeting/ **Shaukat Abdulrazak, Director-TCAF and**

Dongxin Feng Director-NAFA

09:20 – 09:30 Administrative details, outline of the agenda, introduction of the participants and objectives of the meeting. **Ozlem Esengin (TCAF), Maylen Gomez and Marc Vreysen (NAFA)**

SESSION I: Presentations by Experts

09:30 – 10:00 **Imna Malele (United Republic of Tanzania) –** Journey towards Establishment of Tsetse Free Zones Through Area-wide Tsetse Control Interventions Involving SIT in Africa: Status & Way forward.

10:00 – 10:15 COFFEE BREAK

10:15 – 10:45 **Isaiah Ndaburu Kiteto (Kenya) –** Current and past activities in Kenya relevant to tsetse SIT projects.

10:45 – 11:15 **Learnmore Nyakupinda (Zimbabwe) –** Successes of tsetse Control in Zimbabwe.

11:15 – 11:45 **Johan Esterhuizen (South Africa)** – Experiences and perspectives on tsetse fly control, from the south and the east of Africa.

11:45 – 12:15 **Assane Gueye Fall (Senegal)** – Implementation of an area-wide integrated pest management programme to rule out tsetse and Trypanosomosis in the Niayes area, Senegal.

12:15 – 13:30 LUNCH

13:30 – 14:00 **Soumaila Pagabeleguen (Burkina Faso) -** Tsetse fly control in West Africa: optimizing the use of the sterile insect technique.

14:00 – 14.30 **Moussa Sall (ISRA, BAME, Senegal) -** Overview of two socio-economic studies carried out in the context of the fight against the tsetse fly in Senegal.

14:30 – 15:00 **Geoffrey Gimonneau (CIRAD, France)** – On the forefront and in the backstage of the tsetse fly eradication project in Senegal.

15:00 – 15:30 **Weining Zhao and Giuliano Cecchi (FAO, Rome)** – FAO/IAEA collaboration in the framework of the Programme Against African Trypanosomosis (PAAT)

15:30 – 15.45 COFFEE BREAK

15:45 – 16.15 **Adly Abdalla (FAO/IAEA) -** Improvement of Colony Management in Insect Mass-rearing for SIT Applications (CRP).

16:15 – 16:45 **Chantel de Beer (FAO/IAEA) -** Research at the Insect Pest Control Laboratory in support of the tsetse Sterile Insect Technique.

16:45 – 17:30 General Discussion

18:30 – 19:30 Welcome Reception (Salon A, VIC Restaurant)

Tuesday 30 May 2023

SESSION II: Brainstorm on the structure of the Thematic Plan

Chair: Marc Vreysen

09:00 – 10:30 Discussion – setting the scene and outline of the TP.

10:30 – 11:00 COFFEE BREAK

11:00 – 12:30 Discussion – setting the scene and outline of the TP.

12:30 – 14:00 LUNCH

14:00 – 15:30 Discussion – priorities for the TP.

15:30 – 16:00 COFFEE BREAK

16:00 – 16:45 Discussion – priorities for the TP.

16:45 – 17:30 Group Presentation on priorities for the TP.

Wednesday 31 May 2023

SESSION III: Development of the Thematic Plan

Chair: Marc Vreysen

09:00 – 10:30 Individual drafting of the TP

10:30 – 11:00 COFFEE BREAK

11:00 – 12:30 Individual drafting of the TP

12:30 – 14:00 LUNCH

14:00 – 15:30 Individual drafting of the TP

15:30 – 16:00 COFFEE BREAK

16:00 – 17:30 Individual drafting of parts of the TP

16:45– 17:30 Group discussion/presentation on document status

Thursday 1 June 2023

Chair: Marc Vreysen

SESSION III: Development of the Thematic Plan (continuation)

09:00 – 10:30 Group review of the TP

10:30 – 11:00 COFFEE BREAK

11:00 – 12:30 Individual drafting of the TP

12:30 – 14:00 LUNCH

14:00 – 15:30 Individual drafting of the TP

15:30 – 16:00 COFFEE BREAK

16:00 – 17:30 Group discussion on the TP recommendations

Friday 2 June 2023

SESSION III: Development of the Thematic Plan (continuation)

Chair: Marc Vreysen

09:00 – 10:30 Finalising the TP

10:30 – 11:00 COFFEE BREAK

11:00 – 12.30 Finalising the TP

12:30 – 14:00 LUNCH

14:00 – 14:30 Presentation of the TP

14:30 – 15:00 Closing the meeting/ **Rui Cardoso Pereira (NAFA**) **and Ozlem Esengin (TCAF-IAEA)**

Table. List of Participants

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Participants of the Consultants Meeting on Thematic Plan for the Development and Application of Sterile Insect Technique for Tsetse Area-wide Integrated Pest Management Programmes (Vienna, Austria.).