THIRD RESEARCH CO-ORDINATION MEETING
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

Co-ordinated Research Project on

Mosquito Handling, Transport, Release and Male Trapping Methods

Moscamed
Juazeiro, Brazil
12-16 November 2018

Table of Contents

1. Coordinated Research Project (CRP)

1.1. Title

Mosquito Handling, Transport, Release and Male Trapping Methods

1.2. Project

2000023

1.3. Division

NAFA

1.4. Responsible Project Officer

Rafael ARGILÉS HERRERO

1.5. Alternate Project Officer:

Rui CARDOSO PEREIRA

1.6. Summary:

Among the major vectors of human diseases, mosquitoes are the most devastating ones. In addition, urbanisation, globalisation and climate change have further accelerated the spread and outbreaks of new mosquito borne diseases. In view of the problems associated with conventional mosquito control, such as insecticide resistance and adverse health effects associated with abundant use of chemicals, during a Thematic Plan Meeting held in Vienna in June 2014, experts concluded that there is an urgent need to develop new or complementary control techniques such as the Sterile Insect Technique (SIT), for major disease-transmitting mosquito species.

The Sterile Insect Technique (SIT) is an increasingly important component of area-wide integrated pest management (AW-IPM) programmes with great application potential for use against key insect vectors such as mosquitoes. With the toll of vector-borne diseases on human health and mortality increasing year by year, there have been recurring requests from Member States to develop tools and techniques for mosquito SIT to be able to apply the SIT to control mosquito vector populations (Resolution GC(52)/RES/13). The SIT has the ability to suppress, or in specific situations to eradicate existing vector populations and to prevent the establishment of new outbreaks.

Operational use of the SIT against other insect pests continues to reveal areas where new technologies could further improve efficiency and thus lead to more efficacious programmes. In addition to the mosquito SIT package which is being developed by the IPCL, the technologies must be in place for application on an operational level. Key issues to be resolved are handling, transport to the release location and actual release of sterile males without causing significant impact to their survival or post-release performance, as well as a means to monitor their performance after release. Methods for population surveillance of male mosquitoes is also important so that the releases can be scaled to the target population, and in order to enable the progress and impact of the SIT programme to be assessed.

An SIT programme at an operational scale will require several million adult male mosquitoes to be transported to release sites, likely after first being chilled for easy handling, with minimal detrimental impact on the quality and sexual capacity. Some means to transfer adult mosquitoes from emergence cages into temperature controlled containers for transport and ideally for release is required, as well as the ability to maintain a cold chain between the rearing facility, or an emergence centre, and the release site. The scope of this CRP will include all steps of handling and transportation after the irradiation of mosquito pupae, including the containers and cooling systems, and biological

requirements for chilling mosquitoes at sufficiently high densities without impacting survival or performance. Transport of chilled pupae and adults by road and by air should all be considered. The CRP will consider ground release and release from light aircraft, which may be required for certain circumstances, but the focus will be on release from small unmanned aerial vehicles. Again cooling methods will be considered, and the quality of released males is a key concern.

Alongside release methodologies, affordable and efficient trapping and other information collecting devices for field surveillance of the male population are needed prior to, during and following release to improve the evaluation of suppression programmes. Whereas the focus in the past has been on trapping females as the vectors of pathogens, male-specific surveillance will enable programmes to measure male quality, programme progress, and allow necessary adjustments to be made to operational activities. Specific techniques may be needed for surveillance of different species, or in different environmental settings, and so a variety of male-specific or male-targeted trap designs, attractants, automated surveillance tools, and the conservation of samples between trapping and laboratory analysis will all be considered. Although several good solutions are available now for validation and use in project sites, particularly for *Aedes* mosquitoes, no good options for reliably surveying and trapping *Anopheles* mosquitoes are currently available.

Although precise details of release methods will likely be fine-tuned for different species, the technology developed in this CRP should be generally applicable to all species. In addition, though the intention of the CRP is to provide technical advances to support the use of the SIT against mosquitoes, the same or very similar techniques will be of value to other genetic control programmes which rely on the release of mosquitoes.

2. Background Situation Analysis

2.1. Handling and transport to the release site

Helinksi *et al.* transported male *Anopheles arabiensis* pupae and adults from a rearing facility in downtown Khartoum to the site of an irradiation source in Soba, a journey of around 45 minutes by car, and back again, before the irradiated male adults were transported by air and road (5-7 hours travel) to a field site in Dongola. No cooling method was used beyond a moistened towel, and 50 adults were transported in a paper drinking cup covered with mesh and provided with sugar solution. Across three experiments mortality during transportation never exceeded 6% and transported males were seen to be sexually competitive and long-lived in semi-field cage trials. However, more sophisticated tools and methodologies will be required in order to scale up releases to the millions of males required for large scale suppression programmes.

Handling and transport includes all steps in production between irradiation of mosquito pupae to loading mature chilled adult males onto the release vehicle. There are two possible options for the transport of material to the release site:

a. Transport of chilled pupae to an intermediate Emergence Centre, close to the release site, where adults will be allowed to emerge and be kept and packed for release.
b. Transport of adults directly to the release site: this option is preferable as long as adults can withstand ground transportation in crowded conditions without significant mortality or loss of post-release performance.

These two options present different technical and operational challenges depending on whether *Aedes* or *Anopheles* mosquitoes are being considered. Since *Aedes* species can be sexed as pupae prior to irradiation, transport from the Mass Rearing facility will only be of males, and transport of pupae could be suitable in scenarios of shorter distances between Mass Rearing Facility and release site, or if pupae are found to be more resilient to chilling and transport than adult mosquitoes. The transport of *Anopheles* pupae should be avoided, due to the safety risk of transport of a large number of viable (female) disease vectors, and due to the complexity which would then be required of the Emergence Centre, where blood spiking and longer term storage would be required. If reliable screening method could be available to determine the sex of mosquitoes in the egg or pupae phase, they would enable significant cost and risk reduction for SIT and therefore R&D is encouraged in this front.

It is evident that the less handling and less clumping the mosquitoes are exposed to the better, to minimise damage and impact on post-release performance, and operational costs. Therefore, regardless of the release system used, insects would ideally be reared, chilled and transported in, and then released from, the same container preferably unclumped and conscious, or 'release cassette', in the quantities and design required for the release system to be used. Quantities may be very large, in the case of a continuous release system, or much smaller in the case of discrete releases. Care must be taken that neither the release mechanism, aperture, nor trailing turbulence damages the released mosquitoes. Since pupae are concentrated into a small volume for irradiation, and since handling pupae is easier from practical and damage limitation standpoints, pupae would therefore be 'dosed' into release cassettes, or into a cage from which chilled adults could readily be loaded into release cassettes.

Aedes pupae would already have been sex separated, so release cassettes would therefore contain only males, which could be allowed to emerge, mature and receive a sugar meal, then be chilled down for transport and release. In this scenario, transport of *Aedes* pupae, if desirable, would either be *en masse*, for dosing into release cassettes at the Emergence Centre, or pre-dosed, ideally into small containers which would easily be slotted into the cage for emergence and loading of the release cassette. In the case of *Anopheles* male and female adults would emerge and be held for 4 days for female elimination, for example using spiked blood, dead females would have to be removed, and then the males would be chilled down. In this latter case a larger holding cage would be needed, along with a means of efficiently blood feeding cages. The use of Phase Change Materials as a heating system for blood feeding, perhaps in the form of 'hand warmer' units which can be cracked to initiate heating, and then boiled to reset, would allow them to be reused for the following blood feeding.

Whether pupae or adults are transported, insects will need to be immobilised by chilling, to allow transport of a smaller volume in the case of adults, and in both cases to reduce the damage caused during transit by movement and associated metabolic heat generation of mobile insects at high densities. Therefore, aside from the dosing of mosquitoes into containers for transport and loading into the release system, the major consideration in terms of handling and transport of mosquitoes is the maintenance of the cold chain up until the point of release. The optimum temperature at which pupae and adults should be immobilised remains to be defined, as does the maximum time chilling can be applied before survival or performance are impacted. There are a wide range of cooling options from TEC to liquid nitrogen vapour available which may be applicable at different stages throughout the supply chain. Experience of maintaining the cold chain for transport of other species for release, for example tsetse pupae transport for releases in Senegal (Pagabeleguem et al., 2015; Seck et al., 2015) can be used in developing suitable protocols.

In more developed countries refrigerated lorries and vans are widely available and therefore sterile male mosquitoes could be shipped within the existing cold chain network, but where mosquito SIT is applied in less developed countries with less well developed infrastructure, it will be vital to develop a system whereby the integrity of the chilled supply chain is maintained until release. This issue becomes critical if the operational solution chosen comprises a central mass rearing facility to cover a wide areas, countries or regions and transport of material to local Emergence Centres.

The most practical and cost-effective options for ground transport could be "static" PCM based or alternatively "dynamic" cold box options. Compressed Carbon Dioxide (CO_2) could be used for cooling, and should improve heat transfer efficiencies within a cold box to achieve a faster heat exchange process than PCMs, hence, keeping the internal temperature within the desired pre-set temperature levels without the need for moving parts. CO_2 may prove advantageous for cooling, however, the gas should be contained in tubes which do not allow gas to freely flow through the insects which would hinder their respiration.

Research is needed into i) synchronisation of pupation and improved pupal sex separation for *Aedes*, ii) optimal conditions to transport adults over long distances without loss of quality, iii) design of transport/release cassettes, iv) the effect of chilling and crowded transport of adults mosquitoes, and an alternative method for transporting pupae if the quality loss is too great, and v) chilling conditions required to lengthen pupal stage without affecting adult quality.

2.2. Release Methodologies

2.2.1.Ground Release

Robust insects such as screwworm flies or Mediterranean fruit flies are routinely released aerially, but mosquitoes are more fragile as adults and thus easily damaged. Because of this, and the small scale of release programmes for mosquitoes conducted to date mostly in urban areas, ground releases have so far been based on the use of pick-up trucks (or quads) with field staff opening cages/pots and releasing sterile males every 100 or 200m at a fixed and approximate release rate (for example the MOSCAMED programme in Brazil). This approach can be upscaled by the incorporation of an automated release machine mounted on the truck, motor bike or even pedal bike, depending on the nature of the release area, and a release strategy similar to that applied for aerial release may be used to optimize efficiency.

However, there are disadvantages inherent to release by road vehicle or operators on foot, including the risk to the operators of being exposed to a disease endemic area, and the cost, time and expense required make the system difficult to upscale to the routine release of millions of mosquitoes. More specifically, ground release is not well suited to the AW-IPM programme as the releases of sterile males must follow the existing road system and thus are not able to homogeneously cover the area to be treated. Thus, it is recommended that a ground release method be used only when neither drone nor aircrafts are available (due to regulation, legislation, cost…) or for very small suppression trials.

In the case of suppression programmes already using UAVs (such as the Pink Bollworm Eradication Program) or aircraft on a daily basis, the ground release method should be foreseen only as a backup system to disperse the daily production in case of immobilisation of the release vehicle due to meteorological events or maintenance, so that the material is not wasted. More 'advanced' ground release methods than the opening of cups of mosquitoes are available and used in small suppression pilot trials for moths (Canada) or fruit flies (Croatia) and usually consist of small trucks or trailers equipped with a large capacity (up to 10 million flies) ground release machine (GRM). The release machine contains several containers (cylinders) usually made of stainless steel and sufficiently insulated to maintain the insects at the desired temperature and relative humidity. Similarly, to avoid compaction of insects before release, a new container with several compartments has been developed for release of tsetse (Mubarqui Company, Mexico). As weight and space are not limiting factors for ground release, active compressor chilling units are commonly used (working with additional generators or inverters). Weight and compression are critical factors in the conditions during transport and release that must be investigated for their impact on the quality of sterile male mosquitoes, especially with regards to the effects of agitation as an additional passive compression force.

Species specific cooling requirements of a ground release system will be affected t by the driving distances and times, but as neither the weight nor the energy requirements are as critical as for air delivery applications, any of the technologies described above would be applicable. Any delivery technique must take into account the time required by chilled adult mosquitoes to recover and become mobile to ensure that they are able to wake up before hitting the ground. Peltier cooling can be applied to car battery or mains power-driven cold box applications, and might be useful to reduce the weight and space requirements for a road vehicle-based release system, as well as being a low cost option. Conventional compression or mechanical refrigeration should be considered as well, as this technology is well established and many manufacturers around the world can supply large scale as well as miniature refrigeration machinery to match the design requirements.

Existing ground release methods could be adapted for mosquitoes, but further research is needed into i) the design and capacity of the release units, probably by adapting existing solutions to mosquitoes, ii) the chilling methods, which should include a warming period immediately prior to release to allow mosquitoes to recover, iii) a release mechanism which achieves flexible release rates with minimal damage, iv) release software and autopilot that records GPS location of track and release locations, adapted for ground release, v) consider methods for release over waterways, and vi) explore release from public transport, cars, trucks, bicycle or motorcycle, vi) consider ventilation on the insects to avoid condensation and anoxia, which may be monitored by using O_2 and CO_2 meters.

2.2.2.Aerial release

The drawbacks associated with ground release could largely be avoided by employing aerial release, inspired by methods used for screwworm flies, pink bollworm, fruit flies and tsetse, tailored to prevent damage to fragile adult mosquitoes.

The exact nature of the aerial release methodology will depend on the programmatic details of a given SIT release, and releases will vary considerably in scale, from very small pilot suppression trials over a few hectares, to a large scale potential eradication programme in Sudan over tens of square kilometres. The following operational details, however, will always need to be considered for any aerial release solution:

- Chilling will be needed to immobilise and delay emergence of pupae
- Height of release, which is related to recovery time of mosquitoes from chilling
- Density of mosquitoes which can be held without causing damage
- Controlled release rate will be required, and precise release rates will be determined in real time from current population density data
- Volume of mosquitoes to be transported and released will need to be determined based on programme's specifications
- Flight speed - 40 km/hr minimum with fixed wing UAVs, 15-20km/hr with rotary wing drones, 60km/hr for gyrocopter, 30km/hr for multi rotor vehicles, 180 for light aircraft. Mediterranean fruit flies are released at 230 km/hr, 1/2 million per minute, but lower rates will likely be better for mosquitoes.
- Cost of equipment – a balance between reliability and redundancy. Failure in a system can be avoided either by increasing the robustness to reduce the likelihood of its failure or by including duplication of critical elements to maintain releases in the case of failure; both have cost implications.
- Swath width – probably 100 m for *Aedes*, 250 m for *Anopheles*, though these distances could be doubled and releases conducted on alternating paths each flight. A transect study is recommended to calculate the coverage area for various release swaths.
- Measure the effect of shear force and impact with the air during release. The use of a high speed camera for recording the impact on the mosquito from the UAV release device in a wind tunnel may provide valuable insight into how the release mosquitos enter the air column post

release. Also consider optimal release altitude and wind direction/drift. How the mosquito falls from the aircraft depends on many factors including: release altitude, terrain, weather conditions (wind, temperature, precipitation), mosquito internal temperature as it exits the aircraft, local laws regulating flight, and probably many other factors. All these factors affect possible release methods.

- Effect of field sensory conditions, such as light and temperature on mosquito viability - release in the shade, at dawn, at dusk or at night
- Consider ambient conditions, such as temperature, weather parameters and time of release. this will affect optimal time of release and will change with the seasons, latitude and elevation
- Consider ventilation on the insects to avoid condensation and anoxia. May use O_2 and CO_2 meters to collect data

An estimate for release requirements of ~500 g mosquitoes released/week to cover 200 ha can be extrapolated from a need for 4,000 adults per week at typical population densities, which would comprise 9.5 litres (to be confirmed by further experiments), and require 10 km of flight with a 50 m swath, equalling 5 minutes of flight time at 50 km/hr. The release frequency will depend on the local mosquito survival and the length of the gonotrophic cycle, but will likely be twice a week. To maximise the efficiency of an aerial release programme you would aim to release as many insects as possible in one session.

Since continuous release using augers (eternal screws) are not ideal for Mediterranean fruit flies, causing too much damage, and mosquitoes are more fragile, continuous release is unlikely to be suitable for mosquitoes. Discrete, dosed releases are likely to be more suitable, though the use of vibrating conveyors should still be investigated for suitability. Discrete (pulsed) releases would allow males to be dosed into release cassettes for transport to the release site, minimising handling and also the potential for damage caused by complications resulting from large masses of adults being held chilled, immobilised and likely compacted for a period of time.

2.2.3.Aerial release using light aircraft, such as an ultra-light

Aerial releases of Medflies involves the chilling of insects in rearing cages before they are loaded 'in bulk' into release machines which fit into the aircraft, where they are kept cool during the flight. Air may need to be circulated through mosquitoes stored in these conditions to prevent condensation damaging their wings. Cooling systems currently used in fruit fly SIT programmes use conventional refrigeration systems. However when weight requirements are critical, such as in temperature-unregulated UAVs, CO_2 ice (however, a CO_2 atmosphere will kill insects so would have to be contained in cooling coils and not contact the insects) or Liquid Nitrogen vapour-based passive cooling solutions will become necessary.

It is known that aerial releases of sterile insects result in an improved homogeneity of the spatial distribution of the insects throughout the target area. In some situations, the use of light aircraft to release the insects can be a suitable option. The main reasons to select this option instead of UAV would be legal restrictions, since in those countries which have regulated the use of UAVs for civil applications, the operation conditions within urban areas are very restrictive. Light aircrafts can cover large areas in one single flight, and service large scale programs, where the use of light aircrafts with a releasing capacity of tens of millions of mosquitoes per flight could significantly reduce the logistics and become a better, more economic option per unit cost of released insects. However, two main considerations need to be taken into account when selecting this option, namely cost (the operation cost per flying hour of a light aircraft varies between 400 and 800 US$, depending on the selected type of aircraft) and flight height (according to the rules of Civil Aviation authorities, the flight height over populated areas can't be less than 300 m).

The large quantities of mosquitoes to be released from light aircraft recommend the development of continuous release devices for bulk chilled adults as opposed to the discrete release devices that may be better suited for UAV. Due to the fragile nature of mosquitoes, the technology of vibrating trays seems to be the most suitable to limit mechanical damage to the adults. More research is needed to assess the resistance of adult mosquitoes to mechanical injuries caused by the current release devices developed for fruit flies and tsetse flies and to evaluate other building materials, like nylon, with low friction coefficient, or softer more forgiving surface materials. Variable dose release methods are already in use in current operational fruit fly programmes. Since the spatial distribution of the mosquitoes in the field is not homogeneous, similar systems can be adapted to mosquitoes to allow release of variable doses, either as a predefined dose for each release polygon, as currently being used in the Moscamed programme in Central America, or as a differential release rate varying in real time during the flight as used in the medfly SIT project in Spain. The different chilling options proposed for the UAV can also be valid for light aircrafts, with the major difference that there is no restriction on weight, volume or energy consumption.

2.2.4.Aerial release using unmanned aerial vehicles (UAVs)

Since mosquitoes are very light, a day's release would probably weigh around 500 g, and so it should be possible to minimise the weight of insects, release device and cooling system to be carried such that unmanned aerial vehicles (UAVs) could be used instead of aircraft. The proposed use of phase change materials may provide a passive cooling system. Once the optimal temperature to immobilise adult mosquitoes with minimal damage or loss of quality has been determined, a chamber containing the mosquitoes could be chilled to this temperature, insulated and loaded onto a UAV from which insects could be released. For aircraft delivery the use of compression or mechanical refrigeration may be suitable, using conventional electrically driven refrigeration, which is a well-established technology with manufacturers around the world who can supply a range of scales of solution.

Aerial release using UAVs is an attractive option for mosquito release on any scale, from initial pilot suppression trials of a few tens of hectares, such as that proposed in Pointe des Lascars in Mauritius, up to operational scale suppression or eradication programmes, such as the Sudan programme. Aerial release offers an efficient means to cover an area with sterile males with minimal labour and with technology available to precisely control the release rate to correspond to near real time population surveillance data. There are two possibilities available, release from light aircraft, as currently applied to existing large-scale insect release programmes, and the use of unmanned aerial vehicles (UAVs). This latter approach is likely to be more appropriate for mosquito SIT releases, given the relatively small size of releases envisaged at present, and the relatively small payload of insect material compared to fruit fly or moth SIT. The imperative to release as many mosquitoes in one flying session is less critical for releases from UAVs, which anyway have lower possible payloads and lower operational costs.

Both the biological and technical details of a release programme based on UAVs and the legal restrictions surrounding the nature of vehicles in a target site must be considered, as this provides the framework within which any programme would need to operate. It is worth noting that there is precedence in the case of emergency situations, such as wildfires or disease epidemics, for the relevant authorities granting exemptions for relief programmes to operate outside the legal restrictions. However, since mosquito SIT release programmes are designed to be conducted over long periods of time, often over urban areas, they should be designed to fit the legal framework. Little or no regulations exist in South America, Asia or Africa regarding the use of UAVs, and indeed the regulations in Europe and North America are very recent. All these regulations are trying to be harmonized thanks to the work of Joint Authorities for Rulemaking on Unmanned Systems (JARUS) which includes national aviation authorities from all over the world. In Europe EASA (European Aviation Safety Agency) is working to harmonize national regulations, and it is likely that in the next 6-7 years international guidance will be in place. In the meantime, an important number of European countries have already published a specific UAV regulation (for example, Italy, France, UK, Spain, etc.). Under these regulations, flying long distances (called BVLOS operations) are very restricted and are only allowed, in some countries, to very light UAVs (usually less than 2Kg of total weight). Regarding the pilot qualification, most European countries have similar requirements for this: theoretical knowledge imparted by an ATO (Authorized Training Organization by EASA), practical knowledge and medical certificate. Although there is no homologation of UAV pilots among countries yet.

Considering the operational limits and regulatory restrictions placed upon the flight of different UAV types in different settings, different forms of UAV will be most suitable, and may include fixed-wing or multi-rotor types from different weight classes. A prototyping phase, with combinations of UAV, cooling system and release mechanisms being tested in a European flight testing field, could indicate the suitability of different platforms for different settings. A pilot programme phase could then be conducted in the context of an operational SIT programme, probably using contracted services from a professional UAV operator. Specific approaches could then be fine-tuned, and requirements defined for buying a UAV or contracting a service for a large campaign.

Although the current regulation in Europe and USA is very restrictive for flights in urban areas and for flying long distances, the regulation is still evolving, and it will change in the next years. Then, and due to the great interest of using UAVs for SIT programmes with mosquitoes, it is recommended to start gaining flying experience with some pilot programmes and validate the usefulness of the technology as soon as possible. There are designated UAV test areas in several countries in Europe (ex. Spain, France.) where permission to fly long distances may be relatively easy. It is recommended to start testing the UAV technology in these test locations, although these may not be good mosquito release areas. It may also be possible to receive legal permission to release sterile mosquitoes in generally infested areas in the US, based on positive experience with pink bollworm release from UAV. This will be considered for proof of concept. On the other hand, it is also interesting start working on the operational aspects of the application, and the design of a UAV system that could fulfil not only the application requirements, but also the legal restrictions.

2.2.5.Release devices available for application with UAVs

At present there are several automated solutions for sterile insect release being used in different SIT programmes around the world. Though none is specifically designed for the release of mosquitoes, these may be a starting point for designing customized solutions for the release of mosquitoes. The volumes are small in the case of mosquitoes. A good example is the Mubarqui Smart Release Machine, which is currently applied for aerial release of fruit flies in Mexico and Guatemala. A refrigerated and insulated stainless steel box is used to transport flies from the insect packing centre, where sterile adults are collected daily for release, through ground and air transport to the release area, or polygon. Transport and release containers are equipped with thermometers and hygrometers to monitor the internal conditions and ensure insect quality, as well as vibrating units to circulate air. Release is controlled by a control unit, a device that receives instructions from the ground computer and converts them into actions, such as opening and closing of gates, starting and stopping the release mechanism, vibrating feed intensity, and modulating micro-vibration to maintain precision of release rates whilst minimising damage to insects. This unit is used in conjunction with the MACXNAV navigation system. From field data (trap catches) and GIS terrain information it is possible to develop a density map to establish appropriate release rates or release points of pre-dosed volumes of mosquitoes (continuous or discrete release methods, respectively) on the ground (this is usually performed in the central ground office). This way of working guarantees an optimal and homogeneous distribution of released insects on the ground.

Release devices currently in use for automatic air release of fruit flies are mainly based on three types of devices: endless screws, belt conveyors and linear vibrating conveyors. It is necessary to study the type of impact that such solutions may have on mosquitoes to assess the degree of mechanical injuries and impact on the quality of released mosquitoes. However, due to the smaller volumes of release in the case of mosquitoes compared to fruit flies, it may be feasible to develop solutions based on this type of technology on a smaller scale, and specifically to fit the particular characteristics of the species. In any case several questions need to be resolved, including whether fragile mosquitoes can resist mechanical injuries caused by an auger, conveyor belt or vibrating tray, and the highest column of mosquitoes that can be supported within the holding container of the release machine with acceptable levels of damage or mortality. A viable alternative that ensures minimal damage to insects is the use of discrete release of pre-dosed volumes of mosquitoes. It is necessary to determine the most appropriate dose volume, which will depend on the specific release scenario.

Pre-dosed mechanisms are particularly suitable for small and medium size UAVs because of the simplicity and suitability for small volumes and weights. It is expected that small release areas (100 - 300 ha) and medium size areas (2,000 -2,500 ha) be the most common programme scenarios, feasibly covered by UAV release. The selection of the type of UAV to be used, determined based on project design, will determine the maximum size and weight of the release system. In any case, special attention must be taken in the use of materials and manufacturing techniques to reduce weight, optimizing the dimensions and weight of all the components, including actuators and electronics. Microelectronics involved in the release system should be developed specifically in order to ensure the highest possible level of integration (reducing extra weight) and reliability and lowest possible power consumption. As in most cases the aircraft would be driven by electric motors, the battery capacity is limited and any power consumption results in a reduction of the time of flight, so this is a critical point. Hence the use of small and low power consumption motors, sensors and actuators is highly recommended. For example, micro-servos may be appropriate, integrating in just 9 g the electric motor and the gearbox, and providing enough power at low energy consumptions for this size of releasing mechanisms. 3D CAD (Computer Aided Design) and Rapid Prototyping techniques are well suited for the design of small release systems for these kinds of UAVs, due to the immediacy and economy in the generation of solutions.

The cooling requirements will be entirely dependent on the flight duration and in the case of drones the weight as well as the power requirements required to maintain the temperature limits could be the limiting factor to achieve the target payloads. For payloads of around 50 g of mosquitoes, flight times will be short and additional cooling systems may not be required. In intermediate release volumes it will be necessary to use some kind of on-board cooling system, probably passive systems based on Phase Change Materials (PCM). In these cases, one possibility is somehow to incorporate these materials in the release mechanism itself, probably in the interchangeable cartridges. A system to provide a quick method for loading the mosquitoes onto the UAV is needed, to help to maintain the cold chain among other reasons. This restriction suggests release systems based on removable "cartridges", to ensure a quick insertion and replacement in the aircraft, while reducing manipulation of insects. Of course, the main driving factor for the selection of any type of aerial delivery cooling option would be the weight of the cooling system, having a direct impact on how large a payload can be delivered. Hence, whichever design is selected it may require suitable insulation to minimise heat gain during flight.

A holding/release cassette must be developed and validated to efficiently contain chilled mosquitoes without impacting survival or performance after release, and paired with either a continuous or discrete release mechanism, taking into consideration the metabolic heat produced by chilled mosquitoes and the need for air circulation.

2.2.6.Remaining open questions and R&D related to aerial releases

- *In-situ documentation:* Regardless of the aircraft type, a fully documented and traceable record of each release is necessary. There are no technical barriers of high speed 2D or 3D video recording of the release aperture and machine vision based real-time evaluation of the ejected quantity and quality of SIT releases. Beyond providing positive proof, the immediate availability of quality control information allows timely remedial actions, potentially significantly increasing the chance of success for the SIT program.
- *Aerodynamically stable camera system:* The functional system requires the development of aerodynamically stable camera system, the wireless data transmission protocol, and the machine vision-based data analysis.
- *Population density data:* Population suppression depends on data that reflects population density. Releases must be delivered to the highest need areas. This is a crosscutting theme with the monitoring group.
- *Tracking paths and locations of releases:* Cross-cutting methods for recording track and locations for releases, whether by ground or air - use GPS unit with embedded microcomputer and autopilot with start/stop function. Similar solutions are currently used on UAV and could be used for ground release.
- *Ventilation:* Consider ventilation on the insects to avoid condensation and anoxia. Use O2 and CO2 meters to record what the insects experience during transport and release.
- *Multi-Species:* In areas with infestation of multiple mosquito species of concern, and if they are available as sterile males, release both/all species at once.
- *Release devices for mini and light UAVs:* currently there is one of practical experience in the development of release devices, that of pink bollworm. There is, however, a need to come up with best practices regarding the design of such devices for mosquito releases.
- *Design and validation of UAV systems for aerial release:* design of a UAV system that could fulfill not only the application requirements, but also the legal restrictions. Validation with flight experiments in a pilot programme to evaluate the effectiveness of using UAV in mosquito applications.

2.3. Monitoring

2.3.1.Overall Considerations

National mosquito SIT projects including Sudan (SUD5034), South Africa (SAF5013), Mauritius (MAR5019) and Sri Lanka (SRL5044) are nearing the stage of releasing sterile males for pilot SIT programmes. With the initiation of regional projects RAF5072 and RAS5066, many more countries globally will be working towards implementation of SIT. In all cases surveillance has been conducted over a prolonged period in order to collect baseline population data, to use in the design of releases. However, once releases are started it becomes critical that sufficiently effective surveillance tools and methodologies are available to enable the performance of released males and the impact of releases on the target population to be assessed. In addition, with the availability of GIS systems to design and implement release distributions targeted to the near real-time mosquito population, based on trap data, means that the ability to effectively monitor population distributions and fluctuations in real time is essential for efficient rearing and release activities.

Ovitrapping is a standard method to collect eggs, suitable for measuring natural and induced sterility, but methods for adult trapping will also be required. A range of traps are available, particularly for *Aedes* species, but their effectiveness and suitability for different species, situations and sexes needs to be assessed to advise on the best available techniques, and to identify unmet research and technological needs.

Although many adult traps exist and are used globally for both surveillance and population control of mosquitoes, they were designed for female collection, and so most exclusively or predominantly collect females. Likewise, guidelines have been written for mosquito trapping and surveillance, but male surveillance is not emphasised. While daily survival of males is typically lower than that of females, which would skew the sex ratio of collections, this difference does not often explain the collection disparity. Female population data is important for epidemiological studies, and to monitor a population over time, but for scenarios such as monitoring of survival and dispersal of released males, and especially mark release recapture experiments, it is critical that the traps used are sensitive in collecting males, and new solutions may need to be developed. This may be a matter of producing male specific traps, but may alternatively be a matter of adapting the timing, location or baiting of traps, for example, to target males specifically. For some purposes, for example where the ratio of released to wild males caught in the traps is being estimated, the preservation of the collected individuals in sufficiently good condition for subsequent analysis between checks of the traps will be crucial.

Surveillance systems that provide continuous acquisition of systematically collected information, collated frequently enough that it can be analysed and interpreted to provide operational feedback to programme managers, increase efficient decision-making for SIT programs. There are different surveillance tools currently available with different levels of sensitivity and specificity, and in the future efforts may be required to tailor systems based on the need of the programme. In general, the methods or tools should be practical, uniform or standard and rapid rather than 100% accurate or complete, (which is impossible). In SIT systems the collection of male mosquitoes is important to identify the scale of the production required, their dispersion and longevity, e.g. mark-release-recapture studies, and the over-flooding male ratio during release.

2.3.2.Suitable methods currently available for mosquito population surveillance

The need for traps that can collect male *Anopheles* is more acute than for *Aedes*. The methods for collecting adult Anophelines that can be standardized are variable in their sensitivity in different locations, and some potential candidates are too new to be assessed. One mainstay – CDC light trap – is widely used but is reported to capture few mosquitoes in many locations. Others, such as the Suna trap or sticky resting boxes could be standardized, but too little experience has been gained to know if they will be useful for many Anophelines or males. Some sensitive methods such as animal baited traps and human landing catches have not been standardized. Methods such as swarm captures are very useful in locations and times when swarms can be located, but this capacity is restricted.

The *Aedes* species being targeted will determine the specific trapping approach. For example, *Aedes aegypti* is primarily found indoors in low numbers requiring more intensive sampling efforts, and *Aedes albopictus* is generally more exophilic. Due to the varying reports on trap suitability it is recommended that a pilot study be conducted in each area to determine the efficacy or suitability of the traps and methodology. BG-Sentinel (BG-S) traps have been shown to provide consistent captures of male and female *Ae. aegypti* and *Ae. albopictus*. These traps capture adult mosquitoes in most physiological states (nulliparous, parous, blood-fed, and some gravid females), and the modified BG-S traps with black outer covers are considered by many to be the gold standard in *Aedes* traps currently available.

Male and female *Aedes aegypti* are readily captured by aspirating mosquitoes indoors. CDC back-pack and Prokopack electro-mechanical aspirators are commonly used for this purpose. Estimating the number of mosquitoes per house should provide a means to extrapolate the total number of mosquitoes in a given urban area (absolute population density). However, it has not been clearly established what percentage of all adult mosquitoes are collected by this means. The main disadvantages of this technique are that it is invasive (requires entering houses and having residents' consent), time consuming, labour demanding, and dependent on the skills of the operator.

Several models of sticky gravid traps (SGT) have been developed to attract and catch female mosquitoes looking for containers to lay eggs. Captured female *Ae. aegypti* in autocidal gravid ovitraps (AGO traps) were significantly correlated with captures in ovitraps and BG-S traps. Captures of male *Ae. aegypti* in SGTs are generally small, however, and sticky traps used for monitoring *Aedes* populations generally need to be checked once a week. Oviposition by *Ae. aegypti* and *Ae. albopictus* can be monitored using ovitraps, small, dark containers filled with water or plant infusions and a rough substrate where ovipositing females lay their eggs. Ovitraps can be checked every week depending on the ambient temperature, but large numbers are needed. The Gravid *Aedes* Trap (GAT) is a passive ovitrap designed to capture gravid container-inhabiting *Aedes* without the need for messy adhesives. However, as the GAT uses insecticide to knock down females, the trap may not effectively capture insecticide-resistant mosquitoes, and alternative capture methods should be developed. Captures of male *Ae. aegypti* are generally low (5-10% of the total capture) although occasional high captures suggest that the GAT can be modified to increase capture of males.

2.3.3.Placement of traps and operational considerations

For most purposes, traps should be spaced at distances from each other that reflect the expected dispersal of mosquitoes to avoid spatial auto-correlations that can undermine p-values in statistical analyses. For example, BG-S traps were spaced over 100 m from each other in a study of spatial-temporal changes of adult *Ae. aegypti*. When traps are too close together they tend to provide redundant information. However, if the purpose is to observe the dispersal of marked mosquitoes, then traps can be placed closer together to capture their movement in subsequent days. Traps can also be placed closer together when investigating spatial patterns at fine scales (households) or when models that rely on spatial autocorrelations demand it (kriging).

Before release of sterile males, preliminary studies must be made in order to describe the study site and to validate sampling methods and determine appropriate sample sizes. Detailed maps of the area need to be made. This would include up-to-date satellite imagery and house identification (GPS coordinates, streets and address if possible) and, for some species, potential larval and harbourage sites delineated. If multiple sampling methods are considered, Latin Square design trials that rotate treatments between locations can be used to compare captures between different trap types (e.g. Ritchie et al. 2014) for the target mosquito and sex. The logistics of the sampling method (e.g. whether it requires batteries, main power) will also be assessed during this trial. The logistics of sample processing, including the quality of captured material, is also important. This can include the need for fresh or live material for PCR or molecular analysis. Laboratory studies can be used to determine if older specimens can still be successfully processed and results can inform the choice used for routine population monitoring.

Several criteria must be met when selecting sites for trap placement, which include the willingness of householders to participate in the trapping programme, a power supply where necessary for the trap type, exposure to direct sunlight and rainfall should be avoided, and the site should not have a high level of human activity or be vulnerable to damage by domestic animals and small children. Unexpected factors may arise which affect the placement of traps, for example the presence of aggressive dogs or uncooperative householders may make a certain property unsuitable for inclusion. When considering *Aedes* population surveillance in particular, the heterogeneity of the population distribution must be considered. Very different trap data can be collected from neighbouring properties. If traps are relocated, this may affect the representative character of the estimates obtained and it should be considered whether this is advisable. A common approach when a property is not available is to relocate the trap to an adjacent property.

An important factor in obtaining useful data from traps is the length of time between setting the traps and collecting samples or scoring the individuals caught. The precise purpose of the surveillance will be a factor in deciding this parameter. For example, if analysis of collected individuals is required, the length of time before collections are made should be shorter, particularly in tropical or humid conditions, where samples will deteriorate more rapidly. Where ants or other predatory organisms are present in the environment, samples must be protected, or the traps emptied more regularly. In addition, the trap type will be an important factor in collecting suitable samples. Some will be more destructive, and some more suited to preservation of samples, for example wet traps collecting adults in a preservative liquid for PCR analysis. To minimise labour requirements and to maximise the number of mosquitoes collected from a surveillance tool, the longer a trap can be left before collection the better, though this will be situation dependent and there is no single recommended optimum time. Where non-lethal ovitraps are employed, collections must be frequent enough to prevent the emergence of adults from the eggs collected. A positive relationship which has been established with the residents in the study area will be important, and careful preparation and discussion of the importance of the work beforehand will increase the support from householders and scope in which to work.

2.3.4.Need for new surveillance tools for mosquito SIT programmes and promising avenues for their development

The success or failure of SIT approaches depend strongly on accurate initial information on the local insect population and can be cost/benefit optimized if precise surveys of the population is available through real-time monitoring of the population and the efficacy of intervention. It is essential that a sound understanding of the biology, ecology and population dynamics of *Aedes* and *Anopheles* mosquitoes be known. The behaviour of males can be exploited for more male-targeted trapping, for example males are seen to circle around traps into which females are more likely to enter more directly, and the addition of a sticky ‘wing’ to an existing trap may capture these males. Sound of the same frequency as a female wing beat has been shown to enhance male capture of *Ae. aegypti* in the GAT (Johnson and Ritchie, 2015). Sugar sources, or related floral or fruit scents could be used as male attractants, as could perhaps female acoustic cues, mating pheromones, swarm markers or host cues. It should be noted that the effectiveness of attractants for mosquitoes has been shown to be very variable, and situation specific. In this regard, the following developments remain imperative for future SIT: i) identification of potential new lures that enhance captures of males; ii) determination of the optimal attractive dose and sampling duration; iii) development of a slow release dispensing system; iv) standardized experimental design (Latin Square) for trap comparison; v) guidelines on trap placement; vi) standard operating procedures for use of the recommended surveillance tools; vii) modification of existing traps such as the BG-S to improve efficacy of catches and possibly to lower cost; and viii) investigation of dry adhesives that effectively captures adult mosquitoes that can used in a variety of traps.

The needs for monitoring mosquito populations change during the SIT programme phases, namely *the planning and technology development* phases and *the operational phase*. During the planning and technology development phase, the size of the target site mosquito population needs to be known. Many population monitoring methods provide indices of relative population abundance, however, all existing routine sampling methods require an external method to estimate the absolute populations size contemporaneously and thus ‘calibrate’ the sampling methods. The most direct of these methods is Mark-Release-Recapture (MRR). After release operations begin, capturing males provides an ongoing indicator of the over-flooding ratio and the population size because the number of males released is known. Routine trapping is essentially a continuous-mark release-recapture activity during which sterile males are captured along with unmarked wild males. Unexpected changes in the numbers and ratios of male captures indicate operational issues that may need immediate resolution.

While declining female abundance of disease vectors is the most important indicator of an SIT programme's success, the delay between male release and changes in female abundance do not allow the immediate feedback to production and release activities that male monitoring provides.

Key characteristics of surveillance systems for population monitoring and evaluation of suppression activities are common to both *Anopheles* and *Aedes*. Such requirements include the need for traps to be sensitive, especially for males but which also collects females, cost effective, good quality and durable, light weight, easy to set and collect, and acceptable to householders and the wider community. Traps which are versatile will be needed during the development phase and others which are species specific during the operational phase. Likewise, there may be a need for more complex, expensive traps for research activities as well as low cost passive traps for longer term surveillance.

Some fundamental research has been conducted, though more is needed, into the behaviour of the mosquito, especially the male, in particular, studies on mosquito response to visual, acoustic or odour cues, humidity and swarming behaviour. This knowledge could be exploited to develop new trapping and surveillance tools for improved surveillance. Because capturing females has occupied most attention in studies of mosquito vectors, traps and other collection methods have been considered useful to the extent that they monitor that sex. Indirect methods, such as oviposition traps, only reflect female abundance and no methods have been developed specifically for collecting males except netting males seeking mates near hosts (*Aedes*) or swarms (*Anopheles*). Resting shelters take advantage of the seeking behaviour common to males and females to find cool, humid and shady places. Further investigations of various configurations of resting shelters, specifically for their ability to attract and capture males (and females) are warranted. Preliminary findings from South Africa show that clay pots serve as potential resting sites for *An. gambiae* sl. (Givemore *et al*, unpublished data). Attractive chemicals, sounds and improved resting shelters are all compatible with existing trap designs and could be economical modifications of existing traps. Finally, by analogy with many agricultural pests whose pheromones have been exploited for monitoring and control, it is also possible that pheromones are emitted from males that swarm, which might attract other males or that females seeking mates emit attractive chemicals.

More radical new developments may be possible in the longer term. For example, monitoring of wild-type and sterile insect populations in real time, especially for male *Anopheles*, traditionally had been a challenge both from the human labour and technical viewpoints. Unsupervised intelligent networked traps could automatically count and classify mosquitoes entering a trap. Biogents has developed a remote counting system able to detect and count mosquitoes while entering the trap. It will require further refinements to enable differentiation of mosquito species and possibly the sexes. Highly detailed large data from intelligent traps will not only enable lower cost and successful SIT operations, but shall also provide new insights into the behaviour of disease vectors and agricultural pests worldwide. Cost effective imaging devices are capable of collecting images that enable in-depth analysis on remote computing clusters and basic real-time image analysis locally. MarkaLab demonstrated that imaging of mosquitoes entering traps is feasible allowing machine vision-based approaches in Smart Traps. Solar power is sufficient to supply the few watts of power embedded devices require and any local power system that is sufficient to charge cell phones can easily serve the intelligent trap's computing core. Sometimes the physical collection of specimens is not necessary and flow-through devices can be used instead of traps as the image-based monitoring can provide the information required by the projects. Swarm location and swarm size detection can allow insight into the size of local male mosquito population that is inaccessible to traps. Real time localization and characterization of swarms should be possible through wide area acoustic surveys and possibly UAVs.

2.4. Selected References

Nyasembe VO, Tchouassi DP, Mbogo CM, Sole CL, Pirk C, Torto B. 2015. Linalool oxide: generalist plant based lure for mosquito disease vectors. *Parasites & Vectors* 8:581.

Dritsou V, Topalis P, Windbichler N, Simoni A, Hall A, Lawson D, Hinsley M, Hughes D, Napolioni V, Crucianelli F, Deligianni E, Gasperi G, Gomulski LM, Savini G, Manni M, Scolari F, Malacrida AR, Arcá B, Ribeiro JM, Lombardo F, Saccone G, Salvemini M, Moretti R, Aprea G, Calvitti M, Picciolini M, Papathanos PA, Spaccapelo R, Favia G, Crisanti A, Louis C (2015) A draft genome sequence of an invasive mosquito: an Italian *Aedes albopictus*. *Pathogens and Global Health*. 109: 207-220. doi: 10.1179/2047773215Y.0000000031.

Manni M, Gomulski LM, Aketarawong N, Tait G, Scolari F, Somboon P, Guglielmino CR, Malacrida AR, Gasperi G (2015) Molecular markers for analyses of intraspecific genetic diversity in the Asian tiger mosquito, *Aedes albopictus*. *Parasites & Vectors* 8: 188. doi: 10.1186/s13071-015-0794-5.

Ritchie SA, Brian L. Montgomery, and Ary A. Hoffmann. 2013. Novel estimates of *Aedes aegypti* population size and adult survival based on Wolbachia releases. J. Med. Entomol. 50: 624-631.

Azil, A. H., Ritchie, S. A., & Williams, C. R. (2014). Field worker evaluation of dengue vector surveillance methods: factors that determine perceived ease, difficulty, value, and time effectiveness in Australia and Malaysia. Asia-Pacific Journal of Public Health.

Johnson, B.J. and S. A. Ritchie 2015. The Siren's Song: Exploitation of Female Flight Tones to Passively Capture Male *Aedes aegypti* (Diptera: Culicidae). J Med. Entomol. doi: 10.1093/jme/tjv165

Soumaïla Pagabeleguem · Momar Talla Seck · Baba Sall · Marc Jb Vreysen · Geoffrey Gimonneau · Assane Gueye Fall · Mireille Bassene · Issa Sidibé · Jean-Baptiste Rayaissé · Adrien Mg Belem · Jérémy Bouyer · Long distance transport of irradiated male Glossina palpalis gambiensis pupae and its impact on sterile male yield. Parasites & Vectors 05/2015; 8(1):259. DOI:10.1186/s13071-015-0869-3

Momar Talla Seck · Soumaïla Pagabeleguem · Mireille D Bassene · Assane Gueye Fall · Thérèse A R Diouf · Baba Sall · Marc J B Vreysen · Jean-Baptiste Rayaissé · Peter Takac · Issa Sidibé · Andrew G Parker · Gratian N Mutika · Jérémy Bouyer · Geoffrey Gimonneau ·PLoS Neglected Tropical Diseases 11/2015; 9(11):e0004229. DOI:10.1371/journal.pntd.0004229 ·

Johnson and Ritchie 2015

3. Nuclear Component

All the activities in the CRP relate to facilitating the release and monitoring of sterile mosquitoes within SIT programmes. The SIT relies on the use of ionizing radiation to sterilize large numbers of insects and currently there is no alternative that could replace radiation. As radiation induces random dominant mutations, there are no possibilities of resistance development to this physical process. Therefore radiation-induced sterility provides a very high level of biosafety and can be used in combination with other genetic or symbiont-based methods.

3.1. Explanation / Justification:

Two Consultants Meetings were held, one on "Mosquito handling, transport and release" (8-12 December 2014) and another one on "Mosquito male trapping methods to monitor the efficacy of SIT programmes in the field" (February 16-20 2015) as a basis for the development of this proposal for a CRP.

Participation of Agency's laboratories (Yes)
As few institutions are developing methods to handle, transport, release and monitor male mosquitoes, the CRP needs to be supported through adaptive research and development carried out at the IPCL, FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf as part of Project 2.1.4.3. This R and D will also complement the ongoing mosquito CRP that is addressing the other major bottleneck, which is sexing methods for mosquitoes.

3.2. Assumptions:

Member States continue requesting the development of tools and techniques for mosquito SIT to be able to apply the SIT to control mosquito vector populations (Resolution GC(52)/RES/13) in view that the SIT can contribute to address resistance and other shortcomings of conventional mosquito control, and has the ability to suppress or in special situations to eradicate existing vector populations and to prevent the establishment of new outbreaks.

3.3. Related ongoing TC Projects:

- **INT5155** - Sharing Knowledge on the Sterile Insect and Related Techniques for the Integrated Area-Wide Management of Insect Pests and Human Disease Vectors

- **MHL5001** - Strengthening national capacities for the early and rapid detection of Zika virus infections in the Marshall Islands

- **MEX5031** - Using the Sterile Insect Technique to Control Dengue Vectors

- **PHI5033** - Building Capacity in Using the Sterile Insect Technique against Dengue and Chikungunya Vectors

- **RAF5065** - Promoting the sharing of expertise and physical infrastructure for mass rearing mosquitoes and integration of the sterile insect technique (SIT) with conventional methods for vector control, among countries of the region.

- **RAF5072** - Exploring the Use of Sterile Insect Technique as a Novel Technique for Control of Vector Mosquito for Chikungunya and Dengue (*Aedes albopictus*) in the Indian Ocean Region. Phase I

- **RAS5066** - Promoting the Sharing of Expertise and Infrastructure for Dengue Vector Surveillance towards Integration of the Sterile Insect Technique with Conventional Control Methods among South and South East Asian Countries

- **RER5022** - Establishing Genetic Control Programmes for *Aedes* Invasive Mosquitoes

- **RLA5074 -** Strengthening Regional Capacity in Latin America and the Caribbean for Integrated Vector Management Approaches with a Sterile Insect Technique Component, to Control *Aedes* Mosquitoes as Vectors of Human Pathogens, particularly Zika Virus

- **SAF5014** - Assessing the Sterile Insect Technique for Malaria Mosquitoes in a South African Setting, Phase II

- **SRL5047** - Establishing a National Centre for Research, Training and Services in Medical and Molecular Entomology for Vector-borne Disease Control

- **SUD5038** - Implementing the Sterile Insect Technique for Integrated Control of *Anopheles arabiensis*, Phase II

4. Logical Framework

Overall Objective	Objective Verifiable Indicators	Means of Verification	Important Assumptions
The objective of this CRP is to provide the necessary technical advances in the release and subsequent monitoring of male mosquitoes to enable the application of the sterile insect technique against mosquitoes for reduce vector populations and disease burden of affected Member States.	N/A		Requests by Member States in the area of mosquito control using the SIT are increasing. To make this nuclear technology available to Member States for several mosquito species, the development of suitable handling, transport, release and monitoring methods is required as an essential precondition or to reduce programme costs. Biological material is available.
Specific Objectives	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Objective 1. To explore approaches to perform the necessary handling and transport of irradiated, sex-separated male mosquitoes to the site of release, with minimal impact on survival and quality of released insects, including consideration of pre-release nutritional conditions.	Standardised procedures and necessary equipment developed to handle and transport males prior to release	Reports and or published papers Availability of equipment and supporting guidelines/SOPs	Quality control tests can be applied for each evaluated parameter The quality of sterile male that has been irradiated on pupal and adult stage is different. In terms of mass release, pupal irradiation is easier to handle instead of adult. Availability of standard test for flight ability and field performance, including QC tests before and after chilling: Emergence rate Mating competitiveness Flight Ability (dispersal) Longevity Mortality Body integrity Preparation time

Objective 2. To explore approaches to releasing sterile male mosquitoes in a controlled, traceable and documented manner over a large area, with the ability to target specific areas, ensuring low mortality and high quality in released insects.	Develop standard experimental designs to measure impact of adult and pupae handling conditions (chilling, compaction) including: Release studies at dusk vs. dawn to determine effect of light exposure/intensity on dispersal of males. Wind tunnel to assess effectiveness of various release devices on various species. Mark Release Recapture (MRR) concentric sectors and transects studies to assess dispersal and swath width of aerial release. Develop ground-based methods utilizing public transportation, car, bicycles, and or motorcycles, ensuring uniformity of release. Quality control of insects pre and post release to improve release methods. Directly video record releases as they exit the craft or vehicle and cross reference video to GPS and other spatial and time indicators.	Reports and or published peer reviewed and conference papers Uniformity of results (insects delivered to target area) between similar methods, confirmed by multiple reporting/measurement tools Releases performed in such a way to allow efficacy of SIT programme	Adoption of technology is possible: Standardised protocols and technology adopted and accepted by different facilities Mechanical processing in release machine is possible without damage to the males. Survival rate after release is understood and sufficient for effective population suppression
Objective 3. To explore different monitoring systems for surveillance of the target population of an AW-IPM programme with an SIT component, and to follow the performance of released males and the efficacy of population suppression.	Develop novel lures for male mosquitoes. Develop standard operating procedures for testing traps (and lures) in the field. Compare cost-effectiveness of available surveillance systems GUIDELINES FOR: Develop methods to calibrate results from trap catches with actual population densities within a given geographic region. Develop release solutions in coordination with trap monitoring techniques. Periodic MRR to audit release procedures based on trap resolution.	Prototype traps and lures made available and verified in a range of field settings CRP progress reports and/or published papers Analysis of investment and running cost of the recommended surveillance system/s available Increase in male captures relative to the present standard	Cooperation among member states with facilitation by the agency (IAEA) Good communication achieved with the trap developer during and after initial field trials High-resolution molecular markers become available (SSRs and SNPs)

	Characterise SIT strains using high-resolution molecular markers to facilitate assessment of male survival, dispersal. Development of protocol to genotype sperm transferred to wild females to assess insemination capacity of released males.	Increase in ratio of captured male:female relative to the present standard	
Objective 4. To encourage and attract participants to the CRP in the fields of insect handling, surveillance, cooling and release technologies, and with experience of insect population monitoring.		Agreements and or contracts issued and signed	Relevant scientists can be identified and encouraged to join the CRP
Outcomes	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Outcome 1. Methodology and necessary equipment for chilling, handling and transport of mosquitoes developed and tested for impact on insect quality.	Assess the impact of chilling on mosquito species for extended durations of time Assess impact of agitation as it relates to transportation of chilled sterile mosquito over bumpy roads over extended periods of time. Assess the optimum relative humidity and temperature to perform this activity for each target species, including consideration of the occurrence and impact of condensation and need for ventilation. Design and test the container and optimise the volume for each species, using a cassette design so that handling/transport container fits into release device.	Establish experimental design to assess impact of chilling at preset time intervals. Assess longevity, mortality and impact to fitness at preset time intervals as it relates to simulated agitation	Mosquito colonies of different species available for the design and testing of the equipment. SOP for QC of emerged males available.

Outcome 2. Equipment and technique for release of sterile mosquitoes developed and tested in laboratory and validated in a range of field settings for ability to disperse mosquitoes in a controlled, documented, and targeted manner.	Develop standardised methods for release of adults by ground, water and air Develop and validate method for release of pupae Develop uniform experimental designs to assess biological impact of release in regards to, Initial Mortality, Longevity, - Biological Fitness as it relates to body integrity and flight ability - and mating responsiveness	Reports and or published papers, participation in conferences Methodologies for adult and pupal release developed and available for programmes to use	Mosquito colonies of different species available for the design and testing of the equipment. SOP for QC of emerged males available.
Outcome 3. Equipment and technique for recording, documenting, and validating the release of mosquitoes developed and validated.	High speed video recording of mosquitoes leaving the release device Method for counting of mosquitoes leaving the release device Cross reference and combine data from all sources of data for corroboration and calibration: GPS, ground visual clues, and release counter videos	Agreement between release plan and release recording data demonstrated	Mosquito colonies of different species available for the design and testing of the equipment. SOP for MRR available.
Outcome 4. Mosquito population monitoring systems and any necessary male surveillance/trapping devices developed and validated in laboratory and field.			Mosquito colonies of different species available for the design and testing of the equipment. SOP for MRR available.
Outputs	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Output 1. Methodology developed for marking large numbers of sterile mosquitoes for operational programmes	Protocol for marking large number of sterile mosquitoes develop, transferred and tested in different countries with different species of mosquitoes	Reports and peer reviewed publications. SOP for male marking developed.	Mosquito colonies of different species available for the design and testing of the equipment. SOP for QC of emerged males available
Output 2. Methodology developed for emergence of irradiated male pupae directly into, or for transfer of chilled adults into, containers	Male-only cages designed, built and tested with optimised vertical resting surface and other relevant parameters.	Reports and peer reviewed publications. SOP for male handling developed. Equipment (male-only cages) available for use.	Mosquito colonies of different species available for the design and testing of the equipment. SOP for QC of emerged males available

suitable for transport to the release site in a temperature controlled environment.			
Output 3. Equipment available to maintain suitable environmental conditions (both temperature and relative humidity) between the rearing facility and the release site.	Optimal conditions for transport of chilled and non-chilled insects from the mass rearing facility to the release point assessed. Equipment developed and tested.	Reports and peer reviewed publications SOP for pupae/adult transport developed. Equipment available for use.	Mosquito colonies of different species available for the design and testing of the equipment. SOP for QC of emerged males available.
Output 4. Release device/s which is suitable for ground, water, and air release from ground vehicles, watercraft, UWVs, UAVs and light aircraft, developed and tested both in the lab and in the field.	Device designed, manufactured and tested in different mosquito species and countries. Capacity to release differential release doses according to predefined distribution maps.	Reports and peer reviewed publications Equipment available for use	Mosquito colonies of different species available for the design and testing of the equipment. SOP for QC of emerged males available. SOP for MRR and dispersion available. Standardised protocols developed for validation of new and existing surveillance tools
Output 5. Suitable aerial release UAV identified or built for application with appropriate release device/s.	Specifications of the equipment defined. Optimal aerial platform, ground station and communication settings identified for the different mosquito species and project scenarios.	Reports and peer reviewed publications. Equipment available for use.	International regulations on the operation of RPAS are approved and allow the required flight operation.
Output 6. Direct documentation, validation, and analysis of the quantity of mosquitoes released from release device.	Appropriate electronic sensors installed and connected to the autopilot system, recording a track-log file with telemetry of the flight.	Reports and peer reviewed publications. Track-log files available.	Mosquito colonies of different species available for the design and testing of the equipment.
Output 7. Trap/s developed to monitor male mosquito population of the different mosquito species in the field.	Traps and lures (sound and chemical attractants) developed and verified in the lab and tested in the field in different countries Cost-effective, efficient and sensitive traps that incorporate appropriate effective lures developed.	Reports and peer reviewed publications. Equipment available. Guidelines developed for the effective use of available and newly developed traps for population surveillance, assessment of sterile male performance and impact of population suppression efforts	SOP for MRR available. Expertise and engineering available to develop new traps.

	Development of a method/tool for passive/automated counting of mosquitoes.		
Output 8. Male surveillance methods not requiring trapping.	Swarm sampling methodology standardized	Reports and peer reviewed publications.	Procedure to compare these methods with other trapping methods available.
Output 9. Methodology and equipment in 1-7 tested in sites of current or proposed SIT pilot trials.		Reports and peer reviewed publications	Network between the participants of the CRP created and sufficient funding available.
Output 10. Examples of good practice community engagement opportunities identified and documented, since it is crucial in enabling seamless releases		Reports and peer reviewed publications	
Output 11. Survey of legal requirements for use of all release vehicles considered for use.		Reports and peer reviewed publications.	International regulations on the operation of RPAS are approved and allow the required flight operation.
Activities	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Activity 1 Submit CRP proposal.	Consultants meetings on “Mosquito Release Methods” and “Male Mosquito Trapping Methods” held in 2015.	Report of Consultants Meeting and CRP proposal prepared.	CRP proposal approved by IAEA committee.
Activity 2 Announce project amongst established vector entomologists, mosquito trapping specialists and experts on insect release methods	CRP announced, and research contracts and agreements submitted, evaluated and forwarded to IAEA committee.	Issued contracts and agreements.	Suitable proposals submitted and approved by IAEA committee.
Activity 3 Organize first RCM to plan, coordinate and review research activities	1st RCM held.	Working material printed and distributed for 1st RCM.	Research activities started. Reports published and distributed following each RCM.
Activity 4 Carry out R&D.	Research carried out by contract and agreement holders.	Reports and publications.	Renewal requests and continued funding of RCM’s and CRP.

Activity 5 Second RCM to analyse data and draft technical protocols as required	2nd RCM held.	Working material printed and distributed for 2nd RCM; Research published in scientific literature and disseminated to member states and scientific community.	Research activities continue, progress satisfactory.
Activity 6 Continue R&D.	Research carried out by contract and agreement holders.	Reports and publications.	Renewal requests and continued funding of RCM's and CRP.
Activity 7 Review the CRP after its third year.	Mid-CRP review carried out.	Report of mid-CRP review.	Mid-CRP review by Agency committee is positive.
Activity 8 Convene third RCM to evaluate and standardize protocols.	3rd RCM	Working material printed and distributed for 3rd RCM; Research published in scientific literature and disseminated to member states and scientific community.	Research activities continue, progress satisfactory.
Activity 9 Continue R&D. holders.	Research carried out by contract and agreement	Reports and publications.	Renewal requests and continued funding of RCM's and CRP.
Activity 10 Hold final RCM to review data and reach consensus.	Final RCM held.	Final CRP report.	Research and dissemination activities concluded.
Activity 11 Evaluate the CRP and submit evaluation report.	CRP evaluation carried out.	CRP evaluation report.	CRP evaluation by Agency committee is positive.
Activity 12 Summarize and publish advances of CRP in a series of joint publications.	CRP members submit papers summarizing activities.	Publication in scientific literature.	Manuscripts accepted and published

.

5. Future Activities

5.1. Handling, transport and release

Research Contract 19120 (BRA):

CSI: Michelle Cristine Pedrosa, Biofábrica Moscamed, Brasil. (Collaborators: Jair Virgínio, Margareth L. Capurro)

Title: Evaluating mosquitoes transport and trapping methodologies.

Summary: This project aims to test the best way to handle and transport *Aedes aegypti* pupa or adults from the Production Facility to the release sites and to improve the current methodology of transport. We will also evaluate different adult traps for monitoring the adult population.

Research Contract 19115 (CPR):

CSI: Xiaoying Zheng, Sun yat-sen University, China (Collaborators: Zhiyong Xi, Cui Yang, Wei Qian)

Title: Developing a high effective system to transport, release and monitor *Wolbachia*-infected *Aedes* mosquito for Dengue control in China

Summary: The goal of this project is to develop a high effective system to transport, release and monitor *Wolbachia*-infected *Aedes* mosquito for dengue control. Toward this goal, we will compare different approaches to transport *Aedes albopictus* HC mosquito into field sites, including those areas with long distance to our mass rearing facility. Different release methods, including pupae and adult release, will also be compared. In addition, we will develop improved approaches to monitor the performance of male mosquitoes.

Research Agreement 19131 (FPL):

CSI: Hereiti Petit, Institut Louis Malardé, French Polynesia (collaborators: Hervé Bossin, Jérôme Marie, Michel Cheong Sang)

Title: Mosquito Suppression Pilots in French Polynesia - Improving handling, transport, release and monitoring of sterile *Aedes* males

Summary: The efficacy and sustainability of the *Wolbachia* incompatible insect technique (IIT) to control the disease vector *Ae. polynesiensis* is being evaluated through open release trials in French Polynesia. Prior results established through proof of concept trial on the island of Raiatea (O'Connor, 2012) and calibration trial on the atoll of Tetiaroa (2012), supported the pursuit of field evaluation. *Ae. polynesiensis* is an exophilic, day biting mosquito which uses a wide range of domestic and natural larval containers (e.g. rat-chewed coconuts), making classical control impractical, expensive and short lasting. To improve the control of this mosquito a suppression trial is being implemented on Tetiaroa, a populated atoll in the Society islands, French Polynesia. The study investigates the efficacy of an integrated approach combining environmental management (partial removal of breeding containers) and the incompatible insect technique (IIT) to suppress this naturally isolated mosquito population.

In the absence of a genetic sexing strain for *Aedes*, sex separation currently exploits developmental (time to pupation) and mechanical (pupae size) differences between males and females. Males are currently transported as pupae to the release site and allowed to emerge in a dedicated facility before release. Better tools and procedures will be necessary to allow scale up for much larger, "island-scale" *Aedes* suppression trials. To that end, we propose to collaborate with CRP participants to i) test different systems for handling, and transport of adult males, ii) to explore various ground and aerial release systems, and iii) to test different monitoring systems, including smart traps, for surveillance of the target population, to follow the performance of released males and the efficacy of population suppression.

Research Contract 19098 (INS):

CSI: Beni Ernawan, National Nuclear Energy Agency, Indonesia (Collaborators: Tri Ramadhani)

Title: Maintaining the Quality of Radiation Induced Sterile Male: The Effect of Land Transportation and Possibility of Aerial Release

Summary: In Indonesia gamma ray irradiators do not exist in every province. Irradiators only exist in Jakarta (the capital). Meanwhile, the requests for an SIT program to control the *Ae. aegypti* population came from the state government of the cities that are located a long distance from the capital (12-18 hours by land transportation), and from the state government on another island. All processes that are involved, starting from the irradiation process to the male releases are potentially reducing the quality and competitiveness of sterile male. The process of moving sterile males from an emergence cage to the release device using aspirators could damage or even eliminate the fine scales on the wings of the mosquito. Plastic cups/jars/containers use as release devices could also cause problems, as there is no resting surface for mosquitoes and the slippery surface of the plastic could cause loss of energy. Moreover, the availability of a sugar meal during the transportation also needs to be studied.

Research Agreement 19133 (MEX):

CSI: Roberto Angulo Kladt, Servicios Aéreos Mubarqui, Mexico. (Collaborators: Rubén Leal de la Garza, René Cano, Rubén Leal Mubarqui).

Title: Development for Aerial Mosquito Release Machine and Quality Assurance using Web Based System MACX under the Chilled Male Adults Method for SIT

Summary: Thanks to the experience gained during decades of releasing sterile fruit flies and small and fragile parasitoids such as *Diachasmimorpha longicaudata*, Mubarqui will develop a chilling release machine for mosquitos to be installed in light aircrafts. New technologies for aerial vehicles such as UAVs will also be considered. Special attention will be paid to dosing devices in order to minimise mechanical injuries to the insects.

Research Contract 19132 (SEN):

CSI: Assane Gueye Fall, Laboratoire National de l'Elevage et de Recherches Vétérinaires, Dakar-Hann, Senegal

Title: Evaluation of two automatic release machines in *Aedes aegypti*

Summary: The abusive and uncontrolled use of insecticides has led to insecticide resistance phenomena in insects and it is a major public health problem. Facing this, vector control strategies against flies increasingly turns towards biological control methods, including the Sterile Insect Technique (SIT) which is one of the major alternatives to insecticides.
The SIT developed for a long time against fruit flies and tsetse flies is being developed against mosquitoes, particularly *Aedes albopictus* and *Anopheles gambiae.* In this project, we propose to test two machines used for automatic tsetse release for *Aedes aegypti*. If one of these machines is effective, we also offer a size adaptation for the release by drones.

Research Agreement 19095 (SPA):

CSI: Antidio Viguria, Center for Advanced Aerospace Technologies (FADA-CATEC), Spain.
Title: Transport and Release of Mosquitos Using UAS
Summary: The goal of this project is to:

- Develop the operational procedures for mosquito's releases using UAVs and the first design of a suitable UAV configuration for this type of operation.
- Design the release mechanism adapted for mosquitoes and for the selected type/model of UAV.
- Complete a first prototype validated in flying tests.

Research Agreement 19115 (SPA):

CSI: Ignacio Plá Mora, Empresa de Transformación Agraria S.A. (TRAGSA), Spain (Collaborators: Carles Tur Lahiguera).
Title: Design of male only handling cages and systems for transporting mosquito sterile pupae from mass rearing facility to release facility
Summary: The design of the male-only cages is key in an SIT project since they are the most abundant piece of equipment in both the mass rearing and release facilities. The design of the male-only cages must ensure the optimal quality of the males, minimum handling, minimum workload, adequate ergonomic during male handling, chilling and washing. Ideally, the cages should also be inexpensive and potentially manufactured by different suppliers.

Research Agreement 19106 (USA). Part 1:

CSI: Nathan Moses-Gonzales, M3 Consulting Group., Dayton, OH, USA. (Collaborators: Michelle Walters, Nicole Culbert, Hervé C. Bossin Szabolcs Marka, Dan Kline, Luke Alphey, Ring Cardé).
Title: Build and Initial Testing of an Unmanned Aircraft System (UAS) for the Release of Sterile Mosquitos in Support of Mosquito Population Suppression and Eradication Efforts.
Initial Development and Exploration of an Unmanned Aircraft System and associated Release Mechanism for the Release of Sterile Mosquito in Support of the International Atomic Energy Agency's Mosquito SIT CRP
Summary: Unmanned Aircraft Systems (UAS) provide an accurate, low impact means of releasing sterile insects into generally infested areas, are capable of operating in environments that are dangerous to humans and can be deployed as an aerial release component

with minimal setup and teardown time. The efficacy of UAS as an aerial release component, however, is highly dependent upon its ability to deliver and release sterilized insects with minimal impact to an insect's physiology. The goal of this project is to explore the biological impact and aerial release potential of irradiated sterile mosquitoes in support of international SIT programs.

Research Agreement 19106 (USA). Part 2:

CSI: Michelle Walters, United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine, Center for Plant Health and Technology, USA. (Collaborators: Dustin Krompetz, Dan Kline, Luke Alphey, Ring Cardé)

Title: Build and Initial Testing of an Unmanned Aircraft System (UAS) for the Release of Sterile Mosquitos in Support of Mosquito Population Suppression and Eradication Efforts.
Acquisition of Certificates of Authorization (COA) and PPQ Permits for release of sterile male mosquitoes (*Aedes aegypti* and others) in the for a pilot UAV mosquito sterile release program

Summary: The goal of this project is to provide the legal means to test unmanned aircraft systems in the United States, for later use in the US and other countries. The UAS will be equipped with specially designed release mechanisms for sterile male mosquitoes (see Part 1 of Contract 19106). Our ability to conduct test flights facilitates quantifying attributes of mosquito transport that are potentially harmful to the mosquitoes.

5.2. Monitoring

Research Agreement 19046 (AUL):

CSI: Scott Ritchie, James Cook University, Cairns Queensland. Australia

Title: Sound Lures for the Capture of Male *Aedes*.

Summary: Our laboratory has provided proof of concept testing that demonstrates that male *Ae. aegypti* can be readily captured in the Gravid *Aedes* Trap (GAT) by addition of the sound of the female wingbeat frequency (Johnson and Ritchie 2015). In semi-field and field trials we demonstrated that captures of male *Ae. aegypti* are highest using pure tone of 485 Hz, with catches dropping at harmonics of higher frequency. We demonstrated that addition of an MP3 card and cheap speaker captures male *Ae. aegypti* inside and outside homes using loudness of 10-15 db above background (55-65 db). Our colleagues at Emory University also demonstrated that addition of 540 Hz played at 65 db significantly increased capture of male *Ae. albopictus* in the field. In partnership with industry we will develop a simple inexpensive sound lure to capture male *Aedes* in the passive GAT and other traps. It will be used in field trials at multiple sites to compare captures of male *Ae. aegypti*, *Ae. albopictus* and potentially *Ae. polynesiensis* against BG-sentinel traps and other adult sampling methods. We will also analyse BGS *Ae. aegypti* collections from Wolbachia releases in Cairns from 2011-2013 to see if relative increases in mosquito capture method (Ritchie et al. 2013) can be used to estimate the field population of male *Ae. aegypti*.

Research Contract 19061 (BKF):

CSI:	Dabiré Kounbobr Roch and colleagues at IRSS/Centre Muraz Bobo-Dioulasso, Burkina Faso.
Title:	Trapping the *Anopheles arabiensis* males based on the manipulation of swarming cues and visual markers
Summary:	Our work aims to improve the *An. arabiensis* male population monitoring method within swarms and also to track males in their resting places. We have already identified and mapped swarming markers and physical cues throughout our study area in an urban setting. We have also started testing the influence of two contrasts (dark and white clothes) on the size and the behaviour of *An. arabiensis* swarms both in semi field and field conditions. Further, attractiveness of airvents traps placed in houses neighbouring the swarming places are being evaluated to capture living males. We require tse-tse like traps to be developed and deployed around identified swarming places characterised by specific markers and physical cues with the goal to improve captures of males. Furthermore, the team of IRSS/Centre Muraz would be interesting to participate to the evaluation of *Aedes* traps in the field. This is very important to compare the efficacy and sensitivity of such traps following different savannah settings.

Research Agreement 19051 (FRA):

CSI:	Louis-Clément Gouagna, IRD Reunion Island, France.
Title:	Assesment of *Aedes albopictus* Male Trapping Systems in Support of the Sterile Insect Technique on Reunion Island.
Summary:	The goal of field studies planned in Reunion Island is to improve and evaluate the effectiveness of existing sampling tools for collecting adult male *Aedes albopictus* for use in research and control programs based on SIT. *Progress to date:* An initial study in three urban areas in Reunion Island has been attempted to assess the ratio of male to female *Aedes albopictus* attracted to BG sentinel traps, by comparing the efficacy of mice as baits in relation to the standard BG lure as control. Preliminary results showed that the mosquito captures increase with the number of mice present in the trap. Overall, BG trap baited with 2-3 mice attracted more *Aedes albopictus* males than 1 mouse or BG-lure. *Future Plan (2015-2018):* The contribution of Reunion Island will not be restricted to the evaluation and improvement of BG-S traps. Further emphasis will be (1) to compare the efficacy, through field surveys of other potential sampling tool or new prototype developed by other research groups for adult male mosquitoes, 2) to include CO2 (dry ice) in future field trials to delineate the possible effect of host odours, CO2 and heat and their combinations, 3) to validate the accuracy of the most effective male trapping systems in mark-release-recapture experiments utilizing both sterile and non-sterile males. Synthetic host odour blends (matching live mice) could be used with CO2 or other existing attractants to improve collection rates of male mosquitoes in BG-S

Research Agreement 19126 (GFR):

CSI:	Martin Geier, Biogents. Germany
Title:	Development of Low Cost Traps for Male and Female *Aedes albopictus* and *Aedes aegypti* Mosquitoes

Summary: Biogents aims to modify the BG-Sentinel trap design to lower significantly production costs while maximizing trap captures. This will include a ventilator that does not destroy mosquitoes while they are sucked through the ventilator. There are further modifications such as a rain shield and trap stand to improve trap performance and handling. Initial trials shows that addition of a rain shield improves trap captures of *Ae. albopictus* by almost 2-fold (Unpublished data). Biogents will develop a battery operated sound lure for the BG-GAT trap that can be tested also for BG-Sentinel traps. By adding this sound generator that imitates the wing beat frequency of females, we intend to improve the catching rates of both traps for male mosquitoes.

Specifications of the sound lure:

1. Up to 3 different sound frequencies (female wing beat frequencies for *Ae. aegypti*, *Ae. albopictus* and *Ae. polynesiensis*)
2. Operation only during day-light
3. Operation mode: continuous vs interval
4. Battery operated with the option of using solar power and 12V battery from the BG Sentinel trap

A second line of research aims to develop remote counting devices with optical sensors that automatically detect, count and identify insects entering a trap through bioacoustic analysis. The collected data are transmitted wirelessly to a cloud server, where it will be automatically processed and presented in statistical and GIS (Geographical Information System) format. We have already developed a prototype system that is tested in the field at the moment. Our aim is to achieve an accuracy level of > 90% in discriminating mosquitoes from other insects. Future work in the next year includes field testing and evaluation of the counting system.

Biogents will also manufacture sample lures of candidate attractants on request of collaboration partners depending on the nature of the attractant. Biogents has already developed a methodology to manufacture polymer dispensers for long lasting emission or release of odour profiles. Based on this technology, Biogents will provide lures of candidate attractants on request from candidate partners.

Biogents will provide ventilators and collections devices to collect swarms of *Anopheles* mosquitoes as well as new trap designs for collections of malaria vectors (more focused on females probably).

Biogents will also provide samples of glue cards (dry glue with strong adhesive forces to capture mosquitoes when they have just a short contact with the legs) for the use in GAT traps and clay pots.

Research Agreement 19049 (ITA):

CSI: Ludvik Gomulski, University of Pavia, Italy. (Collaborators: Giuliano Gasperi)

Title: Development of Attractants for Male Trapping and the Evaluation of Male Quality and Sexual Capacity in *Aedes albopictus*

Summary: We have two lines of research; firstly to combine molecular approaches and behavioural assays to identify novel attractants that improve captures of male *Aedes albopictus* mosquitoes when utilised within traps, and secondly, to evaluate male quality and sexual capacity of *Ae. albopictus* SIT strains.

For the first line of research, we have identified the olfactory gene repertoire of *Ae. albopictus* (odorant binding proteins and odorant receptors) (Dritsou et al. 2015). Through additional studies comparing male and female antennal transcriptomes, we intend to identify candidate genes that are transcribed preferentially in male mosquitoes. We will express the proteins in a heterologous system and identify the ligands/odorants that they bind using a panel of odorants encompassing a broad range of chemical classes. These will represent attractant candidates. Three dimensional structural studies may also permit the assessment of the docking potentials of different ligands/odorants *in silico* and to identify novel compounds that may act as strong, highly-specific attractants. Using appropriate traps, in collaboration with other interested parties, and in the presence/absence of other cues (CO_2, wing-beat frequency sounds etc), various formulations of these candidate odorants will be assessed in laboratory and semi-field conditions to assess their efficacy in attracting male (and female) *Ae. albopictus.*

For the second line of research, we have developed high-resolution molecular markers (microsatellites or SSRs and single-nucleotide polymorphisms, SNPs) for *Ae. albopictus* (Manni et al. 2015; Gomulski et al. in prep). These markers will be used to characterise SIT strains of *Ae. albopictus*. These genetic fingerprints will enable us to identify released males in captures and to assess the presence of their sperm in the spermathecae of captured wild females in the field. This will permit the assessment of the male quality parameters such as survival, dispersal capacity, insemination capacity and fertilisation success, and the effects of male chilling and irradiation on these parameters.

Research Contract 19048 (MAR):

CSI: Ambicadutt Bheecarry, Vector Biology and Control Division, Ministry of Health and Quality of Life, Mauritius.

Title: Improving the Surveillance of *Aedes albopictus* a Vector of Dengue, Chikungunya and other Diseases, with Emphasis on Male Population Monitoring – with View to Adjust Sterile Male Releases in a Strategy to Suppress the Vector Mosquito Through the SIT.

Summary: There is ongoing study on monitoring of adult populations of *Ae. albopictus* with emphasis on enhancing captures of males. Preliminary results show promise in increasing male trap captures by incorporating aromatic extracts of nine plants- mango, apple, strawberry, banana and others in combination with BG lure both in lab and field settings.

Further investigation includes large scale evaluation of promising plant extracts, and supplementing the BG Sentinel trap with sound cues (produced by a recently developed gadget, CAA/UNIBO (Balestrino et al, Italy)- to attract males *Ae. albopictus*

Research Contract 19044 (PHI):

CSI: Sotero Resilva, Philippine Nuclear Research Institute, Philippines.

Title: Development of Handling, Transport, Release and Trapping Methods of Dengue Mosquito Vector, *Aedes aegypti*, in Philippines.

Summary: A dengue mosquito rearing facility was established only in the last quarter of 2012 at the Philippine Nuclear Research Institute. The mosquito adults were reared on cubicle stainless steel cage housing approximately 5,000 adults. The present stock colony is already on the F29 and being used as source of test insects. Basic studies on quality control monitoring, irradiation and mating competitiveness are continuously being done in the laboratory. Future plans for the next 18 months of the CRP will focus on surveillance and population dynamic studies using different recommended mosquito traps. Flight dispersal studies of sterile male mosquitoes through mark recapture methods will also be conducted in the course of the CRP.

Research Contract 19099 (SAF):

<u>CSI:</u> Givemore Munhenga, Medical Entomology Research Group, Wits Health Consortium, South Africa.

<u>Title</u>: Improving Male Trapping for Anopheline Malaria Mosquitoes and Assessing Mating Performance of Adults after Chilling

<u>Summary:</u> Our group has demonstrated that locally produced African clay pots and modified plastic buckets can be adapted for collection of resting surface seeking wild male and female *An. arabiensis*. Preliminary results showed that these two collection techniques are effective in sampling both male and female wild *An. arabiensis*.

The aim of our contribution to this CRP is to develop and optimize trapping methods for collecting wild male *An. arabiensis*. This will be achieved by addressing the following objectives

- Test new outdoor collection methods for *Anopheles* mosquitoes
- Optimise outdoor collection methods by changing/adapting them to enhance collection success
- Determine the long term sustainability of adult male trapping methods

There are operational and logistical complexities of our developed tools as trapped mosquitoes can only be collected at limited times during a week thereby resulting in loss of mosquitoes through escape from the traps. Additionally, the pots are fragile and bulky and cannot be easily stationed in conspicuous localities where mosquitoes prefer to rest.

Research Agreement 19119 (SWE):

<u>CSI:</u> Jan O. Lundström. Biological Mosquito Control/Nedre Dalven Utveckling AB, Sweden

<u>Title</u>: Testing Male Sampling Strategies for *Aedes sticticus* in Sweden

<u>Summary:</u> Our work focuses on controlling the floodwater mosquito *Aedes sticticus* in Sweden including future plans for SIT. There is a general lack of surveillance methods for sampling male mosquitoes. Although male mosquitoes are observed during emergence, our results from several million mosquitoes collected with CDC-traps over the past 15 years showed that male mosquitoes were extremely rare in the collections. In preparation for future application of SIT against *Aedes sticticus* in Sweden, field surveillance method for males are needed. We are currently evaluating the MosVac mosquito aspirator (a battery operated portable suction device) as a promising new tool for sampling male mosquitoes outdoors.

Results from 2015: Preliminary evaluation shows increase in trap captures of males relative to females following evaluation on days 4, 15, 25 post-emergence while exclusively males were collected on day 1. However, the maximum number of males were captured on day 4 (n=80) and waned by day 25 (n=2). Further evaluation of the MosVac strategy to improve male sampling is required.

Research Contract 19059 (THA):

<u>CSI:</u> Pattamaporn Kittayapong, Go Green Co., Ltd., Thailand

<u>Title</u>: Development of the Moshouse Trap as a Surveillance Tool to Evaluate the Pilot Dengue Vector Control Trial Using Sterile Insect Technique in Thailand.

Summary: Kittayapong et al. has already developed the "MosHouse" traps which were used for surveillance and control of *Aedes* mosquito vectors in Thailand (Kittayapong et al. 2012). This adult trap will be further modified to collect more male mosquitoes. We will evaluate the modified model of MosHouse in semi-field settings. Eventually, we will use this modified model of MosHouse to collect longitudinal baseline data and to monitor *Aedes* vector populations during the pilot dengue vector control trial using sterile insect technique (SIT) at the selected field sites in Thailand.

Research Agreement 19057 (UK):

CSI: Gabriella Gibson

Title: Sound Trap for Males of the Malarial Mosquito *Anopheles gambiae* s.l.

Summary: The aim of this project is to develop a male mosquito trap baited with the 'sounds' of females of the target mosquito species, to provide a new tool with wich to monitor and control malarial mosquito populations outdoors. Our immediate objectives are to design and field test 'sound traps' based on the extreme attraction of male mosquitoes to the flight tones of con-specific females. We will discover the factors that optimise the responsiveness of male malarial mosquitoes (*Anopheles arabiensis, An. gambiae* s.s. and *An. coluzzii*) to a sound trap in the laboratory, and test the most promising models in collaboration with our colleague prof. Kounbobr Roch Dabire at IRSS, Burkina Faso, with the long-term aim of developing a novel mosquito sound trap.

Research Agreement 19112 (USA):

CSI: Szabolcs Marka, Columbia University in the City of New York (Collaborators: Imre Bartos, Zsuzsa Marka, ++)

Title: Enhancement of Mosquito Surveillance, Release, and Trapping through Acoustics, UAVs, Simulations, and SmartTraps

Summary: *Background:* The success or failure of vector control approaches depend strongly on accurate information on the local insect population and can be cost/benefit optimized if precise survey of the population is available through real-time monitoring of the population and the efficacy of intervention. Emerging technologies can enable accurate, cost effective, and robust monitoring of the necessary variables to enable new or improved approaches to vector control.

General aims: Our Team bring to bear Innovation, Emerging Technology, and Automation geared towards the development of vector surveillance and control tools. Our aim is to develop surveillance tools for mosquitoes and mosquito swarms.

Specifically, these new surveillance tools can contribute to the following activities, (a.) generating accurate information on local mosquito population, (b.) mark-release-recapture studies, (c.) real-time population models, and (d.) SmartTraps that are field compatible and can be used to determine parameters of the catch without human service visits at the trap.

6. Agenda

THIRD FAO/IAEA RESEARCH COORDINATION MEETING ON

Mosquito Handling, Transport, Release and Male Trapping Methods

12-16 November 2018, Juazeiro, Brazil

Moscamed

AGENDA

MONDAY, 12 NOVEMBER 2018

08:30 – 08:45 **Chao Tsu Chia, Comissao Nacional de Energia Nuclear (CNEN)**: Welcome statement and Opening remarks.

08:45 – 09:00 **Jair Virginio** and **Rafael Argilés:** Goals of the meeting. Agenda and administrative issues.

SESSION I: Mosquito handling, transport and release methods (Chairperson: Givemore Munhenga**)**

09:00 – 9:30 **Michelle Pedrosa**: Determination of conditions for handling and transport of chilled sterile adult males.

9:30 – 10:00 **Beni Ernawan**: Maintaining the quality of radiation induced sterile male: the effect of land transportation and possibility of aerial release.

10:00 – 10:30 **Assane G. Fall**: Cooling, transport and marking methods in *Aedes aegypti*.

COFFEE BREAK

11:00 – 11:30 **Hae-Na Chung**: Toward implementation of mosquito sterile insect technique: the effect of storage conditions on survival of male *Aedes aegypti* mosquitoes (Diptera: Culicidae) during transport**.**

11:30 – 12:00 **Bheecarry Ambicadutt**: Investigating different methods and cages for the ground transport of chilled adult sterile *Ae. albopictus* males to the field release sites and assessing the dispersal, survival and quality of released sterile males.

12:00 – 12:30 **Glenda Obra**: Development of handling, transport, release and trapping methods of Dengue Mosquito Vector *Aedes aegypti* in the Philippines.

LUNCH

13:30 – 14:00 **Antidio Viguria**: Title to be confirmed. Presentation by video-conference

14:00 – 14:30	**Roberto Angulo**: Advances in Mexico on aerial release of male adults *Aedes aegypti* mosquitoes with drones in pilot area.
14:30 – 15:00	**Rubén Leal Mubarqui**: Recent applications in insect release through the use of drones in Mexico.

COFFEE BREAK

15:30 – 16:00	**Rafael Argilés**: Release machine for ultralight Remotely Piloted Aircraft Systems: design, validation and regulation.
16:00 – 16:30	**Ignacio Plá**: Current status and progress of the SIT project against *Ae. albopictus* in Valencia (Spain).

TUESDAY, 13 NOVEMBER 2018

SESSION II: Male trapping methods (Chairperson: Hervé Bossin**)**

08:30 – 09:00	**Ludvik Gomulski**: Development of attractants for male trapping in *Aedes albopictus*
09:00 – 09:30	**Louis Clément Gouagna**: Evaluating new approaches for surveillance based on adult trapping and for studying key behavioural characteristics of *Aedes albopictus* males.
09:30 – 10:00	**Martin Geier**: Status of the development of low cost traps, sound lures, and remote monitoring devices for SIT.
10:00 – 10:30	**Pattamaporn Kittayapong**: Development of the MosHouse trap as a surveillance tool to evaluate the pilot dengue vector control trial using SIT in Thailand: comparison of *Aedes aegypti* populations during baseline and intervention periods.

COFFEE BREAK

11:00 – 11:30	**Roch Dabiré**: Natural habitats and Trapping systems of males of *Anopheles arabiensis* in relations to genetic control or SIT programmes
11:30 – 12:00	**Givemore Munhenga**: Improving male trapping for Anopheline malaria mosquitoes.
12:00 – 12:30	**Szabolcs Marka**: Swarms from understanding to mitigation (*Anopheles coluzzii*)
12:30 – 13:00	**Gabriella Gibson:** What exactly do mosquitoes hear? Presentation by video-conference

LUNCH

14:00 – 14:30	**Hervé Bossin**: Status of *Aedes polynesiensis* suppression trials in French Polynesia: advancing toward island-scale interventions.

14:30 – 15:00 **Jan Lundström**: Title to be confirmed

14:30 – 15:00 **Joël Gustave (observer):** Guadeloupe SIT project

COFFEE BREAK

SESSION III: General discussion (Chairperson: Rafael Argilés)

16:00 – 17:00 General Discussion

17:00 – 17:30 Selection of Working Groups (including Group Leaders)

17:30 **GROUP DINNER**

WEDNESDAY, 14 NOVEMBER 2018

SESSION IV: Results obtained and research gaps (Chairperson: Group Leaders)

08:30 – 10:30 Working Groups: Evaluation of results obtained since the last RCM and review of research gaps that need to be addressed.

COFFEE BREAK

11:00 – 13:00 Working Groups: Individual research proposal for the next period.

LUNCH

14:00 – 15:30 Working Groups: Review and adjustment of the logical framework matrix per groups.

COFFEE BREAK

16:00 – 17:30 Working Groups: Drafting of the report of the RCM.

THURSDAY, 15 NOVEMBER 2018

SESSION V: Workshop: demonstration of aerial releases using RPAS, including design of a flight mission, flight operation in fully autonomous mode and assessment of the quality of the released mosquitoes using the flight ability test device.

FRIDAY, 16 NOVEMBER 2018

SESSION VI: RCM report (Chairperson: Rafael Argilés and Group Leaders)

08:30 – 10:00 Presentation of the work done by the group on ‘Mosquito handling, transport and release methods’ to the rest of the participants.

COFFEE BREAK

10:30 – 12:00	Presentation of the work done by the group on 'Mosquito male trapping' to the rest of the participants.
12:00 – 13:00	Compilation of the report, discussion on location of final RCM and on the journal for the publication of the Special Issue with the results of the CRP.
13:00	Closure of the RCM

7. Participant abstracts

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: **Natural habitats and trapping systems of males of *Anopheles arabiensis* in relations to genetic control or SIT programmes**

AUTHOR (S): Dabiré K.R.[1], Sawadogo S.P.[1], Poda B.S.[1], Somda N.S.[1], Soma D.D.[1], Maiga H.[1], Diabaté A.[1], Gibson G.[2], Gilles J.[3]

ORGANIZATION:
[1]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso
[2]Nautral Resources Institute/University of Greenwich, Kent, ME4 4TB, UK
[3]Insect Pest Control Laboratory, FAO/IAEA, A2444 Seibersdorf, Vienna, Austria

SHORT SUMMARY OF PAPER

Abstract:

During the year 1 activities of the project performed in 2016 we compared the efficacy of clay pots, pyrethroid spray catch (PSC) to the collection of mosquitoes realised directly within *An. arabiensis* swarms in Dioulassoba, an urban district of Bobo-Dioulasso (Burkina Faso) where this species is predominating. The results showed that in total 488 mosquitoes (95.3% males *vs* 4.7% females) sampled from 12 swarms were identified as 100% of *An. arabiensis* males whereas from the 12 clay pots sampled near the swarming points 300 mosquitoes were trapped composed with 75.6% of males and 24.3% of females. Furthermore, the PSC method permitted to collect within human landing houses few mosquitoes (15) composed by 40% of males and 60% of females and 38 mosquitoes in non-inhabited houses. The main objective for the year 2 in 2017 was to test a trap conceived following the swarms attractive color trough the contact of visual markers. This trap is built according to the frame of tse tse trap but modified by introducing the main colour attracting male's mosquitoes and called tse tse like trap. This trap was then used to collect *An. arabiensis* mosquitoes in comparison with clay pots and another trap already tested in IRSS, the Lehman trap placed in the inner window of human dwelling houses from Dioulassoba. This report presented the preliminary data comparing the efficiency of five mosquitoes collecting methods: tse tse like trap, Lehman entry trap, clay pots collections, pyrethroid spray catch (PSC) and electric aspirators for outdoor collections. The main tendency is that the tsetse like trap allowed to collect more mosquitoes dominated by male's mosquitoes with more than 220 daily collected mosquitoes followed by clay pots (195 mosquitoes/day) and Lehman trap (155). PSC and electric aspirators collected relative low number of mosquitoes. The tree latest methods had collected more females than male's mosquitoes. The tsetse trap shows very promising results and needs to be used in extend localities at different seasons. It can be used both for surveillance and control perspectives.

Key words: tse tse like trap, clay pot, swarms, *An. arabiensis*, Burkina Faso

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: **Application of BG-sentinel baited trap with live mice and combination of CO2 with mouse-odour blend for evaluating the distribution and dispersal of *Aedes albopictus* males in Reunion Island**

AUTHOR (S): Gilbert Legoff[1], David Damiens[1], Jean-Sebastien Dehecq[2], Martin Geier[3], Louis Clement Gouagna[1]

ORGANIZATION : [1] Institut de Recherche pour le Développement (IRD), UM1-CNRS 5290-IRD 224: Maladies Infectieuses et Vecteurs – Ecologie- Génétique, Evolution et Contrôle (MIVEGEC), Montpellier, France ; [2] Service de lutte anti vectorielle, Agence Régionale de Santé Océan Indien (ARS-OI), Saint-Denis, Reunion Island, France ; [3] Biogents AG, Regensburg, Germany.

SHORT SUMMARY OF PAPER

In the search for a new attractant that could allow improving the catch rate of *Aedes albopictus* males in conventional trapping systems, early studies conducted in Reunion Island compared the catch rates of wild *Ae. albopictus* males and females in BG-Sentinel traps baited with live mice, mouse-derived odors and dry ice. After demonstrating that the use of live mice as bait improved BG-S trap efficacy, this system was used to study both the dispersal capacity of laboratory-reared and field-derived *Ae. albopictus* male specimens, and to routinely monitor *Ae. albopictus* populations at candidate sites for future SIT pilot trials. Although the use of BGS traps with mice as bait allowed collecting a relatively high number of mosquitoes, this solution did not seem the most practical for routine *Ae. albopictus* monitoring, mainly because of inherent constraints in mouse production. Following the modification of the existing BGS trap with improved features, the effectiveness of this new design, i.e. BGS2, for capturing *Ae. albopictus* adults was investigated in a standard Latin Square experimental design comparing live mice synthetic lure with or without carbon dioxide. This presentation will provide an account of the results obtained during these successive experiments aimed at improving the sampling male mosquitoes in BGS traps, as well as a brief overview of laboratory experiments carried out to test the appropriateness of using Rodhamine B marker to examine important behavioural characteristics of *Aedes albopictus* males.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: The BG-Counter, the first operative automatic mosquito counting device for remote mosquito monitoring.

AUTHOR (S): Martin Geier, Michael Weber, Ilyas Potamitis, Matthias Geismar, Pancraç Villalonga, João Encarnação, Andreas Rose
ORGANIZATION: Biogents AG

SHORT SUMMARY OF PAPER

Abstract:

Our research aims (1) to modify the existing BG-Sentinel trap design to lower significantly production costs, (2) to develop a low cost sound generator that imitates the wing beat frequency of females to improve the catching rates for male Aedes mosquitoes in passive traps, and (3) to develop remote counting devices that automatically detect, count and classify insects entering a trap and recognize disease vectors down to the species and sex level.

(1) We present the BG-MTS, a new **M**odular **T**rap **S**ystem that can be used similar to many different trap types such as the CDC trap, the EVS trap, the BG-Sentinel trap, or the CDC gravid suction trap. Due to the modular concept production costs of the plastic parts are significantly reduced and new trap configurations can be adapted to the specific monitoring task. For this trap we have also developed a new ventilator that requires very low power (about 1W compared to 3.2W of the Sentinel ventilator). In laboratory room tests the trap performed similar to a BG-Sentinel trap. The new trap will be available for extensive field testing in different regions soon. In addition to the MTS trap we have also developed a new low cost suction trap, the BG-Home, to control *Aedes aegypti* in households. This trap uses heat in addition to visual and chemical stimuli. In laboratory room tests the trap was as effective as a BG-Sentinel trap although it is much smaller and better suited for domestic use. This trap will be available in 2019.

(2) A prototype of a low cost sound generator has been developed and tested with passive BG-GAT traps in room bioassays in combination with different speakers. It turned out that the selection of the right speaker is key for the attraction of male *Aedes aegypti*. There is a clear correlation between the frequency response profile of the speaker and the behavioural response of male *Aedes aegypti*.

(3) We have built a novel 3-D optoaccoustic sensor that analyses the wing beat frequency of a mosquito while it is sucked into a trap. As mosquitoes fly through this sensor, biometric characteristics such as body size, wing shape, wingbeat frequency and wing movement patterns affect the recorded signal. These complex signals can be analyzed using machine learning algorithms or deep learning using convolutional neural networks (CNN) to assess mosquito species and sex. In a free flight situation in the laboratory we were able to discriminate 6 different species with a high precision. At the moment we are building prototypes that can be tested under field conditions next year.

Development of attactants for male trapping in *Aedes albopictus*

Ludvik Gomulski & Giuliano Gasperi
Department of Biology and Biotechnology, University of Pavia, Via Ferrata 9, Pavia, Italy

Abstract:

In the last RCM we described the different approaches that we used to identify potential attractants for trapping male individuals of *Aedes albopictus*. We described how we had collected the volatile emissions produced by individuals of each sex using a static Headspace Solid Phase Microextraction (HSSPME) and that we were analysing the emissions by Gas Chromatography coupled with Mass Spectrometry (GC/MS). Unfortunately, we have so far been unable to detect any significantly different levels of any component compared to the control for either sex at the different physiological stages. Different densities of individuals in different headspace chambers and a set of polydimethylsiloxane fibres were tested without positive results.

Future experiments to identify the volatile emissions will be performed using a dynamic headspace collection chamber and Porapak^{TM} Q (ORBO^{TM} 1103; Porapak^{TM} Q 50/80 mesh; 150/75 mg; Supelco, PA, USA) cartridges. This system will allow us to concentrate the extracted volatiles and thus potentially facilitate the identification of emitted compounds.

We have continued the dual-port olfactometer assays and have increased the number of replicates and the number of odorants assayed from a number of chemical classes. The odorants tested include those known to represent mosquito odorant ligands and components of a putative *Aedes aegypti* pheromone. The chemical compounds were tested at three/four different concentrations.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

Title of Working Paper: **Improving the collection of male *Ae. albopictus* mosquitoes in Mauritius**

Authors: Bheecarry Ambicadutt, Balestrino Fabrizio, Iyaloo Diana P., Elahee Khouaildi B.
Organisation: Vector Biology and Control Division, Ministry of Health and Quality of Life, Mauritius

Abstract

While SOPs were successfully developed in the laboratory for an optimal large-scale production, sex-separation and irradiation of *Ae. albopictus* males in Mauritius, it is equally important that practical transport and release methods of the sterile males from the laboratory in Curepipe to the pilot site in Panchvati (2 h drive) be devised with negligible or reduced effects on their fitness. This study hence investigated the effect of temperature and compaction on the survival and competitiveness of sterile males.

When longevity was assessed for 15 days, compaction (storing 2000 males in a Bugdorm cage (27 000 cm^3) or in a container (1062 cm^3)) and storage temperature (5, 10, 15 or 25ºC for 2 h) did not significantly affect the survival of sterile males. However, when longevity was further assessed over 25 days, the survival of sterile males stored in containers at 5°C, 10°C or 15°C, were significantly lower than control males stored in Bugdorm cages at 25°C (Kaplan-Meier: $P < 0.05$). Moreover, sterile males stored at 5°C survived significantly longer than those stored at 10°C or 15°C (Kaplan-Meier: $P < 0.05$). Considering the fact that sterile males will be released on a weekly basis in Panchvati and that average life expectancy of sterile males was estimated at 9 days during previous Mark-Release-Recapture studies in the village, the long-term negative effect on survival when males are stored at 5°C, may have limited relevance in the field. Furthermore, median survival of 3-day-old sterile males stored at 5°C was 24 days which is comparable or better than that obtained for adult *Ae. albopictus* males maintained under optimum temperature (25 to 30°C) in other studies.

Furthermore, compaction did not significantly affect the survival (assessed over 25 days) of sterile males when the latter were stored at 5ºC for 2 h at a density of 2000 males in cage/containers of three different volumes (1062, 8553 and 27 000 cm^3). However, the competitiveness of the males were significantly affected. At a storage temperature of 5°C, males have been stored in containers for 2 h tended to be less competitive (mean competitiveness index of 0.2) in semi-field competitiveness cages than those stored in the more spacious Bugdorm cages (mean competitiveness index of 0.4). While storing immobile males in the containers, it was observed that legs of *Ae. albopictpus* males tended to 'leg lock' with each other. The 'disentangling' process after the males become active at room temperature is suspected to be a major cause of the reduced fitness observed in the latter and should be further investigated.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

AUTHOR(S): Sotero S. Resilva, Glenda B. Obra, Abigail Mia Javier and Arvin Dimaano
ORGANIZATION: Philippine Nuclear Research Institute, Commonwealth Ave., Diliman, Quezon City, Philippines

TITLE OF WORKING PAPER: Development of Handling, Transport, Release and Trapping Methods of Dengue Mosquito Vector, *Aede aegypti* in the Philippines.

SHORT SUMMARY OF PAPER

Abstract:

Due to the very low activity of the existing gamma cell irradiator, only field activities such as evaluation of dengue mosquito traps and monthly monitoring of dengue mosquito population have been continuously done in Sitio Payong and Villa Beatriz experimental sites. A new gamma irradiator procured thru the IAEA-TC Project PHI5033 with counterpart funding from the Philippine Government will be operational by 2019.

The three trapping devices tested differed in performance regarding their efficacy to trap dengue mosquito vectors. Adult traps such as BG Sentinel and vacuum aspirator tested were less efficient and are not very sensitive for the detection of Ae. aegypti. There was a high number of emerged Ae. aegypti adults from immatures collected from OL traps from January 2016 to June 2018. A very low number of Ae. albopictus mosquitoes were collected on OL traps. Comparing the total number of captured mosquitoes by gender, almost similar number of male and females were collected by these three adult traps.

The mean percentage egg hatch from the two sites were similar. The proportions and similar patterns of high number ofAe. aegypti collected in OL traps in Sitio Payong were also observed in Villa Beatriz (control site). Gradual build ups and declines in dengue mosquito population were observed in the 30-month survey. The dengue mosquito population fluctuation indicates variation according to prevailing climatic conditions with high water rainfall occurring on rainy season causing stagnant water build up on drainage canals which become favorable breeding sites for mosquitoes.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE: Evaluation and optimization of clay pots for mosquito surveillance under a low malaria transmission setting

AUTHOR (S): Givemore Munhenga[1, 2], Leanne N Lobb[2] and Lizette L Koekemoer[2].

[1] Vector Control Reference Laboratory, Centre for Emerging, Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa.[2] Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

SHORT SUMMARY OF PAPER

Abstract:

Use of the sterile insect technique (SIT) as an additional vector control strategy to complement existing tools is under investigation in South Africa. As part of developmental activities to operationalise the SIT programme a low-cost entomological surveillance system capable of sampling both sexes of anopheline mosquito was developed. However, this outdoor-based population monitoring system consisting of African clay pots not fully addressing surveillance requirements needed for an anopheline mosquito SIT programme. Against this background and under the context of a Research Coordination Meeting on "Mosquito Handling, Transport, Release and Male Trapping Methods" our research group is optimizing the use of African clay pots as a mosquito sampling tool to increase their effectiveness. Over the past two years the effect of humidity, clay pot size and temperature on mosquito productivity were investigated. Experiments to increase mosquito catch retention were carried out. Lastly, evaluation on the effectiveness of clay pots against known acceptable standard mosquito collection methods (human landing catches (HLC), window exit traps and CDC light traps (CDC-LT)

Results showed that entry diameter and pot size has an effect on total catch productivity with more mosquitoes collected in smaller pots with narrow entry diameter. Use of adhesive lining inside the clay pots increased mosquito retention, however, the most effective adhesives such as Tanglefoot® and Tangle-trap® Sticky coating resulted in mosquito specimens losing critical morphological parts and or having these obscured making morphological identification challenging. Increasing humidity inside the pots with polyacrylamide beads soaked in water significantly improved mosquito catch productivity. Clay pots which are permanently stationed at the SIT pilot field site in KwaZulu-Natal collected 1,705 anophelines which were identified as members of either the *Anopheles gambiae* complex 1,259 (73.8%) or *Anopheles funestus* group 255 (15%). This information highlights the potential of African clay pots as an anopheline population monitoring tool.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: Development of the MosHouse Trap as a Surveillance Tool to Evaluate the Pilot Dengue Vector Control Trial Using SIT in Thailand: Field Application for Longitudinal Study of *Aedes aegypti* Populations

AUTHOR (S): Pattamaporn Kittayapong[1,2], Rungrith Kittayapong[1], Suwannapa Ninphanomchai[2] and Supaluk Khaklang[2]
ORGANIZATION: [1]Go Green, Co., Ltd., 2nd Floor, Science Building 2, Faculty of Science, Mahidol University at Salaya, Nakhon Pathom, Thailand; [2]Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University at Salaya, Nakhon Pathom, Thailand

SHORT SUMMARY OF PAPER

Abstract:

MosHouse traps were used for both surveillance and monitoring tools during the pilot *Aedes aegypti* population suppression trial in Chachoengsao Province, eastern Thailand. We collected the total numbers of 1,384 and 403 mosquitoes using MosHouse traps during baseline and intervention periods. During the baseline studies, we collected 79% of *Culex quinquefasciatus*, followed by *Ae. aegypti* (14%), *Ae. albopictus* (5%), *Tripteroides* spp. (1%), and 63% were females. During intervention period, we collected 67% of *Cx. quinquefasciatus*, followed by *Ae. aegypti* (23%) and *Ae. albopictus* (10%), and 52% were females. When considered only *Ae. aegypti*, a total of 195 were collected during the baseline studies, of which 59% were females. In contrast, a total of 91 were collected during intervention period and 55% was males. On average, a total of 13 *Ae. aegypti* was collected per month by using MosHouse trap during the baseline studies. The percentages of females collected per house were 2.63%, ranging from 0.99% to 4.00%; while those of males were 1.58%, ranging from 0.40% to 4.00%. Distribution of males and females seemed to follow rainfall; when precipitation increased, the numbers of both *Ae. aegypti* males and females collected in the MosHouse traps were also increased. During the intervention period, an average of 4 *Ae. aegypti* was collected per month, or 3.28% of males and 1.58% of females were collected per house by using MosHouse traps. Higer numbers of *Ae. aegypti* males were collected after the intervention when compared between treatment and control areas. This might cause by continuous release of sterile *Ae. aegypti* males which resulted in more collected male mosquitoes. However, lower numbers of *Ae. aegypti* females were collected due to the release of sterile *Ae. aegypti* males that induced sterility in the wild *Ae. aegypti* females resulting in reduction in *Ae. aegypti* female populations in nature, and hence, less females were collected in the MosHouse traps.

In conclusion, MosHouse traps could be used for collecting both male and female mosquitoes, during baseline and intervention periods of the pilot *Ae. aegypti* population suppression trial. Due to its convenience in handling and no complicated requirement, the MosHouse trap can be employed as a tool for monitoring mosquito populations when the intervention was in place. However, additional tools or methods for collecting mosquitoes should be applied in combination with the MosHouse trap in order to obtain a better output for entomological evaluation.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: Definition of parameters for mass transport of *Aedes aegypti*
AUTHOR (S): Michelle Pedrosa, Illaira Bandeira, Maylen Gómez Pacheco, Luiza Garziera, Jair Virginio, Margareth Capurro
ORGANIZATION: Moscamed Brasil and Universidade de São Paulo

SHORT SUMMARY OF PAPER
Abstract:

Transporting adult mosquitoes without compromising the insect quality is important for the application of the Sterile Insect Technique (SIT). However, for *Aedes aegypti*, the main vector of dengue, chikungunya and zika in Brazil, the conditions for the handling and transport of the chilling adult are not still established. In this context, the present study defined the optimal transport conditions by using the MBR-001 *A. aegypti* strain. Firstly, we determined the "knockdown conditions", evaluating three different range of temperatures (-3−0°C; 2−5°C; 6−9°C), and the duration thresholds for immobilization/handling of sterile male mosquitoes (10; 20; 30; 45; 60 minutes). In a second step the "transport conditions" were assessed being tested three different times (2, 4 and 6 hours). The biological parameters measured were: flight ability, survival under stress (without food for three days) and adult time recovery. In the last step, different mosquito compaction densities (75; 100 and 150 mosquitos/cm3) were evaluated, assessing the same parameters. Regarding the "knockdown conditions", the results showed that the three range temperatures immobilized the adults. However, the 6−9°C range promoted a partial immobilization of the insects. The flight ability rate (> 92.7%) was not affected at any range temperature tested or the duration thresholds of the incubated groups. The same pattern was observed for adult's survival (> 98.4 %). Therefore, we established for the insect knockdown the follow conditions: temperature range (2−5 °C; $\bar{X}$= 3.5 °C) and duration thresholds (45 ±15 minutes). Based on the results obtained in the first step, we used for the transport, a range temperature of: 4−7°C ($\bar{X}$= 5.5 °C). Flight ability (>98.0%) and survival (99.0 %) rates of the chilled sterile males were highest and did not affected by the different transport time tested. Regarding the compaction, the preliminary results showed that density compaction of 150 mosquitoes/cm^3 led the highest rate mortality after three days of incubation. The number of flight mosquitoes was higher than 89% in all chilled groups. Despite that complementary tests such as sexual competitiveness of the sterile males post-handling/chilling will need performed, the results suggest that the incubation temperature 2−5 °C during 45 minutes for the knockdown of insects, and transport at temperature range of 4−7 °C under compaction is feasible for *A. aegypti*. In addition, these results will contribute for the protocols of adults' sterilization and aerial release's (UAVs) of males under chilling conditions.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: Developing Wolbachia-based Population suppression mosquito control strategy in Guangzhou， China

AUTHOR (S): Zheng Xiaoying Qian Wei, Yangcui, Xi Zhiyong

ORGANIZATION: Sun Yat-sen University-Michigan State University Joint Center of Vector

SHORT SUMMARY OF PAPER

Abstract:

As a major vector for arbovirus diseases such as dengue, Chikungunya and Zika, Aedes albopictus is responsible for a huge burden to public health in China. Vector control strategies such as the sterile insect technique (SIT), the incompatible insect technique (IIT), or a combination of both techniques, are currently under development for mosquito control. Both the SIT and IIT are species-specific and environmental friendly approaches for vector control. Any large scale suppression program relies on continuous releases of a large numbers of competitive sterile males, thus developing efficient and effective approaches for transporting and releasing Ae. albopictus mosquitoes is essential.

To develop a high effective system to transport Aedes albopictus mosquitoes, we compared two potential approaches to transport mosquitoes at pupal and adult stages by chilling in laboratory. Various low temperatures have been tested to maintain pupae and adults to evaluate the impact of chilling on mosquitoes. We found that male pupae could be maintained at 18 Celsius for 20 hours without eclosion. No negative impacts were observed on the eclosion rate, adult longevity and mating competitiveness between the chilled and non-chilled male pupae. The recommended chilled temperature for male adults was 10 Celsius for 3 hours. No negative impacts were observed on the survival, longevity, and mating competitiveness between the chilled and non-chilled male adults.

To further investigate the impacts of adult chilling on population suppression efficiency in field trial, we selected two field sites to compare the suppression by using the chilled and non-chilled HC (triple Wolbachia-infected) males. Both chilled and non-chilled male adults were transported by vehicle to release sites and released in different part of tested areas. We found that no significant difference was observed on the suppression efficiency based on the evaluation of egg hatch rate and female adults in the released areas between the chilled and non-chilled HC males in both two tested field sites.

To improve the release efficiency, we have developed a drone with a capacity to release one million male adults per flight. Release method by using drone has been evaluated.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: Successful *Wolbachia* suppression trial against the polynesian tiger mosquito *Aedes polynesiensis* on the atoll of Tetiaroa, French Polynesia

AUTHOR (S): Hervé C. BOSSIN[1], Hereiti PETIT[1], Michel A. CHEONG SANG[1], Jérôme MARIE[1], Benoit STOLL[2]

ORGANIZATION:
1 Medical Entomology Unit, Institut Louis Malardé, Papeete, Tahiti, French Polynesia,
2 GEPASUD Laboratory, University of French Polynesia

SHORT SUMMARY OF PAPER
Abstract:

Aedes mosquitoes (principally *Ae. aegypti*, *Ae. polynesiensis*, and *Ae. albopictus*) severely affect the health and well-being of Pacific island communities by transmitting infectious diseases such as lymphatic filariasis (LF), dengue and chikungunya. Widely distributed across the Pacific, these vector species are also a major source of nuisance affecting local tourism, and the sustainability of Pacific island economies.

Ae. polynesiensis is an exophilic, day biting mosquito which uses a wide range of domestic and natural larval containers (e.g. rat-chewed coconuts), making classical control impractical, expensive and short lasting. A suppression trial was successfully implemented on Tetiaroa, a populated atoll in the Society islands, French Polynesia. The trial involved a public/private partnership between ILM, The Brando Resort and the non-profit Tetiaroa Society. The study investigated the efficacy of the incompatible insect technique (IIT) to suppress this naturally isolated mosquito population.

Entomological and environmental data were collected to characterize and monitor the dynamics of the *Ae. polynesiensis* mosquito before, during and after the IIT intervention. Over 3 million incompatible males were successfully produced, transferred and released at the 75 ha release site between 2015 and 2016. The sustained, inundative release led to the drastic suppression of *Aedes polynesiensis* as determined by adult trap data and ovitrap indices. Mosquito nuisance was almost undetectable for more than a year post-treatment. The target mosquito population ultimately recovered and male releases were resumed in October 2018 for sustainable nuisance control. In view of the success, ILM initiated a second trial on the Island of Taha'a (Society Islands) in partnership with Le Taha'a Resort and Air Tahiti (French Polynesia domestic airline). The extremely high *Aedes polynesiensis* mosquito density (up to 2,500 females/BG trap/d at certain sampling locations) recorded at this study site comes almost exclusively from mosquito larvae developing underground, inside burrows of the terrestrial crab *Cardisoma carnifex*. This contrasted entomological context provides an interesting challenge in which to test the limits of the IIT, and its potential integration with conventional control tools (e.g. source reduction, targeted larvicidal treatment).

Both ILM trials will highlight the benefits and constraints of genetic control strategies under operational conditions. Most importantly, these trials pave the way toward larger, island-scale interventions against this and other *Aedes* vector species (including *Ae. aegypti*). To this end, the government of French Polynesia will be investing next year in the construction of an

experimental male mosquito production facility. This facility will be equipped with an X-ray irradiator to compare the benefits of SIT, and the combined SIT/IIT for suppression of both *Aedes aegypti*, and *Aedes polynesiensis*. French Polynesia islands thus provide an attractive, robust and epidemiologically relevant framework in which to showcase novel production, transport, release and monitoring tools and methods, particularly the use of drones for aerial release.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: Maintaining the quality of radiation induced sterile male: the effect of land transportation and possibility of aerial release

AUTHOR (S): Beni Ernawan, Hadian Iman Sasmita

ORGANIZATION: Center for Isotopes and Radiation Application (CIRA)-National Nuclear Energy Agency (Batan), Indonesia

SHORT SUMMARY OF PAPER

Abstract:

Indonesia facing the fact that gamma irradiator facility does only exist in certain place, whilst the all Indonesia territory endemic for main dengue vector, *Aedes aegypti*. The main challenge of the sterile insect technique (SIT) program against *Aedes aegypti* is maintaining the quality of sterile male mosquito during handling, packaging and transportation. The recent study aimed to evaluate the optimal density of mosquito contained in the featured plastic cup with the goal increasing capacity. In this project, sterile (irradiated with gamma rays dose 70 Gy) male *Aedes aegypti* both in the pupal and adult stage were placed inside featured plastic cup (12 cm diameter; 8 cm high) with varies densities (25; 50; 100; 150;200) mosquito or pupa/cup then transported by land transportation. Then, assessed their mortality, emergence rate, longevity, sterility and mating competitiveness (Fried index). The result showed that there is no density-transportation effects on the emergence rate. It means that all of the pupa can be emerged into adult stage after transportation. On the mortality parameter, the result showed that the mortality have a tendency to increase in line with the high density. The mosquito mortality value were 13.33, 20.00, 38.80, 44.00 and 42.38 with the density 25, 50,100, 150 and 200 respectively. On the longevity and mating competitiveness index, the result showed that the date have a tendency to decrease on the increasing density. While on the sterility parameter, the data showed that there is no density-transportation effect. The sterility value was around 95%.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: **Release Systems using Single Engine Aircrafts for Mosquitoes**

AUTHORS: Leal Mubarqui R., Cano Perez R., Angulo Kladt R., Leal de la Garza

ORGANIZATION: Mubarqui Group, Mexico

SHORT SUMMARY OF PAPER

Abstract:

The pending issue in the matter of release and transport of adults of mosquitoes, has considerable progress based on the coordination reached with the Mexican entities dedicated to the campaign for the control of vectors of the Ministry of Health through the Regional Center of Research in Public Health, located in Tapachula, and the Mubarqui Group.

In coordination with the Institute, we have developed the irradiated sterile adult transport methodology, as well as improved the drone release device, during the tests we have achieved the calibration of the equipment and we have shown that there isn't any kind of damage caused by the release mechanisms.

The design of the transport device consists in a horizontal cage of 10 cm. h, 25 cm l, and 12 cm w., 3 litres, but is loaded just with one liter each one in order to be a layer of 3 centimeters high per cage, the cooling unit is preset to 6 ° C during transport from insectary to the release point.

The drone release machine container is a pipe shape tank, built in aluminium with no active chilling device, due the releases are always less than 12 minutes for 25 hectares and early in the morning.

Finally, through the use of the ground station, the flight pattern is designed, which ensures an accurate trajectory in the area of release.

The pilot project consist in two populations with same conditions and 4 kilometers separation, one of them is 26 hectares and the other is 28 hectares, in the first they are releasing by ground and in the second, we are releasing with the drone.

The project has just started in october 17 2018 so, the results of dispersion of marked adults, are not yet ready to present in this document.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: **Development of Aerial Release Systems for UAS using micro vibration devices.**

AUTHORS: Leal Mubarqui R., Cano Perez R., Angulo Kladt R., Leal de la Garza R.

ORGANIZATION: Mubarqui Group, Mexico

SHORT SUMMARY OF PAPER

Abstract:

Aerial release has been demonstrated to be the best way to release beneficial and sterile insects into the wild, the use of drones is no exception, in the last two years, fruit flies as *Ceratitis capitata* and *Anastrepha ludens*, parasitoids as *Diachasmimorpha longicaudata*, mosquitoes as *Aedes aegypti*, have been released and even the little parasitoid *Tamarixia radiata* in Mexico.

Drones are relatively inexpensive equipment to acquire and have a wide range of applications, loads and autonomy. In particular we will refer to commercial use drones as DJI Matrice 600 plus, since these are available in the civil market.
The drones of great size, cargo and autonomy, are exclusive for the use of the armed forces and although they would be more apt to carry out the release in large extensions, their use is restricted and their price is very high.

Regardless of this, small drones are efficient for short extensions per mission, up to 25 hectares for very specific application sites such as cemeteries, backyards, areas of difficult access and pilot projects in urban areas.
regulations in several countries, everyday restricts more the flights, mainly in payload deploy and flight altitude.

Anyway, important projects as human health are fields where is worth to take advantage of the ability of this tools and defend the use of them in the right applications.
The first issue in small size drones is the payload so release mechanisms has to be as small and light as possible, and autonomy has to be enough to carry out the release in maximum surface.

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: Progress achieved in the coordinated research project Mosquito Handling, Transport, Release and Male Trapping Methods - Senegal

AUTHOR (S): Gorgui Diouf, Assane G. Fall, Mamadou Ciss and Momar T. Seck
ORGANIZATION: Institut Sénégalais de Recherches Agricoles

SHORT SUMMARY OF PAPER

Abstract:

Experiments have been conducted to determine the impact of low temperatures, transport conditions and the marking techniques on male mosquitoes survival. The effects of cooling on pupae and adults were assess to determine the rang of temperatures to be use for mosquito transport. Thus, tests were performed on male pupae and adults exposed to different temperatures (2°C, 4°C, 6°C and 10°C) for different durations (1h, 2h, 4h and 8h). Immediate mortality was recorded after exposure in each box and the remaining pupae and adults were kept in cages at 25°C, 75 ± 5% RH, alternating light / darkness of 12 hours, fed with 10% sugar water solution and followed for 15 days to determine emergence rate and mortality rates.

Thereafter, the AcuTemp brand packaging, use for the transport of tse-tse fly pupae in the tse-tse eradication project in Senegal, was tested both for male Aedes aegypti pupae and adults. One frozen ice pack and two refrigerated phase change material packs (S8) have been used to stabilize the internal temperature of the isothermal box between 6-10 °C. Batches male pupae and adults of Aedes aegypti were placed in plastic boxes, put in the insulated boxes and closed hermetically. The different batches were exposed for 24h, 48h and 72h and stored after exposure at 25°C, 75 ± 5% RH, alternating light / darkness of 12 hours, fed with 10% sugar solution and followed for 15 days to determine emergence rate and mortality rates. Immediate mortlity rate and survival rate were calculated for exposed batches as well as the control one.

Finally, mosquitoes were marked by spraying fluorescent powder for adults and spreading of fluorescent powder in water for marking emergent mosquito from nymphs. The impact of the two techniques was assessed for 15 days for the survival of male mosquitoes. The temporal persistence of the powder on the body of the mosquito and the rate of colored mosquitoes were also calculated.

The results of this investigation will be presented at the third research coordination meeting on "Mosquito Handling, Transport, Release and Male Trapping Methods".

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER: Prototypes of a male only handling cage and a ground release machine

AUTHOR (S): PLA MORA, Ignacio
ORGANIZATION: EMPRESA DE TRANSFORACIÓN AGRARIA S.A. (TRAGSA)

SHORT SUMMARY OF PAPER

Abstract:

The expansion and establishment of *Aedes albopictus* in Spain is rapidly growing during the last years, especially on the Mediterranean coast, where most of the eastern and southern regions of the country have already been colonized.

In the Valencian Region, the governmental company Grupo Tragsa is applying for more than 10 years the SIT against *Ceratitis capitate*, Wied. on an area of more than 140.000 ha, being this project the most important at European level on the implementation of this technique.

Since 2013, we are working to get the SIT is a technically and economically viable option to fight against mosquito vectors of diseases such as the Asian Tiger mosquito. For this reason, we collaborate and are part of the working groups of different IAEA CRPs focused on this purpose.

In 2016, we began experiences at laboratory and semi-field levels related to the application of SIT against *Ae. Albopictus*. During that year, we acquired experience in breeding and developed necessary equipment for the development of mosquitoes under suitable conditions, the irradiation of male, the release of adults in the field, etc.

In April 2018 we started a pilot project to apply the SIT against the Asian Tiger mosquito in the Valencian Region, on a village of close to 50 ha. A nearby village, with similar area and also demographic and socio-cultural characteristics, acts as "control" in order to compare the results when this project finish in December 2020.

Parallel to the above, tests have been carried out with equipment designed for the development of SIT projects against mosquitoes in large areas. Both this equipment and the materials and tools used for the execution of the pilot project will be presented at the Third Meeting of the CRP "Mosquito Handling, Transport, Release and Male Trapping Methods" in Juazeiro (Brazil).

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

Preparing for SIT as part of an Integrated Control Strategy against *Aedes sticticus* in the River Dalälven floodplains, central Sweden.

Lundström JO[1,2], Blomgren E[1,2], Schäfer ML[1], Kittayapong P[3]

[1]Swedish Biological Mosquito Control, NEDAB, Vårdsätravägen 5, 75646 Uppsala, Sweden; [2]Zoonosis Science Center, Medical Biochemistry & Microbiology, Uppsala University, Box 582, 75123 Uppsala, Sweden; [3]Centre of Excellence for Vectors & Vector-Borne Diseases, Faculty of Science, Mahidool University at Salaya, Nakhon Pathom 73170, Thailand.

Abstract:

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Juazeiro, Brazil

12 - 16 November 2018

TITLE OF WORKING PAPER:

Toward Implementation of Mosquito Sterile Insect Technique: The Effect of Storage Conditions on Survival of Male *Aedes aegypti* Mosquitoes (Diptera: Culicidae) During Transport

AUTHOR(S): Hae-Na Chung,[1] Stacy D. Rodriguez,[1] Kristina K. Gonzales,[1] Julia Vulcan,[1] Joel J. Cordova,.[1] Soumi Mitra,[1] Christopher G. Adams,[3] Nathan Moses-Gonzales,[4] Nicole Tam,[5] Joshua W. Cluck,[5] Geoffrey M. Attardo,[5] and Immo A. Hansen,[1,3,6]

ORGANIZATION:

[1]Department of Biology, New Mexico State University, 1200 South Horseshoe Drive, Las Cruces NM 88003
[2]Institute of Applied Biosciences, New Mexico State University, 1200 South Horseshoe Drive, Las Cruces, NM 88003
[3]Department of Entomology, Michigan State University, 579 Wilson Road, East Lancing, MI 48824
[4]M3 Consulting Group, Unmanned Aircraft Systems Initiatives, 10009 Mallet Drive, Dayton, OH 45458
[5]Department of Entomology and Nematology, University of California, Davis, 1 Shields Avenue, Davis CA 95616
[6]Corresponding author, e-mail: immoh@nmsu.edu

SHORT SUMMARY OF PAPER

Abstract:

Sterile insect technique (SIT) is a promising, environmentally-friendly alternative to the use of pesticides for insect pest control. However, implementing SIT with Aedes aegypti (Linnaeus) mosquitoes presents unique challenges. For example, during transport from the rearing facility to the release site and during the actual release in the field, damage to male mosquitoes should be minimized to preserve their reproductive competitiveness. The short flight range (25-200m) of male Ae. Aegypti also requires elaborate release strategies such as release via Unmanned Aircraft Systems, more commonly referred to as drones, in order to increase their dispersal area. Two key parameters during transport and release are storage temperature and compaction rate. We performed a set of laboratory experiments to identify the optimal temperatures and compaction rates for storage and transport of male Ae. aegypti. We then conducted shipping experiments to test our laboratory-derived results in a 'real-life' setting. The laboratory results indicate that male Ae. aegypti can survive at a broad range of storage temperatures ranging from 7 to 28°C, but storage time should not exceed 24 h. Male survival was high at both low and high compaction rates, with mosquitoes surviving at a low compaction rate of 40 males/cm3. Interestingly, results from our 'real-life' shipping experiment showed that extremely high compaction rates (240 males/cm3) were beneficial to survival and preservation of reproductive competitiveness, especially in temperatures lower than 14°C. This study advances key understudied aspects of the practicalities of moving lab-reared insects into the field and lies the foundation for further studies on the effect of transport conditions on male reproductive fitness.
In this article, we explore optimal holding temperatures and compaction rates for Ae. aegypti males to gain information for future transport, storage, and drone-release protocols

Key words: *Aedess, Ae. Aegypti,* sterile insect technique, sterilization, temperature, compaction

THIRD RESEARCH COORDINATION MEETING

On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Jazeiro, Brazil

12-16 November 2018

TITLE OF WORKING PAPER: Swarms from understanding to mitigation (*Anopheles coluzzii*)

AUTHOR (S): Szabolcs Márka and Zsuzsanna Márka et. al.
ORGANIZATION: Columbia University in the City of New York et.al.

SHORT SUMMARY OF PAPER

Abstract:

Just a couple of years ago, Sub-Saharan Africa itself experienced close to half a million deaths from one of the most important vector-borne diseases, Malaria. This malady disproportionally damages the most vulnerable population, including pregnant mothers and small children. Depending on the region, a wide range of *Anopheles* variant are responsible for a significant fraction of the vector based transmission. While insecticide based interventions had been significant help, developing resistance to common classes of chemicals is an emerging problem. Novel survey and control tools that can complement the traditional repertoire of interventions receive more and more attention as it becomes clear that Malaria mitigation and elimination cannot succeed without advances in survey tools, mitigation tools, and sociological changes. Since several major Anopheles vectors of malaria are know to mate in swarms, surveying, understanding, and mitigating swarms and swarming behaviour is critical in breaking the transmission cycle of malaria and in suppressing vector populations. We will highlight advances in the field in West-Africa targeting *Anopheles coluzzii* swarms.

Scientific publications as an output of the CRP

- BALESTRINO F., PUGGIOLI A., CARRIERI M., BOUYER J., BELLINI R. (2017). Quality control methods for Aedes albopictus sterile male production. PLOS. https://doi.org/10.1371/journal.pntd.0005881

- BRIAN J. JOHNSON , SARA N. MITCHELL, CHRISTOPHER J. PATON, JESSICA STEVENSON, KYRAN M. STAUNTON, NIGEL SNOAD, NIGEL BEEBE, BRADLEY J. WHITE, SCOTT A. RITCHIE. (2017) Use of rhodamine B to mark the body and seminal fluid of male Aedes aegypti for mark-release-recapture experiments and estimating efficacy of sterile male releases. PLOS https://doi.org/10.1371/journal.pntd.0005902

- BUHAGIAR T. S., DEVINE G. J., RITCHIE S. A. (2017). Metofluthrin: investigations into the use of a volatile spatial pyrethroid in a global spread of dengue, chikungunya and Zika viruses. Parasites & Vectors2017 10:270 https://doi.org/10.1186/s13071-017-2219-0

- CULBERT N. J., LEES R. S., . VREYSEN M. J.B, DARBY A. C., GILLES J. R.L. (2017) Optimised conditions for handling and transport of male *Anopheles arabiensis*: effects of low temperature, compaction, and ventilation on male quality. Entomologia experimentalis et aplicata. https://doi.org/10.1111/eea.12610

- DAGMAR B MEYER, BRIAN J JOHNSON, KEN FALL, TAMARA S BUHAGIAR, MICHAEL TOWNSEND, SCOTT A RITCHIE. (2017). Development, Optimization, and Field Evaluation of the Novel Collapsible Passive Trap for Collection of Mosquitoes, *Journal of Medical Entomology*, tjx240, https://doi.org/10.1093/jme/tjx240

- DIEVAL, F., J. BOUYER and J.-F. FAFET (2017). An improved extraction method for surface dosage of insecticides on treated textile fabrics. Malaria Journal 16(1):14.

- FIKRIG K., JOHNSON B. J., FISH D., RITCHIE S.A. (2017). Assessment of synthetic floral-based attractants and sugar baits to capture male and female *Aedes aegypti* (Diptera: Culicidae). Parasites & Vectors2017**10**:32. https://doi.org/10.1186/s13071-016-1946-y

- HALL-MENDELIN S., HEWITSON G. R., GENGE D., BURTONCLAY P. J., DE JONG A. J., PYKE A. T., VAN DEN HURK A. F. (2017). FTA cards facilitate storage, shipment, and detection of arboviruses in infected Aedes aegypti collected in adult mosquito traps. The American Journal of Tropical Medicine and Hygiene, Volume 96, Issue 5, p. 1241 – 1243. DOI: https://doi.org/10.4269/ajtmh.16-0981

- HAWKES F. M., DABIRÉ R. K., SAWADOGO S. P., TORR S. J., GIBSON G. (2017) Exploiting Anopheles responses to thermal, odour and visual stimuli to improve surveillance and control of malaria. Scientific Reports volume 7, Article number: 17283 doi:10.1038/s41598-017-17632-3

- LE GOFF, G., DAMIENS, D., PAYET, L., RUTTEE, A. H., JEAN, F., LEBON, C., DEHECQ., J. S. GOUAGNA, L. C. (2016). Enhancement of the BG-sentinel trap with varying number of mice for field sampling of male and female *Aedes albopictus* mosquitoes. Parasites & Vectors, 9: 514.

- PEGUEDWINDE SAWADOGO S., NIANG A., BILGO E., MILLOGO A., MAÏGA H., DABIRE R. K., TRIPET F., DIABATÉ A. (2017). Targeting male mosquito swarms to control malaria vector density. https://doi.org/10.1371/journal.pone.0173273

- SIMÕES P. M. V., INGHAM R., GIBSON G., RUSSELL I. J. (2017). Pre-copula acoustic behaviour of males in the malarial mosquitoes Anopheles coluzzii and Anopheles gambiae s.s.does not contribute to reproductive isolation. Journal of Experimental Biology 2017 220: 379-385; doi: 10.1242/jeb.149757

- SIMÕES P. M. V., INGHAM R., GIBSON G., RUSSELL I. J.. (2018). Masking of an auditory behaviour reveals how male mosquitoes use distortion to detect females. Proc. R. Soc. B 2018 285 20171862; DOI: 10.1098/rspb.2017.1862.

8. List of participants

BRAZIL

Ms Michelle Christine PEDROSA
Biofábrica Moscamed Brazil
Av. C1, 992 – Quadra D13, Lote 15
48.908-000 Juazeiro
Bahia
Brazil
Tel.: 00 55 74 3612-5399
Fax: 00 55 74 3612-5118

BURKINA FASO

Mr Kounbobr Roch DABIRÉ
Institut de Recherche en Sciences de la Santé (IRSS)
399 Avenue de la Liberté
545 Bobo-Dioulasso
Houet
Burkina Faso
Tel.: +226 20981880
Fax: +226 20974868

FRANCE (RÉUNION)

Mr Louis-Clement GOUAGNA
Institut de Recherche pour le Développement (IRD)
Le Sextant – 44 Bd de Dunkerque – CS 90009
13572 Marseille Cedex 02
France
Tel.: +262 262528919
Fax: +262 262483353

FRENCH POLYNESIA

Mr Hervé BOSSIN
Institut Louis Malarde (ILM)
BP30
98713 Papeete
Tahiti
French Polynesia
Tel.: (689) 40 416 452 or (689) 40 533 156
Fax: (689) 40 532 873

GERMANY

Mr Martin GEIER
Biogents AG

Weissenburgstrasse 22
93055 Regenburg
Germany
Tel.: +49 941 5699 21 67
Fax: +49 941 569 921 68

INDONESIA **Mr ERNAWAN Beni**
Center for the Application of Isotope and Radiation Technology
Jalan Lebak Bulus Raya No. 49
12070 JAKARTA
INDONESIA
Email: beni_e@batan.go.id

ITALY **Mr Ludvik GOMULSKI**
Dipartimento di Biologia e Biotecnologie
Università di Pavia
Strada Nuova 63
27100 Pavia
Lombardia
Italy
Tel.: +39 382986435
Fax: +39 382986447

MEXICO **Mr Roberto Angulo KLADT**
Mubarqui Group
Blvd Enrique Cardenas Gonzales 1356
87040 Victoria
Tamaulipas
Mexico
Tel.: +52 8343164921

PHILIPPINES **Ms Glenda Obra**
xxxx

SENEGAL **Mr Assane GUEYE FALL**
ISRA-LNERV
2057 Dakar
Dakar-Hann
Senegal
Tel.: +33 8323679

SOUTH AFRICA **Mr Givemore MUNHENGA**
Medical Entomology Research Group, Wits Health Consortium
8 Blackwood
Parktown
2131 Johannesburg, Gauteng
South Africa

Tel.: +27 118855413
Fax: +27 113866481

SPAIN

Mr Luis Antidio VIGURIA JIMENEZ
Andalusian Foundation for Aerospace Development
(FADA-CATEC)
C/Wilbur y Orville Wright 19
41309 La Rinconada
Seville
Spain
Tel.: +34 954179002
Fax: +34 954115193

SPAIN

Mr Ignacio PLA MORA
Empresa de Transformacion Agraria S.A. (TRAGSA)
Centro de Control Biologico de Plagas
Poligono 9
Parcela 13
Paraje la Cantina
46315 Caudete de las Fuentes
Valencia
Spain
Tel.: +34 962319515
Fax: +34 962319521

SWEDEN

Mr LUNDSTROEM Jan
Vaxmyravägen 15
19444 UPPLANDS VÄSBY
SWEDEN
Email: jan.lundstrom@mygg.se

THAILAND

Ms Pattamaporn KITTAYAPONG
Go Green Co., Ltd.
2nd Floor, Science Building 2, Faculty of Science,
Mahidol University, 999 Phutthamonthon 4 Road
73170 Nakhon Pathom
Salaya, Phutthamonthon
Thailand
Tel.: +66 24410227
Fax: +66 24410227

UNITED KINGDOM

Ms Gabriella GIBSON
NRI University of Greenqich
Central Avenue
Chatham Maritime
Kent
ME4 4TB
United Kingdom
Tel.: +44 1634883457

Fax: +44 1634880077

UNITED STATES OF AMERICA

Ms Hae Nan
xxxx

UNITED STATES OF AMERICA

Mr Szabolcs MARKA
Columbia University in the City of New York, Physics Department,
538 West 120th Street
10027 New York
USA
Tel.: +1 2128543263
Fax: +2128543379

OBSERVERS

FRANCE (GUADELOUPE)

Mr Joel GUSTAVE
xxxxxx

MEXICO

Mr LEAL Ruben Luis
Mubarqui Group
Blvd. Enrique Cardenas Gonzalez 1356
87040 VICTOIA, TAMAULIPAS
MEXICO
E-mail: correoderl@gmail.com

UNITED STATES OF AMERICA

Ms Julia Vulcan
xxxx

BRAZIL

Ms Maylen Gómez
xxx

SCIENTIFIC SECRETARY

IAEA

Mr Rafael ARGILÉS HERRERO
Insect Pest Control Section
Joint FAO/IAEA Division. Vienna International Centre
1400 VIENNA
Austria
r.argiles-herrero@iaea.org