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

Co-ordinated Research Programme on

Mosquito Handling, Transport, Release and Male Trapping Methods

Instituto Valenciano de Investigaciones Agrarias (IVIA)
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1. Coordinated Research Project (CRP)

1.1. Title

Mosquito Handling, Transport, Release and Male Trapping Methods

1.2. Project

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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 unclamped 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 Mediterranean fruit fly involve 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 considered 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 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 fulfil 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 can collect 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

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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" (16-20 February 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 can 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 Increase in ratio of captured male:female	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.	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 present time intervals. Assess longevity, mortality and impact to fitness at present 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 regard 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 suitable for transport to the release site in	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

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. Development of a method/tool for	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.

	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	2nd RCM held.	Working material printed and distributed for 2nd RCM; Research published in	Research activities continue, progress satisfactory.

technical protocols as required		scientific literature and disseminated to member states and scientific community.	
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

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5. Agenda

SECOND FAO/IAEA RESEARCH COORDINATION MEETING ON

Mosquito Handling, Transport, Release and Male Trapping Methods

24 - 28 April 2017, Valencia, SPAIN

Instituto Valenciano de Investigaciones Agrarias (IVIA)
Moncada, Valencia, SPAIN

AGENDA

MONDAY, 24 APRIL 2017

09:00 – 09:15	**Rogelio Llanes,** Director General de Agricultura: Opening remarks and welcome statement.
09:15 – 09:30	**Nacho Pla** and **Rafael Argilés:** Goals of the meeting. Agenda and administrative issues.

SESSION I: Mosquito handling, transport and release methods (Chairperson: Nathan Moses-Gonzales**)**

09:30 – 10:00	**Michelle Pedrosa**: Definition of parameters for mass transport of *Aedes aegypti*.
10:00 – 10:30	**Beni Ernawan**: Maintaining the quality of radiation induced sterile male: the effect of land transportation and possibility of aerial release.

COFFEE BREAK

11:00 – 11:30	**Ignacio Plá**: Prototypes of a male only handling cage and a ground release machine.
11:30 – 12:00	**Roberto Angulo**: Release systems using single engine aircrafts for mosquitoes.
12:00 – 12:30	**L.A. Viguria**: Current status and insight of future approach of UAS civil regulation.
12:30 – 13:00	**Nathan Moses-Gonzales**: Advancements in Unmanned Aircraft Systems for use in Sterile Insect Technique applications.

LUNCH

14:00 – 14:30	**R. Leal Mubarqui**: Development of Aerial Release Systems for UAS using micro vibration devices.
14:30 – 15:00	**Hervé Bossin**: Successful *Wolbachia* suppression trial against the polynesian tiger mosquito *Aedes polynesiensis* on the atoll of Tetiaroa, French Polynesia.

15:00 – 15:30 **Assane G. Fall**: Progress achieved in the coordinated research project 'Mosquito Handling, Transport, Release and Male Trapping Methods', Senegal.

COFFEE BREAK

16:00 – 16:30 **Szabolcs Marka**: title of the presentation to be confirmed.

16:30 – 17:00 **Jan Lundström**: Preparing for SIT as part of an integrated control strategy against *Aedes sticticus* in the river Dalälven floodplains, central Sweden.

17:00 – 17:30 **Zheng Xiaoying**: Developing a *Wolbachia*-based population suppression mosquito control strategy in Guangzhou, China.

TUESDAY, 25 APRIL 2017

SESSION II: Male trapping methods (Chairperson: Martin Geier**)**

09:00 – 09:30 **Brian J. Johnson**: Recent advances in male sound-lures for *Ae. aegypti* and the development of a novel marking technique for male-based mark-release-recapture experiments.

09:30 – 10:00 **Ludvik Gomulski**: Development of attractants for male trapping in *Aedes albopictus.*

10:00 – 10:30 **Louis Clément Gouagna**: 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.

COFFEE BREAK

11:00 – 11:30 **Martin Geier**: The BG-Counter, the first operative automatic mosquito counting device for remote mosquito monitoring.

11:30 – 12:00 **Pattamaporn Kittayapong**: Development of the MosHouse trap as a surveillance tool to evaluate the pilot dengue vector control trial using SIT in Thailand.

12:00 – 12:30 **Sotero S. Resilva**: Development of Handling, Transport, Release and Trapping Methods of Dengue Mosquito Vector, *Aedes aegypti* in the Philippines.

12:30 – 13:00 **Dabiré K.R**: Natural habitats and trapping systems of males of *Anopheles arabiensis* in relation to genetic control or SIT programmes.

LUNCH

14:00 – 14:30 **Givemore Munhenga:** Evaluation and optimization of clay pots for mosquito surveillance under a low malaria transmission setting.

14:30 – 15:00 **Gabriella Gibson:** title of the presentation to be confirmed.

COFFEE BREAK

SESSION III: General discussion (Chairperson: Rafael Argilés)

15:30 – 16:30 General Discussion

16:30 – 17:00 Selection of Working Groups (including Group Leaders)

17:00 **GROUP DINNER AT EL PALMAR**

WEDNESDAY, 26 APRIL 2017

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

09:00 – 10:30 Working Groups: Results obtained and review of research gaps that need to be addressed.

COFFEE BREAK

11:00 – 13:00 Working Groups: Continued review of results obtained and research gaps that need to be addressed.

LUNCH

14:00 – 15:30 Working Groups: Continued review of results obtained and research gaps that need to be addressed.

COFFEE BREAK

16:00 – 17:30 Visit to the mosquito insectary in IVIA.

THURSDAY, 27 APRIL 2017

SESSION V: Review of the individual proposals gaps (Chairperson: Rafael Argilés and Group Leaders)

09:00 – 10:30 Working Groups: Review of individual research proposals for the different working areas.

COFFEE BREAK

11:00 – 13:00 Working Groups: Review of individual research proposals for the different working areas.

LUNCH

14:00 – 15:30 General discussion group on 'Mosquito handling, transport and release methods': review of individual research proposals for the different working areas.

COFFEE BREAK

16:00 – 17:30 General discussion group on 'Mosquito male trapping': review of individual research proposals for the different working areas.

FRIDAY, 28 APRIL 2017

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

08:30 – 09:00 Review and adjustment of the logical framework matrix.

09:00 – 10:00 Agreement on content of the RCM report, and drafting and compiling of the RCM report.

10:00 – 10:30 Agreement on information exchange mechanisms, on location of 3rd RCM, and closure of the RCM

COFFEE BREAK

11:00 – 14:00 Visit to the medfly mass-rearing facility in Caudete de las Fuentes

6. Participant abstracts

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

TITLE OF WORKING PAPER: **Recent advances in male sound-lures for *Ae. aegypti* and the development of a novel marking technique for male-based mark-release-recapture experiments.**

AUTHOR (S): Brian J. Johnson[1], Tim Prachar[2], Kyran Staunton[1] and Scott A. Ritchie[1]
ORGANIZATION: [1]Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia. [2]Verily Life Sciences, South San Francisco, CA 94080.

SHORT SUMMARY OF PAPER

Abstract:

Recent interest in male-based Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT) programs to control *Aedes aegypti* and *Aedes albopictus* populations has revealed, among many things, a need for **1)** the development of novel lures to enhance male monitoring before, during and post release, and **2)** economical, rapid diagnostic tools for determining dispersion and mating success of sterilized males in the wild. Our report highlights advance in both areas, specifically the development of an affordable, long-lasting sound lure that enables passive collection of male *Ae. aegypti* and the development of a novel marking technique for male-based mark-release-recapture (MRR) experiments.

Last year, our team presented on the successful exploitation of male attraction to female flight tones to passively capture male *Ae. aegypti* by the addition of a sound lure (484 Hz, 60dB) to Gravid *Aedes* Traps (GAT). Although successful in both semi-field and field trials, the lure was expensive (>$20), drew too much power for long-term surveillance, and did not easily allow for the incorporation of multiple flight tones or playback strategies. To overcome these issues, we have developed an Arduino-based sound lure that is affordable, long-lasting (>5 months, 3 AA batteries), and highly programmable. Recent field trials conducted in Cairns, Australia revealed the Arduino lure to be as effective as the previous lure and increased male collections in GATs to be comparable to those in concurrently operated Biogents Sentinel traps. Additional results on playback strategies will also be discussed.

In addition to a general lack of male sampling methods, there is a need for rapid diagnostic tools for determining dispersion and mating success of sterilized males in the wild. Previous reports from other insects indicated rhodamine B, a thiol-reactive fluorescent dye, administered via sugar-feeding can be used to stain the body tissue and seminal fluid of insects. We have recently adapted this technique for male *Ae. aegypti* with no significant impact on male survival or mating competitiveness and small-scale field releases in Cairns, Australia have demonstrated successful transfer of marked seminal fluid to wild females, thus allowing for the direct measure of competitiveness (mating success) of released males. These results reveal rhodamine B to be a potentially useful evaluation method for male-based SIT/IIT control strategies without the negative side-effects of traditional marking methods.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

The male population's surveillance systems are a crucial need which allows to monitor the male's populations to be released in the field for SIT or GMO prospects. That is possible if basic studies are performed on male ecology from swarming systems to resting habitats. But only few data are available for *An. arabiensis* that is the targeted species by SIT programme. The current study aimed to test different tools able to monitor *An. arabiensis* males' populations. The different methods included males sampling from swarms to the clay pot trapping systems.

Males of *An. arabiensi*s populations were monitored in Dioulassoba, a district of Bobo-Dioulasso city in Burkina Faso (West Africa) during 10 consecutive days in December 2016. The swarming places and markers were mapped and the male mosquitoes were sampled within the swarms. Then twelve clay pots previously wetted in their inner linings were placed 2 to 5 meters apart from the swarming places indoor and outdoor human courtyards and also within non-inhabited houses (abandoned houses). They were visited each morning from 6 to 9 am to collect all mosquitoes resting. In parallel indoor spraying catches (PSC) were performed during into 10 concessions thorough the swarming sites. Ten outdoor resting sites such as domestic wastes, outdoor compiled stones and wood piles were also sampled using electric aspirators. The data were then compared to evaluate the more efficiency collection method compared to the swarm samples.

The preliminary 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. Clay pots put within courtyards (outdoor) permitted to collect 79 mosquitoes reaching 82% of males' *vs* 18% of females while only 21 mosquitoes were sampled indoor with 43% males for 57% 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 other outdoors resting sites yielded 57 mosquitoes with 75% of males.

The clay pot trapping method showed very promising results that can complete the swarm sampling technique to track male populations in the field. However the results are very preliminary and needed to be extended the second year to confirm the main tendency.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain, 24-28April2017

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

Improved methods to better estimate *Aedes albopictus* male population size or monitor their populations are crucial in order to calibrate the releases of sterile males into wild populations and to evaluate the efficacy of SIT interventions. Among currently available mosquitoes sampling devices, the most commonly used for *Aedes albopictus* (and *Aedes aegypti*) has been the BG-sentinel (BGS) traps, which have been shown to provide consistent captures of females of several mosquito species in areas where they thrive. As part of the FAO/IAEA Co-ordinated Research Programme (CRP) on "*Mosquito handling, transport, release and male trapping methods*" we have proposed (1) to improve and evaluate through field surveys the effectiveness of old and newly developed prototype trapping tools for sampling focal male populations, and (2) to validate the accuracy of the most effective male trapping systems in Mark-Melease-Recapture (MRR) experiments utilizing both wild and laboratory-produced males in La Reunion Island. In a preliminary study, we have demonstrated that the use of BGS traps baited with varying number of live mice could significantly increase their efficiency to sample more *Ae. albopictus* males than BGS traps with the BG-Lure alone. BGS baited with 2-3 mice appeared to work better in situation of very low mosquito density. Additionally, the use of similar mouse-baited BG-sentinel traps during MRR experiments allowed accurate estimation of *Ae. Albopictus* population density and its seasonal fluctuation.

These finding prompted further development and investigations into the effect of new attractants derived from mouse odour blend, possibly in association with a CO2 source, on the performance of BGS for estimating *Ae. albopictus* adult male abundance in the wild. Subsequent field study was recently carried out in which different baits including live mice, a novel mouse odour-blend derived from litter pellets and CO2 were used for field sampling of male and female *Aedes albopictus* mosquitoes. An experiment using a Latin square design was undertaken in one candidate field-testing site previously selected for pilot release of sterile *Ae. Albopictus* males on La Reunion Island. Four types of attractant were tested in two sets of 4 baited BGS traps: (1) the generic BG-Lure, the live bait consisting of three mice and (2) CO2 alone in the form of dry ice, CO2+ litter material, CO2+ lure from litter odor absorbed on porous polypropylen pellets. The presentation will provide detailed results on the differential capture rates of *Ae. albopictus* males recorded both for each of the baited BG-sentinel trapping trials and MRR experiments, with an emphasis given to practical application of these finding, relating specifically to vector population monitoring in SIT planning, implementation and evaluation.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

As part of an autonomous mosquito trapping station, the BG-Counter automatically differentiates captured mosquitoes from other insects, counts them, and wirelessly transmits results to a cloud server for further analysis. Build-in sensors continuously collect and transmit environmental data such as temperature, relative humidity and ambient light intensity. The location is logged using GPS. The user can display and analyse the collected data in real-time on a cloud-based dashboard accessible from PCs, tablet, and smart phones. The data can also be exported to Excel. The dashboard provides data review and management capabilities for multiple devices, including remote set-up of measurement windows and the controlled release of attractants such as CO2. Vector control professionals can now follow the mosquito situation with unprecedented data density, overcoming constraints associated with the laborious manual setting and retrieval of traps and counting of trap contents.

At the moment the BG-Counter cannot differentiate between male and female mosquitoes, but we are working on advanced versions of the BG-Counter, which are able to do so.

In our presentation, we describe the technical background of the BG-Counter and show examples of data sets generated in the field. We also present initial results from the advanced version of a BG-Counter with lab recordings of *Aedes aegypti* males and females. These signals allow a clear differentiation based on the recorded wingbeat frequency spectrum.

The development of the BG-Counter was partly supported by the EU's 7th Framework Programme (grant 306105, acronym MCD), the continuation of the development is being supported by the EU's Horizon 2020 programme (grant 691131, acronym REMOSIS).

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:

Three independent approaches are currently being attempted to identify attractants for trapping male individuals of *Aedes albopictus*. Firstly, as part of a larger transcriptome study to identify mating and blood-feeding-responsive genes in the antennae of *Aedes albopictus*, the antennal transcriptomes of virgin male and female individuals were compared. Considering transcripts that showed at least 2 fold differences in expression (and an FDR of 1%), 1284 transcripts were differentially expressed in the male and female antennae. Among the 528 up-regulated transcripts in the female antenna, the most abundant gene ontology category was related to odorant binding, including 22 odorant binding proteins and 54 odorant receptors. In the male antennae, 756 transcripts were up-regulated, none of which were apparently related to chemoreception. Our initial rationale was to identify chemosensory genes up-regulated in male individuals, express and purify the corresponding proteins and to determine their ligands, which would then be tested for their attractive properties.

Secondly, using a dual-port olfactometer we are assaying a number of odorants from a range of chemical classes, to identify potential male attractants.

Thirdly, we have collected the headspace emissions produced by individuals of each sex and we are currently analysing the components of these emissions. One or more of these components may represent potential attractants.

SECOND RESEARCH COORDINATION MEETING
On 'Mosquito Handling, Transport, Release and Male Trapping Methods'
Valencia, Spain
24 – 28 April 2017

<u>Title of Working Paper</u>: **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

Monitoring the abundance, distribution and movements of wild fertile males is a fundamental requirement for the successful management of the *Aedes albopictus* control programme integrated to an Sterile Insect Technique (SIT) component. Most of the existing traps wilfully attract the female adults. Effective traps to monitor non-biting males are practically rare or were less investigated. Studies were conducted to improve the capture of adult male *Ae. albopictus* in the field.

In one of the studies, the response of *Ae. albopictus* males to various sound stimuli produced with different volumes and frequencies in association with visual cues, were evaluated within laboratory conditions at the Medical and Veterinary Entomology Department, Centro Agricoltura Ambiente, Crevalcore, Italy. While the production of modulated frequencies continuously varying within the typical female sound emission range (500–650 Hz) showed the best results for a sound pressure level between 75 and 79 dB measured at the speaker level, the black colour of the trap used also seemed important in attracting males in the vicinity of the sound traps. Subsequently, as a second part of this study, a plastic sound trap prototype capable of producing the most effective sound stimuli within the laboratory of the Vector Biology and Control Division, Mauritius, were evaluated in Notre Dame, a rural village in Mauritius and demonstrated a good *Ae. albopictus* males' attractiveness in the field despite a reduced use of colour stimuli.

In another study, BG Sentinel^{TM} traps baited with BG Lure^{TM}, were visually modified into four different black-and-white color combinations and their trapping efficacy were evaluated in three rural (Notre Dame, Panchvati and PDL) and one urban (Curepipe) localities in Mauritius. The impact of trap colour and trap location on *Ae. albopictus* collection differed among localities. In the rural localities, completely black BGS traps collected significantly more *Ae. albopictus* males than the other traps (*Post hoc* Tukey tests, $P < 0.05$) while in Curepipe, no significant difference was noted among the latter (*Post hoc* Tukey tests, $P > 0.05$). Furthermore, in Notre Dame, completely black BGS traps collected significantly more *Ae. albopictus* females than the other traps (*Post hoc* Tukey tests, $P < 0.05$) while in the other localities no significant difference in trap performance was noted (*Post hoc* Tukey tests, $P > 0.05$).

Studies are currently underway to assess the competitiveness, dispersal, survival of radio-sterilized males and their transportation to the pilot sites, including packaging at different temperatures.

Second Research Co-Ordination Meeting of the Co-Ordinated Research Project on "Mosquito Handling, Transport, Release and Male Trapping Methods (D4 40 02)"
24 – 28 April 2017,
Valencia, Spain
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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:

Three different traps were compared their effectiveness to collect mosquitoes to be used as monitoring tool for surveillance of dengue mosquito vector population in Sitio Payong (pilot site). Traps evaluated in the study included the BG Sentinel trap, Ovicidal/Larvicidal (OL) trap and the Vacuum aspirator. *Aedes aegypti* and *Culex quinquefasciatus* were the only two mosquito species collected and identified using the three monitoring devices. OL trap out-competed the two other devices by trapping more *Ae. aegypti* from January to December, 2016. Both BG Sentinel and Vacuum aspirator devices are more specific and caught mostly of the *Culex* species.

The relative abundance of dengue mosquitoes was studied in Villa Beatriz (control site) by continuous trapping using the OL traps. High dengue mosquito population density was also observed in the control site. Gradual build ups and declines in dengue mosquito population were observed in the 12-month survey. Two population peaks occurred during the year, one at the start of the rainy season between May and June, and the other one in August and September. The dengue mosquito population fluctuation indicates variation according to season. The peaks in dengue mosquito population coincided on the prevailing climatic conditions with high water rainfall occurring from May to October causing stagnant water build up on drainage canals which becomes favorable breeding sites for mosquitoes.

Initial findings on dye retention and survival of sterile male mosquito adults in field cages revealed that the test insects was not greatly affected by marking method were only 39 – 53 % of individual males died in all treatments after 7 days. In addition, fluorescent dust applied on sterile male mosquitoes remain intact on their bodies after corresponding days.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

As South Africa moves from malaria control to elimination there was a need for complementary vector control strategies capable of targeting outdoor biting vector populations which are now responsible for the on-going residual malaria transmissions. The sterile insect technique (SIT) targeting *An. arabiensis* was selected as potential interventions which can target both the outdoor and indoor biting populations. Feasibility studies on applicability of this technique were initiated and are under way. Since the inception of SIT feasibility studies a number of key aspects central to the successful implementation of a mosquito SIT programme are under investigations. One such aspect under development and falls within the framework of this CRP is the development of mosquito sampling tools for pre and post release population monitoring in an area targeted for releases.

As part of CRP objectives a mosquito surveillance system consisting of two novel trapping methods: traditional African clay pots and modified plastic buckets were set up in Mamfene, northern KwaZulu-Natal a site targeted for SIT pilot releases. Two year mosquito population surveillance has shown that these sampling tools are effective in collecting mosquitoes outdoor and indoor as well as male and female mosquitoes compared to traditional methods which are being used by the National Malaria Control Programme. During the surveillance period clay pots produced the greatest numbers of *An. arabiensis* catches (n = 960) compared to modified buckets (n = 221), and window exit traps (n = 170). Despite clay pots effectiveness in collecting mosquitoes they are not yet fully addressing the surveillance requirements for an SIT programme and are facing a number of operational challenges. As part of our CRP objectives experiments to optimize use of clay pots as a mosquito sampling tool were suggested. Preliminary results of lining clay pots with glue panels and or coating with insecticide providing a "trap and kill" mechanism increased their mosquito collection productivity by 10%. Despite progress made in optimising clay pots as a mosquito surveillance tool, they are yet to be evaluated against other known standard mosquito collection methods. During the next 18 months experiments to evaluating effectiveness of clay pots against CD light traps, window exit trap and human landing catches will be carried out under field settings.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

First, we evaluated the efficacy of an original MosHouse in trapping *Ae. aegypti* males and the proportion of male and female trapped within MosHouse, both indoor and outdoor, during field applications. Our results showed that MosHouse can collect several species of mosquitoes including *Ae. aegypti*, i.e., 38% out of 13,684 mosquitoes collected in Chachoengsao, and 36% out of 1,089 mosquitoes collected in Nakhon Sawan. In Chachoengsao, it was found that 23.49% (n=5,172) of *Ae. aegypti* collected were males, while only 15.09% (n=391) of *Ae. aegypti* males were collected in Nakhon Sawan of which 74.58% (n=59) were collected indoor.

The efficiency of MosHouse applied as a surveillance tool to study longitudinal populations of *Ae. aegypti* was evaluated in Chachoengsao, eastern Thailand. The sticky panels inside each MosHouse were collected monthly for one year to check for trapped mosquitoes. Collection of the remaining mosquitoes in households and surrounding environment were conducted using locally made portable vacuum aspirators (MosVac). Results showed that both *Ae. aegypti* males and females could be collected all year round by using both MosHouse and MosVac. However, significantly higher number of males ($p<0.05$) could be collected from MosHouse during the dry months (Dec.-May) when compared to those from the wet ones (Jun.-Nov.). However, females showed an opposite trend as significant higher number of females ($p<0.05$) were collected in the wet season when compared to those from the dry ones. It is possible that our MosHouse trap may provide more favorable humid environment for *Ae. aegypti* males during the dry season.

In conclusion, MosHouse could be used to collect various species of adult male and female mosquitoes including *Ae. aegypti* in the field. Therefore, it can be considered as an alternative eco-friendly mosquito trapping tool especially when applied in households.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

For the application of autocidal control, having a facility near each release area is not always possible or economically feasible. Therefore, it is necessary to transport the produced males to the treated areas. Regarding this, Moscamed Brasil implemented an efficient Aedes aegypti pupae transport methodology. This method was tested for pupae transport of up to 4 hours and in addition, which requires the instalation of an adult emergence center in the release area. If adult transport is feasible, it would be a great gain for the application of this control strategy. Two biossays are currently being carried out with MBR-001 Ae. aegypti strain: 1) pupae transport and 2) adult transport. For both, different temperatures and incubation time (to simulate the transport), for all biossays, a "plastic jewelry box" with wells for packaging was used. In the first experiment, adult males were knocked out and (~1000) were packaged in filter paper niches. The niches were inserted into of C-25. The incubations time was 2, 4, 8, 12 and 24 hours at low temperature (1, 2, and 3°C) to keep mosquitoes asleep. After the incubation period, mosquitoes were discharged into Bugdorm® cage and received sucrose solution (10%) as food for 24 hours. The adult mortality was assessed every day, for five days. We carried out test to evaluate the minimum lethal temperature for the pupa transportation, and the maximum temperature supported without emergence. Approximately 1000 males pupae were placed in each wells with water. The groups were incubated for 16, 18, 20 and 22 h at temperatures between 8 to 18°C. After the incubation period, pupae mortality and emergence rate were evaluated. The newly emerged adults were fed with 10% sucrose solution, the containers were drained, and the mortality was evaluated. Twenty-four hours later, the survivors were released in cages and were fed a sugar solution for 24 hours. The evaluation mortality was similar to the one found with the adults transported. The first results showed that the extreme temperatures tested (8 and 18 °C) provoked a larger mortality of pupae (5% and 3.6%, respectively) than the temperatures of 12°C (1,5%) and 14 °C (0.8%), while the mean mortality in the control groups was 0.3%. About the adults transported, the preliminary results reveals that the initial mortality of adults (24 hours) incubated at 3 °C (2, 2, 2, 12 and 9%) was lower than those incubated at 1 °C (21, 51, 67, 50 and 100%) and 2 °C (16, 8, 6, 32 and 42%) regardless of the time in which they remain incubated (2, 4, 8, 12 and 24 hours respectively). The control groups have a mortality rate of less than 1%. The time of incubation appears to be related to temperature, since 100%, 42 e 9% of mosquitoes incubated during 24 hours died in first day of evaluation and at 1, 2 and 3 °C, respectively. Although further tests will still be performed, preliminary results indicate adult transport may be possible and pupae transport during longer periods is feasible.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

TITLE OF WORKING PAPER: Developing Wolbachia-based Population suppression mosquito control strategie 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:

Aedes albopictus is main vector of Dengue in mainland China. Currently, there is no drug therapy or vaccines available to combat these diseases. "Vector control" (VC) is the primary method of intervention where such activities as habitat modification and spraying insecticides are used. However, certain aspects of the VC method are questioned, especially as it relates to the use of insecticides and the negative impact on the environment and a growing resistance from the mosquitoes to the chemicals. Novel natural control strategies are proposed through the use of the endosymbiotic bacterium Wolbachia. One of such methods is referred to as "population suppression". By release of Wolbachia infected males to induce sterile matings, mosquito vectors population would be reduced to a level below the epidemiological threshold, or even complete elimination in certain condition.

We start releasing HC mosquito (wPip Wolbachia infected) from March 2015 and a significant suppression effect on wild *Aedes albopictus* population was observed. In 2016, we release mosquito at two field sites: Shazai island (Nansha District, Guangzhou) (about 200 thousands to 1 million per week) and Dadaosha (Panyu District, Guangzhou) island (about 200 thousands to 500 thousands per week). Mosquito surveillance in two trial sites, control sites have been performed since April 2014 and will last during and after the releasing period. Ovitrap and BGtrap have been used to observe the ovitrap index, egg hatch rate and adult index before and after release. Community engagement have been performed since August2014 and will last for the whole release period int wo field sites.

The production of mosquito facility in Guangzhou rose from 1million to 5 millions per week. An X-ray irradiator was used in sterilizing HC females.

SECOND RESEARCH COORDINATION MEETING
On "Mosquito Handling, Transport, Release and Male Trapping Methods"

Valencia, Spain

24 - 28 April 2017

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. To improve the control of this mosquito an unprecedented suppression trial was recently implemented on Tetiaroa, a populated atoll in the Society islands, French Polynesia. The study investigated the efficacy of the incompatible insect technique (IIT) to suppress a 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. Incompatible (*Wolbachia*) males were released at a rate of ca. 57,000/week for population suppression and possibly elimination for a period of 13 months. In total, over 3 million incompatible males were successfully produced, transferred and released over a 75 ha islet on Tetiaroa. These sustained, inundative releases resulted in the drastic suppression of the targeted *Aedes polynesiensis* population as determined by adult trap data and ovitrap indices compared to adjacent, no-release control islets. *Wolbachia*-induced sterility was detectable within a few weeks of treatment, and reduction of the adult female population became manifest after only 4 months of intervention. The target population had almost completely disappeared by the end of the release operation. Six months after the treatment, only a residual population remains despite the favorable conditions offered for mosquito re-infestation by the rainy season. Stemming from this success, our vision is to eliminate mosquitoes vectors of diseases from the Society Islands and beyond over the next decade. To that end, we propose to collaborate with CRP participants to i) test different packaging, transport and monitoring devices, and ii) to explore various ground and possibly aerial release systems. Tropical islands form excellent showcase of sustainable development that provide useful data for more complex continental systems.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

The SIT program against *Ae. aegypti* in Indonesia is facing a new challenge, knowing that the gamma-irradiator facilities only does exist in Jakarta (Java Island), while the incidence of DF/DHF could be occurred in entire territory of Indonesia. Then we undertook a study to investigate the effect of handling and transport on the quality of irradiation sterilized males. The study was divided into 4 main experiments: (1) Comparing the effects of irradiation on pupal and adult stage; (2) The used of featured plastic cup/container for packaging mosquito; (3) The effects of banana bark to maintain the humidity inside the container; and (4) Mark-release-recapture trial of irradiated males inside Pasar Jumat Nuclear Facility Area (12 ha), Jakarta.

Based on the experiment (1), our sterilization protocol has changed from sterilizing adult into pupal stage for easiest handling system. The emergence, longevity, sterility and mating competitiveness were not significantly different between stages tested. Preliminary test on experiment 2 revealed that the little amount of water (1 and 3 pipette drops – to minimize mortality and physical damage on adult) was giving undesired result with only 43.3% and 94% emerging rate. Experiment (2) was related to experiment (3). In term of keeping the adult survive, the cup was only keeping 65.22% irradiated-transported adults alive after transportation, significantly different compared to irradiated-untransported male (98.67%) and unirradiated-transported male (91.86%). The banana bark addition has no effect to all parameters except the survival of males, 94.34% survive with banana bark and 93.17% survive without banana bark. The average longevity of unirradiated-untransported male was 61 days, drastically decreasing for unirradiated-transported male (14 days) and irradiated-transported (13 days). Experiment (4) was preliminary trial to investigate the flight distance and distribution of irradiated male of *Ae. aegypti*. Fluorescent-marked-sterile males (n=3000) were release in three different points (1000 males each), then ten BG sentinel were deployed randomly over the area. The data showed that the sterile male had flight distance 200 m and 500 m.

These present study gives us an important information toward the procedure of handling, transport and release sterile males of *Ae. aegypti*. The future study will be addressed to enhance the capacity of cup and also adding some features of container to maintain the quality of sterile males.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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 main advantage in the use of commercialy available aircrafts, beside the payload capacity, is the flight time autonomy, and the speed of the vehicle.

Speed, swath and time, is coverage in surface , so multiplied for selected density, is equal to load in adults needed, for the mission to be completed.

Is a common mistake to believe that due its low weight, it can be loaded and released in a small size dronne, when the main issue is to cover the surface in a short time, in order to give the released insects the best conditions to ensure the survivance, during the suitable conditions of humidity, light and temperature in the day.

This document has the aim to highlight and understand the relation betwen load and release, in a fixed rate for aerial insect release, thus this, find the best option for a project, evaluating all parameters involved in SIT

A technically successful project needs to be calculated in the understood of the sterile insect production capacity, the required surface of treatment area, as well as to perform the release, in suitable conditions for the specie to be released, to asure its survivance, and not just in the weight of the insect to be released.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

Following the theoretics of the use of micro vibration used actually in high capacity release machines as Mubarqui Smart Release Machine (Mubarqui et all 2014), where it was demonstrated the damage avoidance in the insects, and the good biological material flow thru the device, to the outside to be released, and with this, the presition and homogenial distribution in live sterile insects deployed in the field, it was designed and constructed the Unmanned Aircraft System Smart Release Machine, under the same premise, the UASSRM.

The UASSRM can be carried in MultiCopters as DJI matrice 600 that is a commercial and available vehicles.

The Release Mission control for this system is developed by Mubarqui Group with same priciples as big systems, precise rate, automatic on/off in release devise as open/close action in release mode, Release polygon and exclussion areas, in a independient controller from the UAS hardware, but subordinated to the navigation mission control.

Actually exists two versions of UASSRM one for six liter capacity 133.500 Anastrepha ludens or 265.000 Ceratitis capitata. And the small insect version for Tamarixia radiata. With 1 litre volume enough for more than a million adults.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

The control of rearing conditions is a crucial step to the development of mosquito mass production. To this aim, we estimated larval food rationing and larval density effects on the development of *Aedes aegypti*. Three larval food rations (R1 = 0.2g/2 days, R2 = 0.5g/2 days and R3 = 1g/5 days) and three larval densities (D1 = 1000 larvae/1500ML, D2 = 1500 larvae/1500ML and D3 = 2000 larvae/1500ML) were tested. We also estimated adults emergence and pupae mortality rates in function of pupae exposure to different temperatures (2, 4, 6 et 10°C) for different durations (1h, 2h, 4h and 8h).

Our results show that larval food rationing has no effect on their survival but significantly affects ($P < 0.05$) larval development duration, pupation, survival and fertility of females. They also show that a high larval density affects the duration of their development and the weight of adults without significantly affecting larval survival ($P > 0.05$). The temperature has no effect on the pupae's mortality rate after exposure and on the rate of adults emergence. However exposure of pupae to low temperatures for longer duration increases their rate of mortality after exposure (R^2=0.561; $P<0.001$). Results suggest to use 1000 larvae in 1500ML of water and the application of R2 for efficient breeding. They also suggest that 10°C can be considered optimal for many hours pupae storage.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

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:

A considerable increase in the spread of invasive mosquito species has been observed within Europe since the late 1990s, with the Asian tiger mosquito *Aedes albopictus* continuously expanding its distribution. In addition to being a biting nuisance, *Aedes albopictus* is an important vector of several viruses such as chikungunya, dengue (Gratz 2004) and also the Zika virus disease declared by the WHO as a Public Health Emergency of International Concern (PHEIC) in February 2016.

In Spain, this species has colonised the majority of the coastal Mediterranean region since its arrival in 2004 (Aranda et al. 2006). At present, the mosquito is established and widespread in the Catalonia Region, Valencia Region, Murcia and the Balearic Islands. Moreover, it is threatening to colonise the region of Andalusia (including the Atlantic watershed) and the Basque Country (EID Atlantique 2014).

Mosquitoes are becoming increasingly resistant to intensive insecticide use. Sustainable and environmentally safe methods of controlling invasive species of mosquitoes in Europe are needed and SIT can contribute towards a more environment-friendly control of the disease vector.

In Valencia (Spain), TRAGSA has been implementing an operational AW-IPM programme during the last 10 years against the Mediterranean fruit fly, integrating SIT as the main component.

During the last three years, TRAGSA has been working on some preparatory activities to launch a mosquito pilot project in 2018, such as the establishment of a seeding colony with individuals obtained locally, development of equipment for cost effective mass rearing (adult colony cages, larval racks and laser based pupae sexing system), and monitoring of the populations in selected places.

Regarding this CRP and according to our goals in it, the following prototypes have been developed and are currently at the testing phase:

- Prototype of a cost effective and low labour intensive male only cage with a capacity of 16.000 sterile adult males. After chilling, males are collected in a cassette fitting in the release machine

- Prototype of a release machine loaded with the cassettes from the male only cages for release in the target area using ground vehicles like vans, public bus service, taxis, etc.

Due to the fragility of the mosquitoes and with the aim to reduce the damaged caused during manipulation, the same recipient where the adults are collected is used for the release machine.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

TITLE OF WORKING PAPER: Current status and insight of future approach of UAS civil regulation.

AUTHOR: Antidio Viguria
ORGANIZATION: Center for Advanced Aerospace Technologies (FADA-CATEC), Spain.

SHORT SUMMARY OF PAPER

Abstract:

In the last two years, a lot of changes have been developed regarding UAS/RPAS/drones regulation worldwide. In this presentation, an overview of the future regulation framework regarding UAS will be presented. Especially, it will be commented in detail the situation in Europe and how it is the status of the current harmonization effort in order to have a unique UAS/RPAS regulation in Europe by 2019. Moreover, it will be also presented the main types of applications that will be commercially viable using the new regulation framework. Finally, a short insight on the types of requirements that it is expected that the UAS operators have to fulfill in these new types of applications: urban environments and BVLOS at low altitudes (below 500ft), will be presented.

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]

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Abstract:

The recent progress in adapting the Sterile Insect Technique (SIT) for controlling *Aedes aegypti* and *Aedes albopictus* indicate a potential for controlling other *Aedes* mosquito species. In Sweden, the floodwater mosquito *Aedes sticticus* is causing unbearable nuisance in mainly the River Dalälven floodplains. The species utilize temporary flooded grassland environments for the larvae, while the adult females spread many kilometers from the larval habitats to the surrounding areas for blood-feeding. Using the biological larvicide VectoBac G, containing Bti, we have been able to provide efficient and environmentally sound control. Because a request from the Swedish Government, we propose a plan for reduced dependence on VectoBac G using SIT as an alternative toxin-free control method for this super-abundant floodwater mosquito. A 150 ha study area producting *Aedes sticticus* is identified. With these mosquitoes dispersing at least 5 km, they cause heavy nuisance in at least 15 000 ha of surrounding landscape. The suggested alternative method is local elimination by SIT followed by release of smaller number of sterile males to maintain control. Highly focused and easily located larval habitat makes *Aedes sticticus* suitable for SIT, but there is a need for initial population size reduction. This requirement is met by our VectoBac G treatments shown to strongly reduce larval and adult abundance, and even a reduction of the mosquito egg bank. We have also shown that mating occurs among deciduous bushes and trees near larval habitats, and that males remain mainly near the larval habitats and are short lived. Thus, the arena is pawed for a pilot study with focused aerial release of a relatively low number of sterile *Aedes sticicus* males aiming to achieve local elimination of this highly productive nuisance species. Our recently established colony of *Aedes sticticus* will be used for development of suitable transportation and release technology. This is a promising and challenging project, with potential to break ground for a novel, efficient, sustainable and environmentally safe control method against superabundant nuisance *Aedes* mosquito species in natural wetland environments.

SECOND RESEARCH COORDINATION MEETING

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

Valencia, Spain

24 - 28 April 2017

TITLE OF WORKING PAPER: Advancements in Unmanned Aircraft Systems for use in Sterile Insect Technique Applications

AUTHOR (S): Nathan Moses-Gonzales and Michelle Walters
ORGANIZATION: M3 Consulting Group and the United States Department of Agriculture

SHORT SUMMARY OF PAPER

Abstract:

Unmanned Aircraft Systems (UAS) offer a means of releasing sterile insects aerially, can be deployed on short notice in a rapid response scenario, are less expensive and offer a safer means to release sterile insects than their traditional, manned aircraft counterparts. Technologically, UAS affords researchers the ability to rapid prototype systems and components with considerably less financial burden than their manned counterparts and biologically, provide a high quality released insect accurately targeted at the release site. UAS provide a high level of precision and can be deployed as an aerial release component with minimal setup and teardown time. UAS have successfully released pink bollworm (*Pectinophora gossypiella*) and codling moth (*Cydia pomonella*) with studies showing the sustained competitiveness and longevity of the insects post-release. Recently, the refinement of autopilot technologies for use with small UAS applications led to the possibility of using several UAS to autonomously fly as a "swarm" to achieve greater coverage and address payload limitations. Swarm technology provides control and monitoring of several UAS from a single computer and pilot. In 2017, M3 Consulting Group and Institut Louis Malardé began a study with New Mexico State University to assess how UAS and swarm technology may be deployed in support of mosquito SIT programs. Here, we discuss advancements in UAS technology, and how swarm technology may increase the effectiveness of the release of sterile mosquitoes.

7. 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.

- 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

- 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

- 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.

- 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,

- 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.

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- 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.

- 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

- 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.

- 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

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- 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;

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