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Mosquito handling, transport and release methods

Report of a Consultants Group Meeting held in Vienna, Austria, 8-12 December 2014.

NOTE

FOREWORD

The sterile insect technique (SIT) package for *Aedes* and *Anopheles* mosquitoes is being developed by the IAEA, including equipment and standard operating procedures for mass rearing, stage- and sex-separation and irradiation of males, to support the development of SIT programmes in several Member States. There is, therefore, an imminent need for a functional release system adapted to the specific requirements of an AW-IPM mosquito suppression programme with an SIT component, with suitable methods for handling and transporting chilled, irradiated male pupae and adults prior to release.

This consultants group meeting was therefore convened by the Insect Pest Control Subprogramme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture to advise the Agency on possible designs for aerial release systems suitable for the specific requirements of mosquito SIT programmes, with associated handling and transport systems, and to suggest possible companies or individuals who could develop the design concepts into full engineering design and prototype testing. The consultants were selected from companies and programmes involved in the development or use of equipment and systems which could be of use to mosquito release, including aerial release systems for fruit flies SIT, prototype equipment for handling and releasing more fragile insects, unmanned aerial vehicles and cooling systems.

The meeting was held in Vienna from 8-12 December 2014. The authors of this report and IAEA staff members responsible for the meeting are Mr Rafael Argilés Herrero, Mr Jeremie Gilles and Ms Rosemary Lees.

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1. INTRODUCTION

Among the major vectors of human diseases, mosquitoes are the most devastating ones. Urbanisation, globalisation and climate change have further accelerated the spread and increasing number of outbreaks of new mosquito borne diseases. In view of the problems associated with conventional mosquito control, such as resistance and lack of available vaccines, experts of a Thematic Plan Meeting held in Vienna in June 2014 concluded that there is an urgent need to develop new or complementary control techniques, including the 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 potential also for key insect vectors such as mosquitoes. With the increase in vector-borne diseases and their toll on human health and mortality, there have been recurring requests from Member States to develop tools and techniques to be able to apply the SIT to control mosquito vector populations (Resolution GC(58)/RES/18). The SIT has the ability to suppress or in special situations to eradicate existing vector populations and to prevent outbreaks of disease.

The Insect Pest Control (IPC) Subprogramme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture is involved as part of its programme in the development of the SIT for the control or elimination of mosquito disease vectors. Several mosquito SIT programmes are under development with the assistance of the IAEA and some of these programmes are anticipated soon to reach the stage of operational release of sterile males.

In addition to the mosquito SIT package which is being developed by the IPC laboratory (IPCL), the technologies must be in place for application on an operational level. 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. Key issues to be resolved are handling, transport to the release location and actual release of sterile males, which must all be achieved without causing significant impact to their survival or post-release performance.

Currently AW-IPM programmes with an SIT component have been successfully implemented for several very important fruit fly, screwworm, and tsetse lepidopteran species, and the experience of these programmes may help to inform development of relevant technologies and techniques for mosquito programmes. There is a need for methods to transport and release several million adult male mosquitoes, which must be assessed for any detrimental impact on their quality or sexual capacity. Fruit flies and screwworms are normally released from the air as chill-immobilized adult insects. Chilling the adults allows large numbers to be handled easily so that large areas can be covered in one release flight, and prevents damage to insects while held at high densities. Several different release machines have been used successfully in many programmes around the world, and there is considerable experience in handling and transport of huge numbers of insects. Equivalent systems applied to mosquito SIT would considerably increase the possible size of suppression programmes using sterile males, and the development of ground and aerial release systems would allow males to be released in a closely controlled manner into geographic locations which are currently difficult to access. Similar principles of the systems used for fruit fly or tsetse release could be adapted for mosquito release, though the more fragile nature of adult mosquitoes must be taken into consideration.

This consultants group meeting was, therefore, requested to review current systems, and those currently in development, and consider systems which may be suitable for release of chilled adult male mosquitoes, after irradiation as pupae, with or without the transport of chilled pupae to emergence centres close to the release site or sites. There are several mosquito species that are vectors of different pathogens in different countries. Although any new technology should be generally applicable to all species, precise details of release methods will likely be fine-tuned for different species. One major consideration in this regard is the absence of a method to sex separate *Anopheles* pupae, meaning that adults must be kept for a period of days before release so that females can be removed by spiked bloodmeals, whereas male *Aedes* adults can be released 24 hours after emergence, a period sufficient for sexual maturation and administration of a sugar meal.

2. TECHNICAL REQUIREMENTS FOR HANDLING AND TRANSPORT OF MOSQUITOES

2.1.Overall considerations

Helinksi *et al.* (2008) 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 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.

This meeting considered handling and ground transport to include 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 from the mass rearing facility 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 from the mass rearing facility 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; the processes and timings involved for the two options in either genera are shown in Appendix III. 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 if pupae are found to be more resilient to chilling and transport than adult mosquitoes. Currently, there is no suitable sexing method to sort *Anopheles* species before the adult stage, and until appropriate genetic sexing strains become available, 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.

It is evident that the less handling 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, transported in and then released from, the same container, 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. 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, allowing them to be reused for the following blood feeding.

2.2.Suitable methods available to chill mosquitoes for handling and transport

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 available which may be applicable at different stages throughout the supply chain as illustrated in Figure 1.

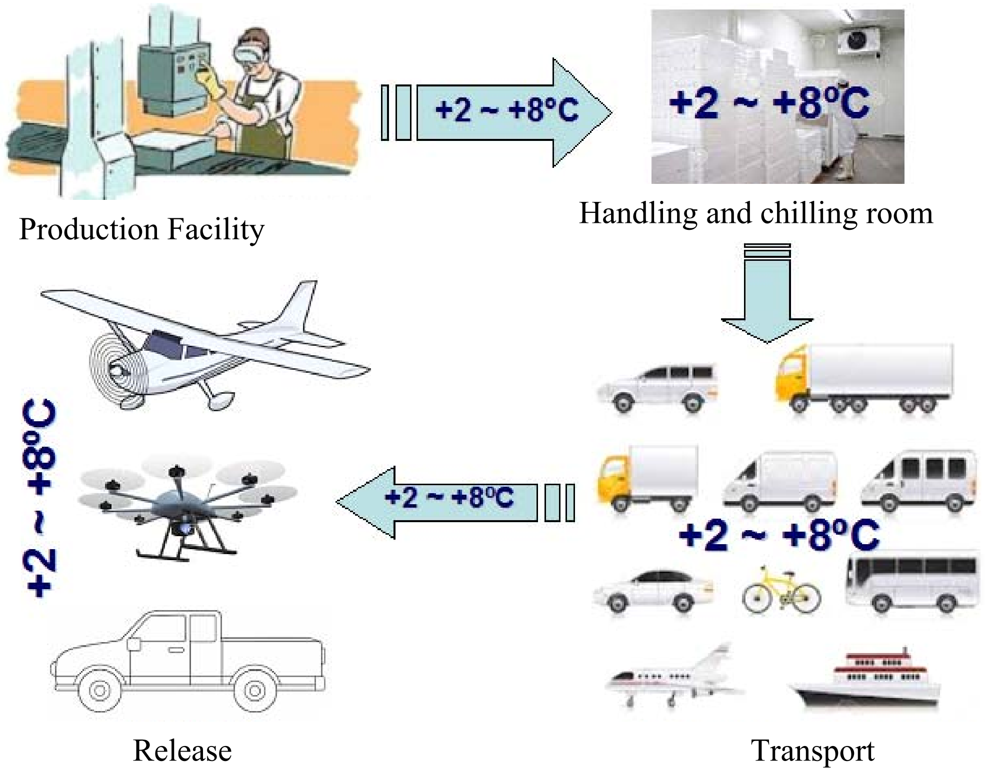

Figure 1. Representation of the supply chain from mosquito mass rearing facility to release site, identifying the key steps requiring chilling solutions.

In more developed countries refrigerated lorries and vans are widely available and therefore mosquitoes could be shipped within the existing cold chain network. However, considering that large numbers of mosquitoes can be transported, both at pupal or adult stages, in relatively small containers, other solutions can be developed using technologies that require less well developed infrastructure. This issue becomes critical if the operational solution chosen comprises a central mass rearing facility to cover wide areas, countries or regions and long distance 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 as illustrated in Figure 2. 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. If compressed CO_2 is selected as a cooling option, attention must be paid to the potential impact of atmospheres with high concentration of CO_2 on the quality of the insects. These basic concepts should be explored as an attractive cooling option for ground transportation.

Static Systems Dynamic Systems

Figure 2. Delivery box options for transporting release cassettes containing chilled mosquitoes by ground.

An alternative would be the use of Peltier cooling, which could make use of a car battery during transportation and have low weight and space requirements, which would also make it an attractive possibility for ground and air based release systems, as long as a source of electrical energy is available. Technically compressed air vortex cooling could be an option but this type of system requires a significant and continuous compressed air supply which can be supplied by various sources such as an electric compressor or engine driven compressed air pump to cool the system or compressed air cylinders for mobile applications.

Although for release from UAVs Compression or Mechanical Refrigeration may not be applicable, for ground transport and handling and maybe also for aircraft release conventional electric refrigeration could be considered. As this technology is well established and there are many manufacturers around the world who can supply large scale products, as well as miniature refrigeration machinery if and when required, the supply source can be explored to match the design requirements.

Based on the temperature range required, use of a PCM during handling and transportation, and even release, of insects may be suitable in terms of volume and weight requirements. PCMs can be produced to produce any desired temperature. However, this type of cooling is a passive cooling option and it has to be charged prior to use so that the stored energy can be released back into the chamber. However, PCM cooling provides the most cost effective and simple cooling option and therefore this PCM based delivery cooling concept should be further investigated in particular for air release systems. Packaging for mosquitoes could contain either a sensible heat capacity block such as light weight metal casing or a latent heat capacity material such as PCM material, or the combination of both, as part of the individual packaging. Thus, once chilled the required temperature could be maintained over short periods of transport, or during a short release flight, for example. PCM-chilled cassettes could be transported over longer distances in insulated or chilled delivery boxes containing sufficient PCM ice-packs to maintain low temperatures as long as needed. The most practical and cost effective way to transport and handle chilled and packed mosquitoes would be to use commercially available temperature controlled distribution boxes and ice packs as illustrated in Figure 3, which can be supplied by manufacturers worldwide.

Figure 3. Typical cold boxes and ice packs, which could be used to maintain the cold chain during ground transport of release cassettes of chilled mosquitoes.

2.3.Research required to inform the design process

Element for consideration	Options	Research needed
Age of pupae at irradiation	20 hours for *An. arabiensis*, 30 hours for *Aedes* (range +- 5 hours)	More synchronized pupation; pupal sex separation for *Aedes* (when to perform the sex sorting) Anoxia may improve insect quality
Transportation from mass rearing facility to release site	Transportation of pupae or adults	Assessment of the less fragile stage of mosquitoes for transportation: pupae or adults, taking into consideration that the release site might be more than 3 hours far from the mass rearing facility. Assess the optimal conditions to transport adults long distances: chill for knock down, conditions (temperature, crowding) during ground transportation. If adults are found to be more suitable, transportation will be done to the release site directly in cassettes ready to fit in the release UAV/aircraft Adults can be transported in relaxed non-crowding conditions although this will require very large volumes to be transported, mainly air
Pupal chilling	Chilling of pupae to extend time to emergence	Determine optimal temperature of chilling and during irradiation and effect on the extension of pupation time. Order of chilling and irradiation.
Transportation of chilled pupae as a slurry	Medium for pupal transport	Assessment of optimal humid medium: wet filter paper, wet sponge, free water layer
Age of pupae during transport	As soon as possible after irradiation	Chilling and other methods to be defined to stop pupal development in order to avoid premature adult emergence
Temperature during transportation	Between 6 and 12 degrees, to be confirmed	Optimal to be assessed, related to the required duration of chilling
Type of pupal transport container	Design of container	Highest possible density of pupae per unit surface area of water without increasing mortality or damage, used to design pre-dosed individual containers with the amount of pupae needed to load a emergence cage and/or release cassette.
Cooling system	Method for chilling mosquitoes	Define different cooling system for the : 1.- production: last step of rearing (pupae and adults) 1.a pupae 1.b adult chilling 2.- Transport: 2.a pupae shipment to emergence facility 2.b adult shipment directly to release site 3.- Release One option to be considered is to have 1+2 altogether in the mass rearing facility, as long as adults are found resistant for long duration transport. Rate of metabolic heat generation of pupae or adults held at the selected immobilisation temperature must be determined so that the cooling systems can be designed to compensate, and efficiently tailored to cool the desired payload without redundancy which would increase the weight and cost of the cooling system. Establish the optimum size of the cold boxes for various pay load and release cassette designs, ideal PCM temperature range, and economic requirements Develop a simple chart to calculate the amount of PCM ice pack required for a given time to ensure safe chilled chain operation all the way from the production plant to the delivery point

Volumes and weight to be transported	Required volume of insects for transport	Number of pupae to be transported will double in the case of anopheles until a sexing system is in place. Volumes and weight to be transported will also be larger in the case of adult transportation, although adults can be transported in bulk if resistant to chilled crowding conditions.
Holding time	Estimated sexual maturation time: 24 hours for both *Anopheles* and *Aedes*	What is the minimum time needed until males reach sexual maturation (*Aedes* 24 hours) or females are killed by blood spiking (*Anopheles*). How much sugar needs to be supplied to the males before release?
Holding cages	Design of cages	Holding cage design needs to be developed to reduce the labour required during collection and loading the release container. Release container should be the same than transport containers and should easily fit into the release machine in the UAV/aircraft to allow fast reload operations. Bottom of the cage is the cassette for the releases Different cages design for different mosquitoes. Cages have to support the different steps: - pupae loading after being irradiated - adults emergence - sugar feeding - blood spiking (in the case of *Anopheles*) - removal of dead females (in the case of *Anopheles*) - chilling - collection Different resting surface will be needed for different species (length of sleeves, diameter). Different volume size of the bottom cassette will be needed for different species.

3. DEVELOPMENT OF AN AERIAL RELEASE SOLUTION FOR MOSQUITO SIT PROGRAMMES.

3.1.Operational technical requirements

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, though this is less critical for releases from UAVs, which anyway have lower possible pay loads and lower operational costs. 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. However, the following operational details will always need to be considered for any aerial release solution:

- Chilling will be needed to immobilise adults
- Height of release, which is related to recovery time of mosquitoes from chilling
- Density of mosquitoes which can be held without causing damage
- Variable release rate over the target area will be required, and precise release rates will be updated according to current population density data obtained from field monitoring
- Volume of mosquitoes to be transported and released will need to be determined based on programme's specifications
- Flight speed - 40 km/h minimum with fixed wing UAVs, 15-20 km/h with rotary wing UAVs, 60 km/h for gyrocopter, 30 km/h for multi rotor vehicles, 180 km/h for light aircraft. Medflies are released at 200-230 km/h, 1/2 million per minute, but probably the mosquitoes will require lower flight speeds to avoid being damaged by the air at the exit of the releasing device.
- Cost of equipment – a balance between reliability and redundancy
- Swath width – probably 50 m for *Aedes*, 250 m for *Anopheles*, could double and release on alternating paths each time

During discussions the meeting participants came to an estimate for release requirements of ~500 g mosquitoes released/week to cover 200 ha, extrapolated from a need for 4,000 adults per week per hectare at typical population densities, which would comprise approximately 10 litres (though density and weight of chilled adults need to be assessed with higher accuracy for both *Aedes* and *Anopheles*), and require 20 km of flight with a 100 m swath, equalling 24 minutes of flight time at 50 km/h.

Participants also came to the conclusion that since continuous release using auggers (eternal screws) are not ideal for medflies, 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 releases would allow males to be dosed into release cassettes for transport to the release site, minimising handling and also the potential for crush damage resulting from large masses of chilled adults being held.

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, and so are currently released by operators from the back of trucks, mostly in urban areas, for example in the PAT programme in Brazil. There is the possibility to upscale this effort by the incorporation of an automated release machine mounted on the truck, or motor bike, depending on the nature of the release area. Oxitec has conducted some modelling to maximize the efficiency of coverage using such a release strategy, to minimize the road distance which must be covered by a driver whilst still ensuring sufficient coverage, based on mosquito dispersal data. 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.

The consensus from this consultants meeting was that aerial release is the most 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 regularly updated 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 me 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 SIT.

3.2.Aerial release from unmanned aerial vehicles (UAVs)

Before considering the biological and technical details of a release programme based on UAVs, the legal restrictions surrounding the nature of vehicles which can be used in different scenarios 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 wild fires 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 as a starting point. 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. Appendix IV provides a summary of the regulations in Spain as they fit the likely requirements of mosquito release systems, which broadly reflect those existing for other European countries. JARUS (Joint Authorities for Rulemaking on Unmanned Systems), a group of experts from the National Aviation Authorities (NAAs) and regional aviation safety organizations, is working to harmonize national regulations, and it is likely that in the next 6-7 years international guidance will be in place. Considering the operational limits and regulatory restrictions placed upon the flight of different UAV types in different settings, the following is a list of possible scenarios where mosquito SIT releases may be required, and the most suitable form of UAV to deploy.

1. *Release flights beyond visual line of sight (BVLOS) in rural areas in countries with no regulation:*

   a. Recommended type of aerial vehicle: fixed-wing (plane)
      i. Preferably launching method by a catapult and landing over its belly. This will increase the operational capacity of the system since we do not a runway. However a flat terrain, usually at least 100 meters, will be needed for landing operations.
      ii. MTOW (maximum take-off weight) of the aerial vehicle should be less than 25 kg in order to defend in front of the local authorities that it is a safe platform and worldwide nobody is asking for official registration or certification of airworthiness for UAV (unmanned aerial vehicle) below this weight.

   b. Operational limits: no real limit on the height although it is important to fly always in non-controlled airspace (usually below 300 m from the ground). Flying in controlled airspace means communications with ATC (air traffic controller) and this will complicate and make much more expensive the system. Regarding the range, maximum 30-40 km from the GCS (ground control station) because of datalink limits and depending on the geography of the surroundings. It is important to take into account that from 5 km distance, the further the UAV flies, the higher it has to be in case it is required to maintain the communication with the UAV. However, it seems that this could not be mandatory for the insect deployment application in countries without UAV regulation.

   c. Requirements coming from the regulator authorities: effectively none since we will operate in a country without UAV regulation. However it could be useful, to convince the local authorities that the system is safe, that the UAS implements a parachute in case of a major failure. Also it could be interesting to operate a UAS that implements the regular emergency modes (see the list at the end of this document).

   d. Payload capacity: 1 kg for a 8 kg platform, 2 kg for a 15 kg platform, 4 kg for a 25 kg platform

   e. Endurance: usually between 45 minutes (for platforms around 8 kg MTOW) up to 3-4 hours or more (for platforms with 25 kg MTOW). The flight endurance will also depend on the final payload weight integrated into the platform. The less payload weight, the more endurance we will have.

   f. Speed: 60-90Km/h.

   g. Price: more than 50 k€ if you use a professional platform. The one that it is shown in Figure 4 costs 150 k€ (the complete system). Much cheaper (10 k€) if you use a RC hobby one (usually around 8-15 kg MTOW).
   h. Example case: this could be the case of the proposed SIT pilot site in Sudan.

Figure 4: Fixed-wing UAS with MTOW < 25kg.

2. *Release flights with visual line of sight (VLOS) in rural areas/city or populated areas in countries with restrictive regulation:*

   a. Recommended type of aerial vehicle: Multirotor.
      i. Preferably with 8 rotors to increase the safety level of the system (with an octocopter you can maintain a safe flight even when a single motor fails) (Figure 5).
      ii. Vertical take-off and landing offers large flexibility from the operation point of view, even more in a urban scenario.
      iii. The MTOW of the vehicle should be less than 10 kg due to Spanish regulation (4 kg for France regulation, however it will be extended to 8 kg in the short future).

   b. Operational limits: 500 m from the pilot in rural areas and 100 m from the pilot in cities. UAV should fly always below 120 m height.

   c. Requirements coming from the regulator authorities: the UAS should be designed to fulfill all the requirements imposed by the local civil aviation authorities of the country where it will be operated. It is important to have an official letter stating this fact. Also it will be probably mandatory to operate a UAS that implements the regular emergency modes in order to get authorization from the regulator (see the list at the end of this document).

   d. Payload capacity: 2.5 kg with a 10 kg platform, 1 kg with a 5 kg platform, 200 g with a 1.5 kg platform

   e. Endurance: usually 15 minutes or more depending on the final payload weight integrated into the platform. The less payload weight, the more endurance we will have.

   f. Speed: 30-40 km/h

   g. Price: 2 k€ to 15 k€ for a 1.5 kg platform, 10 k€ to 30 k€ for a 5 kg platform, 15 k€ to 50 k€ for a 10 kg platform

h. Example case: Urban area in Valencia (Spain)

Figure 5. Octocopter UAS.

3. <u>*Release flights beyond visual line of sight (BVLOS) in rural areas in countries with restrictive regulation:*</u>

   a. Recommended type of aerial vehicle: fixed-wing (plane) (Figure 6)
      i. Preferably launching method by hand and landing over its belly. This will increase the operational capacity of the system since we do not need a runway. However, a flat terrain (usually at least 100 meters will be needed for landing operations).
      ii. The MTOW of the aerial vehicle should be less than 2Kg to fulfill the Spanish and French regulations.
      iii. The main problem of this system is their resistance to wind. However, since it seems that insects will not be deployed in windy conditions, it is not a major factor for this application.

   b. Operational limits: 120 m height during the whole flight. Regarding the range, maximum 5-10 km from the GCS (Ground Control Station) because of datalink limits. This range is lower than the BVLOS case in countries without regulation since it will be pretty difficult to find a datalink that can offer a greater range just flying at 120 m from the ground. It is important to take into account that the further the UAV flies, the higher need to be in order to maintain the communication with the pilot. Also, in this case that we are operating in a country with a UAV regulation it will be required to always maintain the communication with the UAV. If the communication is lost, the national authorities will require that the UAV automatically activates the return to home or the flight termination system immediately.

   c. Requirements coming from the regulator authorities: front visual camera and continuous videolink up to the pilot on the ground. Also it will probably mandatory to operate a UAS that implements the regular emergency modes in order to get authorization from the regulator (see the list at the end of this document).

   d. Payload capacity: very low, maximum 400-500 g or less.

   e. Endurance: 45 minutes maximum. Usually 30 minutes depending on wind conditions

   f. Speed: 40-70 km/h depending on the specific platform

g. Price: between €5,000 and 10,000

h. Example case: the middle of the countryside in France or Spain.

Figure 6. 2 kg MTOW UAS.

The UAVs in these scenarios could be combined with available or proposed cooling and release solutions in the following ways:

BVLOS in rural areas in countries with no regulation

- *Proposed cooling system:* due to flight duration it will probably require dynamic cooling systems.
- *Proposed release device:* continuous release system maybe including a vibration unit.

VLOS operations in rural areas/city or populated areas in countries with restrictive regulation:

- *Proposed cooling system:* passive for short flight durations. In case, of longer flight durations (>30 minutes) may require additional active cooling by means of either compressed gas or peltier heat pump.
- *Proposed release device:* discrete release system.

BVLOS in rural areas in countries with restrictive regulation

- *Proposed cooling system:* passive cooling or even no cooling because of weight restrictions and flight duration.
- *Proposed release device:* discrete release system.

The proposed way to proceed regarding development of UAVs for aerial release is divided into two phases. Firstly, a prototyping phase, during which a cheap UAV could be used and different cooling and deployment methods tested. These tests can be done in Europe (for cost and logistical reasons) either in VLOS operations (500 m from the pilot and 120 m in height) or even BVLOS (several km form the pilot and 300 m or more in height) but in a proper place prepared for flight testing with UAVs (there are a few around Europe specifically oriented to UAV flight testing, listed at: http://icatestsites.org/). Tests should be performed with two types of platforms that best fit this application (multicopters for urban and fixed-wing for rural and semi-rural releases), to get a feeling for when it is better to use each of the platforms.

Secondly, in a pilot programme phase it seems best to contract services from a UAV operator or applied research centre with operational experience. This pilot program should be performed in a country without UAS regulations (for example Sudan). It is important for this phase to have a working prototype of the cooling and deployment device (produced as a result the prototyping phase) since each change in the integration will mean an incremental increase in the budget. Also the cost of each flight in the final location will not be low, so it is important to have confidence that the system will work properly. For that reason, it would also be interesting to perform the integration of the device and some flight testing before shipping everything to the location where the pilot program will be. Finally, if possible from a budgetary point of view, to perform the pilot programme with both types of platform (multicopter and fixed-wing UAV). These two phases will allow the programme to gain the experience to properly define the requirements for buying a UAS or contracting a service for a large campaign.

3.2.1. Release devices available for application with UAVs

Although there are some release programmes targeting mosquitoes, currently releases are performed by ground using manual ground techniques. At present there are several automated solutions for sterile insect release being used in different SIT programmes around the world, but none specifically designed for the release of mosquitoes. Most of these programmes work with different species of fruit flies, such as *Ceratitis capitata*. In many cases, these programmes have developed their own specific equipment, normally oriented to aerial release of large volumes of irradiated male flies over large areas, although there are some programmes in which sterile insects are released by ground (normally small scale operations). Even in large programmes where aerial release is the main release method, ground releases is an alternative option when weather conditions do not permit safe flight over the release area. It is expected that current technology for aerial release of fruit flies, already validated in SIT programmes for decades, may be a starting point for designing customized solutions for the release of mosquitoes, while taking into account that the volumes are likely to be smaller in the case of mosquitoes and their biological characteristics, such as resistance to mechanical damage or chilling requirements.

A good example is the Mubarqui Smart Release Machine, which is currently applied for release of fruit flies in Mexico and for tsetse flies in Senegal. 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 fan units to circulate air through the insects. Release is controlled by a control unit, a device that received instructions from the ground computer and convert 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 (Figure 7), the instrument responsible for guiding the pilot to the release polygon and follow a predesigned flight path within it, and also for measuring, logging and automating the release mechanisms. The release device is installed in the aircraft, and designed based on vibrating feeders, automatic gates and linear actuators, with a duct to the outside of the aircraft which avoids venturi suction, increasing precision. It is also equipped with video cameras for monitoring.

Figure 7. MACXNAV navigation system, used in conjunction with the Mubarqui Smart Release Machine.

This Smart machine operates using software developed by Mubarqui in Mexico, which is installed in a tablet using an Android operating system, with the advantages of GPS technology. This device communicates via Bluetooth with the control unit, performing all actions required for release, as well as automatic calibration, increasing or decreasing the release rate for each polygon, based on outbreaks or exclusion areas. On the tablet screen the pilot has all the information needed to navigate from the airport to the release area, and once the aircraft is flying over the release polygon it will compare speed, maximum and minimum release altitude, swath of flight lines, and the smart release machine will start the release operation. The navigator provides alerts of position, course, heading, altitude and speed, and logs all release and flight conditions automatically. Before a flight commences, the tablet received via internet connection the release programme for the day, and potential alternate polygons, which can be released into if some unexpected event causes the intended flight path to become impossible, presented as a 'release flight order'. Immediately the pilot can activate the system, and the navigation programme instructions will be shown sequentially to each of the polygons. As soon as the aircraft arrives at a polygon release will start and be calibrated according to the current programme, considering flight speed. In the case that it is impossible for any reason the complete the planned flight, the pilot will activate the alternate polygons. As soon as the release flight is finished, the pilot will synchronise with the internet and download the flight files to the MACX system's interactive website, so that they are immediately available for review and analysis by technical staff on the ground.

The 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 other insects, 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, such as: Can fragile mosquitoes resist mechanical injuries caused by an auger, conveyor belt or vibrating tray?; What is 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 pre-dosed release. This is a discrete release method in which pre-dosed volumes of mosquitoes are released, especially interesting in the case of small volumes being required. 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. In general, these are suitable sizes to be covered by UAV release. Release in small areas can be performed using small UAVs with a weight of up to 2 kg (maximum take off weight, or MTOW), for which legal restrictions are less stringent. Such vehicles offer a payload of about 300 g, and should transport a payload of about 50 g of mosquitoes, so the release mechanism (including all components) should be as light as possible, not exceeding 250 g. For medium sized scenarios, UAVs with a weight of at least 25 kg will be needed, which usually have a payload capacity of approximately 5 kg. To justify these larger applications, about 1kg of mosquitoes will need to be released, so the release mechanism should not exceed 4 kg in weight. Thus, 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 kind of UAVs, due to the immediacy and economy in the generation of solutions. These 3D models are stored in STL or OBJ files, and can be 3D printed at any time and in different types of 3D printers, even remotely. If the 3D model of the aircraft is also available, or at least of the cargo space, it would be possible to optimize the integration of the release system into the fuselage of the plane. Fused Deposition Modeling (FDM) 3D printers, mainly using thermoplastics, are the most suitable technology for the manufacture of such small release devices, because the final object has a high mechanical strength and sufficient accuracy of production. Figure 8 shows a fully functional prototype of a discrete releasing machine designed by CAD and then printed in ABS (dimensions: 23 x 12 x 14 cm).

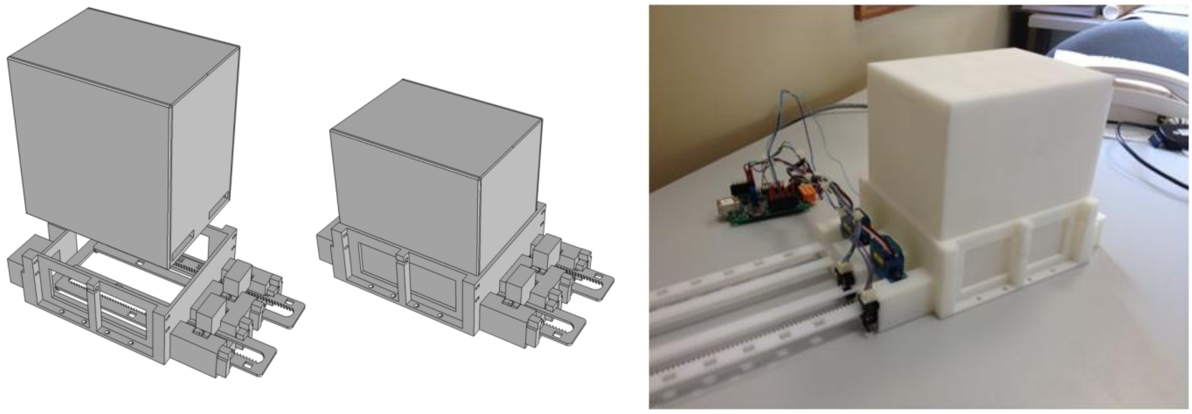

Figure 8. (Left) 3D model of a prototype of a discrete releasing machine. (Right) A real and fully functional 3D printed prototype of the machine in ABS.

Many of the printers based on this kind of technology offer an interesting printing mode, originally conceived to save on extrusion material. It involves the use of high densities of material to build the external surfaces of the objects while using a light structure in the interior. This printing mode provides enough resistance to the objects, while maintaining a very low weight. Additionally it provides a very interesting additional advantage, as objects retain an air layer between the outer surfaces. This attribute allows that a 3D printed release cartridge behave like an insulated container, retaining the cold inside once chilled. 3D CAD and 3D printers offer the possibility that the system designer and the 3D printer can be located at remote locations. For example, the 3D printer could be located in the production or release facilities of the SIT programme, and the designers could remotely send STL files to be printed with updated designs produced according to new requirements.

3.2.2. Cooling systems for use with UAV release

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 pay loads. For payloads of around 50 g of mosquitoes, flight times will be short and additional cooling systems may not be required. In intermediate release volumes it will be necessary to use some kind of on-board cooling system, probably passive systems based on Phase Change Materials (PCM). In these cases one possibility is somehow to incorporate these materials in the release mechanism itself, probably in the interchangeable cartridges. A system to provide a quick method for loading the mosquitoes onto the UAS 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. Whether the delivery uses a fixed or a rotary wing UAV, the weight of the cooling system will have a direct impact on how large a pay load can be delivered. Hence, whichever design is selected it may require suitable insulation to minimise heat gain during flight. Considering cooling options, a dynamic peltier heat pump system may be an option for larger UAVs, and this technology can be a small as a single soft drink can, but for smaller UAVs with limited spare battery capacities this dynamic cooling option would be very difficult to operate. Hence, for smaller UAVs either PCM-based or CO_2 cylinder-based cooling options may be more suitable. Zeolite could be used with a vacuum chamber to produce a relatively light weight cooling system, and it well proven technology for mobile cooling applications. As the cost of the Zeolite is between € 0.75 ~ 3.00 per kg, depending on the quality and quantity, this water evaporation option certainly satisfies the need for a low cost cooling option, and can be considered as an environmentally acceptable option. The technical ability to achieve and maintain material at the required temperature range needs to be tested. Based on the temperature range and the required heat gain, a PCM storage requirement could be developed to fit the volume and weight requirements and may satisfy both the cost and weight limits. This type of cooling is a passive cooling option and therefore has to be charged before use for the stored energy to be released back into the chamber, and this additional operational complexity must be taken into account. However, PCM cooling provides the most cost effective and simple cooling option for delivery cooling applications and should be considered for inclusion in transport and release systems as they are developed.

3.2.3. GIS dose release method for aerial release

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.

Some UAS automatic guidance systems allow the definition of a set of sequentialised waypoints (Figure 9), generating an event whenever the vehicle flies over one of them. Usually this kind of system generates an electrical signal (typically a square pulse) each time they cross a waypoint. Some systems are even able to send/receive serialized data to the terrestrial base, given more information about the waypoint that has been crossed. In the simplest case, where the system generates a square pulse each time it crosses a waypoint, this signal could be used to activate an on-board dosing system, so that every time a pulse is received it releases a dose of mosquitoes. This mode of operation is best suited for discrete release systems, although it could also be implemented for continuous release. Even with such a simple system it could be possible to perform complex release strategies simply by varying the frequency of waypoints as a function of information extracted from the map of population density. Higher frequencies (waypoints close together on the map) will result in a higher dosage over a given area. The position of the waypoints is defined previously in the office using GIS software.

If the system could be built to offer better communication capabilities it would be interesting to consider the implementation of more complex releasing strategies, such as Differential Release Systems, particularly in the treatment of large areas with large volumes of insects, using continuous release mechanisms. It would be interesting to investigate the potential use of small computers with dimensions similar to a credit card, "Raspberry Pi" or similar, to be used as an on-board Differential Release System. In these cases good field information is required, extracted from a dense enough trap network.

Open source systems such as Ardupilot support this functionality and other systems based on professional platforms would also be expected to support it. In any case it is highly recommended that a self-guided system that somehow supports this functionality is chosen. The biggest advantage of choosing an open source platform lies in the capacity for customization. Ardupilot could be considered as a suitable platform for low cost ultra light drones, since it provides good capabilities at a reduced cost. However, special attention must be paid to the local regulations due to the fact that liabilities in the case of any incident may not be accepted by an Open Source platform. There are also hybrid solutions on the market, in which companies guarantee the platform support, but still offer some customization capabilities. It would be interesting to further explore the possibility of using solutions of this type.

Figure 9. Example graphic showing a set of waypoints over a path, from the 3.2.3. GIS dose release method for aerial release.

In the case that a problem arises during the course of a release flight, the flight and navigation systems must have some protocols built in so that the vehicle can be recovered safely, and most importantly does not lose control and risk injury to people or property. The following are the main rescue options:

- *Return to home:* the UAV returns to home automatically following an emergency flight plan or in straight line to the point where the pilot is. This mode is activated usually when the UAV lost the communications with the pilot or the GCS is not working any more.

- *Flight termination system:* the UAV finishes the flight. There are several options: parachute, explosion and destruction of the UAV or automatic landing. This mode is activated when the UAV lost the communication with the pilot and the GPS. Also, when the batteries of the UAV run down or the pilot lost control of the UAV.

- *Assisted mode:* the UAV stabilizes itself but the pilot indicates where to go (up, down, left and right) and its speed. This mode is activated when the GPS fails but we still have communications with the pilot.

3.3.Aerial release by light aircraft

3.3.1. Suitability of aerial release by light aircraft

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 UAS would be as follow:

- Legal restrictions: the legislation on the civil use of UAS is not developed in many countries. In those who have regulated the use of UAS for civil applications, the operation conditions within urban areas are very restrictive, specially reducing the range of the flight operation and forcing to adopt a strategy of increased number of short flights. This is not the case for light aircrafts, which can cover large areas in one single flight, simplifying the logistics at this level.

- Large programmes: in the case of large programmes targeting areas of hundreds of square kilometers, the use of light aircrafts with a releasing capacity of tens of millions mosquitoes per flight can also significantly reduce the logistics and become a better, more economic option per unit cost of released insects.

However, the following considerations need to be taken into account when selecting this option:

- Operating cost: the operation cost per flying hour of a light aircraft varies between 400 and 800 US$, depending on the selected type of aircraft, which is high compared to the option of UAS. Although the cruise speed of a light aircraft during the releases of fruit flies can reach up to 220 km/h (3-4 times more than that of a UAV), which could reduce the cost per released insect, further research is needed to ensure that this speed is suitable in the case of mosquitoes.

- Flight height: according to the rules of Civil Aviation authorities, the flight height over populated areas can’t be less than 300 m. This is the optimal height in the case of fruit flies, although mosquitoes are less strong fliers and might require a less high release altitude (more research is needed to support this).

3.3.2. Solutions for cooling adult mosquitoes for aerial release by light aircraft

As already mentioned, large experience in compression refrigeration systems for insect release machines hosted in light aircrafts is available from fruit fly SIT programmes currently operating in a number of countries. Similar systems relying in mechanical/compression chilling can be adapted for mosquitoes, which is a well-established technology with manufacturers around the world who can supply a range of scales of solution.

The different chilling options proposed for the UAS can also be valid for light aircrafts, with the major difference that there is no restriction on weight, volume or energy consumption.

However, since the use of light aircraft for mosquito releases will likely entail large amounts of insects loaded per flight, an effective ventilation system to circulate air through the chilled adults will be critical to prevent condensation damaging their wings.

3.3.3. Release devices for light aircraft

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 UAS.

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.

3.3.4. GIS dose release method

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.

3.4.Research required to inform the design process for aerial release

Element to be considered	Options	Research needed
Delivery vehicle	Fixed wing drone, rotative wing drone, piloted aircraft	Developing of a decision making matrix on the optimal solution taking into account the different circumstances and conditions: 1.- legal constraints. Consider the possibility of being exempted from the legal constrains of civil aviation in a country by country basis if considered a priority by High level Official (at the ministry level) 2.- cost-efficiency decision . Return of investment (ROI) 3.- size of the programme 4.- technical considerations to define the payload and endurance needed from vehicle: a) swath width to get optimal distribution b) release altitude to warm up the insects before getting to the ground c) optimal storage conditions to maintain quality: temperature, RH and ventilation d) maximum flight duration to avoid waking up the insects in predefined temperature conditions
Holding cassette	- one layer vs several layers - horizontal vs vertical compartments - sliding layers vs opening cells (grid system) - one single compartment with bulk mosquitoes vs compartimented cassette - single cassette vs multiple cassettes	Maximum compaction tolerated by chilled bulk mosquitoes to define the heigh of the column. - Interaction of flight duration, temperature and the resistance to compaction - density of adults (number of adults per volume unit) - metabolic heat generated by bulk adults - materials: as light as possible and explore PCM as in built option. Easy to manufacture and scaling (modular design) - assess the need for air circulation - ease of loading into cassette - ease of placing cassette into release system - ease of cleanning cassette
Release device	Predosed cartridges vs bulk insect	Assess if discrete or continuous release of bulk adults result in acceptable level of damage in the quality and dosing accuracy: 1.- Continuous a) vibrating conveyor b) sliding gate c) other methods to be developped 2.- Discrete a) predosed boxes b) predosed avoiding releasing containers c) other methods to be developped Selection of materials as light as possible

Cooling system	Static (PCM) vs dymanic (peltier, heat pumps, compressed air, dry ice, conventional A/C) vs hybrid	Establish cooling requirements based on the final cassette design and optimising weight v cooling efficiency, as well as up front and operational cost aspects of the designs Determine the metabolic heat rate produced by the mosquitoes in different temperature environment - determine the minimum temperature causing no damage to the insects during release - determine the behaviour during warming - determine the need for air circulation to avoid stratification - insulation of cassette Compare cooling efficiency of statics vs dynamic against weight of payload - static PCM: a) optimize the selection of melting point to balance the poor conductivity; increase conductivity; encapsulate PCM into aluminium containers b) investigate the amount of internal divisions with PCM c) overchilled material with heat transference regulated by air flow - dynamic: a) investigate energy needs in relation to flight duration (for peltier) (medium drones) b) investigate cartidges of compressed air (liquid CO2)(medium drones) c) mechanical refrigeration for aircrafts
Control of release rates	Discrete release rates vs differential release rates	Development of software to control rate of release according to varying release densities. Software to be run from the ground station and information sent in real time to the drone

4. DEVELOPMENT OF A GROUND RELEASE SOLUTION FOR MOSQUITO SIT PROGRAMMES

Robust insects such as Medfly 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, 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 (example of the MOSCAMED programme in Brazil, Figure 10). There is the possibility to upscale this effort 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. Oxitec has conducted some modelling to maximize the efficiency of coverage using such a release strategy, to minimize the road distance which must be covered by a driver whilst still ensuring sufficient coverage, based on mosquito dispersal data. 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.

The ground release method is not well adapted to the AW-IPM programme as the releases of sterile males must follow the existing road system and thus are not able to homogenously cover the area to be treated. Thus, it is recommended that a ground release method be used only when neither UAS nor aircraft are available (due to regulation, legislation, cost…) or for very small suppression trials.

Figure 10. Illustrations of ground release methods of Oxitec and Moscamed in Brazil, consisting of members of field staff team opening cups of adult mosquitoes as the pick-up drives through the release area.

In the case of suppression programmes already using UAVs 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 ground release machine (GRM). The GRM capacity of the red and white pick-ups shown in Figure 11 is 2 and 10 million *Ceratitis capitata*, respectively.

Figure 11. Illustration of pick-ups used to carry automated ground release systems for *Ceratitis capitata* in Croatia and Mexico.

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 (Figure 12). Recently, to avoid compaction of insects before aerial release, a new container with several compartments has been developed for tsetse (Mubarqui Company, Mexico), a system which could also be adapted for ground release. As weight and space are not limiting factors for ground release, active compressor chilling units are commonly used (working with additional generators or invertors).

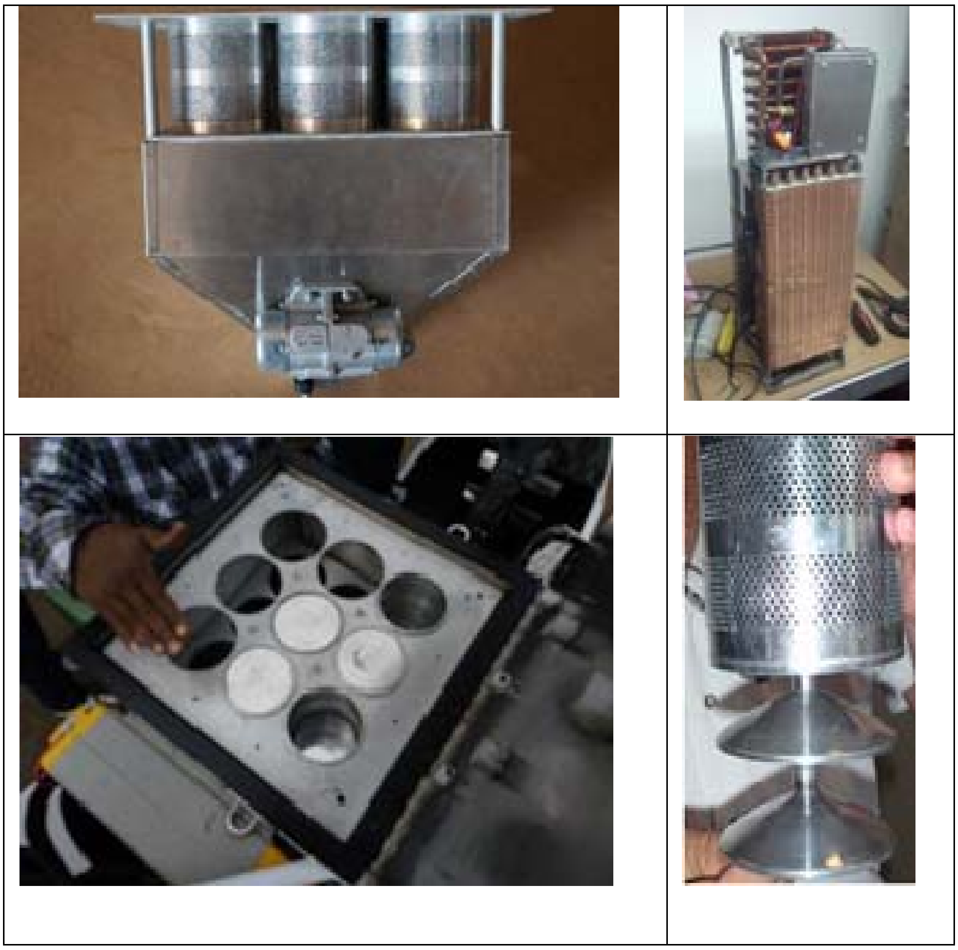

Figure 12. Illustrations of release machine mounted on pick-ups for ground release of *Ceratitis capitata*, including multiple cylinders to carry flies without compaction.

The cooling requirements of a ground release system will be entirely dependent on 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. This leads to an apparent contradiction: bulk mosquitoes must be kept in cold conditions in order to endure the duration of the ground release; however, they must warm up as soon as are released to enable them flying. Therefore, a two-phase release machine might be considered, including a pre-warming step right before release. 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.

4.1.Research required to inform the design process for ground release

Existing ground release methods could be adapted for mosquitoes but further research is needed into the following:

- Required volume and number of units
- Development of new containers with smaller compartments suited to mosquitoes
- Choice of chilling methods (several steps with different temperatures: 6°C for handling and transport and 15°C just prior to release)
- Testing of vibrating conveyors for flexible release rates and to minimise damage
- Improvement of release paths (optimization of area covered) associated to the best release rate (reflecting the mosquito density) using modern technology (GIS software)

Element for Consideration	Options	Research needed
Delivery vehicle	Pick-up, quad, others	Identification of suitable vehicles available in each target area.
Holding cassette	Chilled insects using technology developed for aircrafts and UAS vs (pots/boxes)	Maximum compaction tolerated by chilled bulk mosquitoes to define the height of the column. - Interaction of flight duration, temperature and the resistance to compaction - density of adults (number of adults per volume unit) - metabolic heat generated by bulk adults - materials: as light as possible and explore PCM as in built option. Easy to manufacture and scaling (modular design) - assess the need for air circulation - ease of loading into cassette - ease of placing cassette into release system - ease of cleaning cassette
Release device	Predosed cartridges vs bulk insect associated to blower with pre-warming step	Assess if discrete or continuous release of bulk adults result in acceptable level of damage in the quality and dosing accuracy (see in previous table) Determine optimal temperature for the pre-warning step Determine optimal fan speed for releasing competitive insects Develop non-invasive system ('silent')
Cooling system	Static (PCM), refrigerated boxes or conventional A/C	Determine the metabolic heat rate produced by the mosquitoes in different temperature environment - determine the minimum temperature causing no damage to the insects during release - determine the behaviour during warming - determine the need for air circulation to avoid stratification - insulation of cassette - compare cooling efficiency of statics vs dynamic As the cooling requirements will be entirely depended on the delivery machine and operation once a reliable system is under development, a suitable cooling system must be developed to match the required capacities and energy sources.
Control of release rates	Discrete release rates vs differential release rates	Development of software to control rate of release according to varying release densities. Software run from the car and information sent in real time to the release machine.

5. RECOMMENDATIONS TO THE IAEA

1) Initiate a CRP to develop technologies for handling, transport, release and monitoring of chilled mosquitoes, and invite suitable participants to join

2) Strengthen relationships with industrial partners, developing collaborations where possible, and finding means to provide larger research grants where necessary for the development of expensive technology by commercial partners

3) Identify sources of funding to support procurement for private sector partners, for example by supporting grant applications by private sector partners to develop new release methods, particularly for the use of UAVs

4) Identify collaborators with good scientific and programmatic experience who can validate the proposed solution in a programmatic environment, including insect quality control tests

5) Obtain the biological information outlined in sections 2.3, 3.4 and 4.1 to help engineers to provide suitable technologies

6) Provide more support to the IPCL to assist in their efforts to answer the large number of research questions remaining

7) Develop a consultancy to assist in elaborating different release scenarios and identifying sources of guidance in developing appropriate release technologies for mosquito SIT programmes

8) Programme frequent communication exchange between those groups developing prototypes and Agency technical staff. This is a key issue for increasing the likelihood of finding a realistic solution

6. POTENTIAL PARTICIPANTS FOR A CRP (EQUIPMENT DEVELOPMENT/MANUFACTURE, FIELD TESTING)

- All participants of this meeting
- MOSCAMED facility in Juazeiro, Brazil – Danilo Carvalho (carvalhodanilo85@gmail.com) and Margareth Capurro (mlcapurro@gmail.com)
- Vector control unit, Ministry of Health and Quality of Life, Pointe des Lascars, Mauritius – Ambicadutt Bheecarry (ambicabheecarry@gmail.com) and Diana Iyaloo (dianaiyaloo@yahoo.com)
- Zhiyong Xi and team, suppression programme using Wolbachia-modified *Aedes albopictus*, China - liyongjun_sysu@126.com; zhengxy@mail.sysu.edu.cn; xgchen2001@hotmail.com; xizy@msu.edu
- Steve Dobson, suppression programme using *Wolbachia*-modified *Aedes albopictus* in American Samoa and USA - sdobson@uky.edu
- TRAGSA, SIT pilot project against *Aedes albopictus* in Valencia, Spain – Carolos Tur (ctur@tragsa.es)
- Center for Advanced Aerospace Technologies FADA-CATEC, Seville, Spain - info@catec.aero
- Pilot trial in Sri Lanka (Wimal Abeyewickreme wabeyewickreme@yahoo.com), Thailand (Pat Kittayapong pkittayapong@gmail.com), Indonesia (Hadian Iman Sasmita hisasmita@batan.go.id), Malaysia (Dr Nazni Wasi Ahmad nazni@imr.gov.my), Sudan (Badria El Sayed badriab2@yahoo.com), and South Africa (Lizette Koekemoer lizettek@nicd.ac.za)

Since quality control tests will be performed in Seibersdorf and in Member States with ongoing pilot SIT programmes, we believe that it is very important that the design team has easy contact with these participants.

7. APPENDIX I: CONSULTANTS GROUP MEETING AGENDA

"DEVELOPING STERILE MOSQUITO TRANSPORT AND AERIAL RELEASE METHODS"

8-12 December 2014 Vienna, Austria

Vienna International Centre (IAEA Headquarters), Room MOE100 and Room A2311

MONDAY, 8 DECEMBER 2014. Room MOE100

08:00 – 09:00 Identification and registration at VIC Gate (next to subway station U1)

09:00 – 09:10 Jorge Hendrichs: Welcome statement and goals of the meeting.

09:10 – 09:20 Jérémie Gilles: Agenda and administrative issues.

09:20 – 10:00 Rafael Argilés: Overview of current insect transport and aerial release technologies in pest control programmes with an SIT component.

COFFEE BREAK

SESSION I: Presentations by Consultants

10:30 – 11:30 Roberto Angulo: Current insect aerial release technology used in the Moscamed Programme in Mexico: the 'SMART' release machine. Its use for fruit flies (Mexico) and tsetse flies (Senegal) and its potential for mosquitoes SIT programmes.

11:30 – 12:30 Gustavo Salvador: Current insect release technology used in the Medfly SIT programme in Valencia (Spain), focusing on the GIS based differential release rate. Developing a prototype light drone for tsetse aerial releases.

LUNCH

13:30 – 14:30 Zafer Ure: Applications of phase change materials and its potential as passive cooling system in aerial releases of sterile mosquitoes.

COFFEE BREAK

14:30 – 15:30 Anibal Ollero: Overview of applications and types of Unmanned Aerial Vehicles: its potential for being used for aerial releases of sterile mosquitoes.

15:30 – 16:30 Discussion

TUESDAY, 9 DECEMBER 2014. Room A2311

SESSION II: Conditions for Transport and Release of Mosquitoes

8:30 – 9:00 Jérémie Gilles: Conditions for transport and release of mosquitoes from the point of view of mosquito biology.

9:00 – 09:30 Rafael Argilés: Conditions for transport and release of mosquitoes from the point of view of SIT operations: Target features to be met by the transport & release technology.

SESSION III: General Discussion

09:30 – 10:00 Identification of biological research issues that need to be addressed.

COFFEE BREAK

10:30 – 12:30 Current status of technology suitable for mosquito transport and release at the different component level: cooling system, release device, differential release rate and transportation.

LUNCH

13:30 – 15:00 Identification of appropriate manned and unmanned technology for mosquito transport and release for different landscape scenarios: urban, semi-urban and rural.

COFFEE BREAK

15:30 – 17:00 Evaluation of identified options for manned and unmanned mosquito transport & release from technical, legal and cost-efficiency point of view.

WEDNESDAY, 10 DECEMBER 2014. Room A2311

SESSION III: General Discussion (Cont.)

8:30 – 10:00 Evaluation of identified options for manned and unmanned mosquito transport & release from technical, legal and cost-efficiency point of view (cont.).

COFFEE BREAK

SESSION IV: Development of a Research Proposal

10:30 – 11:30 Background situation analysis and overall objective.

11:30 – 12:30 Specific research objectives.

LUNCH

13:30 – 15:00 Expected research outputs

COFFEE BREAK

15:30 – 17:00 Expected research outcomes.

THURSDAY, 11 DECEMBER 2014. Room A2311

SESSION IV: Development of a Research Proposal (Cont.)

8:30 – 9:30 Activities.

9:30 – 10:30 Assumptions.

COFFEE BREAK

11:00 – 12:30 Logical framework (verifiable indicators and means of verification)

LUNCH

13:30 – 15:00 Logical framework (verifiable indicators and means of verification)(Cont.)

COFFEE BREAK

15:30 – 17:00 Explanation/Justification and other resources required.

FRIDAY, 12 DECEMBER 2014. Room A2311

SESSION IV (Cont.): Development of a Research Proposal

8:30 – 10:00 List of potential participating countries and institutions

COFFEE BREAK

10:30 – 12:00 Summary and finalizing of research proposal.

LUNCH

13:00 – 15:00 Group review of final research proposal.

COFFEE BREAK

15:30 – 17:00 Group review of final research proposal.

8. APPENDIX II: LIST OF PARTICIPANTS

I.1. Consultants

Prof. Anibal Ollero - Unversity of Seville and Head of the GRVC, Scientific Advisor of the Center for Advanced Aerospace Technologies, Co- Chair of the IEEE Technical Committee on Aerial Robotics and Unmanned Aerial Vehicles, Coordinator of the Aerial Robotics Topic Group of euRobotics (Email: aollero@us.es).

Roberto Angulo - (Servicios Aereos, Biologicos Y Forestales Mubarqui). He has over 10 years of experience with fruit fly aerial release systems in different countries and have recently developed a new release machine for tsetse flies that can be installed in a gyrocopter. (Email: rakoperations@yahoo.com.mx and rlmubarqui@yahoo.com.mx).

Gustavo Salvador - University CEU-Cardenal Herrera, Escuela Superior de Ensenanzas Técnicas). He is currently developing a tsetse release machine for light drones and has developed in the past a medfly release machine with differential release rates based on real time GPS readings for the SIT programme in Valencia-Spain. (Email: gsalva@uch.ceu.es).

Zafer Ure (PCM Products). Expert on phase change materials that can be used as passive cooling in drones. (Email: z.ure@pcmproducts.net; ure5hs@btinternet.com).

Antidio Viguria Jiménez - (Center for Advanced Aerospace Technologies FADA-CATEC). He is currently Head of Avionics and Unmanned Systems Department. (aviguria@catec.aero).

IAEA Staff:

Mr Aldo Malavasi
Mr Jorge Hendrichs
Mr Rafael Argilés Herrero
Mr Jérémie RL Gilles
Ms Rosemary S Lees
Mr Jesus Reyes Flores

9. APPENDIX III: OPTIONS FOR TRANSPORT AND RELEASE BY GENUS

Options for Transport and Release of *Aedes* spp. Male Mosquitoes				
Production, Sexing, Irradiation **(and Emergence / Holding / Collection)**	1. Sexing of ***Aedes*** pupae (by size or sexing strain) ↓			**Mass-rearing Facility** ↓
	2. Irradiation of chilled male ***Aedes*** pupae (CO_2?) ↓ ↓			
		3. Transfer into cages for adult emergence ↓		
		4. Feeding and maturation of male adults ↓		
		5. Chilling of mature male adults ↓		
		6. Chilled males falling into transport and release cassettes ↓		
Shipment	3. Transport of chilled male pupae in containers with water ↓	7. Transport of chilled male adults in cassettes ↓		**Transport by Truck or Plane**
Emergence / Holding / Collection	4. Emergence of male adults into holding cages ↓			**Mosquito Emergence and Release Facility** ↓
	5. Feeding and maturation of male adults ↓			
	6. Chilling of mature male adults ↓			
	7. Collection of chilled male adults into release cassettes ↓			
Release	8. Release of chilled male adults from cassette release system	8. Release of chilled male adults from cassette release system		**Airport or mobile release platform (cold truck) with filled cassettes**

Options for Transport and Release of *Anopheles arabiensis* Male Mosquitoes				
Production, Sexing, Irradiation **(and Emergence / Holding / Collection)**	1. Irradiation of chilled ***Anopheles*** pupae (CO_2?) ↓ ↓		Twice per day, minimum 5 hours before emergence Day 1	**Mass-rearing Facility** ↓
		2. Transfer to cages for emergence of male and female adults ↓	Day 1	
		3. Poisoned blood feeding to kill females and then removal of dead females ↓	Day 2 to day Day 5	
		4. Feeding and maturation of males ↓	Day 2 to day Day 5	
		5. Chilling of mature male adults and falling into transport and release cassettes ↓	Day 5	
Shipment	2. Transport of chilled pupae in containers with water Day 1 ↓	6. Transport of chilled male adults in cassettes ↓	Day 5	**Transport by Truck or Plane**
Emergence / Holding / Collection	3. Transfer to emergence and holding cages Day1↓			**Mosquito Emergence and Release Facility** ↓
	4. Poisoned blood feeding to kill females and then removal of dead females Day 2 to day 5↓			
	5. Feeding and maturation of males Day 2 to day 5 ↓			
	6. Chilling of mature male adults and falling into release cassettes Day 5 ↓			
Release	7. Release of chilled male adults from cassette release system Day 5	7. Release of chilled male adults from cassette release system Day 5		**Airport or mobile release platform (cold truck)with filled cassettes**

10. APPENDIX IV: SUMMARY OF THE SPANISH UAS/RPAS REGULATION

<table>
<tr><th>MTOW/ Operation</th><th>VLOS (rural)</th><th>EVLOS (rural)</th><th>BVLOS (rural)</th><th>VLOS (city or populated area)</th><th>BVLOS (city or populated area)</th></tr>
<tr><td><2Kg</td><td rowspan="3">500m from the pilot
120m height
Standard documentation*</td><td rowspan="3">500m from the pilot
1Km extra per observer
120m height
Standard documentation*</td><td>>500m from the pilot
120m height
Forward visual camera
Publish a NOTAM before each operation
Standard documentation*</td><td rowspan="2">100m from the pilot
120m height
Standard documentation* plus a much more detailed aeronautical safety study</td><td>Not allowed</td></tr>
<tr><td>2Kg<MTOW< 10Kg</td><td rowspan="2">Only in segregated airspace or with a certified sense&avoid system

Always in non-controlled airspace</td><td>Not allowed</td></tr>
<tr><td>10Kg<MTOW <25Kg</td><td>Not allowed</td><td>Not allowed</td></tr>
<tr><td>25Kg< MTOW< 150Kg</td><td>Certification of airworthiness issued by the National Civil Aviatiofn Authority
Official registration of the aerial vehicle as any manned aircraft</td><td>Certification of airworthiness issued by the National Civil Aviation Authority
Official registration of the aerial vehicle as any manned aircraft</td><td>Certification of airworthiness issued by the National Civil Aviation Authority
Official registration of the aerial vehicle as any manned aircraft
Certified sense&avoid system will be required</td><td>Not allowed</td><td>Not allowed</td></tr>
<tr><td>MTOW> 150Kg</td><td>Certification of airworthiness issued by EASA
Official registration of the aerial vehicle as any manned aircraft</td><td>Certification of airworthiness issued by EASA
Official registration of the aerial vehicle as any manned aircraft</td><td>Certification of airworthiness issued by EASA
Official registration of the aerial vehicle as any manned aircraft
Certified sense&avoid system will be required</td><td>Not allowed</td><td>Not allowed</td></tr>
</table>

*Standard required documentation for UAS below 25Kg:

- Pilot official approval:
  - Medical certificate (type 2 or LAPL)
  - Theoretical knowledge:
    - 60 hours specific RPAS course (only for MTOW below 25Kg)
    - PPL or ultralight license
  - Practical knowledge: training certification issued by the manufacturer or the operator
- Operation and maintenance manuals
- Description of the UAS or RPAS (aerial vehicle configuration, main characteristics, performances, etc.)
- Aeronautical safety study
- Accreditation that the system has successfully performed the required test flights to validate that the operation of the system is safe (minimum of 5 flights in a controlled and non-populated area)
- Third party liability insurance