**AIM COST**

**WG 2. CONVENTIONAL and INNOVATIVE CONTROL TOOLS**

**Task 2.1 Review, of current control options.**

# Community involvement

Aedes Invasive Mosquitoes (AIM) such as *Aedes albopictus*, *Aedes aegypti*, *Aedes koreicus* and *Aedes japonicus* are container breeder, searching for oviposition small and mostly organic-rich water collections which can be encountered in abundance in the urban environment and namely in the close surroundings of private households.

In the frame of Integrated Vector Management (IVM) programs therefore, involvement of the community is considered as a crucial point for the improvement of the effectiveness of control projects. (ECDC, 2017, Cameron, Bell and Howard, 2012).

Baseline practices for public awareness raising are the distribution of flyers and posters, presentations to schools, public institutions, companies and parishes, distribution of information through social media, telephone lines, informative talks and attending community meetings. However, in order to achieve the active engagement of the community in control efforts and especially in source reduction, more direct and continuous communication tools are necessary. The most frequently used methods in this context are the citizen science approach and door-to-door interventions.

## Citizen science

According to the Citizen Science Association (CSA), ”citizen science is the involvement of the public in scientific research – whether community-driven research or global investigations” (CSA, no date). In Europe there are several citizen science projects in relation to mosquitoes running (European Commission - Joint Research Centre, 2019) the Spanish “Mosquito Alert” (<http://www.mosquitoalert.com/en/>) being the widest known and used beyond the country’s borders. These projects allow citizens to identify and report mosquitos through a mobile application which notes their position by means of global positioning system (GPS) together with other detailed information, which is checked and validated by a team of experts and then published on a map.

Citizen science projects constitute an important tool for the increase in public awareness about mosquito-borne diseases and prevention and contribute essentially to the education of the general public in mosquito control (Switters and Osimo, 2019). The main benefit of this tool is the relatively economical extension capability for geographical and timely monitoring coverage for AIM (and other species) which is extremely costly using conventional monitoring tools such as adult- and ovitraps. Results of these projects can help stakeholders in decision making for other, more drastic control measures to be implemented in time and space.

## Door to door interventions

However, for the achievement of efficient control of AIM populations in infested areas, core intervention in IVMs focusing on AIM are door to door visits and site inspections which aim primarily in community education in terms of elimination of breeding sites, and when combined with other control actions such as larviciding and perifocal adulticiding applications seem to show major efficacy (Baldacchino *et al.*, 2015).

Source reduction is based on removing or turning over temporary water containers and covering permanent water containers which has been proven to be able to suppress temporarily larvae populations of *Aedes albopictus* and other container breeder species as well (Baldacchino *et al.*, 2015). Difficulties in this approach concern mainly the accessibility of private premises, difficulties in reaching the whole population of a given region and the high operational costs. In areas where AIM populations are installed, the door-to-door approach should be integrated in any IVM as long-term strategy and regularly repetitions of inspections are needed in order to maintain a constant dialogue with the communities.

# Trapping

The use of traps for the spatio-temporal surveillance of adult mosquito populations and pathogen detection constitutes a standard method implemented in all IVMs. Besides the qualitative and quantitative evaluation of mosquito populations and the presence of pathogens there have been several attempts to evaluate their efficacy as control method, either at local or at wide-area approach.

Adult trapping methods target either gravid females as in the case of ovitraps or gravid traps, or host seeking female adults as in the case of BG-sentinel® traps (Baldacchino *et al.*, 2015). A mass trapping experiment at household level against *Aedes aegypti* in Brazil showed some encouraging results which however underlied seasonal alterations and restrictions (Degener *et al.*, 2014).

The use of lethal ovitraps, which are ovitraps modified to be lethal to gravid females and larvae, seems to have more encouraging results as commented by Takken and Van Den Berg, 2019, but need still to be tested for operational use in Europe.

However, it has been stated by WHO Regional Office for Europe, 2013, that trapping methods that are unsuitable for widespread municipal use may still be useful for individual residences.

# Biological Control

Biological control involves the addition to aquatic breeding sites of organisms that are predators of, compete with or parasitize larvae and pupae. As with larviciding biological control can be used in habitats where source reduction is not possible. The most common tools for biological control that have been applied in the field are larvivorous fish and cyclopoid copepods with the latter being the more effective predator.

## Larvivorous fish

The mosquito fish *Gambusia affinis* which is the most commonly used larvivirous fish. As itis not native to Europe EMCA have banned the use of this and other non-native fish in Europe due to concerns about the ecosystem should it be accidentally introduced in to local water bodies (ECDC, 2017). Native fish are therefore preferred although some countries may allow non-native fish to be used in isolated water bodies where there is no risk of escape into the local ecosystem.

More widely used for malaria prevention, larvivorous fish can also be used in water storage tanks for container breeding *Aedes* although the addition of fish to small water filled containers is not feasible.

## Copepods

Cyclopoid copepods have proven effective against mosquito larvae including *Aedes* spp. outside of Europe. Semi-field trials in Italy on the effectiveness of *Macrocyclops albidus* controlling *Ae. albopictus* produced favourable results (Veronesi et al. 2015) as have laboratory studies using *Macrocyclops* spp. against *Ae. koreicus* (Baldacchino et al 2017). Despite their effectiveness copepods do however require a food supply and are vulnerable to breeding habitats that are at risk from drying out.

As with most control tools biological control can form part of an integrated mosquito control programme. Copepods predate first instar larvae so given the specificity of Bti with its non-toxicity towards non-mosquito organisms it could be applied to water containers that have been treated with copepods to kill later stage larvae.

# Larviciding

Larviciding involves the application of insecticides to aquatic breeding sites with the goal of killing larvae and thereby reducing mosquito abundance. There is currently a scarcity of studies that have demonstrated the effectiveness of larvicides as a standalone method for vector control. Cryptic containers left untreated could reduce the effectiveness of larviciding. Nonetheless larviciding is recommended as a complementary method, for example alongside source reduction for control of breeding sites of *Aedes* that cannot be removed or are used for water storage. As the effects are temporary, repeated treatment of breeding sites is required.Three classes of larvicides are used for mosquito control; bacterial insecticides, insect growth regulators and chemical insecticides (WHO, 2018).

## Bacterial insecticides

Bacterial protein toxins produced during sporulation proves to be lethal when ingested by larvae. Two bacterial insecticide products are used one based on *Bacillus thuringiensis* var. *israelenis* called Bti and a product based on a mixture of Bti and *Lysinibacillus (bacillus) sphaericus* (Lsph) called Bti + Lsph. The major benefit of bacterial insecticides is that they are specific to mosquito larvae with non-toxicity to other insects, fish mammals and humans. Furthermore, as Bti produces four toxic proteins the risk of resistance developing is reduced compared with chemical insecticides. Lsph on its own produces only one toxin which can increase the risk of resistance however the risk can be reduced with Bti + Lsph. The non-toxicity of Bti to non-mosquito organisms make it suitable for peri-domestic habitats. A disadvantage of Bti is it’s limited residual activity which is further reduced in direct sunlight but this can be countered by using Bti + Lsph. Bti is widely used in Europe and Bti + Lsph can be applied as a granular formula but there is evidence that it is more effective when applied as motorised misters (ECDC, 2017).

The spinosyn class of insecticides have the active ingredient Spinosad which consists of two neurotoxins (A and D) produced during the fermentation of *Saccharopolyspora spinosa.* Although it is non-toxic to vertebrates, Spinosad it is less specific than Bti which requires further study but does have longer residual activity and performed well in an experimental car tyre experiment in Mexico against container-breeding mosquitoes (Marina *et al.* 2012).

## Insect growth regulators (IGRs)

IGRs which mimic juvenile hormone (e.g. pyriproxfen and methoprene) stop the development of larvae and pupae into adults, whilst chitin synthesis inhibitors (e.g. diflubenzuron) kill the larvae when they moult. IGRs sit between bacterial insecticides and chemical insecticides in terms of specificity as they affect other insects and have a low risk to human health. Some have longer residual activity than Bti.

## Chemical insecticides

Chemical insecticides have been the traditional tool for mosquito control but are now less favoured compared with bacterial insecticides and IGRs as they have acute toxicity and are non-specific making them a risk to humans and the environment. Insecticide resistance is also a growing global problem particularly in *Aedes aegypti* (Dusfour *et al.* 2019). Whilst organophosphates temephos and primiphos-methyl are used as larvicides worldwide, temephos is banned from use in mainland Europe.

# Adulticiding

Adulticiding is one of the most drastic and direct control measures which is used mainly in epidemic situations when active transmission of pathogens through mosquitoes is threatening public health (ECDC, 2017). The European regulatory framework for the use of biocides is defined through Directive 98/8/EC (Biocidal Products Directive) and EU Regulation 528/2012 (Biocidal Products Regulation) and in accordance to these, most available adulticides for vector control are of the pyrethrins and pyrethroids group. The use of pyrethroids is relatively safe for humans and they have a high insecticidal potency at low dosages resulting in a rapid knock-down effect but they are also toxic to a broad range of non-target insects, aquatic invertebrates and fish (Baldacchino *et al.*, 2015). Depending on the application method, two different treatment types are distinguished: space treatments and surface treatments.

## Space treatments

Adulticiding space treatments against AIM in Europe is mostly implemented by ground ULV (Ultra Low Volume) applications with hand-carried or vehicle-mounted thermal or cold foggers. These foggers produce a mean droplet size of around 20μm which is capable of hovering in the air for a certain time and penetrate effectively shrub and tree vegetation where adult mosquitoes may hide. Important for this kind of treatment is its implementation during the high-activity period of targeted adult mosquitoes which is difficult in the case of *Aedes albopictus* e.g. because of its daytime activity (Baldacchino *et al.*, 2015). Main active ingredients used for ULV space treatments are deltamethrin and α-cypermethrin and a combination of permethrin–tetramethrin–piperonyl butoxide had shown to be efficient even if applied during nighttime (Farajollahi *et al.*, 2012).

## Surface treatments

Surface treatments of pyrethroids aim in the highest possible residual effect of adulticides on surfaces such as vegetation and shaded surfaces of buildings (ORS), interior walls of houses (IRS) and / or other Insecticide treated surfaces (ITMs).

Outdoor residual spraying applications are implemented either in public areas such as recreation parks, shrubby playground enclosures, surfaces of public buildings or other known mosquito resting harbourages, or on the outskirts of private premises. Applications take usually place using conventional Low Volume (LV) spraying equipment and aiming in 100% surface coverage and avoidance of runoff of the insecticide solution. Mainly used adulticides are deltamethrin and α-cypermethrin.

Indoor residual spraying applications are indicated in cases of anthropophilic infective vectors entering dwellings before and after blood feeding (endophilic) which is a behavior observed mainly in *Aedes aegypti* rather than *Aedes albopictus* or other AIMs (ECDC, 2020). This method consists in total surface coverage of the inner walls of dwellings with adulticides characterized by a high residual effect which can last up to six months depending on the type surface sprayed, the active ingredient and formulation used, as well as on the maintenance behavior of residents.

Whereas Insecticide Treated bed Nets (ITNs), are a standard method for malaria control, targeting endophagic anopheline mosquitoes, the basic principle of this approach, attract and kill host seeking or, in the case of AIMs, breeding site seeking adult females, can also be effectively used for the control of AIM populations as for instance by use of impregnated nets to cover water barrels or lethal traps (pyrethroid impregnated ovitraps, GAT traps or others) (Baldacchino *et al.*, 2015).

In any case, when scheduling the implementation of adulticide treatments for vector control, an assessment of the existing pyrethroid resistance in local AIM populations should be envisaged, especially for *Aedes aegypti* for which pyrethroid resistance has been proven in several places (Dusfour *et al.*, 2019), but also for *Aedes albopictus*, even if pyrethroid resistance seems to be limited to very few focal points. Due to the widespread use of pyrethroids in agriculture an effect on nearby AIM populations cannot be excluded.

Possible “strategies for IRM [Insecticide Resistance Management] include insecticide rotation, mosaic, and mixture/combination as well as the use of nonchemical alternatives in the frame of IVM, but there have been few tests of different strategies for efficacy on *Aedes* populations and mosquitoes generally” (Dusfour *et al.*, 2019).

# Autodissemination

Autodissemination is a control method which utilizes female mosquitoes as insecticide-carriers to contaminate their own breeding sites.

This method is not fully understood because many parameters concerning the abundance/competition of the breeding sites in the field, the behaviour of the females of the targeted species, choice of the insecticide and its capacity to be fixed on the insects etc. are involved. Different traps (called stations in autodissemination) or prototypes were developed (Gaugler *et al.*, 2011) and are as yet not fully defined to demonstrate how this method could be applied in the field for a successful control of vectors; for example shape of the station, number of stations per surface unit, sorts and formulation of the insecticide etc.

Autodissemination can be used for the control of mosquitoes using small containers as breeding sites, like *Aedes aegypti* or *Aedes albopictus* but also *Anopheles arabiensis* and others (Lwetoijera *et al.,* 2014)*.* The process involves three steps:

**Step 1:** The aim is to attract gravid female mosquitoes while they are searching for small water collections to lay their eggs, and entrap them. Prototypes usually involve a container whose size/color/shape has been shown as very efficient to attract these females (males can also be collected, attracted by females or humidity). For most of the stations, the surface of the water is not reachable due to the presence of a mosquito net 5-10 cm above the water.

**Step 2:** While they are in the station, the females try to reach the water surface. Once they realise it is impossible, they try to find a way out of the station. Females can then be contaminated by the insecticide while they are flying or walking inside the trap, or on their way to leave the station, walking along a funnel contaminated with powder or insecticide and getting some slicked on their legs, or walking first in oil and then on the powder when in a contamination chamber before leaving the station. Any insecticide permitted for use in a country could theoretically be used since it is efficient at very low doses but Pyriproxyfen (an agonist of Juvenile Hormone) powder is the most commonly used in published studies.

**Step 3:** The insecticide is released when females exit the trap and find a “natural” breeding site and start to lay their eggs. As females touch the water surface with their legs during the egg-laying the insecticide is released into the natural breeding container.

The efficacy of this method has not been critically evaluated, more research and development of the methods is required (Unlu *et al.,* 2020). Questions remain as to how, where, and when this strategy can be used, however the results of the studies already published are promising.

# Sterile Insect Technique

The Sterile Insect Technic (SIT) is a method the objective of which is to decrease the global population of a targeted species by releasing sterile males which would inseminate the wild female with sterile sperm or sperm which lead to incompatibility with the development of the embryo or the larvae. The efficiency of such strategies has already been demonstrated against insects, for pest control since the 1960’s or, more recently against vectors like glossinidae vectors of trypanosomiasis. This strategy is particularly efficient against populations of isolated insects, and/or for species with a low fecundity. For mosquitoes, this technique showed promising results on “test areas” but is not yet used routinely for vector control. Since it doesn’t require chemicals or insecticide, several programs are now running in Europe to study more in detail its cost and benefit.

SIT requires the production of a large number of sterile males. For mosquitoes three methods could be used: irradiation of wild populations reared in a laboratory at level that doesn’t affect the fitness of the sterilized males, the use of Wolbachia (an endosymbiont of mosquito cells which have the particularity not to allow the development of embryo when the strains of the male and the female are not compatible (Harris *et al.,* 2012)), or the Release of Insects carrying Dominant Lethals (RIDL) technique (see section 8). This last technique recently showed that their previous experiments at large-scale weren’t successful nor as well controlled as they should be and lead to the release of surviving GMO in the field, which could be a problem of ecological concern.

The Wolbachia strategy needs to be adapted for each region, depending on the wild strains of Wolbachia present in the field before choosing an incompatible one carried by the mosquitoes which would be released to obtain sterile descendants (Ayatame *et al.,* 2012). When mosquitoes are inoculated with non-mosquito-specific strains of Wolbachia, the obtained colonies are often considered by the bioethical authorities as GM insects, which could decrease the acceptance of this technique by the human population. The irradiation methods have the advantage that the populations of wild mosquitoes from the field are then reared in the laboratory and thus, in case of a problem during the sterilization process, not to release anything else that irradiated mosquitoes that belong to the same population as the wild one.

To be effective, wild female mosquitoes must be inseminated by reared sterile males, which requires that a large amount of mosquitoes have to be released (at least ten times more sterile males realized than wild male naturally present) which must be reared in efficient mosquito-production units. Not every target species can be reared, so this strategy is limitated to the stenogamous ones (ex : *Aedes albopictus, Aedes aegypti etc*.) . The biology and ecology of the targeted species must be well-known, because releases must occur when and where mosquitoes are emerging, which requires regular and large scale operations; the population of wild mosquitoes can also be lowered before male release with insecticides to decrease the number of required males that need to be released. This strategy is particularly adapted to isolated areas, and/or species with low invasive capacity, to obtain eradication of the target for a consistent period of time. If the objective is not eradication, then releases must be conducted during all the mosquito activity period. These kinds of operations (production and releases) require significant quantities of trained personnel and could be costly compared to more standard control strategy. As this method is increasingly studied and evaluated, parts of the production process have already been well standardized, and their associated costs decreased.

# Autocidal (genetic)

Whilst there are a small number of potential genetic modification (GM) of mosquitoes for control (e.g. RNAi and homing endonuclease genes) only the RIDL technique has been applied in the field (Baldachino, 2015). RIDL is similar to SIT with it’s female -killing effects. With RIDL the female acting transgenes are delivered to the wild female population via mating with the genetically modified males and lead to death of the offspring leading to population suppression (Alphey *et al.,* 2010). Field trials of GM mosquitoes have been conducted in Brazil, Cayman Islands and Panama using Oxitec’s OX513A *Ae. aegypti* males (<https://www.oxitec.com/en/public-health>). This approach, which is not self-sustaining and requires large production facilities for the repeated release of GM males, has not been deployed in the field in Europe.

# References

Alphey, L., Benedict, M., Bellini, R., Clark, G. G., Dame, D. A., Service, M. W., & Dobson, S. L. (2010). Sterile-insect methods for control of mosquito-borne diseases: an analysis. *Vector-Borne and Zoonotic Diseases*, *10*(3), 295-311. [doi.org/10.1089/vbz.2009.0014](https://doi.org/10.1089/vbz.2009.0014)

Atyame, C.M., Labbé, P., Lebon, C., Weill, M., Moretti, R., Marini, F., Gouagna, L.C., Calvitti, M. and Tortosa, P., (2016). Comparison of irradiation and Wolbachia based approaches for sterile-male strategies targeting Aedes albopictus. *PLoS one*, *11*(1). doi: 10.1371/journal.pone.0146834

Baldacchino, F. *et al.* (2015) ‘Control methods against invasive Aedes mosquitoes in Europe: A review’, *Pest Management Science*, 71(11), pp. 1471–1485. doi: 10.1002/ps.4044.

Cameron, M. M., Bell, M. and Howard, A. F. V. (2012) *Handbook for Integrated vector management*, *WHO*. doi: 10.1564/v24\_jun\_14.

CSA (no date) *About - Citizen Science Association*. Available at: https://www.citizenscience.org/about-3/ (Accessed: 2 March 2020).

Degener, C. M. *et al.* (2014) ‘Evaluation of the Effectiveness of Mass Trapping With BG-Sentinel Traps for Dengue Vector Control: A Cluster Randomized Controlled Trial in Manaus, Brazil’, *Journal of Medical Entomology*, 51(2), pp. 408–420. doi: 10.1603/me13107.

Dusfour, I. *et al.* (2019) ‘Management of insecticide resistance in the major Aedes vectors of arboviruses: Advances and challenges’, *PLoS Neglected Tropical Diseases*, 13(10), pp. 1–22. doi: 10.1371/journal.pntd.0007615.

ECDC (2017) *Vector control with a focus on Aedes aegypti and Aedes albopictus mosquitoes Literature review and analysis*. Edited by E. C. for D. P. and Control. doi: 10.1098/rstb.2011.0215.

ECDC (2020) *Mosquito factsheets*, *Fact Sheets*. Available at: https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets (Accessed: 2 March 2020).

European Commission - Joint Research Centre (2019) *European Alien Species Information Network (EASIN)*. Available at: https://easin.jrc.ec.europa.eu/easin/CitizenScience/Projects (Accessed: 2 March 2020).

Farajollahi, A. *et al.* (2012) ‘Effectiveness of Ultra-Low Volume Nighttime Applications of an Adulticide against Diurnal Aedes albopictus, a Critical Vector of Dengue and Chikungunya Viruses’, *PLoS ONE*, 7(11). doi: 10.1371/journal.pone.0049181.

Gaugler R, Suman D, Wang Y. (2012) An autodissemination station for the transfer of an insect growth regulator to mosquito oviposition sites. Med Vet Entomol, Mar;26(1):37-45. doi: 10.1111/j.1365-2915.2011.00970.x. Epub 2011 Jun 20.

Harris, A.F., McKemey, A.R., Nimmo, D., Curtis, Z., Black, I., Morgan, S.A., Oviedo, M.N., Lacroix, R., Naish, N., Morrison, N.I. and Collado, A., (2012). Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature biotechnology*, *30*(9), pp.828-830. doi.org/10.1038/nbt.2350

Lwetoijera, D., Harris, C., Kiware, S., Dongus, S., Devine, G. J., McCall, P. J., & Majambere, S. (2014). Effective autodissemination of pyriproxyfen to breeding sites by the exophilic malaria vector Anopheles arabiensis in semi-field settings in Tanzania. *Malaria journal*, *13*(1), 161. doi: 10.1186/1475-2875-13-161. [doi.org/10.1186/1475-2875-13-161](https://doi.org/10.1186/1475-2875-13-161)

Marina, C.F., Bond, J.G., Muñoz, J., Valle, J., Chirino, N. and Williams, T. (2012). Spinosad: a biorational mosquito larvicide for use in car tires in southern Mexico. *Parasites & vectors*, *5*(1), 95. <https://doi.org/10.1186/1756-3305-5-95>

Switters, J. and Osimo, D. (2019) *Citizen Science in the Surveillance and Monitoring of Mosquito-Borne Diseases. Open Science Monitor Case Study*. Brussels: European Union. doi: 10.2777/431775.

Takken, W. and Van Den Berg, H. (2019) *Manual on prevention of establishment and control of mosquitoes of public health importance in the WHO European Region (with special reference to invasive mosquitoes)*.

Unlu, I., Rochlin, I., Suman, D. S., Wang, Y., Chandel, K., & Gaugler, R. (2020). Large-Scale Operational Pyriproxyfen Autodissemination Deployment to Suppress the Immature Asian Tiger Mosquito (Diptera: Culicidae) Populations. *Journal of Medical Entomology*. [doi.org/10.1093/jme/tjaa011](https://doi.org/10.1093/jme/tjaa011)

Veronesi, R., Carrieri, M., Maccagnani, B., Maini, S., & Bellini, R. (2015). Macrocyclops albidus (Copepoda: Cyclopidae) for the biocontrol of Aedes albopictus and Culex pipiens in Italy. *Journal of the American Mosquito Control Association*, *31*(1), 32-43. <https://doi.org/10.2987/13-6381.1>

WHO Regional Office for Europe (2013) ‘Regional framework for surveillance and control of invasive mosquito vectors and re-emerging vector-borne diseases 2014-2020’, pp. 1–26.

# World Health Organisation (2018). Manual on prevention of establishment and control of mosquitoes of public health importance in the WHO European Region (with special reference to invasive mosquitoes). Geneva, WHO. <http://www.euro.who.int/__data/assets/pdf_file/0004/392998/mosquito-manual-eng.pdf?ua=1>