Review

Received: 29 December 2014 Revised: 19 April 2015 Accepted article published: 21 May 2015 Published online in Wiley Online Library: 26 June 2015

(wileyonlinelibrary.com) DOI 10.1002/ps.4044

Control methods against invasive *Aedes* mosquitoes in Europe: a review

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Abstract

Five species of invasive *Aedes* mosquitoes have recently become established in Europe: *Ae. albopictus*, *Ae. aegypti*, *Ae. japonicus japonicus*, *Ae. koreicus* and *Ae. atropalpus*. These mosquitoes are a serious nuisance for people and are also competent vectors for several exotic pathogens such as dengue and chikungunya viruses. As they are a growing public health concern, methods to control these mosquitoes need to be implemented to reduce their biting and their potential for disease transmission. There is a crucial need to evaluate methods as part of an integrated invasive mosquito species control strategy in different European countries, taking into account local *Aedes* infestations and European regulations. This review presents the control methods available or in development against invasive *Aedes* mosquitoes, with a particular focus on those that can be implemented in Europe. These control methods are divided into five categories: environmental (source reduction), mechanical (trapping), biological (e.g. copepods, *Bacillus thuringiensis* var. *israelensis*, *Wolbachia*), chemical (insect growth regulators, pyrethroids) and genetic (sterile insect technique and genetically modified mosquitoes). We discuss the effectiveness, ecological impact, sustainability and stage of development of each control method.

Keywords: vector control; *Aedes*; integrated vector management; field trial; insecticide; Europe

1 INTRODUCTION

The increasing globalisation of trade and human movement, along with environmental change, facilitates the introduction and establishment of invasive mosquito species (IMS) outside their native geographical areas.[1−3] *Aedes* (Diptera: Culicidae) mosquitoes have a high invasive potential, as their eggs can withstand desiccation for many months and survive long transportation times. Five species have already been established in Europe: *Ae. albopictus*, *Ae. aegypti*, *Ae. japonicus japonicus*, *Ae. koreicus* and *Ae. atropalpus*.[2] Introduced into Albania in 1979, the Asian tiger mosquito *Ae. albopictus* is the most widely spread IMS in Europe and has now colonised almost all Mediterranean countries.[2,4] In comparison, other IMS have been introduced into Europe more recently: *Ae. aegypti* in Madeira Island (Portugal) and around the Black Sea,[4] *Ae. j. japonicus* to Central Europe (Germany, Switzerland, Austria, Slovenia, Croatia),[5] *Ae. koreicus* to Belgium and Italy[6,7] and *Ae. atropalpus* to France, Italy and the Netherlands.[2,8] However, *Ae. albopictus* and *Ae. aegypti* have been reported in some European overseas territories since the beginning of the century.[9−11]

IMS are defined by their ability to colonise new territories and impact on human health, with negative consequences on the environment and the local economy.[4] Owing to their aggressive biting behaviour, *Aedes* mosquitoes, especially *Ae. albopictus*, are a major nuisance for people, who consider that they affect their social life and outdoor activities (up to 81 landing female *Ae. albopictus* $human^{-1}$ 15 min^{-1} counted in Rome; Caputo B, private communication).[12,13] They are also competent vectors of several exotic pathogens such as the dengue and chikungunya viruses, and increase the risk of epidemics in Europe through their establishment and the introduction of these pathogens by infected travellers.[14] An outbreak of chikungunya occurred in Italy in 2007, with more than 200 cases confirmed, and even a large number of autochthonous cases of dengue and chikungunya were reported in Europe between 2007 and 2012.[15,16] In different locations, *Ae. albopictus* (Italy, France, Croatia) and *Ae. aegypti* (Madeira Island) were implicated as vectors. Therefore, some European countries are now highly vulnerable to mosquito-borne diseases (MBD) owing to the continuous reintroduction and spread of *Aedes* mosquitoes.[4]

To face the growing risk of MBD epidemics, the European Centre for Disease Prevention and Control (ECDC) has established a network of medical entomologists and public health experts (VBORNET) and produced guidelines to support the implementation of IMS surveillance in Europe.[1,2] Currently, national surveillance

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systems are in place in France, the United Kingdom, the Netherlands and Germany, and a pilot IMS surveillance programme following ECDC guidelines has been conducted in Belgium.[1] In Italy, regional entomological surveillance has been initiated, for example, in the Emilia-Romagna region and surroundings following the chikungunya outbreak.[17] Yet no specific guidelines have been established for implementing IMS control measures in Europe. The World Health Organisation (WHO) has published a handbook for integrated vector management (IVM), which provides an operational framework for planning and implementing vector-borne disease (VBD) control according to IVM.[18] However, there is a crucial need in Europe to assess IMS control measures taking into account local *Aedes* infestations and European regulations with the aim of lowering biting rates and reducing mosquito populations to an infestation level below the epidemic-risk threshold in case of the introduction of an exotic pathogen.[2,19] To date, only a few studies have evaluated the effectiveness of integrated IMS control strategies in Europe. In Spain, Chebabi Abramides *et al.*[20] demonstrated a reduction in *Ae. albopictus* abundance by source reduction and insecticide application. In Italy, Caputo *et al.*[21] showed a reduction in *Ae. albopictus* abundance during the major phase of population expansion after insecticide application (della Torre A and Caputo B, private communication).

This review presents the IMS control tools available or in development, with a particular focus on those that can be implemented in Europe. Following the WHO handbook, we divided available control methods into four categories: environmental, mechanical, biological and chemical.[18] A fifth category including genetic control methods was also considered. We describe the effectiveness, ecological impact, sustainability and stage of development of each control method (Table 1), focusing on those targeting *Aedes* mosquitoes with the aim of reducing their abundance (Fig. 1). Personal protection methods such as repellents, treated clothes or mosquito screens are not discussed in this review, although they are effective in bite prevention, and their large adoption by the public might protect against pathogen transmission.[22–24] To conclude, we discuss considerations regarding the implementation and evaluation of an integrated IMS control strategy.

2 ENVIRONMENTAL METHODS

Source reduction consists of preventing *Aedes* mosquitoes from using potential breeding sites, which include a wide range of containers, from bottle caps to water tanks. This strategy is based on removing or turning over temporary water containers and covering permanent water containers. It is often the first control method for mosquitoes such as *Ae. albopictus*[25] that breed in artificial containers, and source reduction campaigns generally achieve temporary suppression of immature *Ae. albopictus*.[20,25] This method may also affect the distribution of native mosquitoes such as *Culex* sp. in a locality, by limiting the available sites for oviposition.[25] In a suburb of Washington, DC, source reduction practices by residents to decrease the number of containers used by *Culex pipiens* also affected the number of *Ae. albopictus*.[26]

IMS can find a wide variety of breeding sites in urban, suburban and rural areas. *Ae. albopictus* mostly prefers small- or medium-sized artificial containers.[27] In urban and suburban areas, this includes catch basins and plant saucers in homes or cemeteries; in rural areas, this includes buckets and drums in vegetable gardens. *Ae. koreicus* and *Ae. j. japonicus* prefer natural and artificial aquatic containers, the latter species being highly tolerant to organic concentrations.[7,28] *Ae. atropalpus*, a rock hole mosquito, frequently occurs in tyres.[8,29] *Ae. aegypti* proliferates in artificial containers placed in or near homes.[30] As invasive *Aedes* sp. and native *Culex* sp. mainly select medium-sized containers, it has been suggested that *Ae. albopictus* is displacing *Cx. pipiens* from some of its habitats.[13,31–33]

The type of container available in a specific area is closely related to mosquito production because certain breeding sites can be highly productive for some species. For instance, the most productive breeding sites for *Ae. albopictus* are corrugated extension spouts in New Jersey,[34] catch basins in northern Italy[27] and basins, tanks and tyres on La Réunion Island (Indian Ocean).[35] Moreover, aggregations of containers create 'hot spots' of mosquito production and serve as sources for the infestation of neighbourhoods.[36] As a result, the most time-saving and cost-effective approach may be to focus on the most productive breeding sites. In Brazil, a source reduction campaign against *Ae. aegypti* was conducted using nylon net to cover water tanks and metal drums, both previously identified as the most productive breeding sites in the study area.[37] After two interventions, a long-term reduction in female mosquito density was observed, supporting the effectiveness of targeting key containers. Mapping can also be done at very high spatial resolution using satellite data to facilitate locating these key containers.[38] Unfortunately, this does not help in locating cryptic breeding sites, which are hidden and/or more unreachable sites used by *Aedes* mosquitoes, such as natural reservoirs (e.g. leaf litter) and artificial receptacles (e.g. rubbish).[39]

Effective source reduction, especially for *Ae. albopictus*, requires scrupulous and repeated cleaning or treatment of containers for everyday use, so relies on extensive homeowner collaboration.[31] As private residences are important sources of *Ae. albopictus*, public education campaigns to help people identify and eliminate small water containers from their property have become a basic element in mosquito control programmes, even if this is not always sufficient in motivating residents to reduce backyard mosquito larval habitats.[40,41] Nonetheless, a community-based approach to improving source reduction by targeting containers around the home is an effective long-term strategy that could significantly reduce the cost of control measures.[41] In Spain, a community-based approach associated with insecticide application within the framework of a programme to control *Ae. albopictus* has had promising success.[20] In New Jersey, volunteer-based peer education in source reduction led to a significant reduction in container habitats for *Ae. albopictus* larvae.[42] In Thailand, health education volunteers were trained to conduct biological vector control using copepods (see Section 4.2) and *Bacillus thuringiensis* var. *israelensis* (*Bti*) (see Section 4.3).[43] This community-based vector control programme resulted in a significant reduction in *Ae. aegypti* density. However, voluntary approaches may be limited by the regional culture, resulting in ineffective efforts and loss of public money. Another approach is to support community programmes using paid specialists: for example, in California, mosquito control is efficiently managed through local abatement districts that employ technicians directly involved in surveillance, education and vector control strategy and who interact with and educate the public.[44]

3 MECHANICAL METHODS

Traps are widely used for the survey and monitoring of mosquito populations. Mass trapping using odour baits has been suggested as a means to reduce adult populations of mosquitoes.[45] Available trapping methods for *Aedes* mosquitoes target gravid females

Table 1. Methods to control IMS mosquitoes[a]

Control method	Effectiveness and specificity	Impact on non-target species	Sustainability	Stage of development	Target IMS species
Environmental methods					
Source reduction with or without public education	Container-inhabiting mosquitoes	No	No resistance selection	Operational	*Ae. aegypti*,[190] *Ae. albopictus*,[20,42], *Ae. j. japonicus*,[191] *Ae. atropalpus*[84]
Mechanical methods					
Lethal ovitraps	*Aedes* mosquitoes	No	Resistance to insecticides	Large-scale field trials	*Ae. aegypti*[47]
Sticky or gravid ovitraps	*Aedes* mosquitoes	No	No resistance selection	Large-scale field trials	*Ae. aegypti*[56,57]
BG Sentinel traps	Non-specific	No	No resistance selection	Large-scale field trials	*Ae. aegypti*[59]
Biological methods					
Entomopathogenic fungi	*Aedes* mosquitoes	No	Resistance to fungi infections	(Semi-)field experiments	*Ae. aegypti*,[65,66,68] *Ae. albopictus*[66]
Copepods	Container-inhabiting mosquitoes	No	Escape from predators	Large-scale field trials	*Ae. aegypti*,[70] *Ae. albopictus*[70,71]
Bti with or without *Lsph*	Non-specific	No	No resistance to *Bti*	Operational	*Ae. aegypti*,[83,85,190] *Ae. albopictus*,[85,88] *Ae. j. japonicus*,[82] *Ae. koreicus*,[81] *Ae. atropalpus*[84]
Spinosad	Non-specific	Yes	Resistance selection but no cross-resistance with other insecticides	Large-scale field trials	*Ae. aegypti*,[83,93] *Ae. allbopictus*,[93] *Ae. j. japonicus*[82]
Essential oils	Non-specific	Unknown	Low risk of resistance	Laboratory experiments	*Ae. aegypti*,[95] *Ae. albopictus*,[192] *Ae. j. japonicus*[97]
Wolbachia	Species specific	No	Potential resistance to *Wolbachia* infection	Large-scale field trials	*Ae. aegypti*,[109] *Ae. albopictus*[103,108]
Chemical methods					
Insect growth regulators					
Direct application	Non-specific	Yes	Resistance to insecticides	Operational	*Ae. aegypti*,[115] *Ae. albopictus*[116]
Autodissemination	*Aedes* mosquitoes	Yes	Resistance to insecticides	(Semi-)field experiments	*Ae. aegypti*,[117,121] *Ae. albopictus*[118,119]
Pyrethroids					
Space spraying	Non-specific	Yes	Resistance to insecticides	Operational	*Ae. aegypti*,[130] *Ae. albopictus*,[20,134] *Ae. atropalpus*[84]
ATSB	Non-specific	Yes	Resistance to boric acid or eugenol unknown	(Semi-)field experiments	*Ae. aegypti*,[193] *Ae. albopictus*[149]
IRS and ITMs	*Aedes aegypti*	Yes	Resistance to insecticides	Operational	*Ae. aegypti*[142–144]
Genetic methods					
SIT	Species specific	No	Low mating competitiveness of released males	Large-scale field trials	*Ae. albopictus*[154,155]
dsRNA	Species specific	No	No resistance selection	Laboratory experiments	*Ae. aegypti*[159]
RIDL	Species specific	No	Potential resistance to genetic modification	(Semi-)field experiments	*Ae. aegypti*[160]
RNAi	Species specific	No	Pathogen resistance to RNAi-based blocking	Laboratory experiments	*Ae. aegypti*[165]
HEGs	Species specific	No	Potential resistance to genetic modification	Laboratory experiments	*Ae. aegypti*[167]

[a] IMS: invasive mosquito species; *Bti*: *Bacillus thuringiensis* var. *israelensis*; *Lsph*: *Lysinibacillus sphaericus*; ATSB: attractive toxic sugar bait; IRS: indoor residual spraying; ITMs: insecticide-treated materials; SIT: sterile insect technique; dsRNA: double-stranded RNA; RIDL: rearing of insects carrying a dominant lethal allele; RNAi: RNA interference; HEGs: homing endonuclease genes.

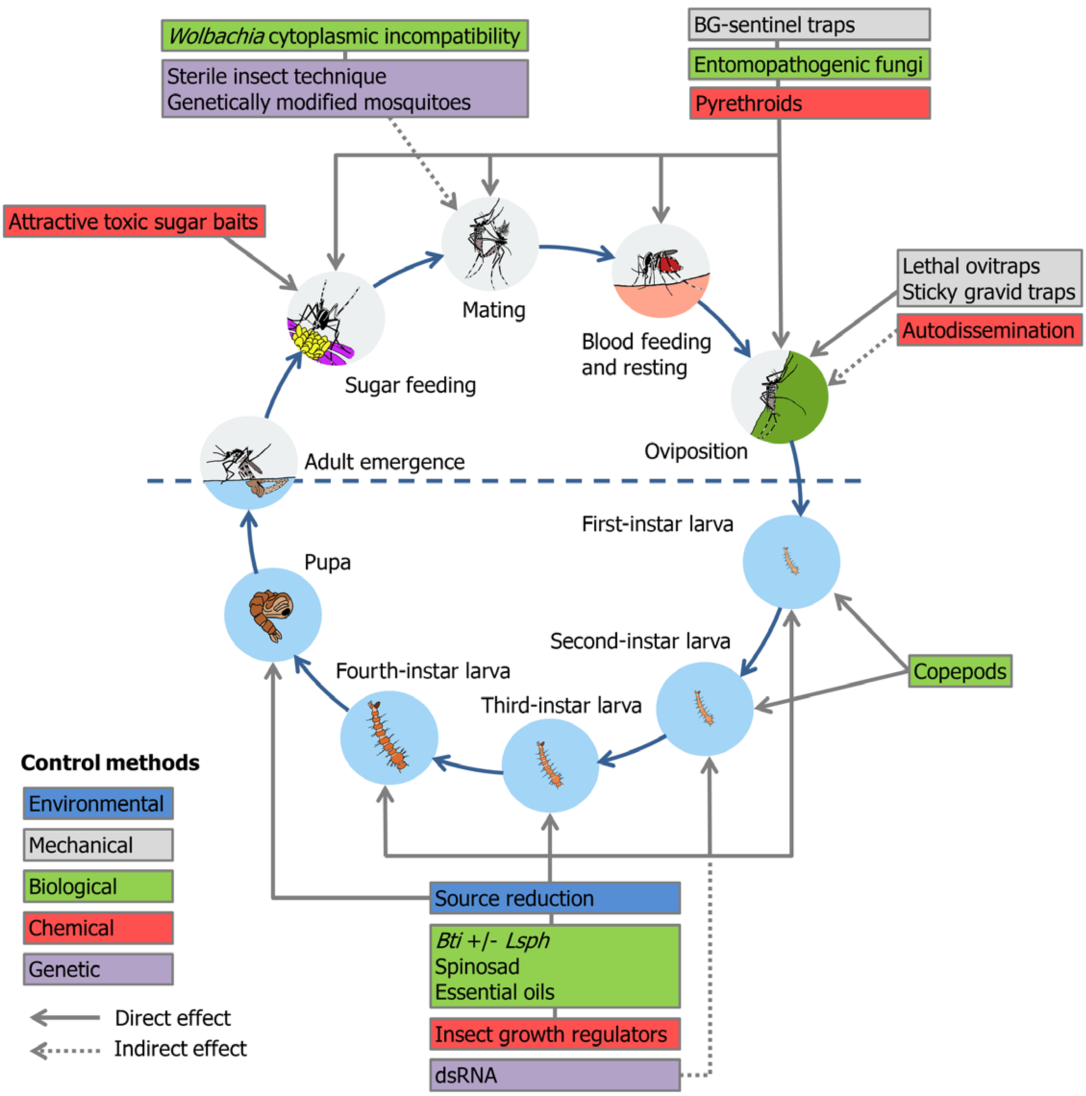

Figure 1. Control methods available against *Aedes* sp. (*Bti*: *Bacillus thuringiensis* var. *Israelensis*; *Lsph*: *Lysinibacillus sphaericus*; dsRNA: double-stranded RNA)

(e.g. ovitraps or sticky/gravid traps) or host-seeking females [e.g. BG-Sentinel (BGS) traps] (Biogents AG, Regensburg, Germany).

Ovitraps exploit the propensity of *Aedes* mosquitoes to lay their eggs in small containers. They are used as a sensitive, inexpensive, passive surveillance tool for detecting the presence of container-breeding mosquitoes and for assessing the adult population dynamics.[26] The addition of a larvicide or an autocidal mechanism allows the long-term use of an ovitrap with minimal risk of its becoming a productive source of adult mosquitoes.[46] Lethal ovitraps have been tested using egg-laying strips treated with insecticide (e.g. permethrin, deltamethrin).[47] Field trials using lethal ovitraps conducted in Brazil, Peru and Thailand have shown an efficient reduction in *Ae. aegypti* population density, although lower effectiveness was observed in Thailand, probably owing to the presence of numerous water containers around homes.[47,48] In Australia, field studies have also shown that lethal ovitrap control programmes have a significant impact on *Ae. aegypti* populations, coupled with high public acceptability.[49] Organic infusions such as grass, hay or oak, as well as NPK (nitrogen–phosphorous–potassium) fertilisers, can be added to ovitraps to improve their attractivity.[50,51] The development of oviposition stimulants could lead to even better control of mosquito populations using these traps.[52]

Sticky ovitraps and gravid traps fitted with adhesive surfaces have also been developed in order to survey gravid females, and various designs have been evaluated in the field to monitor the abundance of *Aedes* sp.[53–55] In order to improve collections, MacKay *et al.*[46] designed a large autocidal gravid trap that provides a more conspicuous visual target and a greater release rate of water vapour and other volatile attractants. In Puerto Rico, a control programme combining gravid ovicidal traps, source reduction and larvicide applications has shown a higher reduction in *Ae. aegypti* females in areas with traps than in areas without traps.[56] In Singapore, gravid traps have also been deployed to complement source reduction efforts in controlling dengue transmission and have been effective in collecting *Ae. aegypti*.[57] Nevertheless, early deployment and a large number of these traps are needed to have an impact on *Aedes* populations.[57]

Studies have shown that BGS traps, especially with a CO_2 source, are effective for collecting *Aedes* sp.[58,59] In northern Italy, BGS traps baited with BG lure were evaluated as a control tool against *Aedes albopictus*; intervention sites with a trap density ranging from one trap per 150 to one per 350 m^2 showed a decrease in human biting rates in comparison with control sites.[60] In Brazil, Degener *et al.*[61] found that mass trapping using BGS traps without any lure significantly reduced the abundance of adult *Ae. aegypti*. Although the possibility of using BGS traps is limited by their requirement for electrical power, the authors consider these traps to be a promising tool that can be used in IMS control programmes or as a push component in a push-pull strategy. As push-pull strategies, combining a repellent with an attractive stimuli in tandem, have proved to be effective against various agricultural pests, they have been proposed as a control method against mosquitoes.[62] In Thailand, push-pull control of *Ae. aegypti* is currently being evaluated for effectiveness and acceptability.[63] It exploits the spatial repellent and contact irritant actions of minimal doses of insecticides used conventionally in public health interventions through indoor residual spraying (IRS) or insecticide-treated materials (ITMs) (see Section 5.2). These indoor treatments are combined with BGS traps positioned in the outdoor environment. Semi-field experiments showed that exposure of *Ae. aegypti* females to pyrethroids did not significantly reduce the attraction of BGS traps.[64] This finding supports the potential effectiveness of a push-pull strategy to reduce *Ae. aegypti* adults inside and outside homes. However, it is important to highlight that IRS and ITMs do not target exophylic species such as *Ae. albopictus*.

4 BIOLOGICAL METHODS

4.1 Entomopathogenic fungi

Entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* show considerable promise as an alternative mosquito control method.[65] In laboratory conditions, *B. bassiana* reduced *Ae. aegypti* longevity, and semi-field experiments demonstrated a reduction in fecundity, adult survival and blood feeding in infected *Ae. aegypti*. Larvicidal and adulticidal activity of *M. anisopliae* against *Aedes* mosquitoes is well established.[66] Moreover, the US Environmental Protection Agency (EPA) has found no risk to humans using *M. anisopliae* products and no adverse effects on non-target species.[67] Various delivery methods for infecting adult mosquitoes have been tested, such as fungus-impregnated cloth or applying fungi on screens around the home.[68] Paula *et al.*[68] found that black cotton cloth impregnated with *M. anisopliae* reduced the survival of *Ae. aegypti* under simulated intradomicile conditions. Survival rates were even lower when *M. anisopliae* was combined with imidacloprid at 10 ppm. The ovicidal activity of *M. anisopliae* was also demonstrated on *Ae. aegypti* eggs, particularly with oil-and-water-formulated conidia.[69] The application of oil-based fungal formulations onto oviposition substrates appeared to be more effective at infecting eggs than direct fungal application, and required less fungal material. This novel approach could be a promising basis for practical and economical strategies to reduce populations of viable eggs of *Aedes* mosquitoes.

4.2 Copepods as a natural enemy

Cyclopoid copepods have proved to be the most effective invertebrate predators of mosquito larvae. The mass production of copepods is relatively easy and inexpensive.[70] Large copepod species are more effective on *Aedes* larvae than on *Culex* larvae. The most effective species can kill more than 40 *Aedes* larvae copepod^{-1} day^{-1}. Most field experiments have focused on container-inhabiting mosquitoes in the Americas, Asia and Oceania. In New Orleans, *Ae. albopictus* populations in tyre piles were eliminated for 3 years after the introduction of *Macrocyclops albidus*.[71] In Vietnam, *Mesocyclops* spp. used in large-scale control campaigns have locally eradicated *Ae. aegypti* in many villages and have been included in community-based strategies.[72–74] In Florida, *Mesocyclops longisetus* was evaluated for its potential in reducing container-inhabiting mosquitoes in residential environments.[75] Populations of *M. longisetus* peaked 2–3 months after introduction, depending on the size of the container, and numbers of *Ae. albopictus* significantly decreased when numbers of copepods were high. During the first 2 months after copepod introduction, control of mosquito larvae is incomplete because copepods generally attack first-instar larvae. Therefore, *Bti* (see Section 4.3), which is harmless to copepods, can be applied during the initial period to reduce mosquito production. As part of an integrated IMS control programme, copepods are a promising tool for biological control of container-inhabiting mosquitoes, but it should be noted that they can only survive in containers with water and food, in addition to mosquito larvae. If the containers dry out, copepods cannot survive.[70] The presence of copepods also seems to attract female mosquitoes for oviposition.[70] Thus, it could be helpful within the framework of an integrated IMS control programme to inoculate large, permanent, water-filled containers with copepods to create egg traps. In Europe, further evaluation of European copepod species is merited. In semi-field experiments conducted in Italy, *Macrocyclops albidus* showed promising results to control *Ae. albopictus*.[76]

4.3 *Bacillus thuringiensis* var. *israelensis* and *Lysinibacillus sphaericus* as microbial larvicides

The microbial larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) is extensively used for the control of mosquito species.[77] *Bti* can be associated with another microbial larvicide, *Lysinibacillus sphaericus* (*Lsph*), formerly known as *Bacillus sphaericus*. Various formulations of *Bti* or *Bti* + *Lsph* are available in commercial products.[77] In Europe, *Bti* is increasingly used for selective control of larval mosquitoes, particularly in habitats such as floodplains and coastal wetlands where aerial spraying (only authorised for *Bti*) is commonly carried out.[78] The larvicidal activities of *Bti* and *Lsph* are due to toxins acting synergistically. These toxins are activated in the gut of the larva and disrupt the cell membranes.[79] This complex mechanism involves many proteins, preventing the selection of resistance in mosquitoes.[79] *Bti* has short-term residual activity, especially in polluted or organically enriched water, while *Lsph* persists for longer, recycling through infected larvae. *Lsph* is mainly active on *Culex* sp. and less active on other mosquito species. As *Lsph* produces only one toxin, resistant populations of *Culex quinquefasciatus* have been recorded and rapidly selected.[80] As a result, commercially available combinations of *Lsph* with *Bti* are more effective because of the synergistic action between their toxins on a wider range of mosquito hosts.

Bti alone or in combination with *Lsph* has proved to be effective against IMS.[81–85] Field experiments, mainly conducted in Asia and South America, involving *Bti* as a control method for dengue vectors have been reviewed by Boyce *et al.*[79] These studies show that, in targeted containers that received treatment, *Bti* eliminated all immature forms within 24 h. The efficacy of *Bti* in comparison with other larvicides (spinosad, diflubenzuron and pyriproxyfen; see Sections 4.4 and 5.1) has been evaluated in only one large-scale European field trial (conducted in Martinique) for the control of *Ae. aegypti*.[83] *Bti* showed residual efficacy for 4 weeks, while spinosad and diflubenzuron were active for 16 weeks. To extend the duration of *Bti*'s residual control, dry formulations have been tested at high doses against *Ae. aegypti* in small containers without water. The product remains active for at least 2 months before the container is flooded.[86]

However, in some field studies, *Bti* intervention alone did not significantly reduce vector abundance compared with educational and/or environmental interventions.[87] The presence of untreated cryptic containers may explain this failure. *Bti* application by a backpack mist blower has been shown to be effective against discrete *Aedes* breeding sites up to 16 m in dense bushland, and larval mortality was sustained for up to 9 weeks post-misting.[39] In Singapore, applying *Bti* on vegetation by motorised backpack and vehicle-mounted sprayers significantly reduced *Ae. albopictus* populations.[88] Further investigations to test the effectiveness of *Bti* and *Lsph*, applied by various techniques and associated with other control methods, still need to be made using cluster randomised controlled trials.[79]

4.4 Spinosad as a biorational larvicide

Spinosad is a product derived from the fermentation of a naturally occurring soil actinomycete, *Saccharopolyspora spinosa*. It contains two insecticidal factors, A and D, which are active against all mosquito species tested thus far.[89] Some formulations have been approved for use in organic farming and as a mosquito larvicide in human drinking water.[89,90] Currently, no larvicidal formulations are commercially available in Europe. Spinosad acts primarily on the postsynaptic nicotinic acetylcholine receptors and secondly on GABA receptors. It possesses a unique mode of action, and no neurotoxic insecticide cross-resistance to spinosad has been described in insecticide-resistant populations of *Ae. albopictus*[91] and *Ae. aegypti*.[92] The larvicidal efficacy of spinosad can be negatively affected by adsorption onto particulate matter and/or by exposure to sunlight (on account of photolysis). Therefore, the level of pollution and organic matter in target habitats should be considered to determine use rates and retreatment intervals, and several controlled-release formulations have been developed to mitigate the impact of ultraviolet light degradation.[89]

The efficacy of spinosad has been evaluated in several field trials. In Mexico, spinosad treatment of car tyres provided 6–8 weeks of effective control of *Ae. aegypti*, *Ae. albopictus* and *Culex* sp. larvae.[93] In Martinique, spinosad had a residual efficacy of 16 weeks on *Ae. aegypti* populations that exhibited a high level of resistance to temephos and a tolerance to insect growth regulators (IGRs) (see Section 5.1).[83] In Connecticut, the application of spinosad to individual catch basins significantly reduced the total numbers of larvae of *Ae. j. japonicus* and *Cx. pipiens* for 5 weeks.[82]

In comparison with *Bti*, spinosad treatment has a longer residual effect, but it also affects non-target aquatic insect species such as *Toxorhynchites theobaldi*, a predatory mosquito species.[93,94] Given the growing use of spinosad as a larvicide, the issue of non-targeted effects merits further investigation.[94] Until then, its usage should be limited to artificial breeding sites with no other insect fauna.

4.5 Essential oils as botanical larvicides

Essential oils (EOs) comprise a complex mixture of constituents such as monoterpenes, phenols and sesquiterpenes, which could act synergistically and are more active than individual compounds.[95] EOs might interfere with insect feeding behaviour, act as insect growth regulators or have a neurotoxic mode of action. Several larvicidal mechanisms of toxicity could be involved, such as protein denaturation, enzymatic inhibition or membrane disintegration, and it is likely to be very difficult for the insect to develop an adaptation that leads to resistance.[95] Therefore, EOs commonly used as mosquito repellents have great potential as larvicides.[95] Dias and Moraes[95] reviewed 361 EOs from 269 plant species tested for their larvicidal activity against *Ae. aegypti*. One of these, neem oil from *Azadirachta indica*, was also found to be successful against *Ae. albopictus* and *Ae. j. japonicus*.[96,97] Moreover, binary mixtures of some EO constituents with *Bti* were observed to be more active against *Ae. albopictus* larvae than *Bti* alone.[98]

The production of EOs is generally cheaper than that of individual compounds that must be isolated or synthesised. Apart from being economically viable, plant-based larvicides are obtained from a renewable resource and are widely accepted by the population.[95] However, the toxicity of EOs against mosquito larvae may vary significantly, depending on the plant species, the vegetative parts used, the age of the plant, the chemotype and the environmental conditions of growth. This could lead to contrasting and non-repeatable results in larval bioassays. Thus, selection of chemical markers is essential for quality control of botanical products.[95] Moreover, no standard criteria have been established for determining the larvicidal activity of EOs, in spite of WHO guidelines for laboratory and field testing of mosquito larvicides.[95,99] EOs have mostly been investigated in laboratory conditions; a small number of patents have been applied for to regulate the production of larvicidal formulations, and no studies have assessed the efficacy of such formulations in the field. Moreover, the ecotoxicity of EOs on non-target species such as aquatic invertebrates is not fully understood, and this must be studied before the commercialisation of plant-based larvicides.[100]

4.6 *Wolbachia*-induced cytoplasmic incompatibility

Wolbachia pipientis is an endosymbiotic α-proteobacterium naturally present in many mosquito species, including *Ae. albopictus* and *Cx. pipiens*.[101] It infects the gonads and is transmitted to the next generation from female adults to their eggs. The success of *Wolbachia* is due to its ability to manipulate diverse functional systems of its hosts, particularly their reproductive properties.[102]

Cytoplasmic incompatibility (CI) is the most commonly detected type of *Wolbachia*-induced reproductive alteration.[103] In unidirectional CI, crosses between uninfected females and infected males are sterile because of embryogenic lethality; all other crosses are fertile.[102] In bidirectional CI, all crosses between individuals infected with different *Wolbachia* strains are sterile. For instance, *Ae. albopictus* populations can be naturally single- or double-infected with *w*AlbA and *w*AlbB.[104] It has been demonstrated that single infection is incompatible with an uninfected host, and that double infection is incompatible with both single-infected and uninfected hosts.[105]

In addition, *Wolbachia* can also reduce the ability of certain pathogens to replicate in insects.[101,106] The presence of *Wolbachia* interferes with the development of a wide range of pathogens such as nematodes, bacteria, viruses and protozoa. For example, *Wolbachia* infection limits the capacity of *Ae. aegypti* females to transmit dengue, chikungunya and yellow fever viruses.[106]

As a result, *Wolbachia* symbiosis has led to the development of two applied strategies: population replacement, based on unidirectional CI, and population suppression, based on bidirectional CI or unidirectional CI if the population is uninfected.[101,102] In population replacement, females infected with a *Wolbachia* strain are introduced to establish and spread the infection in the target population. The aim is to reduce pathogen transmission by shortening the adult mosquito's lifespan and/or preventing pathogen replication inside the mosquito. In population suppression, large numbers of infected males are repeatedly introduced into a population, and the sterility resulting from mating between released males and indigenous females causes the decline of the population, as in the sterile insect technique (SIT) (see Section 6.1). However, in this case, the introduced *Wolbachia* strain is not established within the target population as males are dead-end hosts for *Wolbachia*, so this method is referred to as the incompatible insect technique (IIT).

Both strategies require the manipulation and generation of new infection types by introducing an infection in mosquito populations.[102] Several *Wolbachia* strains have been successfully established in mosquitoes, such as the *w*Pip and *w*Mel strains in *Ae. albopictus*, respectively originating from naturally infected *Culex* and *Drosophila* hosts.[107,108] Current findings from laboratory or semi-field trials are encouraging for the development of experimental IIT population suppression trials in the field. Calvitti *et al.*[103] have created a new stable symbiosis in *Ae. albopictus* with a strain named Ar*w*P by microinjecting the *w*Pip strain from *Culex pipiens molestus* into the eggs. The Ar*w*P-infected males are fully incompatible when mating with uninfected or naturally double-infected wild females. While *Wolbachia*-based population suppression has not yet been tested in field trials, *Wolbachia*-based population replacement has been demonstrated successfully in field trials with *Ae. aegypti* populations. In Australia, *Ae. aegypti* were infected with *w*Mel, making them less competent vectors for the dengue virus, and then released into natural populations in two locations; they were almost established a few months after the release.[109]

Although *Wolbachia*-based methods have been intensively studied since the 2000s, with promising results, some caution should be applied when considering these methods in a vector control strategy. Firstly, these methods are species specific and they may work only in areas with a single vector species. Then, IIT requires high sexual competitiveness of artificially *Wolbachia*-infected males and a highly efficient sex separation technique to avoid the accidental release of females, which could result in population replacement instead of suppression. As an example, for *Ae. albopictus*, *Wolbachia* infection does not seem to reduce male competitiveness,[107] and males are currently separated out at the pupal stage by the use of a 1400 μm sieve with 99% accuracy, which is very high but not sufficient.[110] Thus, further semi-field experiments are warranted to evaluate the mating competitiveness of artificially *Wolbachia*-infected males and the risk of releasing artificially infected females in a wild population. Finally, the vector competence of mosquitoes may be affected differently by *Wolbachia* infection. Instead of decreasing the infection and transmission of pathogens, *Wolbachia* might enhance pathogen infection in mosquitoes, as has been shown in *w*AlbB-infected *Anopheles gambiae* with *Plasmodium berghei* and in *w*AlbB-infected *Culex tarsalis* with West Nile virus.[111,112] Therefore, Hughes *et al.*[112] suggested that *Wolbachia*-infected mosquitoes intended for release into nature should be assessed for inhibition of all relevant pathogens.

5 CHEMICAL METHODS

Today, insect growth regulators (IGRs) and pyrethroids are the unique chemicals used in Europe in mosquito control strategies, as larvicides and adulticides respectively. In accordance with Directive 98/8/EC (Biocidal Products Directive) and EU Regulation 528/2012 (Biocidal Products Regulation), certain biocides are banned from use in Europe, such as temephos, widely used around the world for larval control, and all other organophosphates used for adult control. The updated list of approved insecticides is available on the European Chemical Agency (ECHA) website (http://echa.europa.eu/web/guest/information-on-chemicals/biocidal-active-substances).

5.1 Insect growth regulators as chemical larvicides: direct application and autodissemination

IGRs such as pyriproxyfen, methoprene and diflubenzuron are commonly used as larvicides, and various commercial products are available. They also possess ovicidal properties, and can inhibit egg hatching, depending on their mode of action, the dose applied and the mosquito species.[113] IGRs are relatively safe for non-target organisms.[114] They have been widely used as part of integrated IMS control programmes[21,25] and are most effective when targeted at the most productive breeding sites.[115] In Italy, larvicide treatments carried out by public agencies are focused on catch basins. It has been shown that the adult emergence of *Ae. albopictus* and *Cx. pipiens* was strongly inhibited in diflubenzuron-treated catch basins,[21] and that diflubenzuron was more effective and persistent than pyriproxyfen formulations against *Ae. albopictus*.[116] In Colombia, the monthly application of pyriproxyfen in all street catch basins resulted in a decrease in *Ae. aegypti* larvae in the basins and a reduction in the incidence of dengue.[115]

A new approach, known as autodissemination, consists of exploiting wild adult mosquitoes as carriers of insecticide compounds.[117] Female mosquitoes can be contaminated by the insecticide using treated nets or dissemination stations made from modified ovitraps. This method (using pyriproxyfen as the active compound) has been shown to induce high *Ae. aegypti* and *Ae. albopictus* mortality at the pupal stage in small-scale field experiments carried out in Peru[117] and in Italy[118] respectively. Moreover, an effect on egg production and egg hatchability was also observed under semi-field conditions.[119] Recently, an oil and pyriproxyfen powder dual-treatment autodissemination station has been developped to enhance the transfer of pyriproxyfen to oviposition sites by increasing its attachment and retention on females.[120] Snetselaar *et al.*[121] also designed a novel contamination device with a combination of pyriproxyfen and the entomopathogenic fungus *B. bassiana*. Dissemination of pyriproxyfen led to over 90% larval mortality, and *B. bassiana* increased adult mortality compared with the control under laboratory conditions. However, sprayed applications of conventional pyriproxyfen formulation to treat tyres or vegetation were not effective in autodissemination and were affected by climatic conditions such as high rainfall.[122] The autodissemination technique may be improved by the design of new contamination stations or by the development of specific pyriproxyfen formulations. Another possible approach, suggested by Bouyer and Lefrançois,[123] is to combine autodissemination with the sterile insect technique (SIT) (see Section 6.1) by releasing sterile males coated with pyriproxyfen in order to contaminate females during mating. This might 'boost' the ability of SIT to control mosquitoes.

5.2 Pyrethroids as chemical adulticides: space spraying, indoor residual spraying, insecticide-treated materials and attractive toxic sugar baits

Pyrethroids are sprayed to reduce rapidly the abundance of *Aedes* females, particularly during epidemics.[124] They are mainly used against adult mosquitoes because of their relative safety for humans, their high insecticidal potency at low dosages and their rapid knockdown effects.[125] However, pyrethroids are toxic to non-target insect species, aquatic invertebrates and fish.[124] In Europe, ground applications are mostly carried out to reduce mosquito nuisance, while aerial application is prohibited except in the case of a public health emergency declared by authorities.[126,127] Currently, only Hungary allows aerial application. Ground sprays are applied mainly as thermal fogs or cold fogs, at high volume (HV; $>150\,L\,h^{-1}$), low volume (LV; $18-60\,L\,h^{-1}$) or ultra-low volume (ULV; $0.6-18\,L\,h^{-1}$), using hand-carried or vehicle-mounted foggers.[127,128] While ULV technology is commonly used in the United States, it is rather restricted in Europe.

In Europe, the effectiveness of ground spraying for mosquito control remains poorly studied in field trials, although it is used routinely in summer to limit mosquito populations. In Spain, Chebabi Abramides *et al.*[20] observed that fumigating α-cypermethrin on vegetation in public parks within the framework of an integrated IMS control campaign was effective at controlling *Ae. albopictus*, while Bengoa *et al.*[129] found that a deltamethrin formulation (applied at ULV) showed higher mortality rates against *Ae. albopictus* and had a more effective residual effect on vegetation than an α-cypermethrin formulation. In Italy, Caputo *et al.*[21] observed a reduction in *Ae. albopictus* abundance during the major phase of the population expansion after low-volume application of permethrin and pyrethrum. In French overseas territories, ULV applications of deltamethrin have also been tested against *Ae. albopictus* and *Ae. aegypti*, but pyrethroid resistance in mosquito populations reduced the efficacy of the treatment.[130–132]

The effectiveness of sprays is mostly affected by droplet size distribution (droplet size and flow rate), meteorological conditions (temperature, wind speed and direction), habitat type (vegetation cover, open or secluded locations) and the time of application (flight activity of target species).[124,128,133] This latter parameter is especially crucial for *Aedes* mosquitoes, which are expected to be targeted more efficiently during their diurnal and/or crepuscular flight activity. However, the presence of people represents a constraint for the implementation of diurnal and/or crepuscular sprayings, particularly in urban areas. Interestingly, night-time ULV applications of formulation combining either permethrin–tetramethrin–piperonyl butoxide or sumithrin–prallethrin–piperonyl butoxide significantly reduced *Ae. albopictus* abundance in Italy (della Torre A and Caputo B, private communication) and in New Jersey.[134]

Although spray applications, particularly at ULV, have been successfully used in some integrated IMS control campaigns against *Aedes* mosquitoes,[20,25] this method is debatable because of high costs, slow operational response, low community acceptance, ineffective time of application, rather low efficacy and/or residual effects and potential impact on non-target species.[124,135,136] Furthermore, the development of insecticide resistance in *Aedes* populations remains a challenge for vector control.[121,131,137] In Brazil, pyrethroid resistance levels in *Ae. aegypti* populations increased rapidly after an integrated IMS control campaign including ULV ground spraying of 2% deltamethrin.[138] In the French Caribbean, where *Ae. aegypti* is strongly resistant to pyrethroids, treatments using deltamethrin or pyrethrins did not have any impact on larval or adult densities.[130] Therefore, it is advisable to check pyrethroid resistance in local mosquito populations before using these chemicals, considering that such resistance could arise following the use of pyrethroids in agriculture.[139,140]

The efficacy of indoor residual spraying (IRS) and insecticide-treated materials (ITMs) is restricted to the behaviour of *Ae. aegypti*, which rests inside homes before and after blood feeding, unlike other IMS.[141] IRS and ITMs allow the resting sites of *Ae. aegypti* to be targeted, as spraying outdoor spaces fails to reach indoor areas when houses are closed. In India, indoor thermal fogging of a deltamethrin formulation had a strong adulticidal effect for at least 5 days.[142] In Venezuela, Vanlerberghe *et al.*[143] demonstrated that the deployment of ITMs consisting of curtains and water jar covers can significantly reduce *Ae. aegypti* levels, depending on the coverage attained. In Guatemala, the vector population was reduced by combining ITMs, larvicide treatments and source reduction targeting the most productive breeding sites.[144] In both studies, the insecticide in the ITMs remained effective at least 1 year after use in field conditions. However, in Thailand, ITMs had a low impact on *Ae. aegypti* populations, perhaps owing to the area's open housing structures.[145]

Attractive toxic sugar baits (ATSBs) are a control method that exploits the diet used to sustain a mosquito's daily activities.[146,147] Females and males obtain the sugar essential in their diet from floral nectar or extrafloral nectaries. ATSBs consist of a solution containing sugar and fruit juice blended with an oral toxin (e.g. boric acid, eugenol) or an insecticide (e.g. dinotefuran, spinosad). ATSBs can also use pyriproxyfen to provide additional control of mosquitoes at the larval stage.[148] ATSBs have been tested in stations set near breeding sites and in sprayed applications on vegetation. Sprayed applications appeared to be more effective at controlling *Ae. albopictus* than bait stations.[149] In Florida, spraying applications of ATSB on vegetation resulted in a significant reduction in *Ae. albopictus* populations for 21 days after treatment.[149,150] The negative impact on non-target insects was lower when ATSB was sprayed on non-flowering vegetation.[149]

6 GENETIC METHODS

6.1 Sterile insect technique

The sterile insect technique (SIT) relies on the release of large numbers of sterile males.[151,152] Males are exposed to γ-irradiation or sterilising chemicals, causing large-scale random damage to the insect's chromosomes or dominant lethal mutations in the sperm. SIT requires the production of large numbers of insects and the ability to separate males from females before release. Several SIT programmes have been conducted successfully around the world, such as the elimination of the screw-worm fly *Cochliomyia hominivorax* in the southern United States, Mexico and Central America. In terms of mosquito control, the release of chemosterilised males successfully eliminated *Cx. quinquefasciatus* on an island off Florida, and *Anopheles albimanus* in El Salvador.[153]

Preliminary studies have confirmed the feasibility of using SIT against *Ae. albopictus* to suppress natural populations in Europe.[154–156] *Ae. albopictus* males sterilised by ionising radiation exhibit reduced mating competitiveness. However, a dose of around 30 Gy minimises the potential damaging effects of irradiation, and a 5:1 ratio between sterilised and wild males appeared to be sufficient to reduce, although not to eliminate, the fertility of the female population.[155–157] As the SIT approach requires mass rearing without affecting the mating competiveness of the males, the FAO/IAEA Insect Pest Control Laboratory in Vienna (Austria) has developed a larval rearing unit with a production capacity of 100 000 male pupae $week^{-1}$.[110] In Italy, pilot field trials of SIT have been performed in three villages over 4 years, with the release of around 900–1500 sterile males ha^{-1} $week^{-1}$.[155] The sterility level in the population reached 70–80%, followed by a reduction in the egg density recorded in the ovitraps.

Furthermore, a new approach has been developed to produce non-radiated sterile males using RNA interference (RNAi). RNAi is a gene-silencing mechanism achieved by delivering double-stranded RNA (dsRNA) to cells or organisms.[158] By feeding mosquito larvae with dsRNA targeting the testis genes and a female sex determination gene, Whyard *et al.*[159] produced *Ae. aegypti* males with reduced fertility and a male-biased mosquito population. This technique avoids the debilitating effects of radiation and eliminates the need to sex-sort mosquitoes before release. In the field, vector control might be fulfilled via administration of dsRNA-baited larval food. However, the production of dsRNA is currently too expensive to treat large numbers of mosquitoes.

6.2 Release of insects carrying a dominant lethal gene, RNA interference and homing endonuclease genes

As regards the genetic modification of mosquitoes, there are three main emerging methods: the release of insects carrying a dominant lethal gene (RIDL), RNAi and homing endonuclease genes (HEGs).[101] These genetic methods have been reviewed and well illustrated by McGraw and O'Neill.[101]

The RIDL method operates similarly to SIT, with a focus on female-killing effects. In this method, female-acting transgenes are carried and delivered into the wild population by genetically modified males. These transgenes may induce mortality in pupae or adults, or they may reduce the expression of a gene active in the flight muscle, resulting in flightless females unable to feed and mate.[101] The fitness of males carrying female-acting transgenes is less compromised than the fitness of sterilised males because transgene transcription is driven by female-specific promoters.[101]

This method has been tested successfully in the field in the Cayman Islands.[160] Although genetically modified males showed mating disadvantages, this could be compensated for by releasing them in greater numbers. Moreover, combined releases of adults and pupae seemed well able to maintain long-term suppression of a simulated wild population of *Ae. aegypti*.[161] Field trials have been conducted or are in progress in dengue-endemic regions.[162] In Brazil, the release of RIDL OX513A males led to the suppression of two target wild populations of *Ae. aegypti*.[163]

The RNAi method is aimed at improving the RNAi insect immune response that recognises and degrades invading viral RNA.[101] For instance, *Ae. aegypti* mosquitoes have been genetically modified by constructing an effector gene that targets the dengue virus type 2 (DENV2).[164,165] This resulted in the expression of dsRNA corresponding to an inverted repeat sequence derived from the DENV2 RNA genome. The DENV2-specific dsRNA triggers the RNAi response and blocks the multiplication of the virus in the tissues of the mosquito.[165] In this way, transgenic *Ae. aegypti* females were resistant to dengue virus type 2.

The third genetic method makes use of HEGs, which are selfish genes that can spread rapidly through populations by exploiting cellular repair mechanisms to copy themselves.[166] Discovered in bacteria, HEGs have been experimentally engineered and introduced into mosquitoes for vector control.[101] HEGs encode endonuclease enzymes that recognise and cut specific DNA sequences. In a heterozygote individual, an HEG cuts and inserts itself into the intact copy, converting an HEG heterozygote into an HEG homozygote. This results in an increase in HEG copies in the mosquito population.[101] HEGs are inserted into specific recognition sequences and trigger targeted gene disruptions. HEGs can be designed to target vector competence genes, fertility genes or sex-determining genes, leading to pathogen-resistant females or to population suppression. To date, HEGs have been successfully introduced into *Ae. aegypti*[167] and *Anopheles gambiae*.[168]

Most of these genetic technologies are at an early stage of development, except for the RIDL method, which has already been tested in the field. The control potential of these technologies needs to be tested in natural conditions. Brown *et al.*[169] developed criteria for identifying and evaluating candidate sites for open-field trials of genetically modified mosquitoes (see also Section 7.1). These tests must be prepared and conducted carefully and transparently, following frameworks for environmental risk assessment. The WHO guidelines provide a framework to ensure the quality and consistency of procedures for testing genetically modified mosquitoes.[170]

7 EVALUATION OF CONTROL METHODS IN LARGE-SCALE FIELD TRIALS

7.1 Site selection

Iyaloo *et al.*[171] have provided guidelines for selecting sites for mosquito control trials. Before implementing an areawide integrated IMS control strategy, similar paired sites should be selected according to the vector population (in terms of isolation, density, presence of competing species, etc.) and ecological factors (climate, landscape, etc.). It is recommended to target a vector population that is naturally isolated from immigration, and, if possible, with a sole IMS species. Indeed, the presence of other IMS increases the workload of monitoring based on larval/pupal indices or on egg counts in ovitraps as it implies the identification of *Aedes* species at immature stages (see Section 7.2). The selected sites should also be ecologically representative of the region to potentially expand the integrated IMS control strategy, as well as stable, so that the variability of environmental conditions will not affect the results.[171] In urban or suburban areas, socioeconomic parameters should also be considered. In New Jersey, high poverty and a low education level were positively associated with a high abundance of *Ae. albopictus*.[172] Practical considerations should also be taken into account, such as existing facilities, a manageable site size and access to the whole site. Finally, the social, ethical and legal aspects of the integrated IMS control strategy need to be considered before implementation.[171]

7.2 Monitoring of *Aedes* mosquitoes

The monitoring of *Aedes* mosquitoes is crucial in order to compare infestation levels between different sites and to evaluate the effectiveness of an integrated IMS control strategy. Several indices are traditionally used in developing countries to evaluate *Aedes* populations: house index (percentage of houses with at least one active breeding site), container index (percentage of containers with larvae), Breteau index (number of active breeding sites per 100 premises) and ovitrap index (average proportion of ovitraps with eggs).[38] However, larval indices are of limited value in European countries because of differences in socioeconomic and structural conditions characterising human dwellings and the availability of breeding sites in public areas.[38] The number of pupae per premise (PPI) and the number of pupae per hectare (PHI) seem to be more appropriate for European urban areas, particularly the latter, which is applicable to public and private areas.[38] Moreover, pupal indices exploit the strong correlation between the number of pupae and the number of adults in a defined area, based on the low natural mortality of the pupae.

Ovitraps are the most widely used methods to monitor *Aedes* mosquito populations as they are inexpensive, sensitive and practical for areawide surveys.[20] Indeed, the mean number of *Ae. albopictus* eggs in ovitraps was found to be positively correlated with counts from PPI, PHI and human landing catches in Italy during the chikungunya outbreak in 2007.[19] In areas where several IMS occur, species must be identified from eggs, involving time-consuming labour in the laboratory (egg storage, egg hatching, larval rearing). Alternatively, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has been developed for easy and rapid identification of IMS.[173]

Sticky traps collect ovipositing and resting females, and allow direct identification of *Aedes* sp. Estimates of adult populations from sticky traps were demonstrated to be highly positively correlated with estimates from ovitraps.[53] Sticky traps have been used successfully worldwide for the monitoring of *Ae. albopictus* and *Ae. aegypti*.[53,54,174] In Italy, the efficacy of insecticide applications has been evaluated using sticky traps and mosquito emerging traps, which consist of adhesive traps designed for the collection of adults visiting and emerging from catch basins.[21]

BGS traps, which attract mostly host-seeking adult females, can also be set to monitor *Aedes* mosquito populations during integrated IMS control programmes.[172] In New Jersey, *Ae. albopictus* populations were surveyed weekly with BGS traps and ovitraps to examine the efficacy of active source reduction, insecticide applications and public education.[25] BGS traps were more sensitive than ovitraps to detect *Ae. albopictus* early in the season and to compare treated and untreated sites. However, the deployment of BGS traps over a wide area is costly and unpractical owing to the need for a power supply.

To overcome some of the limitations of entomological indicators, recent studies have been done to develop simple, rapid and highly sensitive complementary indicators to evaluate the level of human exposure to *Aedes* bites and the efficacy of control strategies.[175] When a female mosquito bites, it injects saliva containing highly immunogenic molecules. Human antibody responses, such as IgG, to the saliva of one *Aedes* species can be measured to assess the specific exposure of individuals to this *Aedes* species. Specific biomarkers for *Ae. albopictus* and *Ae. aegypti* saliva proteins are being developed and have been validated in La Réunion Island and in Bolivia respectively.[175,176] Even if these biomarkers are species specific, a cross-reactivity has been observed between *Ae. albopictus* and *Ae. aegypti*, especially in high-immune responders.[175]

7.3 Implementing an integrated IMS control strategy

The implementation of an integrated IMS control strategy in Europe should take into account the target species, its ecology and the public health concern, i.e. nuisance or disease transmission.[177] In the latter case, insecticide treatments and fine-scale removal of breeding sites are recommended in the areas around the reported foci, as has already been implemented in Europe to limit the transmission of chikungunya or dengue by *Ae. albopictus*.[178,179] On the other hand, when the aim of an integrated IMS control strategy is to achieve a medium/long-term population reduction in order to reduce the biting nuisance and the risk of an arbovirus outbreak, the timing and choice of the treatment should be determined by the population dynamic of the target species.[177] For instance, methods such as insecticide spraying are more effective for rapidly reducing high-density mosquito populations or in the phase of major expansion,[21] while genetic methods such as SIT are more effective at controlling low-density populations.[171] The choice of the control method should consider its effectiveness, specificity, residual effect, selection for resistance and ecological impact. For example, the use of larvicides is one of the most effective methods if treatment is focused on the most productive breeding sites in an area; this may differ in urban, suburban and rural areas. Source reduction methods, which are costly and time consuming, should involve the public in a community-based approach. More generally, the success of an integrated IMS control strategy relies on cooperation between political decision-makers, public authorities, scientists and the general public.[177,180] Finally, the implementation of an integrated IMS control strategy has to be in line with financial and human resources.

7.4 Modelling approaches and cost-effectiveness analyses

Recently, modelling approaches have proved to be very helpful in optimising integrated IMS control strategies by testing several control methods at a theoretical level.[161] Modelling studies have investigated the effectiveness of different control methods such as genetic techniques (e.g. RIDL, SIT), source reduction and/or insecticides, applied alone or in combination.[161,181–183] Models have also been used to assess the effectiveness of insecticides in reducing *Ae. aegypti* adult abundance and to predict the evolution of insecticide resistance in mosquito populations.[184] In this way, Luz *et al.*[184] demonstrated that larval and adult controls were optimal at the beginning of the dengue season. In addition, spatial and space–time modelling approaches are very helpful in planning the implementation of an integrated IMS control strategy (e.g. in terms of site selection and timing of treatment) by mapping the spatiotemporal distribution of IMS and exploring the influence of environmental factors.[185] The effects of spatial clustering of integrated IMS control strategies can also be assessed according to different levels of spatial coverage and control method combinations.[186] Finally, cost-effectiveness analyses facilitate comparisons between integrated IMS control strategies and can inform policy decisions.[187] Several studies have underlined the benefits of a real-time vector monitoring system to orientate the vector control campaign alongside a community-based approach with routine vertical *Aedes* control, including source reduction and larvicide and adulticide applications.[188,189]

8 CONCLUSIONS

The implementation and evaluation of integrated IMS control strategies against *Aedes* mosquitoes, especially *Ae. albopictus*, are warranted in Europe, particularly through large-scale field trials. Of the IMS control methods discussed in this review, several have been successfully used against *Ae albopictus* mainly outside of Europe. These include source reduction (Section 2), predation by copepods (Section 4.2), larvicide application (Sections 4.3, 4.4, 4.5 and 5.1), adulticide spraying (Section 5.2) and SIT (Section 6.1). Mechanical methods (Section 3) have been evaluated in large areas, but only against *Ae. aegypti*; lethal ovitraps or gravid traps should also be effective against *Ae. albopictus*. New approaches such as pyriproxyfen autodissemination (Section 5.1), ATSB (Section 5.2) or IIT (Section 4.6) based on *Wolbachia* infection have shown promising results in laboratory conditions or semi-field experiments, supporting their potential for future implementation at a larger scale. Lastly, emerging genetic methods need to be developed for *Ae. albopictus*; so far only *Ae. aegypti* mosquitoes have been genetically modified.

As underlined in previous studies, before implementing an integrated IMS control strategy, entomological surveys are necessary to monitor the IMS and to select similar paired sites for large-scale trials. This allows the efficacy of control methods to be evaluated by determining whether there has been a decrease in the adult population and/or egg oviposition in the treated site compared with the control site. Finally, tools such as mapping and modelling should be developed in order to optimise integrated IMS control strategy, and cost-effectiveness analyses should be carried out to guide policy decisions.

In conclusion, there is a large range of vector control methods against *Aedes* mosquitoes. Traditional methods such as source reduction, public education and insecticide application are routinely implemented by municipalities to reduce *Aedes* populations, but with limited success, probably because of a poor participation of communities, and a lack of coordination and syncronised implementation. Innovative approaches such as pyriproxyfen autodissemination and genetic or *Wolbachia*-based methods have to be sufficiently developed to demonstrate their efficacy and sustainability, and could be considered in programmes of combined implementation afterwards.

As a general rule, an integrated IMS control strategy requires the coordinated involvement of local authorities, private partners, organised society and communities. A high level of public cooperation is necessary from the beginning of integrated IMS control programmes, and only the continued support from both communities and local authorities can achieve a long-term effect. A key to success might be to customise the integrated IMS control strategy to each community according to the local *Aedes* infestations (key containers, infestation level, seasonal activity) and the specific socioeconomic characteristics of the locality.

ACKNOWLEDGEMENTS

This work was funded by the Autonomous Province of Trento (Italy) Research Funds for Grandi Progetti, Project LExEM (Laboratory of Excellence for Epidemiology and Modelling, http://www.lexem.eu). It was also partially funded by EU Grant FP7-261504 EDENext and is catalogued by the EDENext Steering Committee as EDENext337 (http://www.edenext.eu). The contents of this publication are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission.

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