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Safety, regulatory and environmental issues with SIT-based mosquito vector control in European countries

R. Bellini*

Medical & Veterinary Entomology Department, Centro Agricoltura Ambiente "G.Nicoli", International Atomic Energy Agency Collaborating Center, Via Sant'Agata 835, 40014 Crevalcore, Italy

*Corresponding author: rbellini@caa.it

Summary

The globalisation of trade is opening the way to the spread of species in new regions where they may cause negative impacts. Mosquito invasive species such as *Aedes aegypti* and *Aedes albopictus* are raising concern for their capacity to transmit several arboviruses of sanitary and veterinary importance. Currently available integrated vector management measures do not achieve satisfactory results when deployed against these urban mosquitoes. Moreover, insecticides are losing their effectiveness due to the resistance developed by the target species. Policies regulating the use of insecticides are progressively restricting their market availability and this trend is expected to continue. Genetic control methods, such as the sterile insect technique, based on the use of irradiation to sterilise male mosquitoes, are showing good efficacy in pilot trials at local scales in some *Aedes albopictus* colonised urban areas in Europe, without any negative effects. The main limitation is the cost, which may be significantly reduced through the introduction of automation in the mass rearing and drone technology in the field release. These technological advancements require substantial investments at a scale that can only be achieved with centralised production and extensive distribution, which in turn may be granted only if the authorisation frameworks, including the regulation of international transportation and aerial release in an urban setting, are clarified and matured.

Keywords

Aedes aegypti – *Aedes albopictus* – Drone release – Insect sexual sterility –Mass rearing technology – Mosquito genetic control.

Introduction

The human capacity to deal with mosquito borne diseases (MBDs) such as Malaria, Dengue, Lymphatic filariasis, Chikungunya, Zika, Yellow fever, Japanese encephalitis, West Nile fever and many others, leading to millions of cases every year, is largely based on the deployment of integrated vector management (IVM), including the use of insecticides, source removal, community participation and repellents protecting the hosts (1, 2). Mosquito species respond through evolutionary processes to the pressure by the insecticides with increasing evidence of resistance. The phenomenon of resistance to insecticides was observed in several different ecological settings when the use of an insecticide was repeated multiple times over large areas (3). The only case where no evidence of resistance was reported following many years of continuous treatment of mosquitoes may be referred to *Bacillus thuringiensis israelensis*, a microbial insecticide containing at least four toxic proteins with specific modes of action (4). Together with resistance, the insecticides showed other unintentional negative effects, like the impact on non-target organisms, the contamination of the ecosystems and the sublethal toxicity on higher animals. The increasing evidence about the negative environmental effects of insecticides induced the authorities to develop stringent policies to regulate their marketing and use. In this regard in the European Union, EU Regulation No. 528/2012 is considered to be at the forefront (5).

The public in Western countries also is developing a negative perception of the insecticides, with many homeowners forbidding local administrations from spraying insecticides on their properties (6). In countries where MBDs are endemic, the opposition to insecticide use is much less pronounced due to the different balance in cost and benefit, but it may increase in the near future as public awareness and environmental expectations grow. Meanwhile, the number of active ingredients available for mosquito control is rapidly declining on a global scale. The trend is expected to continue with the removal from the market of products showing evidence of negative environmental impact. It becomes more and more difficult for the pesticide industries to develop new products which comply with the current regulations. The research community is, therefore, strongly engaged in finding new tools and methods to be deployed in the endless struggle against mosquitoes and MBDs. Such innovations must be able to avoid natural development of resistance and to be highly effective against the target species, without negative repercussions on wildlife and human health.

Among the proposals for additions to IVM that have appeared in recent years is the sterile insect technique (SIT). This approach is long established in control of agricultural and livestock pest insects, dating back to the 1950s when it was first successfully applied for the suppression of *Cochliomyia hominivorax* in the Southeast United States, prior to expansion of the program throughout the USA, Mexico, and Central America (7, 8). After that success, SIT was applied against several agricultural pest species in different ecological and climatic condition (9, 10). A complete repertoire of the SIT programs attempted and those currently running is reported in the International Database on Insect Disinfestation and Sterilization (IDIDAS) (11). The sterile insect control system has now been extended to mosquito control through advances in mass rearing and delivery techniques that make operational implementation more practical (12, 13, 14, 15).

The SIT application against mosquitoes

While SIT is applied operationally on area-wide scale against several Tephritid fruit fly species, codling moths, screwworm flies and tsetse flies, it is still at the pilot scale on mosquitoes.

The historical reasons behind this apparently difficult to understand situation have been well analysed by Benedict (16). One main point is that, while IVM may impact more than one mosquito species at a time in cases when they are sympatric, by design SIT controls only one species or mating complex. Unless the mosquito mass rearing facility is organised to produce and distribute more than one species, the entire campaign impacts only the target species. This point is not trivial, as in many situations multiple species are involved in disease transmission and should be targeted at the same time.

Another basic assumption allowing an effective genetic control application is that the target species should be easily colonised into artificial environments and mass reared in efficient and safe, confined conditions. In this regard, *Aedes* mosquito species of public health importance, such as *Ae. aegypti* and *Ae. albopictus*, seem to be intrinsically more prone to mate in cages than most of the *Anopheles* mosquito species, due to their specific mating behaviour (17, 18). Other more dispersive *Aedes* species, targeted in Europe for their nuisance value, such as *Ae. vexans* and *Ae. sticticus*, are more reluctant to colonisation in factory production systems (19).

The phenomenon of invasion to new areas by *Aedes* species, while not new to our time (20), is currently raising alarm due to its worldwide scale and surprising rapidity. This puts pressure on the relevant authorities to organise adequate preventive measures (21, 22). *Aedes* invasive species such as *Ae. aegypti* and *Ae. albopictus* should be regarded as particularly suitable for SIT application for several reasons: they are relatively easy to mass rear; their suppression or even elimination from the newly colonised areas pose no threat to local biodiversity, as they do not belong to the local fauna; both these species flourish in urban environments, where contact with humans is frequent and where their limited active dispersal capacity makes it is possible to apply SIT; and finally, the current IVM measures do not guarantee a satisfactory control level and Dengue, Chikungunya and Zika virus epidemics are on the increase in many regions.

Along with the negative impact globalisation is causing on biocenosis and human wellbeing, we may envisage a possible positive side effect: the spread of tropical vector species into more developed temperate countries is mobilising energy, research activities and, more importantly, investment in technology. The rising threat has focused attention on promoting new understanding and technological solutions which are contributing to the advancement in mosquito genetic control worldwide (23). With the help of new technological tools and approaches, we are now in a solid position to overcome the two main obstacles in the way of the broad application of SIT and other genetic based control methods against mosquito vectors: we can achieve efficient production of large quantities of sterile males at a competitive cost, and field release methods that ensure the homogeneous distribution of sterile male mosquitoes in urban areas.

Challenges in area-wide SIT deployment against mosquitoes

Live adult mosquitoes are very delicate; therefore delivery time must be reduced to a minimum, which implies reliance on air shipping companies with express service (24). These companies recognise the difficulties in managing the transport of live mosquitoes and usually do not provide any guaranty about the quality and timing of a delivery.

One question which inevitably arises is: what is the competitive cost for sterile males? Of course, the calculation should include the cost of production and quality control, the cost of delivery from the facility to the target area and the cost of distribution and possibly monitoring after release. The overall cost must compete favourably with the current costs of other components of IVM in similar local contexts, taking into consideration their relative effectiveness in population suppression as well as the financial capacity of entities responsible for funding local control. This is a matter for specialists in cost-effectiveness and cost-benefit analysis (25, 26, 27).

By looking to the mature experience of SIT application in agriculture, the basic difference observed by the author is that the crop production business has an annual budget made available by the marketing of products, while in mosquito control there is no income but rather just the spending capacity of the responsible public administrations that protect public health. A possible methodology assisting the cost-effectiveness analysis in the case of vector borne diseases has been proposed by the World Health Organization (WHO) (25). The relatively small SIT applications in urban settings do not achieve that the economy of scale to allow efficient local production. This key problem can be overcome by more centralised production systems that distribute sterile males within their region, leading to a demand for international shipments in areas such as Southeast Europe or Southeast Asia, where there are many small countries. In this context, clearer regulation dealing with the international trade of sterile mosquitoes will play a useful role in allowing more sustainable operation of SIT facilities.

There is promise for the application of automation technologies, as largely developed in the industrial and logistical sectors, to the rearing of delicate creatures including mosquitoes. A close collaboration between informatics, electronics, mechanical engineers, production flow specialists and entomologists should be established, and a shared language developed between disciplines to achieve this ambitious goal. The challenge remains, however, that to be sustainable such high-tech facilities will require significant investments and then must rely on local-scale commitments exposed to the risk of intermittent contracts. Therefore, the solution may be a large production facility in a regional market (possibly producing multiple species or strains of mosquitoes for different local markets) with an efficient distribution network. The potential for success of this approach, however, is subject to further improvements in the production processes, which are far from being accomplished in the case of mosquito species at present, plus a clear international authorisation path and national or regional regulations that support both cross-border trade and suitable delivery technologies in the control areas.

Regulatory issues

In general, sterile insects can be classified as beneficial organisms, as reported by the International Plant Protection Convention (IPPC) in its International Standard for Phytosanitary Measures (ISPM) 3, “Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms” (28). This document is mainly designed for species of agricultural importance, however, and the conditions for extrapolating it to species of health importance should be better clarified and explained (see paper on ISPM 3, in this issue).

The potential risks related to the intentional/unintentional release of sub-sterile male mosquitoes or residual females together with the sterile males, are specific to public health and should be evaluated within that regulatory perspective.

The current national authorisation procedure to import sterile male mosquitoes differs from country to country, even within the EU. Sometimes the regulatory pathway is unclear, most likely due to the novelty of the case for the relevant authorities. Sometimes, it is even difficult to find out which institution has the specific responsibility and authority to address the issue. Our experience in local urban SIT pilot applications in Italy and several nearby countries has involved both national (environmental and health departments) and local authorities (city and regional governments) providing the authorisations needed for delivery and release of sterile mosquitoes. For example, in the case of Montenegro, a one-year temporary permit was released by the Agency for Environmental Protection; in the case of Albania, the authorisation was provided by the National Institute of Public Health; in the case of Germany the permit was delivered by the state of Baden-Württemberg where the releases were planned; while in the case of Greece, an informal authorisation was obtained through a large stakeholder meeting including the Benaki Phytopathological Institute, the Greek Atomic Energy Commission, the University of Thessaly, the Municipality of Markopoulo, the Prefecture of Attica, the Ministry of Health, the Ministry of Rural Development and Food, and the Greek Pest Control Association.

Table I shows recent experiences of moving sterile male mosquitoes across borders for release in the European scenario. Even the authorisation for conducting pilot feasibility studies may be time consuming and difficult to achieve (29). Consequently, sufficient harmonisation of legislation allowing reasonable investments for this approach to vector control has not yet occurred in Europe.

[Place Table I. here]

Risk analysis specifically related to the international shipments of sterile mosquitoes should be conducted within the context of the overall risk analysis of sterile insect trade, defining the pertinent risk categories and levels (see also Enkerlin & Pereira, and Mumford et al. in this issue [30, 31]). This will assist responsible international authorities to develop guidance assisting the States to adopt more harmonised legislation, including clarification of the condition to be met by entities applying to carry out SIT.

Exotic invasive species may be targeted (and thus sterile ones transported) due to the public health importance of mosquito vector species, thereby introducing nuanced regulatory issues. Transport entities cannot attest to the residual presence of biting females or the males not being fully sterile, two specific issues which must be considered, evaluated, and defined in term of acceptable thresholds. In the case of *Ae. albopictus* in Europe, all the countries involved in SIT field pilot trials decided to use their own field-collected seed material to start the production colony in Italy, to prevent the introduction of non-local genotypes. These questions need to be considered and harmonised where possible.

Regarding the sterile male field release technology two main issues should be considered: the methodology and the timing of a release, which reflects on the costs and the homogeneity of coverage. The release technology is particularly important in an urban setting where artificial barriers act as insurmountable obstacles to the active dispersal of sterile males. From the first evidence (32), drones may be well suited to reduce the costs of release and in the meantime to improve the good coverage of the target area with the desired dose of sterile males. Conditions for the use of drones over urban areas are particularly stringent, for obvious safety precautions, and specific authorisation must be obtained for the defined target area. Common release systems should be used in multiple locations to gain additional efficiency in release operations, which would also improve distribution efficiency if the same packaging systems were used for all or most deliveries.

The release mechanism for sterile mosquitoes may involve specific packaging requirements, such as cannisters for drones. To ensure a rapid and efficient delivery from a central production facility to a field release point, this final release packaging may need to be filled from the production site to avoid the need for additional handling for release, thus affecting the nature of the product being delivered by shipping carriers. This overall system requirement for efficient production and delivery is determined by the model of centralised production for economies of scale and reduced handling for automated aerial release.

A regional factory must be able to meet regulatory conditions for multiple users and make reliable shipments to ensure regular effective releases at a range of destinations. For examples, if marking sterile mosquitoes is required to make them easily distinguishable from wild ones, the currently available marking technologies, mainly based on adding substances, may negatively impact the quality of the males being shipped. In fact, to the author's knowledge, there is no simple method to genotype an insect once made sterile by irradiation. All of these factors interface with regulatory frameworks that generally are not yet mature or harmonised.

The special case of *Aedes aegypti* in Europe

When considering the importance of improved guidance and a clear regulatory pathway for live insect trade, one case that may be useful to examine is the possible establishment of *Ae. aegypti* in Southern Europe. The current climatic conditions are considered suitable for the vector's establishment and this suitability is expected to increase due to the ongoing climate change (33). Moreover, because the species already is well established in a large area bordering the Black Sea, the risk of introduction into the Mediterranean basin is real (34). Would it be possible and convenient to build a preparedness plan with the explicit aim of early detection and prompt elimination of the species in case of introduction? Some good examples of successful SIT deployment to eliminate newly introduced species are available to guide such plan (35, 36).

Conclusions

While the effectiveness of SIT in urban *Aedes* control has been demonstrated in several settings, there remains the need to make the whole process regarding the international framework for trade of sterile male mosquitoes more transparent and widely adopted. This requires agreement among the involved authorities. Consistency and transparency in guidance will have a strong positive effect on the applicability of the SIT to mosquito control, coupled with sound technological development. Thanks to the recent advances in technology, SIT and other possible genetic control methods offer a concrete possibility to open a new era in the struggle against mosquito vectors and the diseases they transmit – an era promising to suppress mosquitoes without harming other organisms. The international authorities responsible for the defining the regulating framework inside which these technologies may operate must give attention and coordinate for the benefits of SIT to be realised in urban settings.

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Table I

Sterile male mosquito shipments from Italy to European countries, 2013-2021

	Recipient	Amount of *Aedes albopictus* sterile males	Delivery time (hrs)	Aim	Financial support
2013	University of Montenegro, Podgorica, MNE	10,000	48–52	Mark-release-recapture	EU FP7 INFRAVEC
2016	KABS, Speyer, Germany	85,600	24–28	Pilot field trial	KABS
2017	Institute of Public Health, Tirana, Albania	60,000	26–48	Mark-release-recapture	IAEA
2017	GFS, Speyer, Germany	265,000	24	Pilot field trial	GFS
2018	University of Montenegro, Podgorica, MNE	234,000	48–50	Pilot field trial	Joint EU Horizon 2020 INFRAVEC2 & IAEA project
2018	KABS, Speyer, Germany	450,000	24	Pilot field trial	KABS

2018	BPI, Kifissia, Greece	120,000	20–24	Pilot field trial	IAEA
2019	University of Montenegro, Podgorica, MNE	180,000	48–50	Pilot field trial	IAEA
2019	KABS, Speyer, Germany	360,000	24	Pilot field trial	Joint EU Horizon 2020 INFRAVEC2 & KABS project
2019	BPI, Kifissia, Greece	740,000	20–24	Pilot field trial	Joint EU Horizon 2020 INFRAVEC2 & IAEA project
2020	BPI, Kifissia, Greece	609,000	20–24	Pilot field trial	EU Horizon 2020 INFRAVEC2
2020	ICYBAC, Speyer, Germany	487,000	24	Pilot field trial	ICYBAC
2021	EID Méditerranée, Montpellier, France	65,000	24	Aerial release trial	EU Horizon 2020 INFRAVEC2

2021	ICYBAC, Speyer, Germany	970,000	24	Pilot field trial	Joint EU Horizon 2020 INFRAVEC2 & ICYBAC project
2021	BPI, Kifissia, Greece	227,000	20–24	Aerial release trial	IAEA

EU: European Union, FP7: 7th Framework Programme, INFRAVEC: Research infrastructures for the control of insect vector-borne diseases, KABS: Kommunale Aktionsgemeinschaft zur Bekämpfung der Schnakenplage, IAEA: International Atomic Energy Agency, GFS: Gesellschaft zur Förderung der Stechmückenbekämpfung e.V, EID: Entente Interdépartementale pour la Démoustication du Littoral Méditerranéen, ICYBAC: subsidiary of KABS, BPI: Benaki Phytopathological Institute.