14. Genetic control of vectors

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Abstract

In a context of tighter regulations on approved insecticide molecules, the spread of insecticide resistance in insect vectors of human and animal diseases and the introduction of exotic vectors to new territories call for the development of new pest control methods and strategies. New genetic control methods, related to the ancestral sterile insect technique (SIT), show particular promise and are being developed in response to increasing health and agricultural challenges. These include the use of symbionts like *Wolbachia* and the use of transgenic insect strains, some of which incorporate gene editing techniques that can lead to transgene spread (gene drive). Here we present the principles, associated opportunities and risks, as well as the degree of advancement of these various techniques for a subset of livestock pests and disease vectors including screwworms, tsetse, mosquitoes and stomoxes. We then present some case studies on recent improvements in the use of the SIT in tsetse and the release of insects carrying a dominant lethal gene, symbiont-based approaches and gene drive in mosquitoes. Finally, we call to speed up the development of genetic control, within a rigorous benefit-risk analysis framework including international public consultation.

Keywords: sterile insect technique, incompatible insect technique, gene drive, pathogen interference, wolbachia

Introduction

Awareness of the toxicity of insecticides to living organisms and ecosystems is leading a growing number of countries to reduce the number of approved molecules (Bouyer 2015). For example, in 2014, the European Union was authorising four classes of vector control insecticides only, and only three new classes are being developed by industrials for operational use no earlier than 2019 (McBeath 2015). Moreover, resistance to pyrethroids, the class most commonly used against insects, is spreading, which could result in its disuse in the short-term. This is particularly true for mosquito vectors of malaria, the most important vector-borne disease causing fatalities globally), for which resistance has been observed in all major vector species throughout Africa (Ranson *et al.* 2011).

These regulations are being tightened in spite of a growing pressure from insect vectors of human and animal diseases. This increased pressure is explained by greater resistance to insecticides, but also by global factors such as climate change or the growth of global trade. These global factors foster the invasion of new territories by exotic vectors. For example, the tiger mosquito *Aedes albopictus* (Skuse), which is native to Asia and a major vector of human arboviruses, caused indigenous cases of dengue, chikungunya and Zika in Europe (Akiner *et al.* 2016; Benedict *et al.* 2007; Rezza *et al.* 2007). Innovations are therefore urgently required in the field of vector control.

Recently, the 2009/128/EC Directive on sustainable use of pesticides (EC, 2009) was adapted to European vectors of animal diseases and SIT appeared as the main alternative biological control method (Durel *et al.* 2015). More generally, genetic control appears as one of the main alternatives to chemical control (Bourtzis *et al.* 2016; McGraw and O'Neill 2013; Suckling *et al.* 2014).

Principles, opportunities and risks associated to genetic control

Genetic control consists in the large-scale rearing of insects – whether genetically modified or not – with the subsequent release of males in order for them to sterilise wild females or to transfer modifications to the progeny of wild females that are either lethal or impede the ability to transmit the pathogen. The genetic modifications correspond to random mutations or to transgenes, whereby foreign genetic material is incorporated in an organism. One variant consists in establishing novel insect-bacterial symbiotic associations which cause the death of embryos in females inseminated by the released males (cytoplasmic incompatibility) or block the transmission of the pathogen (pathogen interference). Symbionts could potentially also be genetically modified to prevent the infection of the vector, an approach called paratransgenesis.

The sterile insect technique (SIT) is the first and ancestral method from which other genetic control methods were derived: the males are irradiated and transmit sperm cells carrying many random lethal mutations to the females they inseminate. It is thus a birth control method that impacts the next generation. This technique has been used successfully in the control of a number of insect pests or vectors, such as species of fruit flies in Mexico (Enkerlin *et al.* 2015), the new world screwworm from the Americas and Libya (Vargas-Terán *et al.* 1994; Wyss 2006) and the tsetse fly in Africa (Vreysen *et al.* 2000). It is generally considered a biological control method in Europe, exempted from the regulation on genetically modified organisms (EFSA 2013), although no specific regulation is available for disease vectors. In the case of plant pests, SIT is also regarded as a biological control technique, the sterile males being assimilated to beneficial organisms (FAO 2015).

The use of genetically modified male insects to transmit a modification to females has the advantage of avoiding the need for irradiation[1] but presents other potential risks associated with the release of transgenes in the ecosystem. Genetic modifications under consideration could block the ability to transmit a pathogen, turn females into inoffensive males (Adelman and Tu 2016), skew the sex ratio in favour of males (Galizi *et al.* 2014, 2016) or even destroy the targeted insect population. However, genetically modified mosquitoes trigger public reluctance because of their potential biological risks, including the possibility of uncontrolled transfer of transgenes to non-targeted insects (EFSA 2013). The risk of horizontal transfer depends on the employed genetic mechanisms, and in particular on their potential for dissemination, but it is overall very low (Alphey 2014). It is even lower for transgenes than for symbionts (Loreto and Wallau 2016a).

The use of transgenic strains for vector control interventions is a recent field of research that has mainly focussed on mosquitoes. Many different mechanisms can be envisioned for control (Alphey 2014) that range from non-diffusive technologies like the release of insects carrying a dominant lethal gene (RIDL) (Lacroix *et al.* 2012) or the use of transgenic genetic sexing strains that can be

[1] Irradiation is often based on the use of radioactive sources but can also be conducted using safer X-ray machines. Besides, irradiation is helpful in treating the blood used to feed mosquito vector colonies to prevent vertical transmission of pathogens, and could be used to secure the mass-rearing of vectors.

irradiated before release to apply SIT, which have already been developed in *Cochliomyia* (Concha *et al.* 2016), to diffusive techniques like transposable elements and gene drive.

Considering the costs, the more diffusive the technology, the less costly it will be since the initial number of insects to release decreases. Techniques that are not diffusive like the SIT and RIDL are the most expensive because they necessitate the continuous release of males if the target population is not eliminated. When genetic modifications and transfected symbionts confer fitness costs which can reduce their diffusion in field conditions, a high initial frequency must be imposed in the target population which is also associated to high costs (Hoffmann *et al.* 2011). Moreover, the diffusion of genetic modifications is also limited by intrinsic dispersal capacities of the target species, which can be very low, for example in the case of *Aedes* species (outside occasional passive transport via human transportation, which will generally be insufficient to establish a modification carrying a fitness cost in a new population). Releases will then have to be carried out all other the target populations and the associated costs will be proportional to the target areas although scale savings are of course possible (Barclay *et al.* 2011). Finally, the use of transgenic insects is generally associated with intellectual property and commercial interests that can highly increase their cost, contrary to SIT.

Whatever the selected technology, genetic control requires the mass rearing, handling and release of sterile or modified insects, which can impact on their quality. Aerial release is recommended to ensure homogeneous distribution of the released insects (Mubarqui *et al.* 2014). It is thereafter critical to assess the competitiveness, survival and dispersal of the released males in the environmental conditions of the target area in order to make sure that they will be able to compete with their wild counterparts (Bellini *et al.* 2013; Sow *et al.* 2012). Finally, the released insects will have to show a similar behaviour as their wild counterparts (Vreysen *et al.* 2011). Below we develop examples of these technologies including SIT, RIDL, *Wolbachia*-based and gene drive approaches against insect pests and disease vectors.

Degree of advancement of the various genetic control methods against vector species

The degree of advancement of the various gene control technologies varies according to the vector group (Bourtzis *et al.* 2016). Table 1 gives an overview of the development of each technique at the time of writing in four families of vectors, ranging from the concept stage to the large scale operational control campaigns.

The sterile insect technique

SIT is the most developed technology for the insect families considered (see below a case study with tsetse flies). It has been used on an operational scale only against screwworms and tsetse flies, despite promising trials against *Stomoxys calcitrans* (L.) within an integrated management strategy in the USA Virgin Islands more than three decades ago (>99% of suppression of the target population) (Patterson *et al.* 1981). It might be useful to reconsider its use in areas like La Réunion island where other techniques failed to achieve a sustainable control of stomoxes (Bouyer *et al.* 2011). In mosquitoes, the technique is actively being developed against *Aedes* vectors of major arboviruses like dengue, chikungunya and Zika but also against *Anopheles* vectors of malaria and it will probably become available at the operational scale within the next five years (Bourtzis *et al.* 2016).

Table 1. Degree of advancement of various genetic control methods against four families of vectors and livestock pest insects.

Technology	Screwworm	Tsetse	Mosquito	Stomoxes
Sterile insect technique (SIT)	operational	operational	pilot field trials	pilot field trials
Incompatible insect technique (IIT)	–	concept (*Wolbachia*)	pilot field trials	–
Replacement (*Wolbachia*)	–	–	pilot field trials, in course of extension	–
Combined SIT/IIT	–	–	pilot field trials	–
Paratransgenesis	–	laboratory evaluation (*Sodalis*)	semi-field trials (*Asaia*)	–
Gene silencing using RNA interference (RNAi)	–	laboratory evaluation	laboratory evaluation	–
Release of insects carrying a dominant lethal gene (RIDL)	laboratory evaluation (genetic sexing strain)	–	pilot field trials, in course of extension	laboratory evaluation
Gene drive	–	–	laboratory evaluation	–
References	Concha *et al.* 2016; Vargas-Terán *et al.* 1994; Wyss 2006	Bourtzis *et al.* 2016; Dicko *et al.* 2014; Vreysen *et al.* 2000; Walshe *et al.* 2009; Whyard *et al.* 2015	Alphey 2014; Bourtzis *et al.* 2016; Gantz *et al.* 2015; Hammond *et al.* 2016; Haut Conseil des Biotechnologies 2017; Mancini *et al.* 2016	O'Brochta *et al.* 2000; Patterson *et al.* 1981

When considering applications of SIT to insect vectors of human disease (as opposed to agricultural pests, for which there is no such concern), special attention must be dedicated to the risk of introducing radiation-induced mutations in the natural insect population. Mutations in mosquito immune genes could render them hyper-susceptible to a human pathogen they can vector; therefore, 100% sterility of the released males must be guaranteed to exclude the possibility of accidentally introducing mutations that could elevate the vector competence of the mosquito population, especially when a strategy of long term reduction is selected, whereby a fraction of the initial population is preserved and might be modified by the intervention.

The incompatible insect technique

The incompatible insect technique (IIT) is an approach akin to SIT based on cytoplasmic incompatibility conferred by certain strains of *Wolbachia* bacteria, and that can be uni or bi-

directional[2]. This technique has been mostly developed against *Aedes* mosquitoes where it is currently tested in pilot field trials to reduce target populations (O'Connor *et al.* 2012). *Wolbachia* induces cytoplasmic incompatibility (CI), i.e. embryonic lethality corresponding to a sperm-egg incompatibility occurring when infected males mate either with uninfected females or with females infected with different, incompatible *Wolbachia* strains. This technique was tested successfully in laboratory cages in La Réunion, with the introduction of highly competitive infected *Culex quinquefasciatus* Say males resulting in a demographic crash (Atyame *et al.* 2011). It is presently being tested in a pilot trial in French Polynesia against *Aedes polynesiensis* (Marks) (H. Bossin, personal communication; O'Connor *et al.* 2012).

One of the important risks associated to this strategy is to release, in addition to the intended males, a few females infected with the *Wolbachia* strain used for control that may quickly establish the *Wolbachia* infection in the target population, thus 'immunizing' this population against further IIT interventions. To curb this risk, it could be beneficial to combine IIT with SIT. Actually, a low irradiation dose suffices to ensure complete sterilization of females, which as a result cannot establish the *Wolbachia* infection in the target population, while male sterility is ensured by the combination of *Wolbachia*-induced CI and irradiation (Bourtzis *et al.* 2016). This strategy is presently tested in a pilot trial in Thailand against *Aedes aegypti* (P. Kittayapong, personal communication) and in China against *Ae. albopictus* (Zhang *et al.* 2015).

Population replacement using Wolbachia

Transinfection consists in transferring specific bacteria from their natural host to a new host. In the case of the *Wolbachia* endosymbiont, this can result in stable novel infections causing cytoplasmic incompatibility and pathogen interference, two properties that can be exploited in vector control. The pathogen interference (PI) strategy has been used successfully to modify local *Ae. aegypti* populations in pilot trials in Australia (Hoffmann *et al.* 2011), even if additional studies will be necessary to demonstrate the epidemiological impact of this modification, particularly on Dengue transmission (see below). A variant of PI is to genetically modify a symbiont (paratransgenesis) to prevent the transmission of pathogens. This has been successfully tested in laboratory conditions on *Sodalis* symbionts of tsetse resulting in fly resistance to trypanosomes (Aksoy *et al.* 2008) but never in field conditions at the time of writing. More recently, large cage tests have demonstrated the ability of genetically modified *Asaia* bacteria to spread in *Anopheles stephensi* Liston and *Anopheles gambiae* Giles mosquito populations, confirming that using *Asaia* to deliver anti-malarial compounds in the mosquito is a valid perspective (Mancini *et al.* 2016).

Gene silencing using RNA interference

Silencing specific gene expression through RNA interference in larvae proved to be efficient in the lab to kill *Ae. aegypti* females by targeting the female-specific isoform of a sex determination gene (*doublesex*), concomitantly with testis-expressed genes, which produced a population of mosquitoes that was both highly male-biased and sterile (Whyard *et al.* 2015). In these experiments, double-stranded RNA was produced in bacteria and delivered to the larvae by feeding, a cost-effective gene silencing method that is potentially compatible with mass rearing with a built-in sex sorting component. The main challenge with this approach will be the homogeneous delivery of the RNAi-triggering molecules under large-scale mass rearing conditions to achieve 100%

[2] Cytoplasmic incompatibility is unidirectional when released males are sterilizing for wild females, and bidirectional when, in addition, wild males would be sterilizing for accidentally released females.

efficiency, but the possibility to produce large amounts of double-stranded RNA using bacteria or yeast makes it a promising option in the near future. Much work is however still needed to explore the field competitiveness of such males, especially when they are produced in mass-rearing conditions, before it becomes available for operational use.

It has also been suggested that the delivery of RNAi elements to mosquitoes through bacteria, algae or yeasts might also be used to silence essential genes to induce a specific larvicidal effect (Kumar *et al.* 2013).

Release of insects carrying a dominant lethal gene

RIDL consists in releasing mosquito males that have been genetically engineered to carry a dominant lethal gene (Figure 1).

The repression of the lethal gene using tetracycline as a dietary supplement has been the subject of criticism. Transgenic *Ae. aegypti* larvae are rescued from lethality by a diet supplemented with this antibiotic, but even in its absence, up to 3-4% of transgenic larvae survive to adulthood (Phuc *et al.* 2007). However, the massive fitness cost conferred by the transgene will lead to its rapid disappearance in the natural environment. The main associated drawback is that this might cause a partial modification of the target population through the 'pollution' of the local strains with some of the genetic background of the transgenic strain (from Asian origin, and colonized 14 years

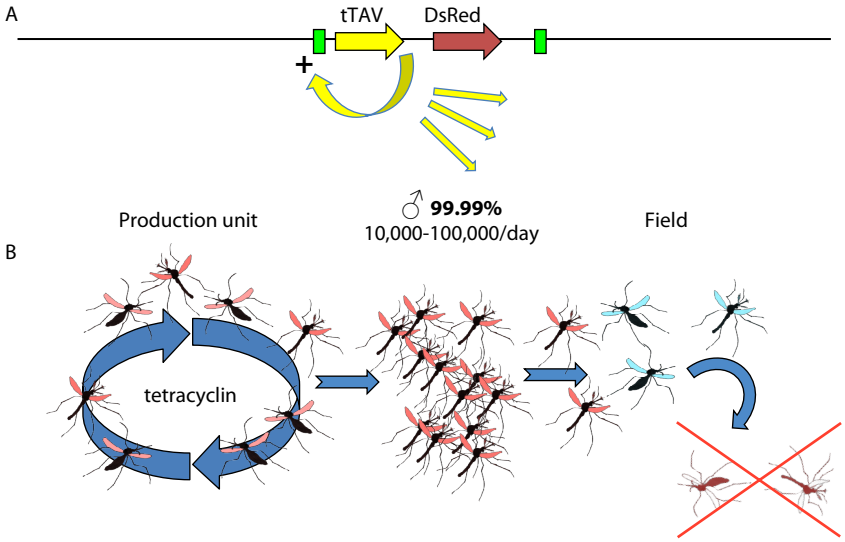

Figure 1. Principle of the release of insects carrying a dominant lethal gene (RIDL). (A) scheme of the transgene. The tetracycline activator variant (tTAV) protein binds to its own promoter, activates its own transcription and perturbs overall gene expression in the cells, resulting in mosquito death, unless tetracycline that binds and inactivates tTAV is provided. (B) During mass rearing in the production unit, mosquitoes develop normally in the presence of tetracycline. For an intervention, males are sorted at the pupal stage (based on the smaller size of male pupae). Once released, they mate with wild females whose progeny will die due to unrestricted tTAV activity.

ago), with unforeseen effects on vector competence for example. Field trials of this technique are presented below.

Gene drive

The most diffusive technology at the time of writing is gene drive based on the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system, which has the potential to invade a full target population from only one or few released individuals (see below) (Adelman and Tu 2016). Gene drive is based on the super-Mendelian inheritance of a homing endonuclease-like gene specifically designed to home into, and disrupt, a specific locus (Burt 2003; Windbichler *et al.* 2011). On cleaving its specific genomic sequence on the homologous (non-transgenic) chromosome in a heterozygous individual, this selfish transgene can replicate by homing with an efficiency reaching 90 to 100% (Figure 2). This underlies its remarkable selective advantage and spread in the target insect population.

A gene drive construct can be designed to carry another sequence (cargo) in its insertion locus. After mating with a released gene drive transgenic male, almost all the offspring of a wild female would thus inherit the construct, theoretically leading to its invasion of the entire population of the target species within a few generations (Esvelt *et al.* 2014). Although this concept is relatively old, the recent advent of the CRISPR-Cas9 system has recently boosted its development in insects

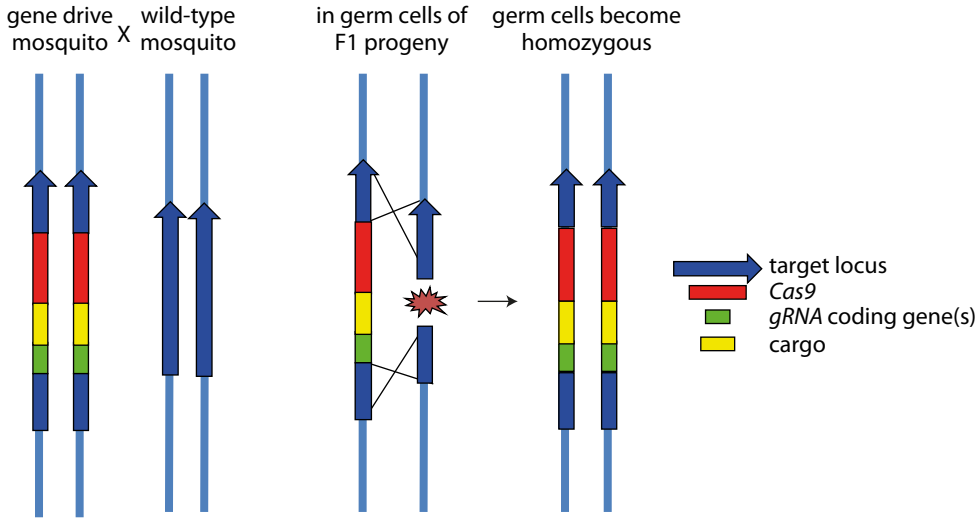

Figure 2. Principle of a CRISPR-Cas9 based gene drive in mosquitoes. A compound transgene composed of Cas9 *and guide RNA (gRNA) coding genes, and in some designs of a cargo and/or selection marker genes, is inserted on a mosquito chromosome exactly inside the target site recognized by the gRNA. In embryos formed when such a transgenic mosquito fertilizes a wild mosquito, the wild chromosome gets cut by Cas9 at the gRNA recognition site. This DNA break is usually repaired by homologous recombination with the intact chromosome. As a consequence, the transgene is copied onto the repaired chromosome, which becomes transgenic. Should this mechanism be 100% efficient, inheritance of the transgene would be 100% instead of 50%.*

(Gantz *et al.* 2015; Hammond *et al.* 2016). Two vector control approaches based on gene drive are envisioned: gene drives aiming at population suppression or even elimination, and gene drives aiming at population modification to decrease vectorial capacity (see below).

Case studies

Recent improvements in the use of SIT against tsetse flies

One of the recent examples of the use of the SIT against vectors is a tsetse fly eradication project that has been implemented in the Niayes area of Senegal, based on an integrated pest management strategy (Vreysen *et al.* in press). In 2005, this country initiated a project entitled 'Projet de lutte contre les glossines dans les Niayes' (tsetse control program in the Niayes) with the aim of creating a zone free of *Glossina palpalis gambiensis* (Vanderplank) in that area. The project received technical and financial support from the IAEA, the FAO, the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), and the US Department of State through the Peaceful Uses Initiative (PUI), and was implemented in the context of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). The project was implemented following a phased conditional approach (PCA), that entails programme implementation in distinct phases in which support to the next phase is conditional upon completion of all (or at least the majority) activities in the previous phase. In the case of the tsetse project in Senegal, the PCA consisted of 4 phases: (1) training and commitment of all stakeholders; (2) baseline data collection and feasibility studies; (3) pre-operational activities; and (4) operational activities.

Activities of phase 2 focused on the collection of entomological (Bouyer *et al.* 2010), veterinary (Seck *et al.* 2010), socio-economic (Bouyer *et al.* 2014) and environmental baseline data, and a population genetics study that proved that the target population was isolated (Solano *et al.* 2010). These data enabled the tsetse infested area to be delimited, the impact of animal trypanosomosis on the farmers' welfare to be quantified, and the formulation of an area-wide integrated pest management strategy to eradicate the isolated tsetse populations from the Niayes that included a sterile insect (SIT) component.

This project benefited from a strong applied research component and each stage involved methodological and technological developments. During the pre-operational phase, a series of activities were carried out that were needed to implement the operational phase.

The operational phase included a reduction of tsetse densities using insecticide traps and insecticide treatment of cattle followed by the aerial release of sterile males. Habitats suitable to the tsetse fly were identified by remote sensing based on a population distribution model (Dicko *et al.* 2014). Using this model, a low trap density of only 1-3.4 per km^2 allowed reducing tsetse populations by more than 95%, thanks to the targeting of the most suitable habitats.

The SIT component included the development of transport methods for long distance shipments of sterile male pupae. These sterile males were actually produced and irradiated in Burkina Faso at CIRDES (Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide), and in Slovakia, by the Slovak Academy of Sciences. They were transported to Senegal by express delivery under regulated conditions. A specific transport method was developed to guarantee their survival (in particular, a temperature of 10 °C) (Pagabeleguem *et al.* 2015) and a quality control method originally developed in fruit flies SIT programmes was transferred to tsetse to ascertain that highly competitive sterile males would be released (Seck *et al.* 2015). They were

then reared for six days in an insectarium at ISRA, Senegal (Institut Sénégalais de Recherches Agricoles). Sterile males were released by an automatic machine loaded onto a gyrocopter (Figure 3). This machine, developed by a Mexican company, is piloted by a geographic information system (GIS) that can adjust the density of sterile males to be released in order to achieve the objective (Mubarqui *et al.* 2014). Installed on an Android tablet, this GIS enables the pilot to concentrate on following release lines, with the machine automatically beginning the release upon entering the target zone, and ceasing upon exit. Release densities of sterile males were thus adjusted depending on the availability of suitable habitat predicted by the distribution model (10 sterile males per km^2 if the habitat is unsuitable; 100 if it is suitable). The competitiveness of sterile males was very good (Fried[3] index ranging from 0.3 to 0.5) (Bouyer *et al.* 2012; Fried 1971).

The innovative elements of this project are currently being transferred to other tsetse fly eradication projects in Africa, like the tsetse eradication campaign in the Deme valley of Ethiopia, within the framework of PATTEC. This successful experience can be used as an example for improving or starting vector control in other parts of the world.

RIDL against mosquitoes

RIDL has been developed (Phuc *et al.* 2007) and tested by the British-based company Oxitec against *Ae. aegypti* in Cayman Islands, Panama and Brazil within small pilot trials (10 to 16 ha) (Carvalho *et al.* 2015; Harris *et al.* 2011; Lacroix *et al.* 2012). It is currently extended to larger areas in Brazil, encompassing 12 km^2 with nearly 60,000 residents which will allow evaluating its potential

[3] Fried index measures the propensity of a wild female to mate with a sterile male if it is given the choice between this sterile male and a wild male. It is calculated using the ratio of sterile to wild males and the induced sterility in the target population. For example, the female will mate twice more often with a wild than a sterile male if the Fried index is 0.5.

Figure 3. A gyrocopter releasing sterile tsetse male Glossina palpalis gambiensis *in Senegal thanks to an automatic release machine (photo by Jérémy Bouyer).*

impact on virus transmission (Servick 2016). In the system tested (OX513A strain), the progeny of transgenic males are killed by the transgene (Alphey 2014). In pilot trials, the target larvae populations were suppressed by 80 to 96% but an overall low competitiveness of the transgenic males was observed in the field, ranging from 3 to 6% (Carvalho *et al.* 2015). This can be partly blamed on the current legislation inappropriately considering transgenic strains as medicines, making it necessary to use the originally registered strain everywhere without adjusting its genetic background (Mexican for OX513A in this case) (Haut Conseil des Biotechnologies 2017), which is better adapted to laboratory conditions than to the environmental conditions in the target areas.

Finally, the cost of this technique is still high, estimated at $ 5 per person per year in Brazil (Servick 2016) while the total treated area remains minuscule compared to the extent of *Ae. aegypti* distribution in this country.

Use of transinfected Wolbachia *to transform mosquito populations*

A successful trial was conducted in 2011 in two natural *Ae. aegypti* populations in Australia, using a strain of *Ae. aegypti* transinfected with the *w*Mel *Wolbachia* strain from *Drosophila melanogaster* (Hoffmann *et al.* 2011). The *Wolbachia* strain, released using infected males at a density allowing to reach the invasion threshold of 30% (which is quite high due to a loss of fitness in the infected adults), successfully invaded the two wild populations, reaching near-fixation in a few months, in the release areas only, following releases (Hoffmann *et al.* 2011). Pathogen interference from this *Wolbachia* strain blocks the transmission of various pathogens including dengue virus, thanks to a mechanism that is still not fully understood at the time of writing.

A follow-up study demonstrated that the strain was still fixed in the Australian target populations in 2013 and 2014 (Hoffmann *et al.* 2014). The strain also did not diffuse outside of treated areas, which is probably due to the low dispersal capacities of *Ae. aegypti* combined with the high invasion threshold necessary to fix *w*Mel *Wolbachia* because of its intrinsic fitness cost. Moreover, *w*Mel mosquitoes collected from these localities in January 2012 and challenged with three dengue virus serotypes still showed little vector competence in comparison to the wild type (Frentiu *et al.* 2014).

These open releases were approved by the Australian Pesticides and Veterinary Medicines Authority (Hoffmann *et al.* 2011). However, these field releases stimulated debates in the scientific community about the potential risks associated with this technique (Dobson *et al.* 2016; Loreto and Wallau 2016b; O'Neill 2016). One of the controversial points was that this particular symbiotic association would have a very low probability to appear without human intervention, even if other *Wolbachia* strains were previously detected in *Ae. aegypti* (Coon *et al.* 2016; Klasson *et al.* 2009; Woolfit *et al.* 2009). Still, the man-stimulated spread of such a symbiotic association in a natural population is currently not raising as much public concern as transgenes. Nevertheless, predicting how transmitted viruses will react to this new selection pressure is difficult, all the more so as the mechanism blocking their transmission is still unknown. It cannot be excluded that virus strains evolve to evade the *Wolbachia*-mediated mosquito resistance, perhaps with increased pathogenicity for vectors or even hosts.

Finally, this technique is also costly at the time of writing, although the Eliminate Dengue program in Brazil plans to bring its cost down to $ 1 per person or less (Servick 2016).

Gene drive to control mosquitoes

The elimination approach was tested by designing gene drive cassettes homing into three different female fertility genes (Hammond *et al.* 2016). In preliminary cage experiments, the frequency of one of the constructs increased from an initial 50 to 75% within 4 generations, consistent with a model of invasion – and suppression- of a wild mosquito population in case of release of this construct. However, the efficacy of these gene drives is currently limited by the unexpected sterility of heterozygous females. The loss-of-function mutations in these fertility genes should be recessive, but due to somatic activity of the drive – in addition to the desired activity in the germ line – females that are initially heterozygous become somatically homozygous and sterile, hampering a female contribution to the spread of the transgene. In addition, gene drive spread in nature is likely to be limited by natural genetic diversity and by the appearance of drive-resistant mutations due to the drive mechanism itself (Champer *et al.* 2017; Unckless *et al.* 2017). Work is in progress to solve these roadblocks and generate more efficient elimination drives. However, it is important to note that decisions regarding field implementation of elimination drives will have to consider the possible undesired ecological consequences of the elimination of an entire species (Hochkirch *et al.* 2017).

In contrast to these elimination drives, an example of a population modifying gene drive has been provided for *An. stephensi*, in which antipathogen effector genes targeting *Plasmodium falciparum* and transcribed in female only thanks to blood meal-regulated expression were successfully transmitted at a very high rate (~99.5%) (Gantz *et al.* 2015). These results illustrate the possibility to transform wild populations of vectors in order to interrupt pathogen transmission.

In *Ae. aegypti*, it was suggested that CRISPR-Cas9 might be used to target the mosquito male determining factor (M factor) to drive maleness to control wild mosquito populations (Adelman and Tu 2016). Ectopic expression of M in genetic females would result in female masculinization or death, whereas males may carry the masculinizing transgene on to their progeny. This would lead to a fast spread of the transgene in the population, ideally each new transgenic generation being constituted of transgenic males only. The M factor has only been recently discovered in *Ae. aegypti* (Hall *et al.* 2015) and even more recently in *An. gambiae* (Krzywinska *et al.* 2016).

Although very attractive – regarded by some as a silver bullet – gene drive strategies offer the possibility to alter entire wild populations and therefore ecosystems, and must include robust safeguards and methods of control (Esvelt *et al.* 2014). Moreover, two primary risks related to undesired spread have been identified. First, rare fertile hybridisation events may allow the drive to affect closely related species (in the *Anopheles* complex for example). It should be possible to mitigate this risk by using precision drives (subspecies-specific sequences) to target sequences unique to the targeted vector. Second, where this method would be employed against invasive species such as *Aedes* mosquitoes, the construct might spread from the targeted invasive populations back into the native distribution range and lead to species eradication. This risk poses ethical concerns about the desirability of eradicating entire species. It will thus be critical that all decisions involving the use of suppression drives involve extensive deliberations including but not limited to ecologists and citizens of potentially affected communities (Esvelt *et al.* 2014). In addition, given that these transgenes have the potential to disseminate through borders, their use will demand international debates and regulations.

A committee of experts appointed by the US National Academies of Sciences, Engineering, and Medicine has recently estimated that current evaluation and regulatory procedures are

not adequate to address the risks and requirements of this technology (National Academies of Sciences, Engineering and Medicine 2016). Therefore, a wide consultation involving regulatory bodies, scientists, medical authorities, vector control agents and the public is needed to establish the risk/benefit evaluation and regulatory procedures that are necessary to move this research from the lab to field implementation (James *et al.* 2018).

Involving public research and industries

Collaboration between public research and the private sector is needed for the large-scale use of genetic methods that require mass-rearing and area-wide releases of insects. Gene drive is a notable exception, since it theoretically requires the release of only few individuals to initiate target population invasion by the transgene.

In the case of SIT, the large-scale use of sterile insects implies mobilising extensive research and development programmes, such as the ones leading to the eradication of the screwworm in North America, Central America and Libya, and of the Mediterranean fruit fly, of agricultural concern, in Mexico (Enkerlin *et al.* 2015). However, public research cannot implement these programmes alone, but can contribute to them by conducting the operational research essential to their optimisation, success and regulation.

Pesticide industries are faced with increasingly strict regulations limiting the number of approved molecules and with the shorter lifespan of these molecules because of the spread of genetic resistance in insects. It is therefore in their interest to invest in the development of new non-toxic control methods, and to deploy these within an integrated pest control framework, taking into account lessons learned from past experience. Public research can make significant contributions to both processes.

Another area of collaboration is the assessment of risks associated with the use of transgenic insects which requires a case-by-case analysis of risks, which depend on the genetic mechanisms used, and in particular on their potential for dissemination. In Europe, this analysis can be based on the European Food Safety Authority recommendations (EFSA 2013).

Although genetic control has potential that must be exploited, no technique should be automatically ruled out, and it is essential to analyse the advantages, disadvantages and risks associated with each one. This implies gaining further knowledge of the ecology of target vector populations, but also of the socio-ecosystems concerned by vector control. It will then be possible to achieve an optimal and integrative combination of several different methods, with the help of modelling studies.

Perspectives

Beyond the emerging genetic methods described above, other integrated strategies have been proposed to amplify the impact of genetic control. For example, implementing SIT requires sufficient quantities of sterile males to overwhelm wild males in the target population, which is generally very costly or even impossible when suppression techniques are not fully efficient, like in the case of *Aedes* mosquito species. To overcome these obstacles, a variant of SIT, named 'boosted SIT', has been proposed (Bouyer and Lefrançois 2014). It aims to reduce by 90 to 99% the quantity of sterile males needed to control or eradicate a target population. In this variant strategy, sterile males are considered as a specific means of contaminating females with a control

agent. This agent may be an active substance, bacteria, a fungus or a virus, or even recombinant versions of these pathogens. An example of proposed association to sterile males are densoviruses (Bouyer *et al.* 2016), that are very specific to the target species or closely related species (Carlson *et al.* 2006). In the case of the AeDNV virus isolated from *Ae. albopictus* for example, only other *Aedes* or *Culex* species are sensitive to the virus, while all other insects or crustaceans are not. The double specificity of action warranted by specific contamination of the target species and larval habitats through the sterile males and the restricted host range of the virus might allow proposing efficient and safe vector control methods in the near future.

Conclusion

Genetic control appears as a major alternative to the use of large spectrum insecticides in the global war opposing humans to pest and vector arthropod species. No technology represents a solution on its own, but the SIT, the ancestor of all the techniques presented in this chapter, represents a biological alternative with validated successes in the past and that appears as the safest alternative at the time of writing. Many opportunities have emerged recently, stimulated by the spread of vector-borne diseases, particularly mosquito-borne arboviruses like dengue, chikungunya or Zika. But these opportunities are also accompanied by ecological and ethical challenges that will necessitate unbiased assessments of potential benefits and risks at an international level before they can be used at an operational scale.

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References

Adelman ZN and Tu Z (2016) Control of mosquito-borne infectious diseases: sex and gene drive. Trends Parasitol 32: 219-229.

Akiner MM, Demirci B, Babuadze G, Robert V and Schaffner F (2016) Spread of the invasive mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Black Sea region increases risk of chikungunya, dengue, and Zika outbreaks in Europe. PLoS Negl Trop Dis 10: e0004664.

Aksoy S, Weiss B and Attardo G (2008) Paratransgenesis applied for control of tsetse transmitted sleeping sickness. In: Aksoy S (ed.) Transgenesis and the management of vector-borne disease. Springer, New York, NY, USA, pp. 35-48.

Alphey L (2014) Genetic control of mosquitoes. Ann Rev Entomol 59: 205.

Atyame CM, Pasteur N, Dumas E, Tortosa P, Tantely ML, Pocquet N, Licciardi S, Bheecarry A, Zumbo B, Weill M and Duron O (2011) Cytoplasmic incompatibility as a means of controlling *Culex pipiens quinquefasciatus* mosquito in the islands of the south-western Indian Ocean. PLoS Negl Trop Dis 5: e1440.

Barclay HJ, Matlock R, Gilchrist S, Suckling DM, Reyes J, Enkerlin WR and Vreysen MJB (2011) A conceptual model for assessing the minimum size area for an area-wide integrated pest management program. International Journal of Agronomy, Article ID: 40932.

Bellini R, Balestrino F, Medici A, Gentile G, Veronesi R and Carrieri M (2013) Mating competitiveness of *Aedes albopictus* radio-sterilized males in large enclosures exposed to natural conditions. J Med Entomol 50: 94-102.

Benedict MQ, Levine RS, Hawley WA and Lounibos LP (2007) Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector-Borne Zoonotic Dis 7: 76-85.

Bourtzis K, Lees RS, Hendrichs J and Vreysen MJB (2016) More than one rabbit out of the hat: radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. Acta Trop 157: 115-130.

Bouyer F, Seck MT, Dicko A, Sall B, Lo M, Vreysen M, Chia E, Bouyer J and Wane A (2014) Ex-ante cost-benefit analysis of tsetse eradication in the Niayes area of Senegal. PLoS Negl Trop Dis 8: e3112.

Bouyer J (2015) Integrated management of insect vectors of human and animal diseases. Developing genetic control. CIRAD Perspectives 34, 4 pp. Available at: https://tinyurl.com/y88phugf.

Bouyer J, Chandre F, Gilles J and Baldet T (2016) Alternative vector control methods to manage the Zika virus outbreak: more haste, less speed. Lancet Glob Health 4: e364.

Bouyer J, Grimaud Y, Pannequin M, Esnault O and Desquesnes M (2011) Importance épidémiologique et contrôle des stomoxes à la Réunion. Bull Epidémiol 43: 53-58.

Bouyer J and Lefrançois T (2014) Boosting the sterile insect technique to control mosquitoes. Trends Parasitol 30: 271-273.

Bouyer J, Seck MT, Pagabeleguem S, Sall B, Lo M, Vreysen MJB, Balenghien T and Lancelot R (2012) Study of the competitiveness of allochtonous sterile males during the tsetse eradication campaign in Senegal. 18th Conférence E-sove 2012, EID, CIRAD, IRD, Montpellier, France.

Bouyer J, Seck MT, Sall B, Guerrini L and Vreysen MJB (2010) Stratified entomological sampling in preparation of an area-wide integrated pest management programme: the example of *Glossina palpalis gambiensis* in the Niayes of Senegal. J Med Entomol 47: 543-552.

Burt A (2003) Site-specific selfish genes as tools for the control and genetic engineering of natural populations. Proc Royal Soc London B: Bio Sci 270: 921-928.

Carlson J, Suchman E and Buchatsky L (2006) Densoviruses for control and genetic manipulation of mosquitoes. Adv Virus Res 68: 361-392.

Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, Malavasi A and Capurro ML (2015) Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. PLoS Negl Trop Dis 9: e0003864.

Champer J, Reeves R, Oh SY, Liu C, Liu J, Clark AG and Messer PW (2017) Novel CRISPR/Cas9 gene drive constructs in Drosophila reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. bioRxiv: 112011.

Concha C, Palavesam A, Guerrero FD, Sagel A, Li F, Osborne JA, Hernandez Y, Pardo T, Quintero G and Vasquez M (2016) A transgenic male-only strain of the New World screwworm for an improved control program using the sterile insect technique. BMC Biol 14: 72.

Coon KL, Brown MR and Strand MR (2016) Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. Mol Ecol 25(22): 5806-5826.

Dicko AH, Lancelot R, Seck MT, Guerrini L, Sall B, Lo M, Vreysen MJB, Lefrançois T, Williams F, Peck SL and Bouyer J (2014) Using species distribution models to optimize vector control: the tsetse eradication campaign in Senegal. Proc Nat Acad Sci 111: 10149-10154.

Dobson SL, Bordenstein SR and Rose RI (2016) *Wolbachia* mosquito control: regulated. Science 352: 526-527.

Durel L, Estrada-Peña A, Franc M, Mehlhorn H and Bouyer J (2015) Integrated fly management in European ruminant operations from the perspective of Directive 2009/128/EC on sustainable use of pesticides. Parasitol Res 114: 379-389.

European Commission (2009) Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. OJ L 309: 1-26.

European Food Safety Authority (EFSA) (2013) Panel on Genetically Modified Organisms (GMO) Guidance on the environmental risk assessment of genetically modified animals. EFSA J 11: 3200.

Enkerlin W, Gutiérrez-Ruelas JM, Cortes AV, Roldan EC, Midgarden D, Lira E, López JLZ, Hendrichs J, Liedo P and Arriaga FJT (2015) Area freedom in Mexico from mediterranean fruit fly (Diptera: Tephritidae): a review of over 30 years of a successful containment program using an integrated area-wide SIT approach. Florida Entomol 98: 665-681.

Esvelt KM, Smidler AL, Catteruccia F and Church GM (2014) Concerning RNA-guided gene drives for the alteration of wild populations. Elife 3: e03401.

Food and Agriculture Organization of the United Nations (FAO) (2015) Glossaire des termes phytosanitaires NIMP 5. FAO, Rome, Italy.

Frentiu FD, Zakir T, Walker T, Popovici J, Pyke AT, Van den Hurk A, McGraw EA and O'Neill SL (2014) Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. PLoS Negl Trop Dis 8: e2688.

Fried M (1971) Determination of sterile-insect competitiveness. J Econ Entomol 64: 869-872.

Galizi R, Doyle LA, Menichelli M, Bernardini F, Deredec A, Burt A, Stoddard BL, Windbichler N and Crisanti A (2014) A synthetic sex ratio distortion system for the control of the human malaria mosquito. Nature Com 5: 3977.

Galizi R, Hammond A, Kyrou K, Taxiarchi C, Bernardini F, O'Loughlin SM, Papathanos P-A, Nolan T, Windbichler N and Crisanti A (2016) A CRISPR-Cas9 sex-ratio distortion system for genetic control. Sci Rep 6: 31139.

Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E and James AA (2015) Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. Proc Natl Acad Sci 112: E6736-E6743.

Hall AB, Basu S, Jiang X, Qi Y, Timoshevskiy VA, Biedler JK, Sharakhova MV, Elahi R, Anderson MAE, Chen X-G, Sharakhov IV, Adelman ZN and Tu Z (2015) A male-determining factor in the mosquito *Aedes aegypti*. Science 348: 1268-1270.

Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, Russell S, Burt A, Windbichler N, Crisanti A and Nolan T (2016) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nature Biotech 34: 78-83.

Harris AF, Nimmo DD, McKemey AR, Kelly N, Scaife S, Donnelly CA, Beech C, Petrie WD and Alphey L (2011) Field performance of engineered male mosquitoes. Nature Biotech 29: 1034-1037.

Haut Conseil des Biotechnologies (2017) Avis du Comité Scientifique en réponse à la saisine du 12 octobre 2015 concernant l'utilisation de moustiques génétiquement modifiés dans le cadre de la lutte antivectorielle. Haut Conseil des Biotechnologies, Paris, France.

Hochkirch A, Beninde J, Fischer M, Krahner A, Lindemann C, Matenaar D, Rohde K, Wagner N, Wesch C and Wirtz S (2017) License to kill? – disease eradication programs may not be in line with the convention on biological diversity. Conservation Lett 11: e12370.

Hoffmann AA, Iturbe-Ormaetxe I, Callahan AG, Phillips BL, Billington K, Axford JK, Montgomery BL, Turley AP and O'Neill SL (2014) Stability of the *w*Mel *Wolbachia* infection following invasion into *Aedes aegypti* populations. PLoS Negl Trop Dis 8: e3115.

Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y, H. Cook, Axford J, Callahan AG, Kenny N, Omodei C, McGraw EA, Ryan PA, Ritchie SA, Turelli M and O'Neill SL (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature 476: 454-457.

James S, Collins FH, Welkhoff PA, Emerson C, Godfray HCJ, Gottlieb M, Greenwood B, Lindsay SW, Mbogo CM, Okumu FO, Quemada H, Savadogo M, Singh JA, Tountas KH and Touré YT (2018) Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in sub-Saharan Africa: recommendations of a Scientific Working Group. Am J Trop Med Hyg 98 (Suppl 6): 1-49.

Klasson L, Kambris Z, Cook PE, Walker T and Sinkins SP (2009) Horizontal gene transfer between *Wolbachia* and the mosquito *Aedes aegypti*. BMC Genomics 10: 1.

Krzywinska E, Dennison NJ, Lycett GJ and Krzywinski J (2016) A maleness gene in the malaria mosquito *Anopheles gambiae*. Science 353: 67-69.

Kumar A, Wang S, Ou R, Samrakandi M, Beerntsen BT and Sayre RT (2013) Development of an RNAi based microalgal larvicide to control mosquitoes. Malaria World J 4: 1-7.

Lacroix R, McKemey AR, Raduan N, Wee LK, Ming WH, Ney TG, Siti Rahidah AA, Salman S, Subramaniam S and Nordin O (2012) Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS ONE 7: e42771.

Loreto ELS and Wallau GL (2016a) Risks of *Wolbachia* mosquito control. Science 351: 1273.

Loreto ELS and Wallau GL (2016b) Risks of *Wolbachia* mosquito control. Science 351: 1273-1273.

Mancini MV, Spaccapelo R, Damiani C, Accoti A, Tallarita M, Petraglia E, Rossi P, Cappelli A, Capone A and Peruzzi G (2016) Paratransgenesis to control malaria vectors: a semi-field pilot study. Parasite Vectors 9: 1.

McBeath J (2015) An industry perspective on the development of new insecticide – based tools for vector control. International Society for Neglected Tropical Diseases – ISNTD Bites, London, UK.

McGraw EA and O'Neill SL (2013) Beyond insecticides: new thinking on an ancient problem. Nature Reviews Microbiology 11: 181-193.

Mubarqui RL, Perez RC, Angulo Kladt R, Zavala Lopez JL, Parker A, Seck MT, Sall B and Bouyer J (2014) The smart aerial release machine, a universal system for applying the sterile insect technique. PLoS ONE 9: e103077.

National Academies of Sciences, Engineering and Medicine (2016) Gene drives on the horizon: advancing science, navigating uncertainty, and aligning research with public values. National Academies Press, Washington, DC, USA.

O'Connor L, Plichart C, Sang AC, Brelsfoard CL, Bossin HC and Dobson SL (2012) Open release of male mosquitoes infected with a *Wolbachia* biopesticide: field performance and infection containment. PLoS Negl Trop Dis 6: e1797.

O'Neill SL (2016) *Wolbachia* mosquito control: tested. Science 352: 526-526.

O'Brochta DA, Atkinson PW and Lehane MJ (2000) Transformation of *Stomoxys calcitrans* with a Hermes gene vector. Insect Mol Bio 9: 531-538.

Pagabeleguem S, Seck MT, Sall B, Vreysen MJB, Gimonneau G, Fall AG, Bassene M, Sidibé I, Rayaisse JB, Belem A and Bouyer J (2015) Long distance transport of irradiated male *Glossina palpalis gambiensis* pupae and its impact on sterile male yield. Parasite Vectors 8: 259.

Patterson RS, LaBrecque GC, Williams DF and Weidhaas DE (1981) Control of the stable fly, *Stomoxys Calcitrans* (Diptera: Muscidae), on St. Croix, US Virgin Islands, using integrated pest management measures: III. Field techniques and population control. J Med Entomol 18: 203-210.

Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G, Fu G, Condon KC, Scaife S, Donnelly CA, Coleman PG, White-Cooper H and Alphey L (2007) Late-acting dominant lethal genetic systems and mosquito control. BMC Biol 5: 1.

Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z and Corbel V (2011) Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol 27: 91-98.

Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, Cordioli P, Fortuna C, Boros S and Magurano F (2007) Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370: 1840-1846.

Seck MT, Bouyer J, Sall B, Bengaly Z and Vreysen MJB (2010) The prevalence of African animal trypanosomoses and tsetse presence in western Senegal. Parasite 17: 257-265.

Seck MT, Pagabeleguem S, Bassene MD, Fall AG, Diouf TAR, Sall B, Vreysen MJB, Rayaissé J-B, Takac P, Sidibé I, Parker AG, Mutika GN, Bouyer J and Gimonneau G (2015) Quality of sterile male tsetse after long distance transport as chilled, irradiated pupae. PLoS Negl Trop Dis 9: e0004229.

Servick K (2016) Brazil will release billions of lab-grown mosquitoes to combat infectious disease. Will it work? Science, Washington, DC, USA. Available at: https://tinyurl.com/h383qek.

Solano P, Kaba D, Ravel S, Dyer N, Sall B, Vreysen MJB, Seck MT, Darbyshir H, Gardes L, Donnelly MJ, De Meeûs T and Bouyer J (2010) Tsetse population genetics as a tool to choose between suppression and elimination: the case of the Niayes area in Senegal. PLoS Trop Negl Dis 4: e692.

Sow A, Sidibé I, Bengaly Z, Bancé Z, Sawadogo GJ, Solano P, Vreysen MJB, Lancelot R and Bouyer J (2012) Irradiated male *Glossina palpalis gambiensis* (Diptera: Glossinidae) from a 40-years old colony are still competitive in a riparian forest in Burkina Faso. PLoS ONE 7: e37124.

Suckling DM, Stringer LD, Stephens AE, Woods B, Williams DG, Baker G and El-Sayed AM (2014) From integrated pest management to integrated pest eradication: technologies and future needs. Pest Manag Sci 70: 179-189.

Unckless RL, Clark AG and Messer PW (2017) Evolution of resistance against CRISPR/Cas9 gene drive. Genetics 205: 827-841.

Vargas-Terán M, Hursey BS and Cunningham EP (1994) Eradication of the screwworm from Libya using the sterile insect technique. Parasitol Today 10: 119-122.

Vreysen MJB, Saleh KM, Ali MY, Abdulla AM, Zhu Z-R, Juma KG, Dyck VA, Msangi AR, Mkonyi PA and Feldmann HU (2000) *Glossina austeni* (Diptera: Glossinidae) Eradicated on the island of Unguja, Zanzibar, using the sterile insect technique. J Econ Entomol 93: 123-135.

Vreysen MJB, Saleh KM, Lancelot R and Bouyer J (2011) Factory tsetse flies must behave like wild flies: a prerequisite for the sterile insect technique. PLoS Trop Negl Dis 5(2): e907.

Vreysen MJB, Seck MT, Sall B, Mbaye AG, Bassene M, Fall AG, Lo M and Bouyer J (in press) Eradication of *Glossina palpalis gambiensis* from the Niayes area of Senegal: a review of operational research in support of a phased conditional approach. In: Hendrichs J, Pereira R and Vreysen MJB (eds.) Area-wide integrated pest management: development and field application. CRC Press, Boca Raton, FL, USA.

Walshe DP, Lehane SM, Lehane MJ and Haines LR (2009) Prolonged gene knockdown in the tsetse fly *Glossina* by feeding double stranded RNA. Insect Mol Biol 18: 11-19.

Whyard S, Erdelyan CN, Partridge AL, Singh AD, Beebe NW and Capina R (2015) Silencing the buzz: a new approach to population suppression of mosquitoes by feeding larvae double-stranded RNAs. Parasit Vectors 8: 96.

Windbichler N, Menichelli M, Papathanos PA, Thyme SB, Li H, Ulge UY, Hovde BT, Baker D, Monnat RJ and Burt A (2011) A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. Nature 473: 212-215.

Woolfit M, Iturbe-Ormaetxe I, McGraw EA and O'Neill SL (2009) An ancient horizontal gene transfer between mosquito and the endosymbiotic bacterium *Wolbachia pipientis*. Mol Biol Evol 26: 367-374.

Wyss JH (2006) Screwworm eradication in the Americas. Ann NY Acad Sci 916: 186-193.

Zhang D, Zheng X, Xi Z, Bourtzis K and Gilles JRL (2015) Combining the sterile insect technique with the incompatible insect technique: I-impact of *Wolbachia* infection on the fitness of triple-and double-infected strains of *Aedes albopictus*. PLoS ONE 10: e0121126.