Trends in Parasitology

CellPress
REVIEWS

Opinion

Phased Conditional Approach for Mosquito Management Using Sterile Insect Technique

Jérémy Bouyer, [1,2,*] Hanano Yamada,[1] Rui Pereira,[1] Kostas Bourtzis,[1] and Marc J.B. Vreysen[1]

Mosquito-borne diseases represent a major threat to humankind. Recently, the incidence of malaria has stopped decreasing while that of dengue is increasing exponentially. Alternative mosquito-control methods are urgently needed. The sterile insect technique (SIT) has seen significant developments recently and may play an important role. However, testing and implementing SIT for vector control is challenging, and a phased conditional approach (PCA) is recommended, that is, advancement to the next phase depends on completion of activities in the previous one. We herewith present a PCA to test the SIT against mosquitoes within an area-wide-integrated pest-management programme, taking into account the experience gained with plant and livestock pests and the recent developments of the technique against mosquitoes.

Highlights

Recent progress on the development of the SIT package against mosquitoes allows envisaging its larger-scale deployment. Four phases are presented, that is, from preparatory activities to operational deployment, with some milestones highlighted that include go/no-go criteria.

Phase 0 is a preintervention phase in which stakeholder commitment is secured;

Phase I includes the collection of all relevant baseline data that are required to develop an appropriate intervention strategy against target mosquito populations;

Phase II includes all necessary activities for a successful small-scale field trial;

Phase III includes the necessary activities to upscale the intervention;

Phase IV corresponds to the area-wide deployment of the intervention (including the release of sterile mosquitoes) that is guided by an adaptive management approach and the evaluation of the SIT programme.

The Need for a PCA to Manage Mosquito Populations Using SIT

According to the World Health Organization (WHO), 17% of infectious diseases are vector-borne, leading to more than 700 000 deaths annually [1]. Mosquitoes account for a large part of these diseases of which malaria, dengue, and Zika are the most devastating. After a period of success in global malaria control in the past decades, no significant progress was made in the period 2015–2017, with an estimated 219 million cases in 2017, due to various threats including the spread of insecticide resistance of the mosquitoes hampering current control strategies [1]. In addition, dengue incidence has increased dramatically, with yearly new infections estimated at 390 million. Zika epidemics in 2015–2016 had dramatic effects in Latin America, and this disease still poses a major threat to human health [2]. Increased concerns about the impact of insecticides on living organisms and ecosystems is driving a growing number of countries to reduce the number of approved active chemicals and overall broad-spectrum insecticide outdoor applications. The resistance of mosquitoes to pyrethroids, the most commonly used class of insecticides, continues to increase. The WHO expresses the urgent need for alternative mosquito-control methods that should be added to existing tools, particularly against *Aedes* spp. [3].

Many alternative methods are being tested [4–6]. Among those methods, the **sterile insect technique** (**SIT**) (see Glossary) is an environment-friendly control method which has been used with great success against other insect pests; for example, the New World screwworm, *Cochliomyia hominivorax*, has been eradicated from Northern and Central America [7]; the tsetse fly *Glossina austeni* from Unguja Island, Zanzibar since 1997 [8]; the Mediterranean fruit fly, *Ceratitis capitata*, from Mexico and the programme is still ongoing to contain its reinvasion from Guatemala [9]; and the codling moth, *Cydia pomonella*, has been suppressed using SIT in British Columbia, Canada, for more than 25 years [10]. The development of SIT against mosquitoes has progressed rapidly in recent years with significant advances made with the development of genetic sexing strains [11–13], mass-rearing [14–19], sex separation [20,21], handling [22], radiation [23], quality control [24], and release technologies [25].

[1]Insect Pest Control Sub-programme, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, A-1400 Vienna, Austria
[2]CIRAD, UMR ASTRE CIRAD-INRA 'AnimalS, health, Territories, Risks and Ecosystems', Campus International de Baillarguet, 34398 Montpellier Cedex 05, France

*Correspondence:
j.bouyer@iaea.org (J. Bouyer).

Existing PCAs for testing genetically modified mosquitoes are not fully applicable to SIT [26,27]. As with plant [28,29] and livestock [30] pests, the implementation of SIT for vector control is challenging, and management intensive [31], and a PCA is therefore recommended to minimize the risks of failure. Although the different phases might be different for different insect groups, the principle remains identical, that is, support or advancement to the next phase is conditional on the completion of all (or most) activities in the previous phase, and the scope, expense, and commitment increase along the process [30]. Based on the accumulated experience of the Joint Food and Agriculture Organization of the United Nations (FAO)/International Atomic Energy Agency (IAEA) Insect Pest Control Subprogramme in developing and providing technical advice and assistance in implementing SIT projects against various insect pests worldwide, we present a proposal for a PCA to assess the feasibility, potential large-scale deployment, and effectiveness of SIT, as a component of an **area-wide-integrated pest management** (**AW-IPM**) [29,32] approach against mosquitoes. Because most of this approach derives from experience gained and concepts used to manage plant and livestock pests, we use the more general term IPM, although **integrated vector management** (**IVM**) is included within this definition.

Our proposal is graphically depicted in Figure 1A. The proposed phases are (0) preintervention, (I) baseline data collection, (II) small-scale field trial, (III) preoperational, and (IV) operational. Testing sites currently implementing SIT against mosquitoes, alone or in combination with the incompatible insect technique (IIT) are mapped by phase in Figure 1B. For each of these phases it is necessary to identify relevant and committed stakeholders who are well informed and fully understand the different steps in the decision-making process for implementing these different phases. Capacity building is also required at each step of the PCA to provide all the necessary skills and expertise for informed decision making and implementation.

Phase 0. Preintervention

Political will and government commitment, including financial support, are essential prior to the initiation of any programme that follows the PCA against mosquitoes. Ideally, any government commitment for integrated mosquito control that includes an SIT component, be it in the pilot trial or operational phase, should be accompanied by a national or regional structure, with the necessary budget, infrastructure, and dedicated trained staff (Table 1, Key Table). The implementation cost of the PCA is incremental, and mitigating risks and/or having milestones conditioning progressive investments will certainly be more attractive to potential donors and investors.

During this phase, the regulatory pathway authorizing release of radiation-sterilized male mosquitoes should be identified and, in some countries, it might have to be developed by the relevant authorities.

Previous SIT pilot trials and operational programmes have clearly shown that such efforts require both managers, who are responsible for the implementation of the programme, and researchers, who carry out operational problem-solving research. Whereas managers should be the driving force of the programme, staff with experience in insect rearing and implementing the field components are needed for technical support [30]. Community support is critical for the success of any SIT trial, especially for the operational phase, and therefore, a communication plan should be developed and be an integral part of such initiatives. For example, in the SIT project against *Aedes albopictus* in La Réunion, communication with the beneficiary populations was initiated from the beginning of the programme (Phase 0), more than 5 years before the first release of sterile males. Of equal importance is the evaluation of the pilot trials/operational programmes that will be based on assessing the vector population dynamics and disease incidence before,

Glossary

Area-wide-integrated pest management (AW-IPM): integrated pest management (see IPM below) applied against an entire pest population within a delimited geographic area [32], with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area. The concept of 'area-wide' refers primarily to a total population in a delimited area, the influence of migration/dispersal on its dynamics, and its ecological relationships within its ecosystem [32]. Area-wide eradication or control refers to elimination or control of a total, discrete, circumscribed population. AW-IPM is a concept of preventive suppression of a mobile insect pest species throughout its geographic range, rather than reactive field-by-field control [29].

Clusters: clusters can be geographical areas (e.g., sectors of a large city), communities (e.g., villages), administrative units (e.g., district, region), institutions (e.g., schools), health facilities, or households. In randomization, clusters are randomly assigned to either control or intervention groups [54].

Integrated pest management (IPM): the careful consideration of all available pest-control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM initially emphasizes the growth of a healthy crop with the least possible disruption in agroecosystems and encourages natural pest-control mechanisms [32]. It was thereafter largely applied to the integrated control of vector species like tsetse [30] where it includes –but is larger than – the IVM definition below.

Integrated vector management (IVM): a rational decision-making process to optimize the use of resources for vector control [60]. The aim of the IVM approach is to contribute to achievement of the global targets set for vector-borne disease control by making vector control more efficient, cost-effective, ecologically sound, and sustainable. Use of IVM helps vector-control programmes to find and use more local evidence, to integrate interventions where appropriate, and to collaborate within the health sector and

during, and after implementation and monitoring the temporal and spatial changes. Thus, the existence of national or regional monitoring systems of entomological and epidemiological data on mosquito-borne diseases will be a great asset for testing and assessing the feasibility of the strategy. Moreover, it is important to collect and compile all historical data on existing mosquito vector species, with a focus on 'local data' as mosquitoes are known to adapt frequently to local ecological situations, and this must be accounted for when designing the next phase. As an example, *Ae. albopictus* populations are structured by habitat in Réunion Island [33], and they outcompete *Aedes aegypti* [34], which is now limited to a few rural sites whereas this species is generally considered as urban.

Phase I. Baseline Data Collection

Implementing an AW-IPM programme with an SIT component against certain mosquito species is not possible everywhere. As an example, the production level of sterile male mosquitoes will have to be proportional to the size of the target area, the local density of the wild population, and the target sterile-to-wild male ratio [35]. Also, if multiple anopheline vectors are involved in malaria transmission in the target area, implementing a field trial that includes the release of sterile males of one species may not make sense. As a consequence, a technical feasibility study, based on baseline data, is necessary prior to the initiation of a trial that will require the collection of entomological (spatial and temporal characterization of wild populations), epidemiological (transmission patterns and dynamics of disease occurrence), socioeconomic, and environmental baseline data.

A risk assessment, to identify risks and containment strategies, is also required in this phase. Any risk that cannot be mitigated might prevent the programme from progressing to the next phase, for example, the risk of releasing disease-transmitting female mosquitoes in case no proper sex-separation technique is available for the target species.

The selection of appropriate field sites is a crucial step and should always be done within an AW-IPM context [36]. Target areas should ideally be isolated and the relationship between the target population and the surrounding ones be taken into consideration. As an example, the selection of a non-isolated target population will require the implementation of buffer areas to reduce immigration from neighbouring populations [37]. Dispersal capacity of females will need to be accounted for in the design of such a buffer zone, as the immigration of gravid females into the release area will reduce the level of induced sterility in the native mosquito population. In such buffer areas, the same IPM strategy may be applied as that in the target area, but the impact of the latter will be moderated by immigrating mosquitoes. The minimum size of the target and buffer areas will determine the minimum required production of sterile males for the field trial [38].

Once the target area has been identified, baseline data should be collected longitudinally in order to characterize the phenology and distribution of the target vector populations [39]. This should be carried out by staff who are knowledgeable in mosquito taxonomy, field data collection and analysis, and the use of standard monitoring tools. Once the control operations start, the population dynamics change, and the density of the target insect population will decline and may reach levels that are difficult to detect with existing trapping devices. Interpretation of zero catches is then subjected to modelling probabilities, which is possible only when the population dynamics has been characterized [40]. The collected data will be used to formulate a control strategy and to adjust the dimensions and borders of the target area. The entomological monitoring system (particularly adult traps) should allow trapping and differentiating sterile from wild males through marking [41] or molecular techniques [42,43].

with other sectors, as well as with households and communities.

Randomized controlled trial (RCT): in an RCT, individuals or clusters (cluster-randomized controlled trial) are randomly allocated to receive either intervention or control. Intervention and control groups are then followed up for the outcome of interest [54].

Semi-field conditions: entomological trials conducted in large-scale cages placed in a realistic environment, either outside or in a greenhouse with controlled humidity, temperature, and light mimicking outside climatic conditions. See [48] for a reference trial.

Step-wedge cluster randomized controlled trial: 'an alternative to parallel cluster trial designs, which are commonly used for the evaluation of service delivery or policy interventions delivered at the level of the cluster. The design includes an initial period in which no clusters are exposed to the intervention. Subsequently, at regular intervals (the 'steps') one cluster (or a group of clusters) is randomised to cross from the control to the intervention under evaluation. This process continues until all clusters have crossed over to be exposed to the intervention. At the end of the study there will be a period when all clusters are exposed. Data collection continues throughout the study, so that each cluster contributes observations under both control and intervention observation periods. It is a pragmatic study design, giving great potential for robust scientific evaluations that might otherwise not be possible.' [57].

Sterile insect technique (SIT): according to the International Standards for Phytosanitary Measures No. 5, SIT is a 'Method of pest control using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species'.

If not available, an insectary has to be set up and the local mosquito population colonized [44]. This will allow: (i) routine maintenance of the mosquito colony [45], (ii) if possible, the development of a genetic sexing strain [12], (iii) characterization of the life history traits of the strain, (iv) an assessment of the vector competence of the strain that may be conducted by specialized partners or subcontractors, and (v) an assessment of potential insecticide resistance in the colonized strain. In addition, easy access to an irradiation facility with a credible dosimetry system is required as this will enable the development of dose–response curves and the determination of the irradiation dose conferring the desired level of sterility [46,47]. A very important component is the study of the mating propensity and competitiveness of the sterile males in

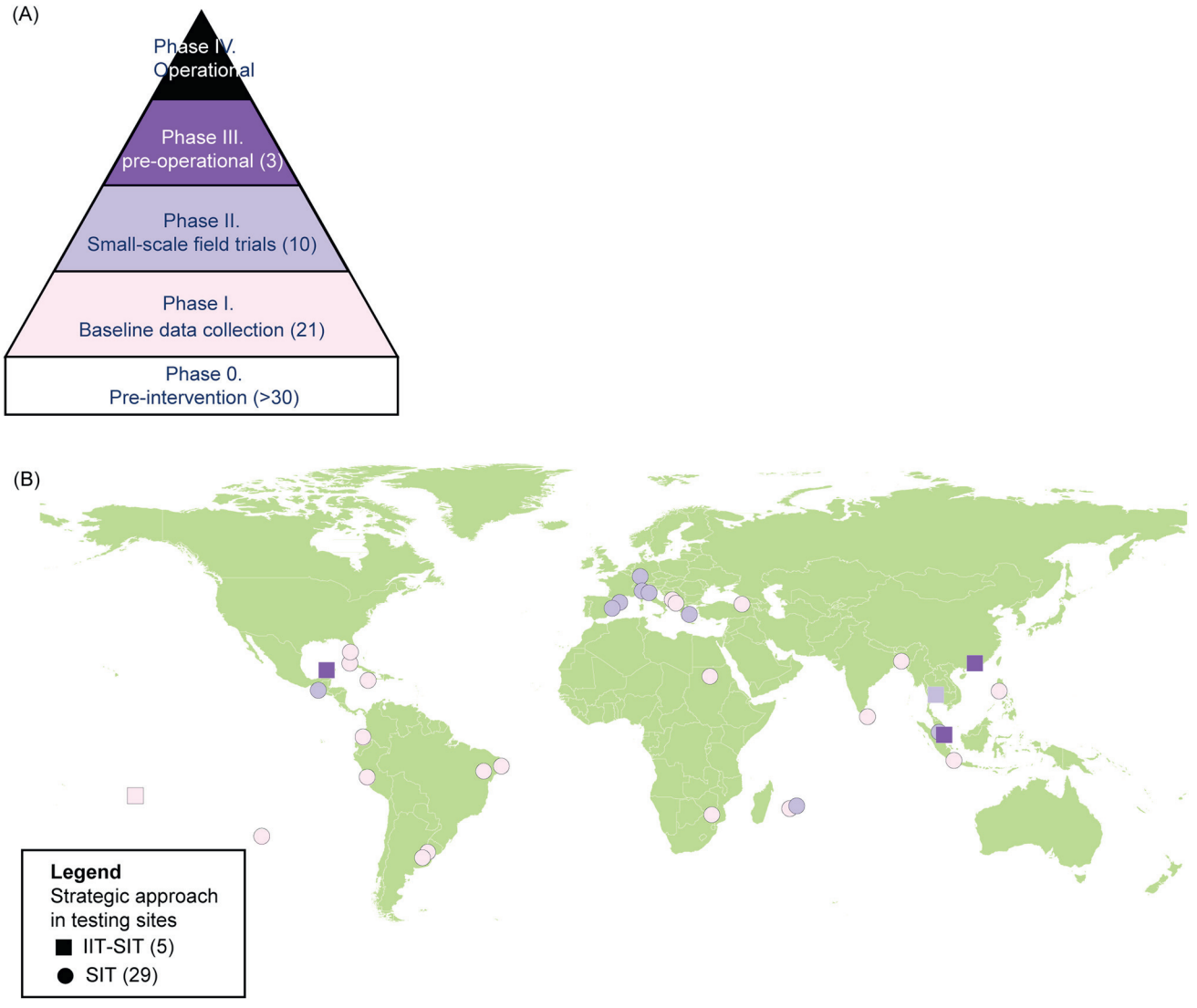

Figure 1. Schematic Representation of the Proposed Phased Conditional Approach (PCA) and Location of the Pilot Sites in Each Phase. (A) The pyramid symbolizes the amount of innovation related to operational research that is needed in the different phases, whereas the volume of activities and investment will, overall, grow in the opposite way. Commitment of the stakeholders will be necessary in all phases, and capacity-building and technology transfer will be specific to each phase. Testing site numbers in each phase are presented in brackets. (B) Distribution of testing sites implementing the sterile insect technique (SIT) against mosquitoes, some of them in combination with the incompatible insect technique (IIT–SIT). Testing site numbers in each strategy are presented in brackets. Phase 0 sites are not shown on the map.

Key Table

Table 1. Milestones and Indicators to Implement a Mosquito Population Management Strategy That Includes an SIT Component

Phase	Milestones	Indicators
Phase 0. Preintervention	Structure or programme dedicated to mosquito control	Number of staff dedicated to mosquito control
	National budget dedicated to test SIT as a new mosquito-control method[a]	Amount of money/year
	Regulatory pathway authorizing release of sterile male mosquitoes[a]	Regulatory pathway identified
	Monitoring system for entomological and epidemiological data at the country scale	Historical entomological and epidemiological data available
	Historical data on the knowledge of the existing mosquito species compiled[a]	Available publications and reports
Phase I. Baseline data collection	Training in mosquito taxonomy and surveillance[a]	Number of staff trained
	Training in mark–release–recapture[a]	Number of staff trained
	Training on irradiation, mass-rearing, handling, and release[a]	Number of staff trained
	Risk analysis of SIT conducted	Risk identified and quantified
	Monitoring systems for entomological data in the selected field site established[a]	Recent data on the phenology of the target populations available
		Density of the target population measured along the different seasonal periods
		System to mark, trap, and differentiate sterile from wild males
	Monitoring systems for epidemiological data in the selected field site established	Recent data on disease prevalence and incidence available
	Colonization of a local strain[a]	Insectarium available
		Local strain colonized and characterized
		Genetic sexing strain colonized or other efficient sexing method available
	Irradiation capacity established[a]	Access to an irradiator warranted
		Dose–response curve established, and dose inducing at least 99% sterility identified
		Competitiveness of sterile males quantified in semi-field conditions and Fried index close to 1
	MRR conducted[a]	Competitiveness of sterile males quantified in field trials and Fried index upon 0.2
		Survival and dispersal of sterile males quantified
		Dispersal of wild females quantified
		Needs in terms of sterile males per hectare per week quantified
	Communication strategy[a]	Communication plan available
	Technical and economic feasibility of the pilot trial assessed	Published data and reports
	External review	Expert recommendation
Phase II. Small-scale field trials	Complementary suppression methods identified[a]	Efficacy of complementary methods assessed
	Pilot field trial designed[a]	Pilot field trial plan including entomological and

(continued on next page)

Table 1. (continued)

Phase	Milestones	Indicators
		epidemiological monitoring
	Regulatory pathway identified[a]	Authorization to release sterile male mosquitoes obtained
	Communication strategy implemented[a]	Communication activities conducted
	Production (or import) capacity of sterile males established[a]	Number of sterile males produced (or imported) per week superior to the requirements considering the field site selected and defined release frequency
	External review	Expert recommendation
Phase III. Preoperational	Establishment/access to mass-rearing, irradiation, and release facilities[a]	Number of sterile males produced (or imported) weekly superior to the needs and reliability of the production well established
	Methods for mass-rearing, irradiation, handling, and release available	Irradiation, mass-rearing, handling & release SOPs available
	Communication strategy[a]	Communication activities intensified
		Community acceptance/ownership
	Cost of the operational programme	Cost-effectiveness of mosquito SIT, and complementary measures quantified
	Quality-control established[a]	QC SOPs available and validated
	Planning of the operational phase	Management plan and structure established
Phase IV. Operational	Management[a]	Adaptive management implemented
		Strong daily communication between field, mass-rearing and management teams
	Irradiation, mass-rearing, handling, and release strategies[a]	Irradiation, mass-rearing, handling & release SOPs applied
	Quality-control strategy[a]	QC SOPs applied
	Monitoring of entomological and epidemiological impacts	Entomological and epidemiological impacts quantified
	Monitoring of release of sterile males[a]	Post-release data on sterile males available (survival, ratio)
	Costing of the operational programme	Cost-effectiveness of IPM strategy monitored and improved through adaptation to the reduced target mosquito population
	External evaluation	Periodic external reviews implemented

[a]These milestones are mandatory to proceed to the next phase while others can be completed at a later phase. Most indicators are qualitative but some are quantitative: for example, an absence of a communication strategy or a field competitiveness of sterile males below 0.2 does not allow implementing a pilot trial because it is increasing exponentially the release rate of sterile males necessary to achieve elimination [61].

walk-in field cages under **semi-field conditions** [48]. All of the above will require well trained staff in laboratory rearing, sex separation, irradiation and quality control based on standard operating procedures (SOPs).

At the end of this phase, staff trained in handling, marking, transport, and release of sterile male mosquitoes need to be available to implement a mark–release–recapture (MRR) protocol based on available SOPs but adapted to local conditions [49]. The objectives of the MRR are to assess the dispersal, survival, and field competitiveness of sterile males (Table 1) as well as to obtain estimates of the actual density of the target population [50]. These parameters will be useful for defining the

release frequency and density of the sterile males, as well as the maximum distance between release points or lines. The results of these tests will be reviewed by experts, during an external review meeting, who will make recommendations whether or not to proceed with a small-scale SIT field trial.

Phase II. Small-Scale Field Trials

Before releasing sterile males, a communication plan needs to be developed to inform the beneficiary communities, assess their perception about the planned trials and, more importantly, solicit their support. This support is essential to the success of the field trial and can extend from simply granting access to their private properties for monitoring activities or for door-to-door campaigns to an active participation through the removal of larval habitats when the selected integrated strategy includes source reduction.

In most ecological settings, mosquito-control methods may need to be integrated with the SIT to reduce the mosquito population to the lowest possible levels before the release of sterile males is initiated. This prerelease population suppression (reduction) is related to the inverse density-dependent efficiency of the SIT (Figure 2). This is one of the many aspects that makes SIT a powerful tool in modern integrated insect pest-control strategies [51]. Before implementing an SIT field trial it is therefore essential to assess the efficiency of the available control methods at a small scale, especially when the target population is present at high densities throughout the year. The same suppression strategy may be applied in the control area to assess the incremental impact of the SIT in the treated area. In some areas (e.g., Palearctic region), it will be possible to use seasonal reduction of the density due to climate conditions to plan SIT instead of additional control methods.

A detailed plan for the small-scale field trials needs to be developed that includes all steps, from the

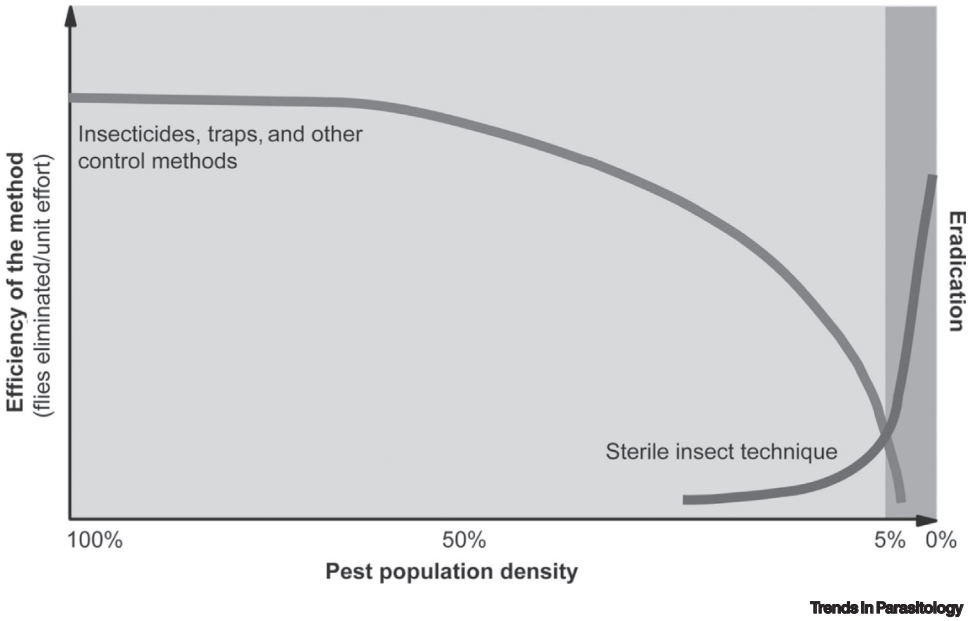

Figure 2. Optimizing the Efficiency of a Vector-Control Intervention. The sterile insect technique (SIT) is more effective at low population densities and is ideal to combine with other control tactics, such as insecticide treatments or source reduction, that are more effective at high population densities. The inverse density-dependent properties are related to the exponential increase of the sterile-to-wild male ratio in each subsequent generation when the target population is reduced. This figure was designed for the particular case of tsetse flies but is fully relevant for mosquito control. Source: [51].

production of the sterile males to the entomological (and epidemiological) monitoring of the vector and the disease. Depending on the size of the target area and purpose of the trial (e.g., prevention in nonendemic countries), it will not always be feasible to monitor epidemiological indicators (see below). This detailed plan will be used to obtain all necessary authorizations to initiate releases (including import permits if production of sterile males is outsourced and a risk assessment analysis if required). A colony of the target species, of adequate size, needs to be available to deliver the required numbers of sterile males, considering the density of the target population (with or without presuppression) or the required numbers of sterile males can be procured from an external supplier (Table 1).

At the end of the field trial, the analysis of the results will define the minimum number of sterile males required for release per surface area for the continued suppression of the targeted wild female population. This will also provide information on the cost effectiveness of the SIT component of an operational programme, provided that the benefits of upscaling the intervention can be quantified [43].

After the field trials, another external review is recommended to get an expert opinion on whether the programme should continue or not. In addition, the stakeholders will have to decide whether they will incorporate the SIT into a larger-scale AW-IPM programme.

Phase III. Preoperational

After deciding to upscale the intervention, programme management will have to establish or warrant access to adequate mass-rearing and mass-sterilization facilities. This will generally require large investments in which the private sector can play a major role. Cost effectiveness of such an upscaling will have to be demonstrated to convince all the stakeholders involved.

Specific storage, handling, transport, release, and monitoring strategies will have to be established, taking into account the upscaling of the production of sterile males and the areas to be treated. Quality-control procedures will be a key activity that should be applied throughout the chain of production and release of sterile males.

At this point, community outreach activities will need to be intensified in the target areas to ensure the acceptance by the general public of a larger-scale operational AW-IPM programme with an SIT component [52].

Finally, a management plan and structure will have to be developed and established to efficiently manage the upscaled AW-IPM programme since the efficient management of all available resources, including personnel, is a key factor for its successful implementation.

Phase IV. Operational

A larger-scale operational AW-IPM programme that includes mass-rearing, handling, irradiation, aerial releases, and monitoring should be implemented using an adaptive management scheme [30]. Preferably, the management set-up should be autonomous and independent from any government structure to retain the required flexibility [31]. In such a scheme, the management structure takes decisions according to the feedback information derived from the continuous monitoring activities in the target areas.

The objective of the programme should be well defined from the beginning (desired vector suppression level, elimination, reduction in disease incidence, etc.) to facilitate evaluation. A regular (weekly) analysis of field data and an assessment of the impact of the releases on the

entomological and epidemiological indicators are necessary to take corrective measures in case of problems. Evaluation of the intervention (both entomologically and epidemiologically) should be conducted by a team independent from the one implementing control in order to provide confidence in the results.

Monitoring will be conducted based on entomological and epidemiological indicators different from those used in the small-scale field trial in order to improve the efficiency and cost-effectiveness; for example, during the small-scale field trials, monitoring of the mosquito population will include traps to sample adults, but during the operational phase the monitoring will mainly use ovitraps or ovi-sticky traps as the deployment of many adult traps will be cost prohibitive. Details of entomological and epidemiological outcomes to be monitored during each phase can be found in [53]. Feedback mechanisms will be implemented between field, mass-rearing, and management teams to ensure a quick circulation of the information and the overall involvement of all staff. Regular external reviews will be organized to monitor and improve the efficiency of the programme.

Concluding Remarks

The PCA presented in this paper has been derived from operational AW-IPM programmes that include an SIT component in other insect models [30]. In addition, the recent knowledge acquired from ongoing small-scale field SIT trials against mosquitoes have been taken into account [25]. The proposal presents some similarities with the 'stages in development of a new vector-control product' scheme used by the WHO Vector Control Advisory Group (VCAG) that assesses the public health value of new vector-control interventions [54] starting from the laboratory (Phase I) via semi-field and small-scale field studies (Phase II) to large-scale programmes (Phase III). However, the presented PCA has the specific objective of guiding governmental institutions to implement an integrated strategy, including the SIT, and focuses not only on disease/epidemiological impact but also on the entomological impact as well. As an example, it will consider additive, synergistic, or antagonist interactions between vector-control techniques when designing the IPM strategy but will not focus on independently quantifying the impact of each control tactic presented as a product. The focus is more on development than research, even if operational research is mandatory throughout the processes.

The SIT against selected insect pests and disease vectors can be successful only when the technology is applied on an area-wide basis [37]. One of the major remaining challenges will be to set up appropriate epidemiological trials to measure the impact of this technology on mosquito-borne diseases (see Outstanding Questions). Current strategies, including **randomized controlled trials**, permit the reduction of contamination between **clusters** that might occur if individuals within the same community receive different interventions [54]. However, if the mosquito population within a cluster is not isolated, for example, in the case of a district within a big city, it will receive contamination not only from other clusters but also from all the surrounding areas through immigration of infected female mosquitoes [55]. For example, in Rio de Janeiro, Brazil, females of both *Ae. aegypti* and *Ae. albopictus* were found to disperse at least 800 m in 6 days, potentially spreading viruses [56]. If a given cluster is much smaller than the female dispersal distance, the SIT may well be very efficient in suppressing the resident mosquito population whereas no, or a scarce, epidemiological signal will be measured with such a design [38]. In Figure 3 we present a theoretical design that can be considered as a **step-wedge cluster randomized controlled trial** [57] within an intervention programme using the wave principle against a pest population with a continuous distribution [37]. Although other step-wedge designs were recently implemented to test other vector-control interventions

Outstanding Questions

Can we use entomological indicators to assess the epidemiological impact of new vector-control techniques?

How can we obtain reliable measures of female mosquitoes' dispersal and of its epidemiological significance? How can this dispersal be accounted for when designing epidemiological trials? In other words, how can we measure the epidemiological impact of the SIT against mosquitoes at a cluster level when this technique can only be successful when applied on an area-wide basis?

How can we measure the efficiency of SIT when it is used in combination with other methods within an integrated strategy? How can we account for additive, synergistic, or antagonist interactions between control techniques?

How can we assess the immediate and long-term benefits of control tactics that are applied on an area-wide basis impacting human health?

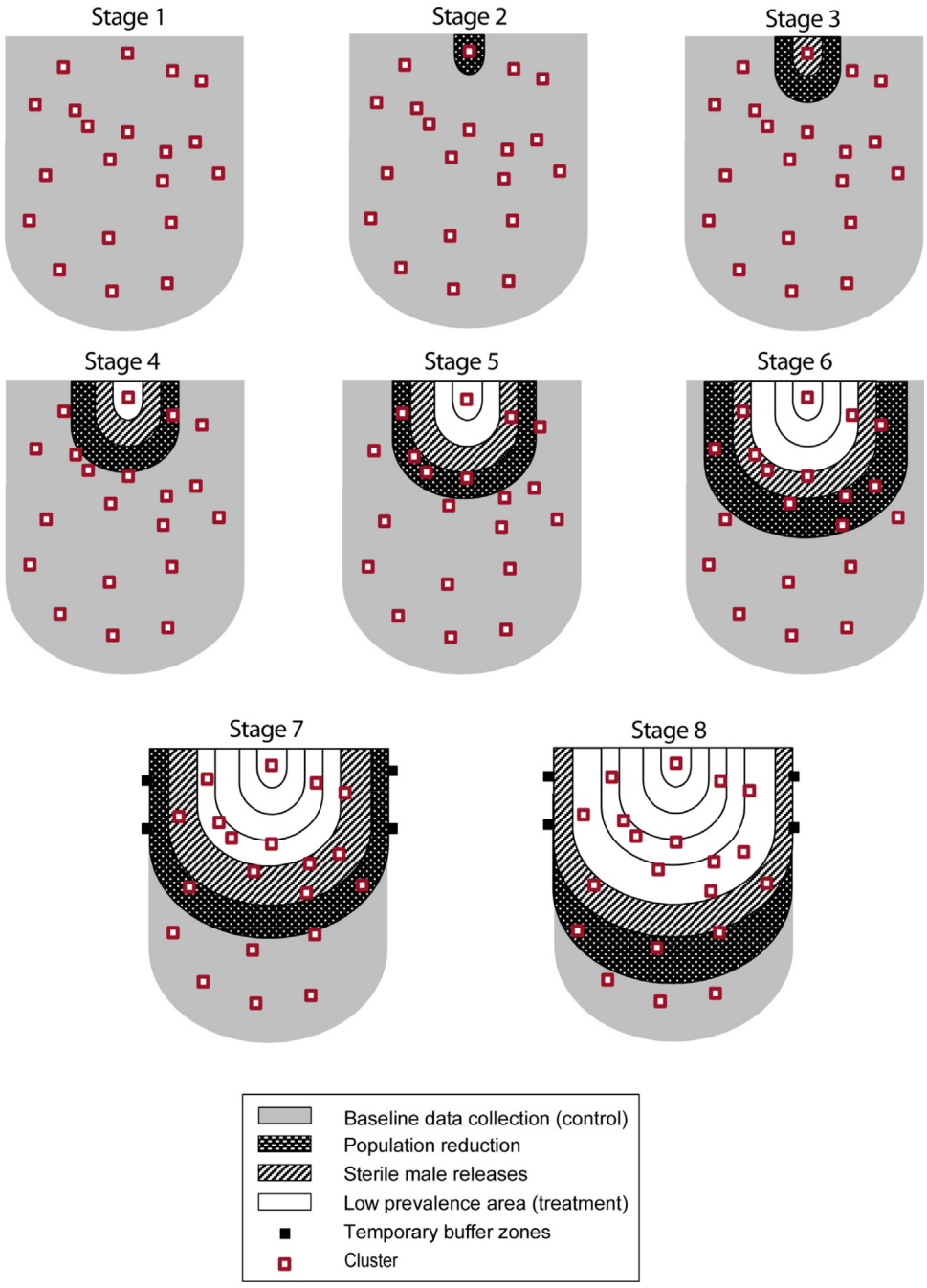

Figure 3. Wave-Based Randomized Epidemiological Trial. Proposal based on the diagram of different phases of an area-wide-**integrated pest management** (AW-**IPM**) programme using the sterile insect technique (SIT) according to the wave principle against a pest population with a continuous distribution [37]. In this theoretical example, the intervention develops along a multidirectional front in stages 1–6, until full production capacity of sterile males is reached. Beginning in stage 7, the intervention continues along a one-directional front, and requires the establishment of temporary buffer zones. Clusters are exposed to various treatment regimens as in a step-wedged epidemiological trial [62] and only the light-grey (control) and white (treatment) areas are in a 'stable' stage. All other areas are in transient situations. In this theoretical situation, the top of the diagram represents a natural barrier (e.g., seashore). Each ring, and the temporary buffer areas, should be designed to account for female dispersal.

[58,59], our design has never been implemented to date but may theoretically mitigate the impact of dispersal of infected females. Another possibility would be to select clusters on isolated islands, as was recently done in China, to demonstrate the ability of the combined incompatible and SITs to suppress two populations of *Ae. albopictus* [43]. It is, however, unlikely to find an area allowing enough randomization units with such characteristics.

In 2019, the IAEA and WHO issued a guidance framework for assessing the feasibility of using the SIT as a mosquito-control tool and thus reducing or eliminating *Aedes*-borne diseases [53]. This guidance covers all processes for decision support – including risk assessment and regulatory aspects, technical aspects (e.g., insect mass rearing), entomological and epidemiological indicators, as well as community involvement, cost-effectiveness and programme monitoring and evaluation. However, some questions are still pending and deserve further research (see Outstanding Questions).

Acknowledgements

This project received funding from the FAO/IAEA and the European Research Council under the European Union's Horizon 2020 - Research and Innovation Framework Programme (grant agreement no. 682387–REVOLINC). This article reflects only the authors' views, and the agency is not responsible for any use that may be made of the information it contains.

References

1. World Health Organization (2018) *World Malaria Report 2018*, WHO
2. Mayer, S.V. *et al.* (2017) The emergence of arthropod-borne viral diseases: a global prospective on dengue, chikungunya and zika fevers. *Acta Trop.* 166, 155–163
3. World Health Organization and UNICEF (2017) *Global Vector Control Response 2017–2030*, WHO and UNICEF
4. Flores, H.A. and O'Neill, S.L. (2018) Controlling vector-borne diseases by releasing modified mosquitoes. *Nat. Rev. Microbiol.* 16, 508–518
5. Bourtzis, K. *et al.* (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
6. Lees, R.S. *et al.* (2015) Back to the future: the sterile insect technique against mosquito disease vectors. *Curr. Opin. Insect Sci.* 10, 156–162
7. Wyss, J.H. (2006) Screwworm eradication in the Americas. *Ann. N. Y. Acad. Sci.* 916, 186–193
8. Vreysen, M.J.B. *et al.* (2000) *Glossina austeni* (Diptera: Glossinidae) eradicated on the Island of Unguja, Zanzibar, using the sterile insect technique. *J. Econ. Entomol.* 93, 123–135
9. Enkerlin, W. *et al.* (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. *Fla. Entomol.* 98, 665–681
10. Thistlewood, H. and Judd, G.J.R. (2019) Twenty-five years of research experience with the sterile insect technique and area-wide management of codling moth, *Cydia pomonella* (L.), in Canada. *Insects* 10, 292
11. Papathanos, P.A. *et al.* (2018) A perspective on the need and current status of efficient sex separation methods for mosquito genetic control. *Parasit. Vectors* 11, 654
12. Lutrat, C. *et al.* (2019) Sex sorting for pest control: it's raining men! *Trends Parasitol.* 35, 649–662
13. Ndo, C. *et al.* (2018) Isolation and characterization of a temperature-sensitive lethal strain of *Anopheles arabiensis* for SIT-based application. *Parasit. Vectors* 11, 659
14. Somda, N.S.B. *et al.* (2019) Insects to feed insects-feeding *Aedes* mosquitoes with flies for laboratory rearing. *Sci. Rep.* 9, 1–13
15. Maïga, H. *et al.* (2019) Reducing the cost and assessing the performance of the adult mass-rearing cage for the dengue, chikungunya, yellow fever and Zika vector, *Aedes aegypti* (Linnaeus). *PLoS Negl. Trop. Dis.* 13, e0007775
16. Mamai, W. *et al.* (2019) Black soldier fly (*Hermetia illucens*) larvae powder as a larval diet ingredient for mass-rearing *Aedes* mosquitoes. *Parasite* 26, 57
17. Mamai, W. *et al.* (2018) Optimization of mass-rearing methods for *Anopheles arabiensis* larval stages: effects of rearing water temperature and larval density on mosquito life-history traits. *J. Econ. Entomol.* 111, 2383–2390
18. Mamai, W. *et al.* (2019) The efficiency of a new automated mosquito larval counter and its impact on larval survival. *Sci. Rep.* 9, 7413
19. Zhang, D. *et al.* (2018) Establishment of a medium-scale mosquito facility: tests on mass production cages for *Aedes albopictus* (Diptera: Culicidae). *Parasit. Vectors* 11, 189
20. Zacarés, M. *et al.* (2018) Exploring the potential of computer vision analysis of pupae size dimorphism for adaptive sex sorting systems of various vector mosquito species. *Parasit. Vectors* 11, 656
21. Bellini, R. *et al.* (2018) Exploring protandry and pupal size selection for *Aedes albopictus* sex separation. *Parasit. Vectors* 11, 650
22. Culbert, N.J. *et al.* (2019) Investigating the impact of chilling temperature on male *Aedes aegypti* and *Aedes albopictus* survival. *PLoS ONE* 14, e0221822
23. Yamada, H. *et al.* (2019) Identification of critical factors that significantly affect the dose-response in mosquitoes irradiated as pupae. *Parasit. Vectors* 12, 1–13
24. Culbert, N.J. *et al.* (2018) A rapid quality control test to foster the development of genetic control in mosquitoes. *Sci. Rep.* 8, 16179
25. Lees, R.S. *et al.* Potential impact of integrating the sterile insect technique into the fight against disease-transmitting mosquitoes. In *Sterile Insect Technique. Principles and Practice in Area-wide Integrated Pest Management* (2nd edn) (Dyck, A.V. *et al.*, eds), CRC Press (in press).
26. National Academies of Sciences, et al *et al.* (2016) *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*, National Academies Press
27. WHO (2014) *Guidance Framework for Testing of Genetically Modified Mosquitoes*, WHO

28. Pereira, R. *et al.* (2019) Area-wide management of fruit flies using the sterile insect technique. *IOBC-WPRS Bull.* 146, 75–78
29. Klassen, W. and Vreysen, M.J.B. Area-wide integrated pest management and the sterile insect technique. In *Sterile Insect Technique. Principles and Practice in Area-wide Integrated Pest Management* (2nd edn) (Dyck, A.V. *et al.*, eds), CRC Press (in press).
30. Vreysen, M.J.B. *et al.* Area-wide integrated management of a *Glossina palpalis gambiensis* population in the Niayes area of Senegal: a review of operational research in support of an operational phased conditional approach. In *Area-wide Integrated Pest Management: Development and Field Application* (Hendrichs, J. *et al.*, eds), CRC Press (in press).
31. Vreysen, M.J.B. *et al.* (2007) Lessons from area-wide integrated pest management (AW-IPM) programmes with an SIT component: an FAO//IAEA perspective. In *Area-wide Control of Insect Pests* (Vreysen, M.J.B. *et al.*, eds), pp. 723–744, Springer
32. Hendrichs, J. *et al.* (2007) Area-wide integrated pest management (AW-IPM): principles, practice and prospects. In *Area-wide Control of Insect Pests, from Research to Field Implementation* (Vreysen, M. *et al.*, eds), pp. 3–34, Springer
33. Delatte, H. *et al.* (2013) Evidence of habitat structuring *Aedes albopictus* populations in Réunion Island. *PLoS Negl. Trop. Dis.* 7, e2111
34. Bagny Beilhe, L. *et al.* (2013) Ecological interactions in *Aedes* species on Reunion Island. *Med. Vet. Entomol.* 27, 387–397
35. Bellini, R. *et al.* (2013) Pilot field trials with *Aedes albopictus* irradiated sterile males in Italian urban areas. *J. Med. Entomol.* 50, 317–325
36. Vreysen, M. *et al.* (2007) *Area-Wide Control of Insect Pests, from Research to Field Implementation*, Springer
37. Hendrichs, J. *et al.* (2005) Strategic options in using sterile insects for area-wide integrated pest management. In *Sterile Insect Technique. Principles and Practice in Area-wide Integrated Pest Management* (Dyck, V.A. *et al.*, eds), pp. 563–600, Springer
38. Barclay, H.J. *et al.* (2011) A conceptual model for assessing the minimum size area for an area-wide integrated pest management program. *Int. J. Agron.* 2011, 409328
39. Iyaloo, D.P. *et al.* (2019) Seasonal changes in the distribution and relative abundance of the dengue vector, *Aedes albopictus*, in two villages in Mauritius: implications for a sterile release programme. *Afr. Entomol.* 27, 218–227
40. Barclay, H.J. and Hargrove, J.W. (2005) Probability models to facilitate a declaration of pest-free status, with special reference to tsetse (Diptera: Glossinidae). *Bull. Entomol. Res.* 95, 1–11
41. Johnson, B.J. *et al.* (2017) Use of rhodamine B to mark the body and seminal fluid of male *Aedes aegypti* for mark-release-recapture experiments and estimating efficacy of sterile male releases. *PLoS Negl. Trop. Dis.* 11, e0005902
42. Pagabeleguem, S. *et al.* (2016) A molecular method to discriminate between sterile and wild tsetse flies during eradication programmes that have a sterile insect technique component. *PLoS Negl. Trop. Dis.* 10, e0004491
43. Zheng, X. *et al.* (2019) Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature* 572, 56–61
44. Maïga, H. *et al.* (2018) *Guidelines for Colonization of Aedes Mosquito Species*, FAO-IAEA, p. 11
45. Maïga, H. *et al.* (2017) *Guidelines for Routine Colony Maintenance of Aedes Mosquito Species*, FAO-IAEA, p. 18
46. Balestrino, F. *et al.* (2014) γ Ray dosimetry and mating capacity studies in the laboratory on *Aedes albopictus* males. *J. Med. Entomol.* 47, 581–591
47. Yamada, H. *et al.* (2019) Guidelines for small scale Irradiation of mosquito pupae in SIT programs. In version 1.0. *Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,* FAO/IAEA, p. 38
48. Bellini, R. *et al.* (2013) Mating competitiveness of *Aedes albopictus* radio-sterilized males in large enclosures exposed to natural conditions. *J. Med. Entomol.* 50, 94–102
49. Bouyer, J. *et al.* (2019) *Guidelines for Mark-Release-Recapture Procedures of Aedes Mosquitoes*, FAO-IAEA
50. Iyaloo, D.P. *et al.* (2019) Dispersal and survival of radio-sterilised male *Aedes albopictus* Skuse (Diptera: Culicidae) and estimation of the wild populations in view of an sterile insect technique programme in Pointe des Lascars, Mauritius. *Int. J. Trop. Insect Sci.* 39, 63–72
51. Feldmann, U. and Hendrichs, J. (2001) *Integrating the Sterile Insect Technique as a Key Component of Area-wide Tsetse and Trypanosomiasis Intervention*, Food and Agriculture Organization
52. Paterson, G. *et al.* (2019) Peri-urban community attitudes towards codling moth trapping and suppression using the sterile insect technique in New Zealand. *Insects* 10, 335
53. IAEA and WHO (2019) *Guidance Framework for Testing the Sterile Insect Technique (SIT) as a Vector Control Tool against Aedes-Borne Diseases*, IAEA and WHO
54. Wilson, A.L. *et al.* (2015) Evidence-based vector control? Improving the quality of vector control trials. *Trends Parasitol.* 31, 380–390
55. Kittayapong, P. Combined sterile insect technique and incompatible insect technique: concept, study design, experience and lesions learned from a pilot suppression trial in Thailand. In *Area-wide Integrated Pest Management: Development and Field Application* (Hendrichs, J. *et al.*, eds), CRC Press (in press).
56. Honório, N.A. *et al.* (2003) Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. *Mem. Inst. Oswaldo Cruz* 98, 191–198
57. Hemming, K. *et al.* (2015) The stepped wedge cluster randomised trial: rationale, design, analysis, and reporting. *BMJ* 350, h391
58. Tiono, A.B. *et al.* (2018) Efficacy of Olyset Duo, a bednet containing pyriproxyfen and permethrin, versus a permethrin-only net against clinical malaria in an area with highly pyrethroid-resistant vectors in rural Burkina Faso: a cluster-randomised controlled trial. *Lancet* 392, 569–580
59. Hiscox, A. *et al.* (2016) Mass mosquito trapping for malaria control in western Kenya: study protocol for a stepped wedge cluster-randomised trial. *Trials* 17, 356
60. WHO (2012) *Handbook for Integrated Vector Management*, World Health Organization
61. Pleydell, D. and Bouyer, J. (2019) Biopesticides improve efficiency of the sterile insect technique for controlling mosquito-driven dengue epidemics. *Commun. Biol.* 2, 201
62. Brown, C.A. and Lilford, R.J. (2006) The stepped wedge trial design: a systematic review. *BMC Med. Res. Methodol.* 6, 54